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Bioluminescence and environmental light drive the visual evolution of deep-sea shrimp (Oplophoroidea)



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Light functions as the universal language in the deep sea (>200 m). Both bioluminescent emissions and downwelling light sources dimly illuminate the water column and can drive sensory system evolution. In pelagic environments, vertically migrating animals can experience drastic changes to their lighting environment across depth, subjecting them to unique selective pressures, possibly to distinguish between changes in ambient light and bioluminescent sources. Here we show that visual opsin diversity across a group of variable vertical migrators -bioluminescent deep-sea shrimp belonging to the Superfamily Oplophoroidea- is higher among species who migrate to shallower waters with more variable light conditions. Further, we provide evidence for adaptive visual evolution among species who have evolved an additional mode of bioluminescence (photophores), including positive selection for a putative mid-wavelength sensitive opsin that may facilitate light source discrimination. Diversification of this opsin appears to play an important role in the visual ecologies of photophore-bearing shrimp with its diversification in Oplophoroidea likely playing a critical role in the fitness and evolutionary success of this group.

Light is the universal language in the deep sea, functioning as a shared sensory signal and cue for a diverse array of animals. This illuminated signal ranges from downwelling surface light to offensive and defensive signaling using bioluminescence. Downwelling light is attenuated with depth and effectively absent by 1000 meters (m) where point sources of bioluminescent (biologically produced) light dominate. In marine habitats, the use of light as a signal ranges from avoidance behavior, diurnal changes¹ and/or functioning as a depth gauge², to predator avoidance via confusion or mimicry (i.e., bioluminescent smoke screen or camouflage), prey attraction (i.e., luminescent lure) and communication between conspecifics. Particularly in the light-limited environments of the deep sea (>200 m), this language of light is necessary for survival in the dark. Here, the primary sources of light are downwelling blue light ($\lambda_{\text{max}} \sim 475 \text{ nm}$)^{3,4} and bioluminescence (most commonly between 460 and 490 nm)^{5–7}. Regardless of habitat type, light serves as a crucial driver of sensory system evolution (e.g., Nilsson⁸; Härer et al.⁹; Sondhi et al.¹⁰; Fröhlich et al.¹¹; Schott et al.¹²) due to the fundamental role it plays in the behavioral responses, fitness, and life history of animals.

Light detection is achieved by a range of photosensitive structures of varying complexity, ranging from simple photoreceptors containing light sensitive opsin protein(s) (e.g., photoresponsive neurons of molluscs¹³ and

larval eye spots of various marine zooplankton¹⁴) to more complex eyes capable of spatial vision (e.g., compound eyes of arthropods and camera-type eyes of cephalopods and vertebrates). Eyes are convergently evolved organs with layered arrangements of photoreceptors, screening pigments and optical lenses¹⁵. The evolution of complex eyes enabled image resolution and acuity, facilitating more complex visually guided behaviors including monitoring and regulating self-motion in relation to surrounding objects (e.g., anticollision behavior, prey and predator detection and mate recognition) and habitat suitability^{8,16,17}. In pelagic environments, where animals navigate both horizontal and vertical (depth) ranges, some organisms traverse depth zones with drastic changes in the light regime among other changing environmental variables (e.g., temperature and pressure). Acute environmental changes are especially prominent in animals that participate in diel vertical migration, where organisms move from deep to shallower waters at night to feed under the relative cover of darkness and return to deeper depths during the day for protection. However, the extent of this vertical movement, or migration strength, can vary greatly as does the subsequent changes to the light environment. Therefore, the visual systems of these migrating organisms may be under unique selective pressures, to distinguish the vertical changes in the ambient light field during migration, as well as between bioluminescent light sources (congener vs. predator).

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Differences among the visual pigment sensitivities of photoreceptors are largely due to amino acid differences among the associated opsin(s) – a seven-transmembrane-domain G protein-coupled receptor protein bound to a retinal-based chromophore. Visual opsin proteins effectively regulate light transduction, subjecting them to adaptive evolution. Previous phylogenomic studies have found multiple opsin duplications and losses across invertebrates (e.g., Porter et al.^{18,19}; Henze and Oakley²⁰), with as many as 46 opsin copies in the arthropod *Daphnia pulex*²¹. Photoreceptors containing opsins of variable wavelength sensitivities enable spectral tuning and possibly color discrimination^{22–25}, both of which can provide an adaptive advantage when navigating dynamic environmental conditions like those experienced during diel vertical migration.

Though non-adaptive forces (e.g., drift) can influence random evolution of duplicated opsin sequences, sometimes resulting in non-functional pseudogenes, adaptive forces (i.e., positive selection) can lead to repeated patterns of opsin diversification and retention^{26–29} which has been observed previously in systems with variable light intensity (e.g., Sander and Hall³⁰; Hauser et al.³¹) and among nocturnal animals (e.g., Tierny et al.³²; Warrant³³; Sondhi et al.¹⁰). Corresponding genetic differences that lead to variations in sensory structures and/or function can cause variations in behaviors that rely on that sensory information¹⁷. It is therefore possible that visual opsin evolution and diversification influenced changes to visually guided behaviors, like vertical migration, enabling deep-water animals to occupy new, shallower depth ranges and light environments. This likely led to changes in fitness that selection could act upon which potentially influenced the migratory strength of deep-sea fauna. As various deep-sea animals also evolved the ability to bioluminesce, it is also probable that fitness associated with this trait further shaped opsin evolution as it also alters the surrounding light environment.

Bioluminescent deep-sea shrimp in the Superfamily Oplophoroidea are vertical migrators that have evolved one (secretory) or two (secretory and light organ) unique modes of bioluminescence. Among the ~70 species, all presumably have a bioluminescent spew^{5,34} that is emitted as a visual signal when they are startled, functioning as a smoke screen to help them evade predation^{5,34–36}. This includes members of the family Acanthephyridae (Spence Bate, 1888) belonging to seven genera according to the World Register of Marine Species (WoRMS): *Acanthephyra* (A. Milne-Edwards, 1881), *Ephyrina* (Smith, 1885), *Heterogenys* (Chace³⁷), *Hymenodora* (Sars, 1877), *Kemphya* (Chace³⁷), *Meningodora* (Smith, 1882) and *Notostomus* (A. Milne-Edwards, 1881) (Fig. 1). Other members belong to the family Oplophoridae (Dana, 1852) comprised of three genera: *Janicella* (Chace³⁷), *Oplophorus* (H. Milne Edwards, 1837) and *Systellaspis* (Spence Bate, 1888)^{38–40}. A major difference among the two families is that some oplophorids have evolved an additional mode of bioluminescence—cuticular light organs called photophores that function in counterillumination and possibly conspecific signaling and light detection⁴⁰. Counterillumination is a form of dynamic camouflage where animals precisely match downwelling light to make themselves less conspicuous to predators below and this behavior has been experimentally demonstrated in other deep-sea shrimp⁴¹. Accurate mimicking of this light is necessary for survival, making it probable that photophore-bearing shrimps have evolved visual sensitivities to a more diverse range of wavelengths to properly counterilluminate in changing light environments. Previous physiological, spectrophotometric and behavioral evidence show that eyes of some photophore-bearing oplophorids possess a near-ultraviolet (390–410 nm) photopigment in addition to the common blue-green photopigment of many deep-sea species^{42–47} possibly to distinguish between different types (secretion vs. photophore) of bioluminescence^{42–44}. For example, photophores emit light that has a slightly different wavelength and narrower spectral bandwidth than that of secretory luminescence^{5,42} and all photophore-bearing oplophorids that have been studied to date possess an additional near-UV photopigment^{42,46}. However, previous phylotranscriptomic studies^{40,48} have identified an even more diverse putative opsin repertoire among photophore-bearing oplophorids, suggesting their visual sensitivities may be even more complex.

It is possible that opsin diversity is further augmented by the shallower migration depths achieved by some species, and the subsequent variability in light exposure and light sources in their surrounding environment. Both shrimp families reside in deep waters and undergo diel vertical migration, though they differ in their migration strength^{49,50}. For some, this migration can be upwards of 1000 m across diverse light environments (i.e., from the bathypelagic or midnight zone >1000 m (bioluminescent light only) to the light-abundant epipelagic or photic zone <200 m), whereas others migrate shorter distances across relatively analogous light conditions (i.e., within the mesopelagic or light-limited “twilight” zone, 200–1000 m). Known as the largest migration on the planet in terms of biomass, these journeys are necessary to find food and mates (due to swarming behavior of many species, per observation) in shallower waters. Various photophore-bearing species within Oplophoridae are considered strong migrators, migrating up to depths of ~100 m at night in the Gulf of Mexico (GoM) where downwelling moonlight is more abundant (including *Janicella spinicauda*, *Systellaspis debilis*, and *Oplophorus gracilirostris*) and UV light intensities are still biologically relevant (min. 200 m^{51,52}). Alternatively, many species of Acanthephyridae only migrate as shallow as the light-limited, mesopelagic between 450 and 750 m^{37,50,53–57} though some *Acanthephyra* spp. have been recorded in photic waters (<400 m). Therefore, aside from distinguishing between bioluminescent sources, a more diverse opsin repertoire may facilitate shallower migrations and successful counterillumination, both of which are crucial to survival.

Adaptive evolution among photophore-bearing oplophorids may be evident in the predicted sensitivities and diversity of their visual opsin repertoire. It is known that certain sites along the opsin sequence are involved in spectral tuning, where associated amino acid changes can shift the corresponding wavelength sensitivity of the opsin. Therefore, it is also probable that these opsins were subject to positive selection as oplophorids evolved bioluminescent light organs and diversified into new, shallower photic habitats. To address these questions, we sequenced the eyes of various species belonging to the superfamily Oplophoroidea with both single and dual-modes of bioluminescence and a diverse range of migratory behaviors and associated light environments. Using a phylotranscriptomic approach, we characterized the visual opsins retained across both families and estimated the ancestral opsin repertoire of Oplophoroidea. We hypothesized that opsin diversity would be higher among species with greater migratory strength due to exposure to more varied light conditions. We further hypothesized that evidence for positive selection exists among the opsin(s) of photophore-bearing species that are affiliated with their unique light-associated behaviors (i.e., counterillumination and/or signaling with conspecifics). Here, we provide evidence for adaptive evolution in the visual system of photophore-bearing species, including positive selection for a putative mid-wavelength sensitive opsin. Our findings also suggest that low opsin diversity is associated with photophore loss or reduction in oplophorids and/or ecological differences associated with reduced migratory strength and depth ranges restricted to deeper waters across this superfamily.

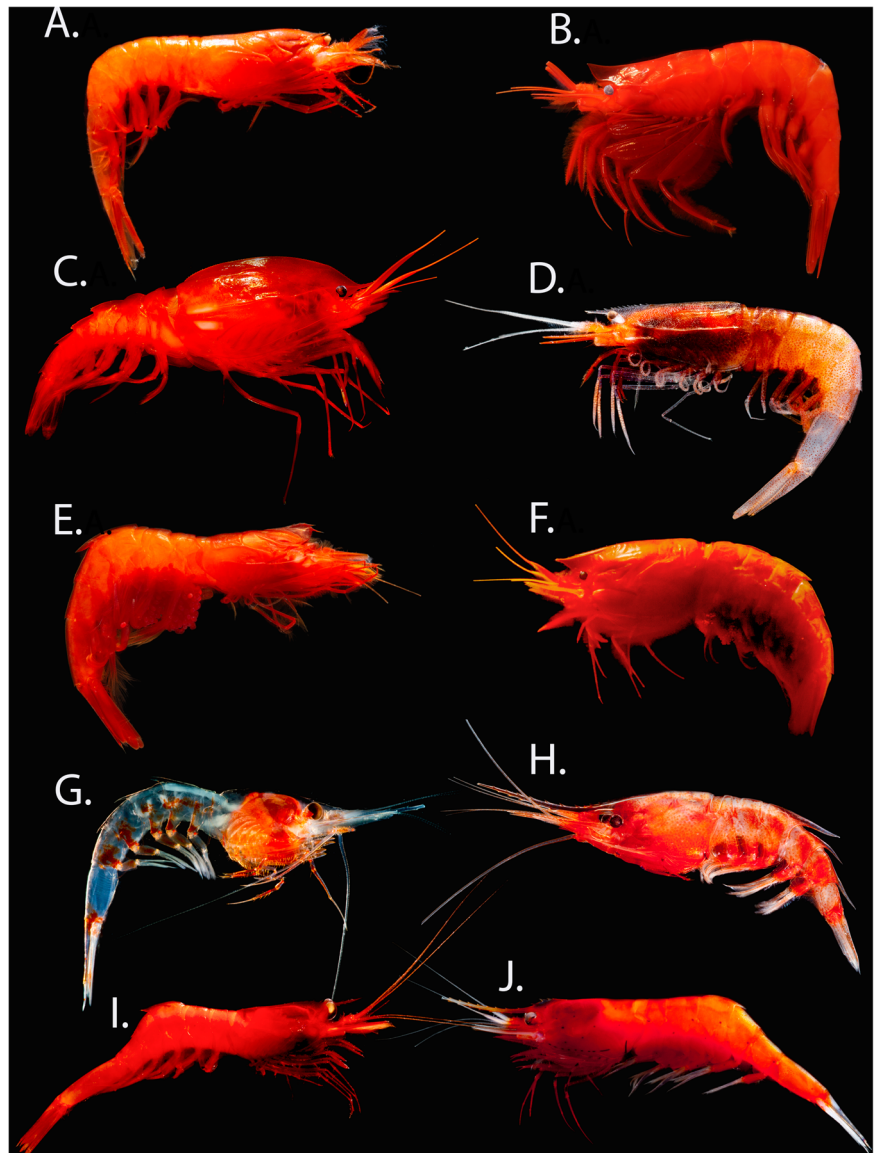
Results

Ten newly sequenced eye transcriptomes⁵⁸ were generated and assembled de novo for nine representatives of seven genera belonging to Oplophoroidea (Fig. 1), and the outgroup species *Nematocarcinus ensifer* (Table S1). The transcriptomes were supplemented with two additional oplophorid visual transcriptomes^{59,60} for downstream analyses, for a total of eight representative genera.

Oplophoroidea tree reconstruction

To investigate patterns of opsin evolution and signatures of selection across Oplophoroidea, particularly for species exhibiting different modes of bioluminescence (single vs dual mode) and migratory lifestyles (migrator vs. non-migrator; strong vertical migrator, weak vertical migrator), an Oplophoroidea tree ($n = 11$ species which included 8/10 accepted genera in WoRMS) was inferred from the transcriptome assemblies. This highly

Fig. 1 | Diversity of Oplophoroidea representing all genera in the study. A *Hymenodora gracilis*, B *Ephyrina benedicti*, C *Notostomus gibbosus*, D *Meningodora vesca*, E *Acanthephyra stylorotris*, F *Acanthephyra* sp., G *Janicella spinicauda*, H *Oplophorus graciliorotris*, I *Systellaspis cristata*, and J *Systellaspis debilis*. Modified from Collins and Bracken-Grisson⁷¹. Photograph credits: D. Fenolio.



supported Oplophoroidea tree was assembled from 622 single copy orthologs using *N. ensifer* as the outgroup ($n = 12$) (Fig. 2). The shrimps fall into two monophyletic clades by family, Oplophoridae and Acanthephyridae, with 100/100 (UFBoot/ShAlrt) percent support, with 100/100 support for all other nodes except one (98.9/97; *S. cristata* and *S. braueri*). The genera groupings are as follows: clade 1—*Janicella*, *Oplophorus*, *Systellaspis*, and clade 2—*Hymenodora*, *Ephyrina*, *Meningodora*, *Notostomus*, *Acanthephyra*.

Visual opsin diversity

The eye transcriptomes were used to characterize putative visual opsins in a phylogenetic context. Opsin diversity varied greatly within and between Oplophoridae and Acanthephyridae. Across both families, Acanthephyridae contained species with both the least ($n = 2$, *H. gracilis*; SWS2 and LWS2 only) and most putative opsin types ($n = 5$, *Acanthephyra* spp.) (Fig. 2, Fig. S1). Two putative SWS opsins (SWS1, SWS2) were recovered in *Acanthephyra* spp., in addition to the two MWS opsins (MWS1, MWS2) and LWS2 opsins previously recovered in oplophorid shrimps⁴⁸. The SWS1 opsin was not recovered in any other genus. *Meningodora mollis* appears to have only one MWS opsin (MWS1).

Less variation was observed in the opsins recovered from Oplophoridae, with at most four types recovered in the strongest vertical migrators (*J.*

spinicauda, *O. graciliorotris*, *S. debilis*), with three types recovered in other species (*Systellaspis* spp.). SWS opsins (SWS2) were only recovered in *J. spinicauda* and *O. graciliorotris*. Another potentially novel opsin type, described here as SWS/MWS due to its placement in between the SWS and MWS opsin clusters (Fig. S1), was recovered from *S. debilis* as well as the acanthephyrid, *M. mollis* and *N. ensifer* the outgroup species (Fig. 2). This opsin formed a separate well supported clade (100/100 support) (Fig. S1), removed from any other crustacean opsin recovered here or in the previously published r-opsin dataset.

Ancestral state reconstruction

Ancestral state reconstruction of the putative r-opsins (presence/absence of expression) was used to estimate the ancestral opsin state and patterns of opsin diversification across Oplophoroidea. Our findings suggest the most recent common ancestor (MRCA) of Oplophoroidea had a diverse opsin repertoire (minimum of $n = 4$ types) including one SWS, two MWS, and one LWS opsin (Fig. 2). This opsin repertoire was retained in the MRCA of Acanthephyridae and Oplophoridae, though with less certainty. Expression of this SWS opsin appears to have been lost at least two times across both families, in the genera *Systellaspis* and *Ephyrina* (Fig. 2). The MRCA of the genus *Acanthephyra* appears to have evolved an additional SWS-like opsin (SWS1) which was retained in the extant species present in this study, while

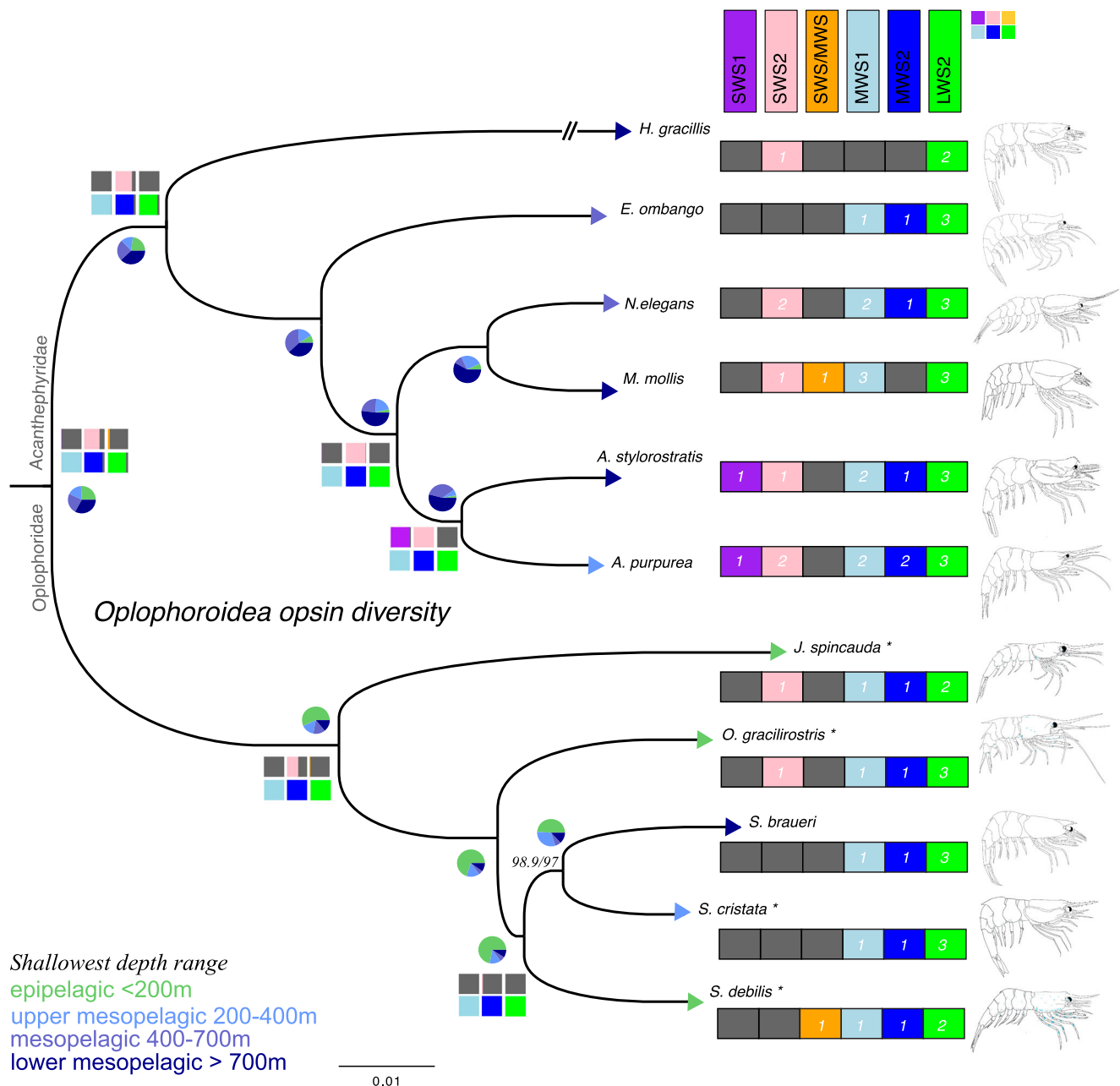


Fig. 2 | Phylogeny of Oplophoroidea with ancestral opsin and lighting environment states, and overall opsin diversity. Putative opsin type was determined based on PIA results and corresponding clade in the opsin gene tree, defined using wavelength categories of maximum absorption (short = SWS, short/mid = SWS/MWS, mid = MWS, long = LWS). Opsin diversity and total opsin (expression) count recovered in each species is shown on the right, belonging to the major families Acanthephyridae (top clade) and Oplophoridae (bottom clade), all nodes have 100/100 support unless noted. Asterisk signifies photophore-bearing species. Species were categorized based on the shallowest depth of their known range in the GoM

(=highest light presence or lowest light attenuation), as either epipelagic <200 m (green), upper mesopelagic 200–400 m (light blue), mesopelagic 400–700 m (dusky blue) or lower mesopelagic >700 m (dark blue); the depth category (color triangle) is shown at the phylogeny tips. Ancestral state reconstructions (ASR) of both opsins (squares) and lighting environment (circles) shown at select nodes were based on the ER model assuming equal rates of trait gain/loss. In the ASR analysis of opsins, a gray shaded box is indicative of absence of expression whereas the ratio of gray to color indicates the % support for the two character states.

the less common SWS/MWS opsin appears to have been more recently acquired in both families.

Species within Oplophoroidea inhabit highly diverse depth ranges, vertically migrating from abyssal depths where light is completely absent, to the light abundant epipelagic. As light availability can exert strong selective pressures on vision, ASR of lighting environment (Table S2) was performed across Oplophoroidea to gain a better understanding of how ancestral depth ranges and associated light availability may influence visual opsin diversity.

Our findings show that the MRCA of Oplophoroidea was equally likely to have migrated to the shallower/brighter (<400 m) or deeper/darker light fields (>400 m) (Fig. 2, Fig. S2, see “Methods” for additional details). The MRCA of the family Acanthephyridae has the highest combined probability of inhabiting a deeper range (>400 m) with a limited light environment, whereas the MRCA of Oplophoridae likely inhabited shallower depth ranges and or more varied light fields, vertically migrating to depths (<400 m) where downwelling light is more abundant.

Signatures of selection

Selection tests were performed to investigate whether the opsins (among other orthologs) recovered from the eyes of photophore-bearing species were more likely to be under positive selection than non-photophore-bearing species. PAML first tested differences between the rates of selection (ω —the ratio of nonsynonymous (dN) to synonymous (dS) nucleotide substitutions) discretely for each opsin type most commonly shared across Oplophoroidea (MWS1, MWS2, LWS2) for photophore- and non-photophore-bearing species. Branch models, used to detect whether photophore-bearing species, set as the foreground, were more likely to have different rates of selection (ω or dN/dS) relative to non-photophore-bearing species (background), revealed all three opsins had a significantly better fit when partitioned into two rates based on photophore presence, than one rate for all branches ($p = 0$, MWS1: $\omega_{\text{photophore}(p)} = 0.1272$, $\omega_{\text{none}(n)} = 0.1352$; MWS2: $\omega_p = 0.2156$, $\omega_n = 0.2563$; LWS2: $\omega_p = 0.2524$, $\omega_n = 0.2393$).

Site and branch-site models were used to test whether particular sites were under positive selection in photophore-bearing species. Site models can detect positive selection that is not foreground-specific (i.e., not specifically due to the presence of photophores), and tests whether any amino acid sites have different ω rates across all branches irrespective of photophore presence. The site model revealed significant sites under positive selection in both the MWS2 and LWS2 opsins ($\omega > 1$, $p = 0.0002$ and $1.47e-07$, respectively) (Table 1). No significant sites were found for MWS1.

Branch-site models were run to specifically address whether the opsins of photophore-bearing species were more likely to be under positive selection ($\omega > 1$) than non-photophore-bearing species and allowed for an ω value that is variable across particular sites and branches. This revealed significant amino acid sites under positive selection in photophore-bearing species for the MWS2 opsin only ($\omega > 1$, $p = 0.002$) (Table 2, Fig. 3). Additional tests partitioning by (1) family and (2) strong vertical migrators (<200 m; where light (i.e., moonlight) is more abundant), also revealed significant results for MWS2 ($\omega > 1$, $p = 0.002$, 0.003) as well as LWS2 ($\omega > 1$, $p = 0.008$, $1.13e-06$).

Additional tests for selection were run in HyPhy using models that examine differences in dN/dS rates in different sites among different branches, as opposed to PAML which looks for rates (ω) greater than 1. These models are thought to be more sensitive for detecting selection^{61–63} and yielded similar results to PAML as well as additional sites under putative selection among the opsins. Based on likelihood ratio tests, BUSTED detected evidence of episodic, diversifying selection ($\omega > 1$) in the MWS2 ($p = 0.01$) and LWS2 opsins ($p = 0.01$), with two to five sites yielding evidence ratios (site-level likelihood ratios) greater than 10 for positive selection, respectively (Table 2, Fig. 3). MEME found evidence of episodic positive/diversifying selection at six sites for MWS2 and 17 sites for LWS2, though these sites are not foreground (photophore-bearing species) specific (Table 2). Evidence of selection was not found for MWS1.

Selection tests (branch-site) conducted on the additional orthologs recovered from the eye transcriptomes ($n = 803$, excluding *N. ensifer*), to identify additional genes and associated pathways under putative selection

in photophore-bearing species, revealed 17 genes with $\omega > 1$, 12 of which had at least one significant site ($p < 0.01$) under selection according to the BEB analysis (Table S3). This included genes coding for ATP biosynthesis, insulin growth factors involved in regulating cell growth and signal transduction, and various mitochondrial proteins that function in electron transport, the mitochondrial respiratory chain complex, translation, ribosome biogenesis and rRNA processing, phosphate carrier proteins and transmembrane transporters (Table S3, Fig. S3). Between family-level comparisons revealed significant results for 17 genes, with 10 genes under putative selection for the shallowest migrators (<200 m) (Table S3, Fig. S3).

Discussion

This study provides a highly supported phylogenomic tree for the superfamily Oplophoroidea and traces visual opsin evolution across this superfamily to identify the visual proteins that were retained across various bioluminescent lineages. We provide evidence that the ancestral opsin repertoire of this diverse superfamily likely included opsins that were sensitive to a range of wavelengths, including short-, mid-, and long-wavelengths, which subsequently diversified or were lost in certain lineages with varied life history traits. Further, our results indicate adaptive evolution is presently working on a mid-wavelength sensitive opsin in lineages that have evolved photophores and whose common ancestor likely had an expanded range into shallower waters with varied light conditions. Taken together, our results suggest this mid-wavelength sensitive opsin enables light source discrimination (abiotic and/or biotic) in the deep-sea and facilitated the migratory lifestyle of these animals.

Opsin diversity across Oplophoroidea was highly variable, particularly for the family Acanthephyridae with opsin types ranging from 2 to 5 as opposed to Oplophoridae which ranged from 3 to 4. Visual opsins are generally divided into classes based on the wavelengths of light that they are sensitive to including ultraviolet light (UV) ranging from ~100–400 nm, and visible light (~400–700 nm) divided into subcategories of short- (SWS), mid- (MWS), or long- (LWS) wavelengths. Approximate wavelengths of maximal sensitivity of the opsins recovered here, based on sequence similarities to opsins with measured absorbance spectra, are approximately: 334 nm for SWS1 and 383 nm for SWS2 (UV-shifted) based on their proximity to *Neogonodactylus oerstedii* UV1 and UV2 opsins⁶⁴ (Fig. S1), 480 nm (blue-green light) for the MWS opsins based on two MWS opsins that were co-expressed in the crab *Hemigrapsus sanguineus*⁶⁵ and 512–520 nm (green light) for the LWS2 opsins based on measurements from *Holmesimysis costata* and *Neomysis americana*⁶⁶. Within each shrimp family, the observed variability in opsin diversity is likely attributed to an amalgam of ecological differences and associated visually guided behaviors, including hunting strategy, photophore loss or reduction with diminished capacity for signaling and camouflaging behaviors, and vertical migratory strength and associated light environments. Within Acanthephyridae, *Acanthephyra* spp. had the most diverse opsin repertoire ($n = 5$) including the putative SWS1 (UV sensitive) opsin not recovered in any other genus across the superfamily. Among Oplophoroidea, *Acanthephyra* is the most diverse genus comprised of ~27 species^{67,68}, some of whom are strong vertical migrators like *A. purpurea* which migrates as shallow as the upper mesopelagic (~300 m). High rates of diversification within the genus as certain species expanded into shallower depth ranges may have further influenced their visual evolution.

Multiple putative opsin losses also occurred in both families relative to the predicted ancestral opsin repertoire of Oplophoroidea. For Acanthephyridae, multiple losses (of expression) were observed for species with the deepest upper range limits (lower mesopelagic, >700 m): *H. gracilis*, *M. mollis*, and *E. ombango*. We predict the multiple putative opsin losses in *H. gracilis* (MWS1 and MWS2) eyes may be correlated with impaired vision or loss of function. Eyes within this species are small and lack pigment (appear yellow instead of black/brown) and other species of *Hymenodora* have a reduced cornea, few to no facets and no pigment⁶⁹. Compared to other deep-dwelling species, *M. mollis* also has reduced eyes and like *H. gracilis*, appears to have subsequently lost the MWS2 opsin under adaptive selection in

Table 1 | Sites that have a probability higher than 95% or 99% (bold) to be under positive selection in photophore-bearing species according to the BEB analysis under the site model M2a

Opsin	Pr($\omega > 1$)	ω (mean $\pm$ S.D.)
MWS1	–	–
MWS2	178	6.347 $\pm$ 1.882
LWS2	243	3.261 $\pm$ 0.832
	359	3.262 $\pm$ 0.830
	224	3.275 $\pm$ 0.819
	225	3.269 $\pm$ 0.823

The ω values (dN/dS rates) associated with each significant site are listed. Sites are numbered based on the first sequence in each alignment.

Table 2 | Amino acid sites in photophore-bearing species identified by (1) PAML, with a probability higher than 95% (or >99% in bold) to be under positive selection ($\omega > 1$) according to the BEB analysis under the Branch-site Model A, (2) BUSTED-MH with an evidence ratio threshold >10 for $\omega > 1$ and by (3) MEME that have significant LRT values (adj. $p < 0.05$) for $\omega > 1$, but are not foreground (photophore-bearing) specific

Opsin	Hypothesis tested	PAML	BUSTED	MEME
MWS1	Photophore-bearing	–	–	83, 92, 134, 161, 165, 187, 252, 310, 368
	Oplophoridae	–	–	
	Shallow migrators (<200 m)	–	–	
MWS2	Photophore-bearing	178, 199, 283	160, 199	124, 155, 160, 199, 350
	Oplophoridae	178	160, 199	
	Shallow migrators (<200 m)	283	–	
LWS2	Photophore-bearing	–	70, 225, 229, 237, 279	12, 60, 70, 106, 139, 150, 161, 168, 185, 224, 225, 229, 233, 279, 283, 309, 364
	Oplophoridae	–	70, 225, 229, 237, 279	
	Shallow migrators (<200 m)	125, 243	70, 225, 229, 237	

Sites are numbered based on the first sequence in each alignment.

photophore-bearing oplophorids. While it is possible that genes coding for MWS2 are present in *M. mollis* and not expressed at the time of sampling for this study, its absence among multiple individuals supports this predicted loss. Similar reduction of visual system plasticity, specifically loss of a UV-shifted SWS opsin was observed among neotropical cichlid fishes evolving under light-limited conditions³¹. It was hypothesized that reduced selective constraint acting on this opsin led to pseudogenization and loss³¹ and it is probable that a similar scenario is occurring here. However, it is possible that the transcriptomes are incomplete and deeper sequencing could recover additional opsins, though the assemblies are from eye tissue only where visual proteins are expected to be more abundant. Other studies have recovered a similar diversity of opsins in other crustacean groups within similar levels of BUSCO completeness (i.e., amphipods, Drozdova et al.⁷⁰). Future genomic data is needed for this group to confirm whether the loss of opsin expression, or its absence across multiple individuals reported here, can truly be attributed to opsin loss.

The mid-wavelength opsin MWS2 was also differentially expressed during vertical migration in the oplophorid *S. debilis*⁴⁸, signifying its functional importance for this photophore-bearing shrimp while navigating shallower waters at night to feed and possibly signal conspecifics. If MWS2 plays a vital role in navigating dynamic light environments, it is likely that selective forces not only facilitated the diversification of this opsin but also its retention in shallower migrators. Given *M. mollis* remains in relatively deep, dark waters (725–5000 m), MWS2 was likely lost due to neutral evolutionary processes (e.g., drift). MWS2 may not be essential in deep environments where only bioluminescent point sources of light exist but may offer a fitness advantage when species must differentiate between light sources (downwelling vs. bioluminescence) and/or varied light conditions. To the best of our knowledge, *M. mollis* appears to have a novel SWS/MWS opsin-like sequence newly recovered in this study and also found in the shallow migrating oplophorid *S. debilis*. While this opsin is lowly expressed in both species, its expression across multiple species (including the out-group *N. ensifer*) and replicates suggests it is not a result of assembly error (Table S4). All species of *Systellaspis* sampled in this study appear to have lost the ancestral (UV-shifted) opsin SWS2 found among related photophore-bearing species and most acanthephyrids. However, the SWS/MWS opsin was recovered for *S. debilis*, which is the only species of the three included to migrate into the epipelagic (shallowest distributions for the GoM among all species of *Systellaspis*) and have numerous photophores across their entire body. Both *S. cristata* and *S. braueri* have reduced or absent photophores, respectively, while *S. debilis* has purple-pigmented photophores⁷¹, which indicates additional, variable selective pressures acting on this genus. Together, these results suggest that this SWS/MWS opsin may be a non-functional copy indicative of a more recent loss of function event in both *M. mollis* (MWS2) and *S. debilis* (SWS2). In the case of *Systellaspis* spp., this

putatively non-functional opsin could have been subsequently lost in other lineages (e.g., *S. braueri* and *S. cristata*) due to neutral processes (e.g., drift). However, SWS/MWS opsins were recently recovered in other distantly related bioluminescent, deep-sea shrimps belonging to the family Sergestidae (Dana, 1852) (unpublished data). Examining opsin diversity among additional species across Oplophoroidea, and more specifically among these two genera, could help determine whether SWS/MWS is a true paralog, though additional protein-level investigations are needed to further elucidate functionality.

The opsin repertoires recovered in this study show some similarities but do not directly correspond to past physiological methods used to characterize the visual sensitivities of Oplophoroidea. Previous electrophysiological studies identified a dual-peak of spectral sensitivity among several photophore-bearing oplophorids including *J. spinicauda*, *O. gracilirostris*, and *S. debilis*. These peaks were in both the blue and UV-shifted wavelength range, while only a single peak in the blue-light range was recovered for several acanthephyrid species⁴². Within Oplophoridae, we are also finding expanded UV-shifted opsin diversity as seen in previous studies, but unlike previous studies we are also finding expanded diversity within Acanthephyridae. Recent in situ hybridization investigations of MWS1, MWS2, and LWS2 are also in accordance with our expression profile within photophore-bearing shrimp⁷². This study revealed ocular expression of MWS2 in the singular R8 (R=reticular) cell while LWS2 was localized to R1–7 of *J. spinicauda*, *O. gracilirostris*, and *S. debilis* with the first evidence for colocalization and expression of both MWS1 and MWS2 in the R8 cell of at least one species (*J. spinicauda*)⁷². MWS (1 or 2) opsin expression was not recovered in the single acanthephyrid representative (*A. purpurea*), though additional replication is needed to rule out false negatives. These results highlight the difficulty in using physiological methods to detect absorbance spectrums of opsins specific to the R8 cell. In addition, overlap among the absorbance spectra of the MWS opsins (1 and 2) and putative SWS opsin(s) recovered here, may be coupling and/or masking the absorbance spectra of additional opsins and obscuring the true opsin diversity of these shrimps. Additional immunohistochemistry work, particularly on the SWS and MWS opsins recovered among many of the species present in this study is needed to decipher function and localization of these putative blue and UV-shifted opsins.

Our results suggest the MRCA of this bioluminescent superfamily had at least four spectral opsin types: LWS, MWS (MWS1 and MWS2) and SWS2, similar to the ancestral photoreceptor diversity posited for vertebrates (discussed in Baden⁷³). It is therefore likely that the spectral sensitivity of the MRCA of Oplophoroidea was shifted beyond the typical 480 nm λ_{max} and most likely covered a broader range of light, like the visual adaptations observed among many deep-sea teleost fish (reviewed in de Brusserolles et al.⁷⁴). Retention of these ancestral opsins was then dependent

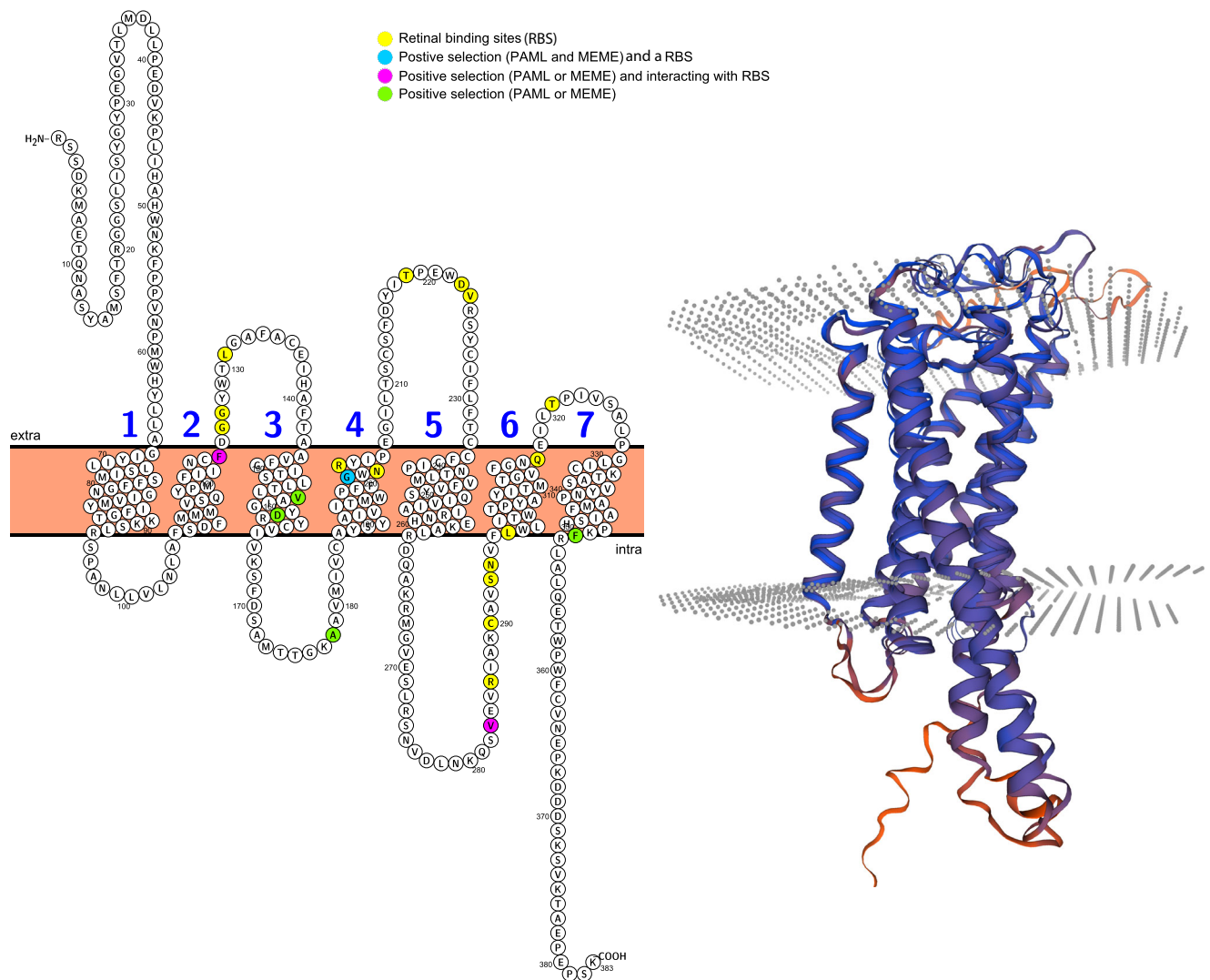


Fig. 3 | MWS2 predicted protein structure and sites under positive selection in photophore-bearing species. Amino acid sites with significant evidence for positive selection from PAML and/or HyPhy (MEME) were mapped onto the predicted protein structure of the MWS2 opsin. Transmembrane helix predictions were

obtained via Phobius and Swiss-model was used for 3D protein structure modeling using the jumping spider opsin X-ray structure with putative retinal binding site (RBS) information as a template (6i9k.1.A). Sites interacting with retinal were within 4 Å of the RBS.

on lineage-specific life history traits. However, Baden⁷³ hypothesized that these different photoreceptor types did not specifically evolve to serve color vision but instead functioned as parallel feature channels to differentially support visual-motor programs⁷³, and color vision was a secondary benefit. It is possible that these different photoreceptor types convergently evolved among invertebrates, including the MRCA of Oplophoroidea, to help visual navigation in the marine environment and across diverse depths and light regimens. The LWS2 opsin in this study is highly conserved across the superfamily which would support its general-purpose function with regard to visual resolution⁷³. Like vertebrates, color vision likely evolved secondarily and may have facilitated the occupation of new, shallower niches.

The ancestral depth range of the MRCA of this superfamily is still unknown, though it is possible it spanned a wide range of depths including both shallower, photic and deep, aphotic environments. However, the ancestral range of the MRCA of oplophorid shrimps appears to be largely shallow (<400 m) relative to its sister clade, with the MRCA of acanthephyrids predicted to have a deeper ancestral range (>400 m). Subsequent diversification due to both neutral forces and adaptive pressures acting on shallower migrators may have facilitated photophore evolution among oplophorids.

In Oplophoroidea, it is possible that the ancestral SWS opsin functioned to improve visual sensitivity, similar to the specialized foreground

system discussed by Baden⁷³. Over time, it may have enhanced the visual acuity of strong migrators and photophore-bearing species to facilitate light source discrimination and congener recognition. A putative loss in *S. cristata* and *S. braueri* would therefore not be surprising given the reduction and or loss of photophores entirely and their overall deeper nightly distribution. Ancestral MWS opsins were further hypothesized to play a regulatory or suppressive role on the feedback from the other (LWS & SWS) systems⁷³. Differential expression of MWS2 in *S. debilis* during diel vertical migration⁴⁸, supports this hypothesis in terms of spectral tuning, regulating both spectral sensitivity and resolution during migration. It is further possible that selective pressures are acting on this regulatory system due to the unique visual fields associated with photophore bioluminescence and signaling. Is it also possible that gene duplication events leading to multiple MWS opsin types, including one that is more UV shifted, further facilitated spectral sensitivity. This could have led to some redundancy, thereby facilitating SWS/UV opsin loss in some species that no longer have photophores (or reduced).

In this study, we provide evidence of adaptive evolution acting on a mid-wavelength opsin of photophore-bearing oplophorids, with predicted sensitivity to blue light. While both the bioluminescent spew characteristic of this deep-sea shrimp superfamily and the photophore emissions of

oplophorids are both blue, the emission spectra is slightly different. Photophores emit light that has a slightly longer wavelength ($\lambda_{\text{max}} \sim 475$ nm) and narrower spectral bandwidth (half bandwidth ~ 55 nm) than that of secretory luminescence ($\lambda_{\text{max}} \sim 460$ nm, half bandwidth ~ 70 nm)^{6,34}. In addition, some oplophorids also have pigmented photophore lenses (reviewed in Collins and Bracken-Grissom⁷¹) that may narrow the emission wavelength and shift the lambda max of the light emission. A recent study that examined the ecological predictors of eye size evolution in other deep-sea shrimp found light organ type was the only significant correlate, suggesting bioluminescence likely plays an important role in vision and possibly conspecific signaling (Schweikert, Thomas et al.⁷⁵). Therefore, it is possible that the MWS2 sequence divergence observed in this study, specifically at the sites identified under positive selection, have facilitated spectral tuning among oplophorids to differentiate the photophore emissions of conspecifics. This is most evident among the sites interacting with the retinal binding pocket, which can change the chemical environment surrounding retinal (e.g., Piechnick et al.⁷⁶). It is also possible that transmembrane sites under selection here (Fig. 3) are influencing the compressibility of this opsin among strong migrators exposed to highly variable pressure conditions, similar to what was identified for the r-opsins of cephalopods from high-pressure environments⁷⁷ and believed to influence the adaptative evolution of crustacean opsins with respect to spectral tuning⁶⁶. Putative selection acting on this MWS2 opsin over time following photophore evolution in Oplophoridae, may have influenced shallower migration ranges for some species (e.g., *J. spinicauda*, *O. gracilirostris* & *S. debilis*). Diversification of this mid-wavelength opsin could have enabled strong migrators to better navigate different light fields offering a fitness advantage as they could more easily find food and mates. This would suggest that the selective pressures associated with mate recognition and or migration/camouflage may act both directly and indirectly on opsin evolution. Regardless, the mid-wavelength opsin MWS2 may play an important role in the visual ecologies of photophore-bearing shrimp with its diversification in Oplophoroidea likely playing a critical role in the fitness and evolutionary success (i.e., high abundances, wide variety of environmental conditions and geographical ranges) of this group.

Methods

Sample collections and processing

Specimens were collected from the Gulf of Mexico and Florida Straits aboard the RV Point Sur (2015–2018), RV Sonne and RV Walton Smith (2016, 2017), respectively. Collections were done by a 9-meter² Tucker trawl fitted with a light-tight, thermally insulated cod-end⁷⁸ or a multiple opening/closing net and environmental sensing system (MOC-10). No ethics approval was required to obtain these samples. Shrimp length varies in size with carapace length typically ranging between 5–25 mm for the individuals collected for this project. All animals were sorted shipboard under dim red light or low light to avoid damaging photosensitive tissues. Samples were preserved in RNAlater, frozen at -20°C before being transported to Florida International University and stored at -80°C . Eye tissues were carefully dissected in RNAlater from three biological replicates for a majority of the shrimp species, except for *Systellaspis braueri* where only two replicates preserved for RNA work were available and the outgroup species *N. ensifer* ($n = 1$) (Table S1), chosen for its placement sister to Oplophoroidea⁷⁹. Each sample was discretely homogenized in TRIzol[®] reagent (ThermoFisher Scientific). Voucher specimens were curated in the Florida International Crustacean Collection (FICC).

Total RNA was discretely extracted from tissues using Trizol/Chloroform reagents and rDNase (Macherey-Nagel) treated following the protocol described in DeLeo et al.⁸⁰. RNA quantity was assessed via a Qubit 2.0 fluorometer (Life Technologies, USA). RNA integrity was assessed via gel electrophoresis and an Agilent Bioanalyzer. RNA sequencing (RNAseq) libraries were constructed from high-quality RNA using the NEBNext[®] Ultra[™] II Directional library prep kit for Illumina[®] using the manufacturer's protocol for use with the NEBNext Poly(A) mRNA Magnetic Isolation Module (NEB #E7490). Libraries had a target amplicon size of

330 bp and contained NEBNext Multiplex Oligos for Illumina[®] dual index adaptors. Purification of cDNA was done using AMPure XP beads. Libraries were pooled and sequenced on an Illumina HiSeq4000 to obtain 150 bp paired-end reads at the GENEWIZ[®] Core Facility (South Plainfield, NJ).

Transcriptome assembly and assessment

Raw Illumina data was quality assessed using FastQC⁸¹ to inform quality and adaptor trimming. Reads were trimmed using Trimmomatic v0.36⁸² (*adapter.clip* 4:30:10, *min.read.length* 30). Reads were then error-corrected using Rcorrector⁸³ prior to assembly. Tissue-specific (eye) reference transcriptomes were assembled de novo for each species with Trinity v2.8.5^{84,85} using in silico read normalization, a minimum contig length of 200 bp and a k-mer size of 23, which has proven to be the optimal kmer size for these crustacean RNAseq datasets (i.e., Pérez-Moreno, DeLeo et al.⁸⁶; Bracken-Grissom et al.⁴⁰). Contamination was subsequently removed from each assembly using Kraken v2.0⁸⁷ with default parameters and the bacteria, archaea and viral database Minikraken2, v2. Duplicate transcripts and rRNA were removed using BBduk and dedupe (BBTools suite, available at: <http://sourceforge.net/projects/bbmap>). Transcriptome quality and completeness was assessed using Transrate v1.0.3⁸⁸ and BUSCO v3.0.2 (Benchmarking Universal Single-Copy Orthologs⁸⁹) using OrthoDB's Arthropoda reference dataset⁹⁰ of orthologous groups ($n = 1066$) (--*confidence* 0.1) (Table S2). All transcriptome analyses were conducted on the Smithsonian's High-Performance Computing Cluster, Hydra (<https://doi.org/10.25572/SIHPC>).

Oplophoroidea tree reconstruction

For each species, Transdecoder v5.5.0 (<https://github.com/TransDecoder>) was used to extract and translate the ORFs from the assembled transcripts, including the previously published transcriptomes of *S. debilis* and *J. spinicauda*^{59,60}. CD-HIT v4.8.1⁹¹ was used to cluster and remove redundant (~ 1.0) peptide sequences prior to running OrthoFinder (v2.5.4) (*-S diamond -M msa -A mafft -T fasttree*) to identify one-to-one orthologous sequences among all species ($n = 12$, including outgroup). Phylotranscriptomic analyses were then conducted using maximum likelihood and a partitioned analysis for the multiple-gene alignments of the single-copy orthologs ($n = 622$) to reconstruct a species tree for Oplophoroidea using IQTREE⁹² (*-m MFP --merge -rcluster 10 -bb 1000 -alrt 1000*). The deep-sea caridean shrimp *N. ensifer* was used to root the tree as done in previous studies and is suggested to be a close evolutionary lineage to the targeted superfamily⁷⁹.

Visual opsin diversity

Assembled transcriptomes were analyzed using the Phylogenetically-Informed Annotation (PIA) tool⁹³, modified for command-line use (PIA2⁸⁶), which characterizes putative visual opsins and phototransduction pathway genes in a phylogenetic context. Briefly, this tool extracts all open-reading frames (ORFs) from each assembly, identifies the light interaction (LIT) genes via BLAST-searches against a database of known visual genes, aligns and subsequently places significant hits into precomputed gene phylogenies to differentiate between false positives and genes of interest. Emphasis was placed on the rhabdomeric phototransduction pathway (rtrans) which contains invertebrate visual opsins, rhodopsins (r-opsins). R-opsin identity was confirmed via structural alignments with PROMALS3D⁹⁴ to Bovine rhodopsin (2.8 Å) template (IF88.pdb)⁹⁵ and the subsequent identification of conserved domains, motifs and residues characteristic of invertebrate r-opsins (as described in Katti et al.⁹⁶). Putative opsins were initially retained only if they contained 2 or more conserved elements and had a minimum of 75% sequence identity to the other r-opsins recovered across Oplophoroidea, to further filter out short fragments and/or divergent sequences. Assembled isoforms that were <99% identical were retained for downstream analyses. R-opsin diversity was further refined and characterized for each assembly by aligning putative opsin sequences with PROMALS3D to a curated reference opsin dataset ($n = 996$ ^{18,20}) that comprises visual opsins across a range of spectral sensitivities as well as non-

visual opsins and related G-protein coupled receptors (GPCR). Phylogenetic tree reconstruction was done with IQ-TREE v2.1.2⁹² using an LG general amino acid replacement matrix, under a FreeRate model with 10 rate categories, and empirical base frequencies (LG + F + R10^{97,98}) as recommended by ModelFinder⁹⁹. Support was assessed with an Ultra-fast bootstrap approximation (UFBoot¹⁰⁰, 1000 replicates) and the Shimodaira–Hasegawa-like approximate likelihood ratio test (SH-aLRT¹⁰¹, 1000 replicates). False positives aligning with non-visual opsins or outgroups were removed before generating a final (arthropod-only) opsin gene tree as previously described in DeLeo and Bracken-Grissom⁴⁸, including curated opsins recovered from previously sequenced eye transcriptomes from the oplophorids *S. debilis*⁵⁹ and *J. spinicauda*⁶⁰.

Ancestral state reconstruction

Ancestral State Reconstruction (ASR) of opsin type (presence/absence of expression) was conducted using stochastic character mapping by sampling ancestral states from posterior probability distributions¹⁰² generated from 100 stochastic character maps using the *make.simmap* function (nsim=100) in the R package *phytools*¹⁰³. Both an “equal rates” model (model = “ER”), assuming equal probabilities of trait gain and loss of expression and an “all rates different” (ARD) model (model = “ARD”) were tested. The best fit model was determined by fitting and comparing extended Mk models (fitMk) for discrete character evolution¹⁰⁴. Akaike information criterion (AIC) values for the fitted models were then compared to determine the model with the lowest AIC score. This was determined to be the ER model assuming equal rates of trait gain/loss (of expression), which was utilized for downstream analysis. *Phytools* was then used to plot one stochastic character map for the expressed opsin traits on the well-supported, rooted Oplophoroidea phylogeny, along with the posterior probabilities (pie charts) at each node.

For the ASR of lighting environment for Oplophoroidea, species were categorized based on the shallowest depth of their known range in the GoM (= highest light presence or lowest light attenuation), as either epipelagic <200 m, upper mesopelagic 200–400 m, mesopelagic 400–700 m or lower mesopelagic >700 m. Because species can exhibit different vertical migration ranges depending on location (i.e., which ocean they are collected) due to environmental factors, all vertical ranges were optimized to collection locality where most of the range data was characterized, and not compiled across all oceanic basins. Depth information for the GoM was obtained from the literature and supplemented (or amended) with depth records from OBIS (obis.org) and metadata from the Florida International Crustacean Collection (FICC). ASR was run as previously described using the ER model which produced the lowest AIC score.

Signatures of selection

The putative r-opsin sequences were further curated to obtain only the longest isoform of each spectral type per species, where applicable. The coding sequences (CDS) for each opsin were extracted from the transdecoder output and used along with opsin protein alignments for each opsin type to generate codon alignments with pal2nal¹⁰⁵ for use with Codeml within the PAML package¹⁰⁶. Only the opsin types recovered in a majority of species (>75% of species) were tested ($n = 3$, MWS1, MWS2, LWS2). Unrooted trees were pruned in R for each opsin category to contain only the species for which a full, or mostly full, length sequence was recovered. The species for which a usable sequence was recovered varied among opsin type, however, representatives (min. of 2) from each family/category were present in all downstream analyses (Table S1).

Additional single-copy orthologous genes among Oplophoroidea species ($n = 11$) were identified with OrthoFinder after excluding *N. ensifer*. An unrooted tree with outgroup *N. ensifer* removed was generated in R for downstream analyses. Prior to running selection tests with Codeml, CDS were extracted for each orthologous gene from the transdecoder output using custom scripts. Both CDS and the multiple sequence peptide alignments (MSA) from OrthoFinder were used as input for pal2nal to generate codon alignments for use with Codeml.

Adaptive evolution for each opsin type (MWS1, MWS2, LWS2) from the Oplophoroidea species that have evolved a secondary mode of bioluminescence (photophores) was evaluated using PAML in Codeml to estimate ω (dN/dS). PAML generated three pairwise model comparisons to identify signatures of selection following Jeffares et al.¹⁰⁷. (1) Branch model comparisons included the *one-ratio* (M0) model, which estimated one average ω across the entire gene and served as the null against the *two-ratio* (M2) model which enabled ω to vary across the foreground and background branches. (2) Site models were used to estimate positive selection ($\omega > 1$) and an ω ratio that was variable among amino acid sites (codons) but remained the same across branches/lineages. The *nearly neutral* (M1a) model served as the null and only allowed two classes of sites (purifying selection $0 < \omega < 1$, neutral selection $\omega = 1$) while the *positive selection* (M2a) model also allowed for positive selection ($\omega > 1$). (3) Branch-Site models which allow partitioning by photophore presence, used the *Model A null* to only allow for purifying or neutral selection ($0 < \omega < 1$, $\omega = 1$) and was compared to the *Model A alternative* which allowed for positive selection ($\omega > 1$) in the foreground species, with the background species restricted to two classes ($\omega = 0$, $\omega = 1$)¹⁰⁸. Additional comparisons were made to determine if the opsins/genes were under selection for (a) the family Oplophoridae ($n = 5$ species) relative to the family Acanthephyridae ($n = 6$) and (b) strong vertically migrating oplophorids who reach the shallowest migration depths among the superfamily Oplophoroidea, the epipelagic (<200 m), ($n = 3$; *J. spinicauda*, *S. debilis*, and *O. gracilirostris*).

Likelihood ratio tests (LRT) were used to determine significant differences between the pairwise model comparisons; whether the data better fit parameters of the null or alternative model^{107,109}. The LRT were conducted following the methods of Santagata¹¹⁰, comparing the likelihood scores of these models ($\Delta\text{LRT} = 2 \times [\ln L_1 - \ln L_0]$) using χ^2 . *P*-values were corrected to limit false discovery rates (FDR) among the multiple comparisons¹¹¹. Adaptive evolution was inferred across each opsin in the (a) branch models—if the LRT was significant ($p < 0.05$) and ω was >1 and (b) in the branch-site and site models if the LRT was significant and sites of positive selection with a posterior probability > 95% were identified by the Bayes Empirical Bayes (BEB) output of those models¹¹². Results were supplemented by running both the BUSTED-MH (v4.5) and MEME packages from HyPhy⁶³ on the Datamonkey server¹¹³. BUSTED-MH tests for gene-wide episodic diversification using a branch-site random effects model¹¹⁴, accounting for site-to-site synonymous rate variation¹¹⁵ and multinucleotide (or multihit, MH) substitutions¹¹⁶, and allows ω to vary from branch to branch. BUSTED was shown to have greater power in detecting sites subject to positive selection than PAML branch-site models¹¹⁷. MEME⁶¹, can detect both episodic and pervasive positive selection at the level of individual sites using a mixed effects model of evolution, though it is not foreground-specific.

For the additional orthologs recovered with OrthoFinder, branch-site models were run with PAML to test for evidence of positive selection in photophore-bearing species. The identity of genes with significant LRT values ($p < 0.01$) with sites of positive selection (posterior probability >95%, identified by BEB) was determined using blastp¹¹⁸.

Site mapping

As both PAML and HyPhy detected sites under putative positive selection in the MWS2 opsin for photophore-bearing (foreground) species, identified sites were mapped onto the predicted protein structure. First, transmembrane helix predictions were obtained using the structural prediction tool Phobius¹¹⁹ using the first sequence in the branch-site alignments. The LWS2 opsin was used for transmembrane predictions as it was the only opsin to recover all seven transmembrane domains in Phobius. Swiss-model¹²⁰ was used for 3D protein structure modeling using the jumping spider opsin X-ray structure as a template (6i9k.1.A) (GMQE, 0.69, identity = 40.8) which contains information on retinal binding sites (RBS). Other putative sites interacting with retinal were within 4 Å of the RBS^{10,121,122}. The protein annotation tool Protter¹²³ was used to highlight sites under selection in a structural context using input from Phobius and Swiss-model.

Statistics and reproducibility

Chi-square tests were used to compare likelihood scores between the pairwise model comparisons testing for evidence of positive selection. Details on LRT comparisons and p-value corrections are described in the “Methods”. Input data, parameters used and support values for the phylogenetic analyses (gene and species trees) and ancestral state reconstructions are mentioned in the “Methods” with additional metadata and coding information supplied in the supplemental files.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

Raw RNA sequencing data used for these analyses are publicly available through NCBI's Sequence Read Archive (SRA) database (BioProject ID: [PRJNA1194204](https://doi.org/10.1038/s42003-025-07450-z)). The tissue-specific eye assemblies and associated metadata for each species, including the ASR coding, are available on the Dryad Digital Repository: <https://doi.org/10.5061/dryad.cz8w9gids>⁵⁸. Putative opsin sequences are also available on Genbank (accessions PQ677825-PW677867). The opsin alignments used for the selection tests are included as Supplementary data 1/2/3). All other data are available from the corresponding author on reasonable request.

Code availability

Support scripts for the data analyses are available on Dryad⁵⁸. All details on how these data were processed are available in the “Methods”.

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Author contributions

The project was conceptualized by D.M.D. and H.D.B.-G. Both authors participated in research cruises and helped with specimen collections. D.M.D. performed all wet laboratory procedures and analyses and wrote the manuscript. Both D.M.D. and H.D.B.-G edited the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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