Opsin gene expression in larval and adult deep-sea fishes supports a conserved cone-to-rod pathway in teleost visual development

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Abstract

Deep-sea fishes show extraordinary visual adaptations to an environment where every photon of light that is captured might make the difference between life and death. While considerable effort has been made in understanding how adult deep-sea fishes see their world, relatively little is known about vision in earlier life stages. Similar to most marine species, larval deep-sea fishes start their life in the well-lit epipelagic zone, where food is abundant and predation relatively low. In this study, we show major changes in visual gene expression between larval and adult deep-sea fishes from eight different orders (Argentiniformes, Aulopiformes, Beryciformes, Myctophiformes, Pempheriformes, Scombriformes, Stomiiformes and Trachichthyiformes). Comparison between 18 species revealed that while adults mostly rely on rod opsin(s) (*RH1*) for vision in dim-light, larvae mostly express green-sensitive cone opsin(s) (*RH2*) in their retinas. Adults of the scombriform and three aulopiform species also expressed low levels of *RH2*, with the latter using different copies of the gene between ontogenetic stages. Cone opsins in adult fishes are rather surprising as most deep-sea fishes have lost their cone photoreceptors in favour of a highly sensitive pure rod retina. The

expression of *RH2* in larvae, on the other hand, shows that even in species that might not have any cones as adults, the larval retina is likely to be cone dominated first, before rod photoreceptors are added through ontogeny. Our study therefore supports a conserved pathway for the cone-to-rod developmental sequence of the teleost or even vertebrate retina.

INTRODUCTION

As animals grow, their morphology, physiology and behaviour change. This is often associated with ontogenetic transitions between environments (Dahlgren & Eggleston 2000, Evans & Fernald, 1990, Grant 2007). For example, many coral reef fishes shift habitat as they mature from open ocean pelagic larval forms to juvenile and adult forms that inhabit coral reefs (Collin & Marshall, 2003). Much effort has been spent in studying vision in adult fishes, how it might differ between (light) habitats and how this might enable them to thrive. However, ontogenetic changes of the visual system and especially on the molecular level remain less understood.

Fish visual systems are very diverse and they vary in morphology, physiology and spectral sensitivity (Hunt et al., 2014, Carleton et al., 2020). Vision in vertebrates is enabled by cone and rod photoreceptors in the retina, which carry light-sensitive molecules composed of an opsin protein bound to a light absorbing, vitamin A-derived chromophore (Lamb 2013). In fishes, there are usually four types of cone opsins (*SWS1*, *SWS2*, *RH2* and *LWS*) used for photopic and colour vision, and one rod opsin (rhodopsin, *RH1* or Rho) for scotopic vision in dim-light conditions. Gene duplications followed by functional diversification resulted in extant teleost fishes having a median of seven cone opsin genes within their genomes (Musilová et al. 2019a). However, unlike for the cone opsins, rod opsin duplicates are rarely found and hence, most teleost lineages have only one or at most two *RH1* copies in their genomes (Pointer et al. 2007, Morrow et al. 2017, Musilová et al. 2019a). An exception can be found in several mesopelagic lineages, which have independently expanded their *RH1* repertoires (Musilová et al. 2019a).

The functional diversification of opsin genes may involve mutations at key-spectral tuning sites, thus shifting the peak spectral sensitivity (λ_{max}) of the photopigment all the way from ultraviolet to the far-red spectrum of light (Nakayama & Khorana 1991, Hope et al. 1997,

Hunt et al. 2001, Yokoyama 2008, Yokoyama & Yia 2020). Changes in the light environment and ecology are thought to primarily drive visual diversity in fishes (Carleton et al. 2020). For example, longer and shorter wavelengths are scattered and filtered out with increasing water depth and consequently fishes living in deep-water habitats such as sculpins of Lake Baikal (Hunt 1997), cichlids in lakes Malawi and Tanganyika (Sugawara et al., 2005) or from small African crater lakes (Malinsky et al., 2015; Musilová et al., 2019b), and deep-sea fishes that live below a depth of 200 m (Douglas et al. 2003), have visual systems sensitive to the blue-green part of the visible spectrum. An alternative to changing key spectral tuning sites and arguably a faster way to adapt to changes in the light environment at the molecular level, is to regulate opsin gene expression itself. This can be achieved when only a subset of opsin genes is expressed, and this subset can be altered among or within species and even within the same individuals during ontogeny (Carleton & Kocher, 2001, Manousaki et al. 2013; Carleton 2016 and references therein) or as co-expression in different types of photoreceptor cells (Dalton et al. 2014, 2017, Stieb et al., 2019).

Ontogenetic changes in relative opsin gene expression between larval and adult fishes have thus far only been studied in a few shallow-water fish species including cichlids (Carleton et al. 2016), dottybacks (Cortesi et al. 2016), zebrafish (Takechi & Kawamura 2005), killifish (Fuller et al. 2010), guppies (Laver and Taylor 2011), flounders (Kasagi et al. 2015), snappers (Robinson et al. 2017) and surgeonfishes (Tettamanti et al., 2019). However, studies on deep-sea fishes are virtually missing and it is not known if they show same patterns of change in expression between forms similar to their shallow water counterparts. Although Musilová et al. (2019) showed that deep-sea fish genomic repertoire in all cases consists also of some cone opsin genes (mostly green-sensitive RH2 and blue-sensitive SWS2), it remains unknown when exactly these cone opsins express, and whether their expression is limited to only certain developmental stages, such as shallow-living larvae. It might be that difference in habitat preferences between larval and adult forms affects the visual system of the deep-sea fish larvae in a similar way seen in other marine fishes (such as coral reef species).

The deep sea is one of the most extreme and challenging environments on earth. In the mesopelagic zone between 200 and 1000 metres the residual sunlight decreases significantly with depth, first eliminating the longer (red) and shorter (UV) wavelengths, resulting in a narrower spectral window of blue–green light (470–490 nm) before ending in a dark zone where sunlight is replaced by bioluminescence produced by the deep-sea inhabitants

themselves. According to the “sensitivity hypothesis”, the selective pressure of capturing any photon of light available in the deep-sea results in adaptations that maximise sensitivity to the ambient light (Denton & Warren 1957, Munz 1958). Therefore, visual pigments of deep-sea fishes are short-wavelength-shifted having absorption maxima that coincide both with the remaining sunlight at depth and bioluminescent emission spectra (Douglas and Partridge 1997, Douglas 1998, Hasegawa, et al. 2008, Turner, et al. 2009).

Generally, adaptations to the deep are found in the morphology, anatomy or physiology of the eye. For example, deep-sea fish tend to have larger eyes, wider pupil apertures (Locket & Crescitelli 1977, Warrant & Locket 2004), aphakic gaps (Warrant et al. 2003), reflective tapeta lucida or more photoreceptor cells converging on fewer retinal ganglion cells (Locket 1977, Nicol 1989), all of which maximise photon capture (Douglas et al., 1998). Moreover, most deep-sea fishes rely on a single rod opsin (*RH1*) for vision with a shorter shifted visual pigment to match the blue-green dominated ambient light conditions (Nakayama and Khorana 1991, Hope et al. 1997, Hunt et al. 2001, Yokoyama 2008, Yokoyama & Yia 2020). Recently, three the deep-sea lineages have also been described with multiple rod opsin proteins (Musilova et al. 2019a). In those species, *RH1* duplicates have diversified to possibly enable colour vision or some sort of colour detection in this dimly lit environment.

An additional adaptation to the deep are pure rod retinas that are either single or multibank (Locket, 1977; Wagner et al., 1998; Land & Nilson 2002). Pure rod retinas are commonly reported in adults in numerous deep-sea lineages, such as deep-sea eels (Hirt & Wagner, 2005), Atlantic argentine (*Argentina silus*), deep-sea smelts (*Bathylagus* and *Microstoma*), barreleyes and spookfishes (*Dolichopteryx*, *Opisthoproctus*, *Rhynchohyalus*, *Winteria*), most Stomiiformes (lightfishes - Gonostomatidae, hatchetfishes - Sternoptychidae), viperfishes - Chauliodontidae, dragonfishes - Idiacanthidae; but see De Busserolles et al., 2017 for exception in the pearlside, *Maurolicus*), slickheads (Alepocephalidae), lanternfishes (Myctophidae), bathylaconids, spiderfishes (Bathypteroidae), sabre-tooth fishes (Evermannellidae), pearleyes (Schopelarchidae), neoscopelids, giganturids, tube-eyes (*Stylephorus*), melamphaids (*Poromitra*, *Melamphaes*), deep-sea anglers (Oneirodidae) and ceratid seadevils (Ali & Anctil, 1976). In some cases, cones persist as individuals mature, but the relative proportion of cones usually decreases throughout the development (Bozanno et al. 2007). Contrarily, pure cone retinas have been detected mostly in larvae of many shallow-water teleosts (e.g. salmons; Ali 1959, plaice; Blaxter 1968, cod; Valen et al. 2016, and others;

Blaxter & Staines 1970), but also in deep-sea living *Bathysauropsis gigas* (Blaxter & Staines 1970) and *Idiacanthus fasciola* (Wagner et al. 1981). Apart from possible developmental constraints, it is thought that the initial formation of cones in deep-sea fish larvae may be stimulated by broad-spectrum sunlight, as they inhabit shallow-water zones, a completely different visual environment compared to adults. Lanternfish larvae, for example, are presumed to be visual predators feeding during the daytime, suggesting a marked ontogenetic shift in their feeding ecology and behaviour (Sabatés et al., 2003). Some deep-sea fishes also exhibit daily vertical migrations (Hopkins & Gartner 1992, Afonso et al. 2014) and those species may therefore benefit from cone cells even as adults. For example, adult deep-sea fish retinas consisting of both cones and rods have been described in *Omosudis* (Frederiksen 1976), *Scopelosaurus* (Munk 1975, Pointer 2002 & 2007), *Lestidiops* (Munk 1989), *Lampanyctus crocodilus* (Bozzano et al. 2007), *Benthosema glaciale* (Bozzano et al. 2007), *Myctophum punctatum* (Bozzano et al. 2007) and *Maurolicus* (cone cells which morphologically resemble rods in this case; De Busserolles et al., 2017).

To elucidate visual developmental pathways in deep-sea fishes, we used transcriptomics and tested 1) whether larval deep-sea fishes start with a pure cone expression profile indicating pure cone retinas, or whether they start with a different composition of opsin genes suggesting either a duplex retina or even a pure rod-retina. Moreover, we 2) characterised the opsin genes that are expressed in adults that have morphological cones and rods, or pure rod retinas. We report the first comprehensive study on opsin gene expression in larval and adult deep-sea fishes and show that on the molecular level, deep-sea fishes change expression from cone-dominated to rod-dominated as they grow and mature.

MATERIALS AND METHODS

The specimens used in this study were collected in the Sargasso Sea during two multipurpose fishery surveys conducted by the German Thünen Institute of Fisheries Ecology onboard of the research vessel *Walther Herwig III* in March to April in 2014 and in 2017. The sampling occurred during both day or night at a depth of 600 – 1,000 m using a mid-water pelagic trawl net (Engel Netze, Bremerhaven, Germ) with an opening of 30 m x 20 m, a length of 145 m, and mesh sizes (knot to knot) from 90 cm decreasing stepwise to 40, 20, 10, 5, 4, 3, 2 cm, with a 1.5-cm mesh in the 27-m-long codend. The larvae were mostly collected using an

Isaacs-Kidd Midwater Trawl net (IKMT; 6.2 m^2 mouth-opening, 0.5 mm mesh size; Hydro-Bios Apparatebau GmbH) at a depth of 0 - 300 m by double-oblique transect tows. Adult fish specimens were flash-frozen at -80 ^{0}C upon arrival on board. Larval samples were fixed in RNAlater™ (ThermoFisher) and stored at -80 ^{0}C until further use.

Total RNA was extracted from the whole eyes using either the RNeasy micro or mini kit (Qiagen) and the extracted RNA concentration and integrity was subsequently verified on an Agilent Bioanalyzer (company). RNAseq libraries for 31samples were constructed in-house from unfragmented total RNA using the NEBNext Ultra II Directional RNA library preparation kit for Illumina, NEBNext Multiplex Oligos for Illumina and the NEBNext Poly(A) mRNA Magnetic Isolation Module (New England Biolabs). Multiplexed libraries were sequenced on the Illumina HiSeq platform as 150 bp paired-end (PE) reads. Library construction and sequencing (150 bp PE) for an additional 10 samples was outsourced to Novogene (Singapore). We additionally re-analyzed 11 retinal transcriptomes previously published in Musilová et al. (2019a). Toegether, then, our dataset comprised 52 samples of which, based on morphology, 25 were classified as larvae, 5 as juveniles and 22 as adults. Sample ID's, number of raw reads and further parameters are listed in Table 1.

The sequence data was quality-checked using FastQC (Andrews 2010). Gene expression was then quantified using Geneious software version 11.0.3 (Kearse et al. 2012). Figure 1. For each sample we first mapped the reads against a general fish reference dataset comprising all visual opsin genes from the Nile tilapia, *Oreochromis niloticus* and the zebrafish, *Danio rerio*, with the Medium-sensitivity settings in Geneious. This enabled us to identify cone and rod opsin specific reads. If present, paralogous genes were subsequently disentangled following the methods in Musilova et al., 2019a and de Busserolles et al., 2017. Briefly, we created species-specific references of the expressed opsin genes and their several copies (Musilová et al. 2019a) and re-mapped the transcriptome reads with Medium-Low sensitivity to obtain copy-specific expression levels. If multiple opsin genes were found to be expressed, we report their proportional expression in relation to the total opsin gene expression (Figure 1).

To check for key amino-acid substitutions in RH1 and RH2 and potential shifts in its absorbance, we first translated the opsin coding sequences into amino acid sequences, and then aligned them with the bovine RH1 (GenBank Acc.No: M12689). We have specifically focused

on the positions identified as key-tuning sites in Yokoyama (2008) and Musilova et al. (2019a). For details, see Tables 2 and 3.

A dataset containing *RH1* opsin gene sequences from our transcriptomes, and additional *RH1*s obtained from GenBank (link to NCBI) were aligned using the MAFFT (Katoh et al. 2009) plugin as implemented in Geneious and a phylogenetic tree was subsequently reconstructed using MrBayes v3.2.1 (Ronquist and Huelsenbeck 2003). Figure 2. Trees were produced using the Markov chain Monte Carlo analysis which ran for 1 million generations. Trees were sampled every 100 generations, and the printing frequency was 1000, discarding the first 25% of trees as burn-in. The evolutionary model chosen was GTR model with gamma-distributed rate variation across sites and a proportion of invariable sites. Posterior probabilities (PP) were calculated to evaluate statistical confidence at each node. We used the same approach with an *RH2*-specific reference dataset to reconstruct the phylogenetic relationship between the transcriptome-derived deep-sea *RH2* genes (Figure 2).

For the purpose of the whole-genome sequencing, we extracted DNA of XXX species using QiaGen DNA Tissue kit. We have purchased the whole-genome sequencing on the Illumina platform as a customer service on the commercial basis (Novogene; Singapore).

In addition to confirm some of our species identification and to assist with the fish larval identification, we first both checked the COI in the transcriptome, and we have also re-sequenced each sample's COI from genomic DNA using the Sanger method. We then used BLAST (Madden 2013) against the GenBank database for confirmation of some COI genes. Genomic DNA used for barcoding purposes was extracted from the tissues using the DNeasy Bloood and Tissue kit (Qiagen). Partial sequences of the mtDNA cytochrome c oxidase subunit 1 (COXI) gene were amplified by polymerase chain reaction (PCR) using a combination of universal primers LCO1490: 5'GGT CAA CAA ATC ATA AAG ATA TTG G3', HC02198: 5'TAA ACT TCA GGG TGA CCA AAA AAT CA3' (Folmer et al., 1994) and FishF1: 5'TCA ACC AAC CAC AAA GAC ATT GGC AC3', FishF2: 5'TCG ACT AAT CAT AAA GAT ATC GGC AC3', FishR1: 5'TAG ACT TCT GGG TGG CCA AAG AAT CA3' and FishR2: 5'ACT TCA GGG TGA CCG AAG AAT CAG AA3' (Ward et al., 2005). PCRs of the COI were carried out in 10 µl reaction mixtures containing 1 µl of template DNA, 5 µl Premix - GoTaq G2 Green Mastermix (Promega, Madison, WI, USA Madison, WI), 3.5 µl of sterile distilled water, and 0.25 µl of forward/reverse primer (final concentration of 20pmol). They

were conducted using a Mastercycler Gradient 96-well system (Eppendorf, Hamburg, Germany) with initial denaturation at 94°C (4min), followed by 35 cycles of 94°C (45s), 52°C (45s), and 72°C (45s). A final extension step of 72°C (5min) was added in each case. Preheating of DNA, water and GoTaq G2 at 94°C (max 1 min) was sometimes applied to the samples. To confirm amplification, PCR products were electrophoresed on 2 % agarose gels and purified using ExoSAP-IT (ThermoFisher). A few samples were sequenced in both directions, to confirm sequencing accuracy and occasionally to obtain a better-quality sequence where the quality of the sequence from the forward primer was not entirely satisfactory.

RESULTS

Retinal transcriptomes of 18 species revealed that deep-sea fishes mainly express rod opsin (*RH1*) or a middle-wavelength sensitive cone opsin gene (*RH2*) (Fig 1, Table 1). While larvae were mostly found to express *RH2*, adults and juveniles mostly expressed *RH1* or a combination of both rod and cone opsins (Fig 1, Table 1). We found none or very low expression of any of the other cone opsin genes: the red-sensitive *LWS* opsin gene was not found to be expressed in any of the studied species, the UV-sensitive *SWS1* opsin was only found in the whalefish, *Gyrinomimus* sp., larva, and the blue/violet sensitive *SWS2* opsin only in the fangtooth, *Anoplogaster cornuta*, larvae (Fig 1, Table 1). The identity of these genes was confirmed by phylogenetic reconstruction which also revealed information about ancestral duplications (Fig 2).

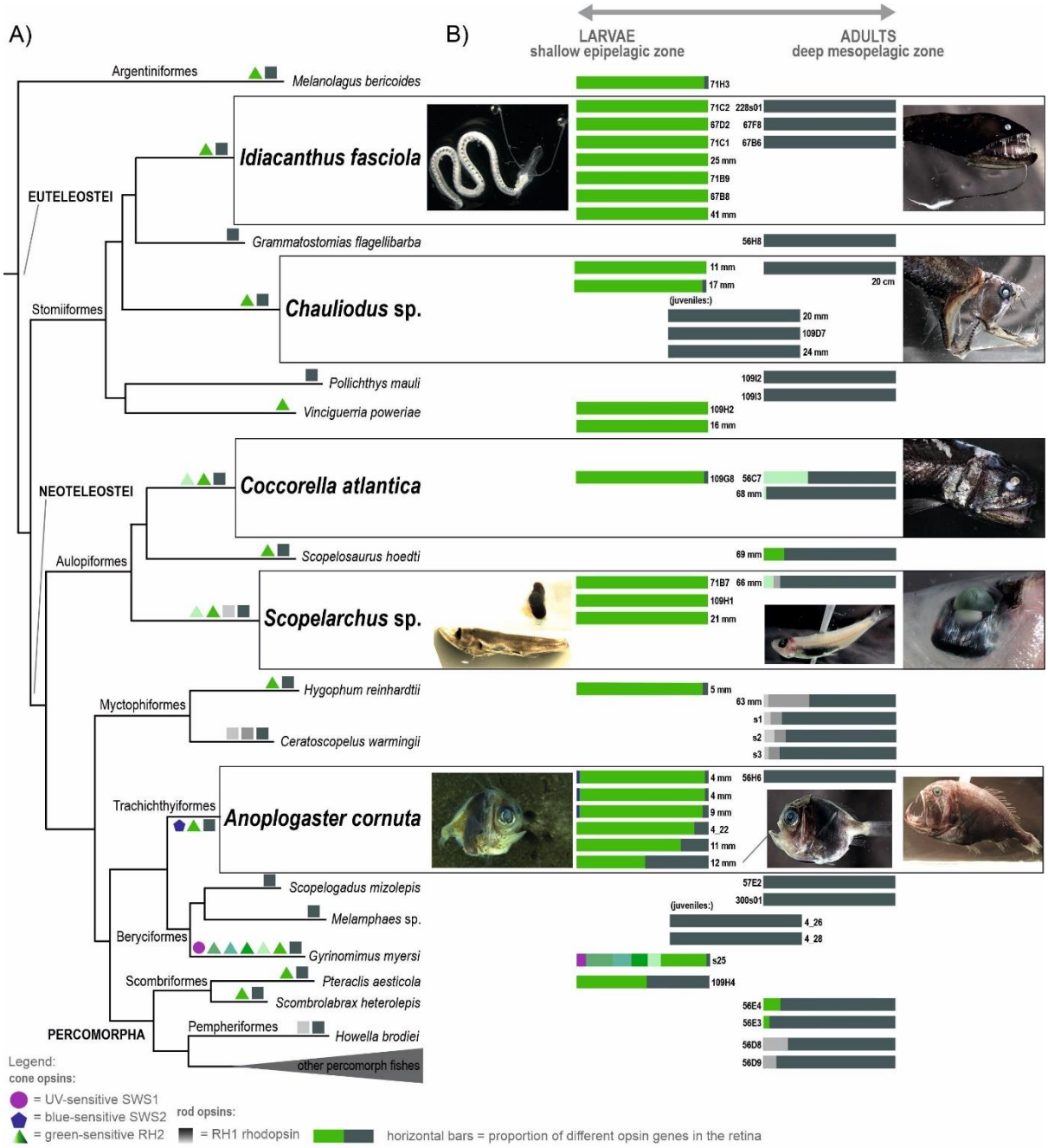

Figure 1: Opsin gene expression in larval and adult deep-sea fishes. *A) Retinal or eye transcriptomes of 52 deep-sea fish individuals were used to characterise the expressed opsin gene repertoires and mapped on the simplified phylogenetic tree after Betancur et al., 2017. B) The proportion of different opsin genes in the retina. One horizontal bar represents one individual. Different colour corresponds to different cone (colours) or rod (shades of grey) opsin genes. The larvae (left column) show a pure-cone or cone-dominated retina, while the adults (right column) have a pure-rod or rod-dominated visual system. The most commonly expressed cone opsin in deep-sea fishes is the green-sensitive RH2 opsin, while the other cone opsin classes, LWS, SWS1 or SWS2 are expressed only marginally or are completely absent. Different shades of colour represent multiple copies of the same opsin genes.*

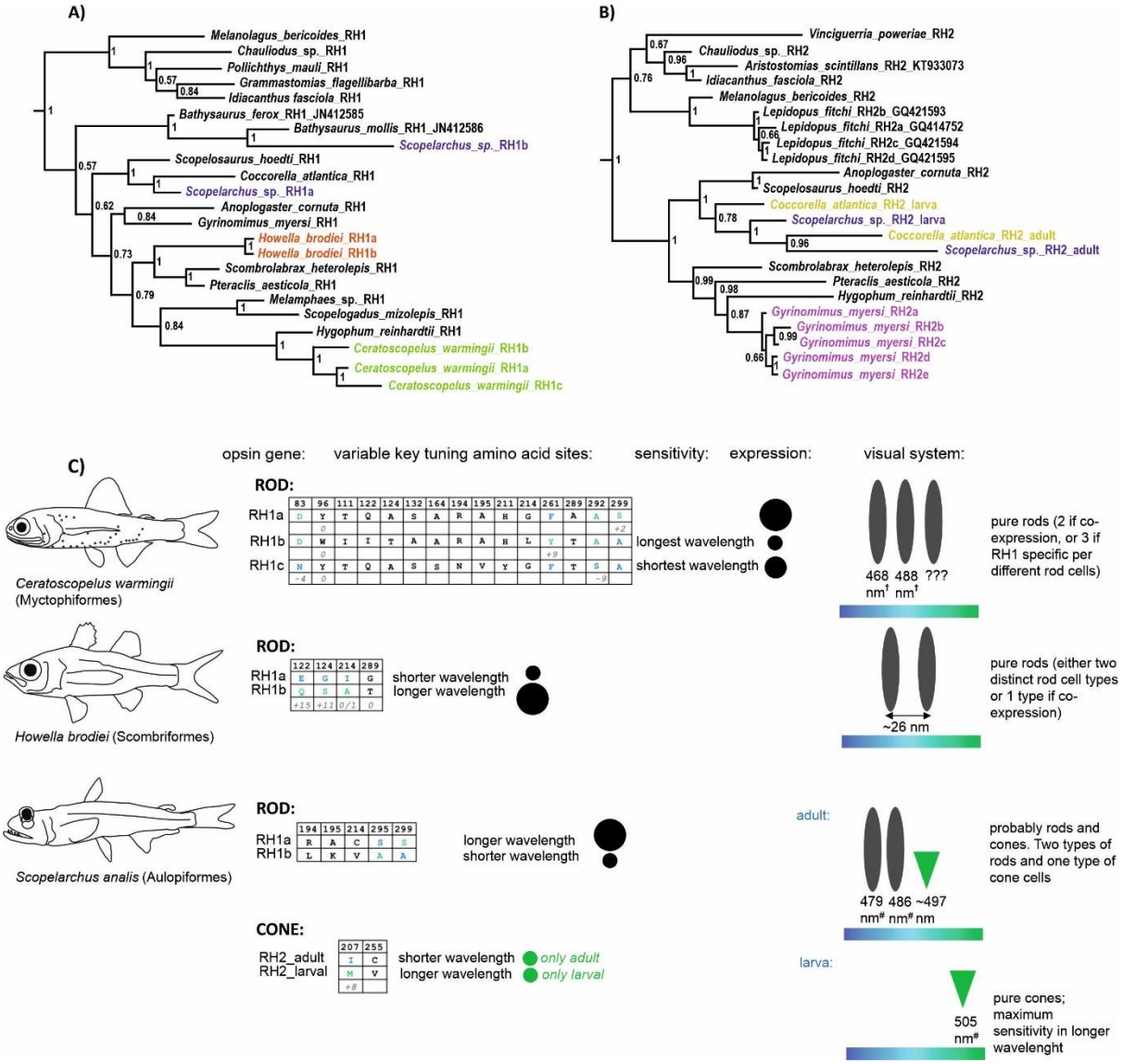

Figure 2*: A) RH1 and B) RH2 gene trees with highlighted species with multiple copies. C - visual system of the species with multiple rhodopsins and cone opsins. Predictions made on the variable key tuning sites following Yokoyama (2008) and Yokoyama and Yia (2020). Multiple rod opsins seem to differentiate in all three species. Measurements by # = Pointer et al., 2007; † = Collin & Marshall, 2003*

When comparing the opsin gene expression in different developmental stages we found the following:

Larval specimens of *Idiacanthus fasciola*, *Scopelarchus sp*., *Vinciguerria poweriae*, and the earliest stage of *Chauliodus sp.* only expressed *RH2* (Fig 1, Table 1). Larval specimens of

Anoplogaster cornuta, *Coccorella atlantica*, *Hygophum reinhardtii*, *Melanolagus bericoides* and *Pteraclis aesticola* and the later larval stage of *Chauliodus sp*., expressed mostly *RH2* with low levels of *RH1* (Fig 1, Table 1). Juvenile specimens of *Chauliodus sp.* and *Melamphaes sp.* only expressed *RH1* (Fig 1, Table 1). Adult specimens of *Idiacanthus fasciola*, *Anoplogaster cornuta*, *Ceratoscopelus warmingii*, *Grammastomias flagellibarba*, *Pollichtys mauli*, *Howella brodiei*, *Scopelogadus mizolepis* and *Chauliodus sp*. only expressed *RH1*, while adults of *Coccorella atlantica*, *Scopelosaurus hoedtii*, *Scopelarchus sp*., and *Scombrolabrax heterolepis* expressed both *RH1* and low levels of *RH2* (Fig 1, Table 1). Furthermore, some species expressed multiple *RH1* copies (*Scopelarchus*, *Howella brodiei* and *Ceratoscopelus warmingii* adults) or multiple *RH2* copies (*Gyrinomimus sp*. larva) (Fig 1, Table 1). Notably, adults and larvae of *Scopelarchus sp*. and *Coccorella atlantica* expressed different copies of *RH2* (Fig. 1, Table 1, Fig 2).

Amino acid resconstructions revealed functional difference between *RH1* orthologs and paralogs at positions 83, 102, 122, 124, 132, 164, 183, 194, 195, 214, 261, 269, 289, 292, 295, 299, 300 and 317 when compared to the Bovine rod opsin (Table 2). Changes in these key-tuning sites might result in a functional change of the protein, shifting the absorbance to either lower or higher wavelengths as detailed in Table 2.

The *RH1* and *RH2* phylogenies revealed that most deep-sea fish visual opsins cluster together by species or order (Fig 2). For example in the whalefish *Gyrinomimus* all expressed *RH2s* are clustered together suggesting lineage/species specific *RH2* duplications (Fig 2). However, there were a few exceptions, suggesting more ancient duplication events (Fig 2). In *Scopelarchus* the two *RH1* copies are not in a sister relationship, and result in different clusters. Also, the *RH2s* in Aulopiformes (*Scopelarchus*, *Coccorella*) cluster by ontogenetic stage (Fig. 2).

DISCUSSION

In this study, we aimed to investigate whether deep-sea fishes, like their shallow counterparts, start their lives with a cone opsin expressing dominated visual system. We were also interested in whether adult deep-sea fishes, which morphologically often have pure-rod retinas, predominantly express *RH1* in their photoreceptors or whether some species still retained features of a duplex retina expressing both cone and rod opsins. We provide evidence that based on opsin gene expression, the visual system in deep-sea fishes undergoes drastic ontogenetic changes which likely correspond to differences in the light habitat between shallow-living larval and deeper living adult forms. Namely, larvae across species and orders mostly (or only) expressed the green-sensitive *RH2* opsin until (or shortly before) reaching metamorphosis, while adults either solely expressed *RH1* or a combination of mostly *RH1* complemented with low levels of *RH2* expression. The expression patterns found here provide a functional link to the somewhat puzzling finding that deep-sea fishes, despite relying almost exclusively on rods as adults, have maintained a high number of cone opsin genes in their genomes (Musilova et al., 2019). We also report three species that as adults express multiple *RH1*s within their retinas. Contrarily, with the exception of the larval whalefish (*Gyrinomimus* sp.) which expressed five copies, all deep-sea fishes relied on a single *RH2* opsin for vision. However, in aulopiforms the *RH2* copy which was expressed differed between larvae and adults, suggesting an ontogenetic switch in cone opsin use in this lineage. Generally, our data is in agreement with previous findings that deep-sea fishes lack the expression of the red-sensitive (*LWS*) opsin gene, which is also supported by the loss of the opsin class with depth in many teleost lineages (Musilova et al. 2019a). *RH2* is the most common (and often the only) cone opsin gene used for vision in the deep-sea fishes we studied here.

The developmental changes in the visual system we uncovered here are best explained by the different habitats larval and adult deep-sea fishes live in. In general, deep-sea fish larvae live in the shallow epipelagic zone (0-100 m) and are active during the day, when ambient light levels are sufficiently high to warrant a cone-based visual system. Once they metamorphose, deep-sea fishes start to submerge deeper and take up live at different depths in the mesopelagic (100 - 1'000 m) or even bathypelagic (below 1'000 m) zone where sunlight is replaced by bioluminescence as the main source of light. In this rather dim environment, rods work at their best and cone photoreceptors would be obsolete for the most part at least.

During the ontogeny of the vertebrate retina, cone photoreceptors are generally first to develop, followed by temporally and spatially distinct rods (Raymond 1995, Shen & Raymond 2004). For example, in zebrafish, photoreceptor progenitor cells start out by first differentiating into cones before rods are added later during development (Sernagor et al. 2006). Similar to our findings, though based on opsin gene expression data, Atlantic cod, *Gadus morhua*, have also been shown to start their lives with pure cones, and rods developing later during metamorphosis (Valen et al. 2016). That the developmental sequence of cone first then rod might be conserved across vertebrates is indicated by the mice developmental pathway that starts as a cone-only, but later on gets inhibited by rod-specific genes (Mears et al. 2001). Since most adult deep-sea fishes rely on a pure rod retina dominated by *RH1* expression (e.g., Musilova et al. 2019a), the question remained whether larval deep-sea fishes would start out with a similar expression profile or whether they would follow a more general vertebrate development with a cone-to-rod sequence. Our findings that larval fishes across orders had a cone opsin dominated expression profile which was successively replaced by rod opsin in larger fishes and adults strongly supports a conserved vertebrate developmental pathway in deep-sea fishes, as previously suggested based on morphological studies (Raymond 1995, Mears et al. 2001, Shen & Raymond 2004).

As for all animals, the ultimate proof for colour vision rests on behavioural experiments showing colour discrimination, something that is exceedingly difficult if not impossible to do in deep-sea fishes. Some species, such as *Scopelosaurus hoedti*, *Coccorella atlantica* and *Scombrolabrax heterolepis* might use multiple opsins to increase sensitivity to light through expressing low levels of *RH2*, suggesting that to some extent, they might be able to detect the residual daylight and can thus be used for vision during the day. In addition, it could well be that when migrating shallower at night, deep-sea fishes might be using cones instead of rods to detect prey, predators and mates in moonlit shallow marine waters; or perhaps cones serve to detect bioluminescent flashes if they are of sufficient strength and duration. It is also possible that cone opsins are expressed in rod-shaped cone photoreceptors as found for the deep-sea pearlside *Maurolicus*, which enables vision during crepuscular hours (de Busserolles et al. 2017). The expression of multiple opsins, if they are located in different photoreceptors and confer different spectral sensitivities would, at least in theory, allow for colour discrimination in dim-light conditions. This might be possible based on the expression of rod and cone opsins in aulopiform and scombriform species, based on the expression of multiple cone opsins in larval fangtooths and whalefish, and based on the expression of multiple *RH1*s in the pearley,

Scopelarchus sp., the Warming's lanternfish, *Ceratoscopelus warmingii* and the pelagic basslet, *Howella brodiei*. It is possible to assume that certain species achieve colour vision by either opsin co-expression, thus making the absorbance spectrum wider, or by summation of differently tuned photoreceptors.

In *Chauliodus*, expression of both opsins can be thought of as a transitional phase between pure cone opsin expressing larvae and pure rod opsin expressing adults, and is in congruence with anatomical data that showed that younger specimens already develop rods which are, however, far less densely packed than those present in multibank rod-only retinae of adults (Locket 1980). In the fangtooth, *Anoplogaster cornuta*, all larvae expressed both *RH1* and *RH2*, with an increasing proportion of *RH1* to *RH2* as fishes grew. These individuals all had traits of larval phenotypes (horns & small teeth; Fig. 1) and were collected relatively shallow between 0-300 m using plankton trawls, while adult specimens rarely occur above 500 m of depth. This suggests that the transition from cone to rod opsin gene expression happens before the morphological metamorphosis and habitat shift occur. A similar pattern has also been reported from shallow water fishes such as European eels (Bowmaker et al. 2008), dottybacks (Cortesi et al., 2016) and surgeonfishes (Tettamanti et al., 2019), where the visual system changes before metamorphosis takes place. Based on our data we cannot say for certain whether fangtooths start their lives with a pure-cone retina or whether low-levels of rod opsin expression are the norm even in pre-flexation larvae, as the smallest larva analysed in this study was 4 mm in total length and it already expressed a small proportion of the rod RH1 opsin gene. A similar pattern was also detected for *Coccorella atlantica*, where a limitation in sample size makes it difficult to conclude whether the larva we sampled is at a transitional phase or not. Multiple copies of larval *RH2* (up to five) have been seen in *Gyrinomimus* sp coding for five similar but not identical opsin proteins (93 - 99% amino acid identity). In addition to the green-sensitive cone opsin *RH2*, *Anoplogaster cornuta* also expresses the blue-sensitive *SWS2* as larva, potentially conferring dichromatic colour vision to the early life stages of this species.

Expression of both RH1 and RH2 in adult specimens has also been detected in three aulopiform species *Coccorella atlantica*, *Scopelosaurus hoedti* and *Scopelarchus sp*., and in a scombriform *Scombrolabrax heterolepis*. Similar to fangtooth, in *Coccorella atlantica* and *Scopelarchus* sp., the ratio of RH2:RH1 expression levels in the adult drops compared to the larval stage, suggesting the cone-dominated to rod-dominated retina transition in these species, which is again in accord with the life history of the deep-sea fishes. These results are also in

congruence with observations made on other deep-sea fish species by Bozzano et al. (2007). This therefore suggests that even in deep-sea fishes, opsin gene expression is dynamic throughout ontogeny.

Additionally, *Scopelarchus* sp. has two copies of *RH1* as an adult (*RH1a* and *RH1b*), which do not closely resemble each other according to the reconstruction of the *RH1* gene evolution (Fig. 2) and differ in 66 amino acids (three amino cites possibly tuning; Figure 2). A similar finding for *Scopelarchus* has also been reported by Pointer et al. (2007). Both *Coccorella atlantica* and *Scopelarchus*, respectively, have different copies of *RH2* expressed between larval and adult stages, and their larval copies are more similar to each other than to their respective adult variant. This indicates a larval and adult specific repertoire similar to what we find in ontogenetic transitions in e.g. cichlids (Carleton et al., 2016), but through a copy change instead of the opsin type. In *Scopelarchus* sp., the cone RH2 opsin gene might be expressed only locally in the ventral accessory retina only, as this is the only tissue with cone cells found in *Scopelarchus michaelsarsi*, while the main retina seems to be composed of rods only (Collin et al., 1998). The accessory ventral retina is therefore ontogenetically different than the larval retina.

Interestingly, as shown by morphological work from Wagner et al. (2019) on a closely related evermannellid species, *Evermannella balbo*, the retinae of adults only appear to have rods. Transcriptomic results on the other hand suggest not only the expression of rod opsin but also the cone opsin, giving support to previous studies by e.g. Underwood (1968), Ma et al. (2001); Simoes et al. (2016); de Busserolles et al. (2017), Schott et al. (2016, 2019) which have shown that it is sometimes difficult to determine the exact properties of photoreceptors as they could at the same time exhibit morphological, electrophysiological and molecular characteristics of both rods and cones. Our results thus allow a further discussion on whether the retinae of aulopiforms, in our case *Coccorella atlantica*, consist of two different populations of rods as suggested by morphology, or perhaps one type of rod cells supplemented by the transmuted cone cells that resemble them (as seen for example in the pearlside *Maurolicus* spp. (de Busserolles et al., 2017). Further investigation of the photoreceptor cells through a combination of in-situ, detailed morphological assessments and genetics, focusing on the phototransduction cascade, would be needed to disentangle the presence of pure rods vs. duplex retina in evermanellids.

Visual system of the species with multiple opsin genes in their retina:

In our data, we found three species with multiple rod RH1 opsin genes expressed and the translated protein differs in multiple amino acids. First, the Warming's lanternfish *Ceratoscopelus warmingii* has three different RH1 genes expressed in the adult retina. This corresponds to previous findings of the novel visual system based purely on multiple rods in several deep-sea fishes, including the glacier lanternfish (*Bethosema glaciale*) (Musilova et al., 2019). The three RH1 copies differ in total in 15 key-tuning amino acid sites (Table 2), and probably the shortest sensitive copy is RH1c (expression level low - 4-33%), longest-sensitive copy RH1b (expression low 1-5%) and the copy with the sensitivity in between - RH1a (expression high - 66 - 95%) suggesting one dominant rod opsin type with the low abundance presence of the shorter and longer sensitive types of rod opsin. Unfortunately, we cannot predict the exact values of the sensitivity, however Collin and Marshall (2003) provide the MSP measurements of 468 and 488 nm. We assume they have measured the two most abundant proteins, i.e. the middle-sensitive RH1a (i.e. 488 nm) and the shorter-shifted variant RH1c being therefore 468 nm. Therefore, we predict, that possibly another longer-shifted variant of RH1 (RH1b) exists in the visual system of this species. In *Howella*, we found two RH1 copies which differ in four amino acid sites (Table 2) and this suggest functional diversification of the rod opsins. Out of the four AA sites, two sites have previously been shown to have major effect: E122Q (RH1a:RH1b) shifting by ~15 nm to the shorter wavelength (based on the coelacanth - 10 nm or bovine - 20 nm in vitro mutagenesis; Yokoyama 2008) and G124S (RH1a:RH1b) shifting by 11 nm similarly to the shorter wavelength. We could therefore assume, that the RH1a of *Howella* has maximal sensitivity in ~26 nm longer wavelength than the shorter-shifted copy, RH1b, which has also higher expression levels (80-93% of all rod opsins). In *Scopelarchus*, the two rod opsins have their max. absorption of 479 and 486 nm (Pointer 2007) and the larval cone opsin is most sensitive in 505 nm (Pointer et al., 2007). The adult RH2 opsin as compared to the larval differ in two key tuning amino acid sites of which at least one is known to cause shift of 8 nm (Yokoyama, 2008). The larval opsin seems to be longer shifted, therefore we assume that the adult RH2 opsin is sensitive around 497 nm and complements the rods (Fig. 2).

In accordance with Collin & Marshall (2003), Pointer et al. (2007), Hope et al. (1997), Douglas & partridge (1997), Hunt et al. (2001), Yokoyama (2008), Yokoyama & Yia (2020), we have also observed the key-tuning AA sites in RH1 and RH2 that are common in species belonging to different fish orders, implying a tendency in deep-living fish to convergently

evolve their visual systems via a shift in absorbance properties of the protein itself (λ_{max}). (Table 2).

Up until now, molecular basis of ontogeny of vision in deep-sea fishes remained unclear and mostly supported by morphological and anatomical investigations. In this study we throw light on the visual system of major deep-sea fish lineages and inform of molecular changes that seem to be driven by shifts in ecology, physiology and behaviour. Although it has been clear that that deep-sea fish genomic repertoire consists also of cone opsin genes, adult eyes often seemed to be rod opsin expressing only. Here, we show that deep-sea fish larvae express cone opsins, most often RH2, and that animal maturation correlates to a developmental change in opsin expression, resulting in a shift from a cone-based vision to duplex, or rod-only vision. Our molecular results strongly support the developmental progression we also see in shallow water fishes, in that cones come first, and rods are added secondarily (Raymond 1995, Shen & Raymond 2004, Sernagor et al. 2006, Valen et al. 2016, Mears et al. 2001).

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References:

Afonso, P., McGinty, N., Graça, G., Fontes, J., Inácio, M., Totland, A., & Menezes, G. (2014). Vertical migrations of a deep-sea fish and its prey. PloS one, 9(5), e97884.

Ali, M. A. (1959). The ocular structure, retinomotor and photo-behavioral responses of juvenile Pacific salmon. Canadian journal of Zoology, 37(6), 965-996.

Ali, M. A., & Anctil, M. (1976). Retinas of Fishes - An Atlas. Springer Berlin Heidelberg.

Andrews, S. (2017). FastQC: a quality control tool for high throughput sequence data. 2010.

Betancur-R, R., Wiley, E. O., Arratia, G., Acero, A., Bailly, N., Miya, M., ... & Orti, G. (2017). Phylogenetic classification of bony fishes. BMC evolutionary biology, 17(1), 162.

Blaxter, J. H. S. (1968). Rearing herring larvae to metamorphosis and beyond. Journal of the Marine Biological Association of the United Kingdom, 48(1), 17-28.

Blaxter, J. H. S., & Staines, M. (1970). Pure-cone retinae and retinomotor responses in larval teleosts. Journal of the Marine Biological Association of the United Kingdom, 50(2), 449-464.

Bozzano, A., Pankhurst, P. M., & Sabatés, A. (2007). Early development of eye and retina in lanternfish larvae. Visual neuroscience, 24(3), 423-436.

Carleton, K. L., & Kocher, T. D. (2001). Cone opsin genes of African cichlid fishes: tuning spectral sensitivity by differential gene expression. Molecular Biology and Evolution, 18(8), 1540-1550.

Carleton, K. L., Dalton, B. E., Escobar-Camacho, D., & Nandamuri, S. P. (2016). Proximate and ultimate causes of variable visual sensitivities: insights from cichlid fish radiations. Genesis, 54(6), 299-325.

Carleton, K.L., Escobar-Camacho, D., Stieb, S.M., Cortesi, F. and Marshall, N.J., 2020. Seeing the rainbow: mechanisms underlying spectral sensitivity in teleost fishes. Journal of Experimental Biology, 223(8).

Collin, S. P., Hoskins, R. V., & Partridge, J. C. (1998). Seven Retinal Specializations in the Tubular Eye of the Scopelarchus michaelsarsi : A Case Study in Visual Optimization. Brain, Behavior and Evolution, 1998(09), 291–314.

Collin, S. P., & Marshall, N. J. (2003). Sensory Processing in Aquatic Environments. Springer-Verlag New York. http://doi.org/10.1371/journal.pone.0067005

Cortesi, F., Musilová, Z., Stieb, S. M., Hart, N. S., Siebeck, U. E., Cheney, K. L., ... & Marshall, N. J. (2016). From crypsis to mimicry: changes in colour and the configuration of the visual system during ontogenetic habitat transitions in a coral reef fish. Journal of Experimental Biology, 219(16), 2545-2558.

Dahlgren, C. P., & Eggleston, D. B. (2000). Ecological processes underlying ontogenetic habitat shifts in a coral reef fish. Ecology, 81(8), 2227-2240.

Dalton, B. E., De Busserolles, F., Marshall, N. J., & Carleton, K. L. (2017). Retinal specialization through spatially varying cell densities and opsin coexpression in cichlid fish. Journal of Experimental Biology, 220(2), 266-277.

Dalton, B. E., Loew, E. R., Cronin, T. W., & Carleton, K. L. (2014). Spectral tuning by opsin coexpression in retinal regions that view different parts of the visual field. Proceedings of the Royal Society B: Biological Sciences, 281(1797), 20141980.

de Busserolles, F., Cortesi, F., Helvik, J. V., Davies, W. I., Templin, R. M., Sullivan, R. K., ... & Kaartvedt, S. (2017). Pushing the limits of photoreception in twilight conditions: The rod-like cone retina of the deep-sea pearlsides. Science advances, 3(11), eaao4709.

Denton, E.J & Warren, F.J. (1957). Photosensitive pigments in the retinae of deep seafish. Journal of the Marine Biological Association of the UK. 36, 651–662.

Douglas, R. H., & Partridge, J. C. (1997). On the visual pigments of deep-sea fish. Journal of Fish Biology, 50(1), 68-85.

Douglas, R. H., Hunt, D. M., & Bowmaker, J. K. (2003). Spectral sensitivity tuning in the deep-sea. In Sensory Processing in Aquatic Environments (pp. 323-342). Springer, New York, NY.

Douglas, R. H., Partridge, J. C., & Marshall, N. J. (1998). The visual systems of deep-sea fish. I. Optics, tapeta, visual and lenticular pigmentation. Prog. Ret. Eye Res, 17(4), 597-636.

Evans, B. I., & Fernald, R. D. (1990). Metamorphosis and fish vision. Journal of neurobiology, 21(7), 1037-1052.

Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3, 294–29

Frederiksen, R. D. (1976). retinal tapetum containing discrete reflectors and photoreceptors in the bathypelagic teleost omosudis lowei. Vidensk. rneddr dansk naturh. Foren. 139: 109-146

Grant, J. B. (2007). Ontogenetic colour change and the evolution of aposematism: a case study in panic moth caterpillars. Journal of Animal Ecology, 439-447.

Hasegawa, E. I., Sawada, K., Abe, K., Watanabe, K., Uchikawa, K., Okazaki, Y., ... & Douglas, R. H. (2008). The visual pigments of a deep-sea myctophid fish Myctophum nitidulum Garman; an HPLC and spectroscopic description of a non-paired rhodopsin–porphyropsin system. Journal of Fish Biology, 72(4), 937-945.

Helfman, G., Collette, B. B., Facey, D. E., & Bowen, B. W. (2009). The diversity of fishes: biology, evolution, and ecology. John Wiley & Sons.

Hirt, B., & Wagner, H. J. (2005). The organization of the inner retina in a pure-rod deep-sea fish. Brain, behavior and evolution, 65(3), 157-167.

Hope, A. J., Partridge, J. C., Dulai, K. S., & Hunt, D. M. (1997). Mechanisms of wavelength tuning in the rod opsins of deep-sea fishes. Proceedings of the Royal Society of London. Series B: Biological Sciences, 264(1379), 155-163.

Hunt, D. M., Dulai, K. S., Partridge, J. C., Cottrill, P., & Bowmaker, J. K. (2001). The molecular basis for spectral tuning of rod visual pigments in deep-sea fish. Journal of Experimental Biology, 204(19), 3333-3344.

Hunt, D. M., Fitzgibbon, J., Slobodyanyuk, S. J., Bowmaker, J. K., & Dulai, K. S. (1997). Molecular evolution of the cottoid fish endemic to Lake Baikal deduced from nuclear DNA evidence. Molecular Phylogenetics and Evolution, 8(3), 415-422.

Hunt, D.M., Hankins, M.W., Collin, S.P. and Marshall, N.J. eds., 2014. Evolution of visual and non-visual pigments (Vol. 4). Boston, MA: Springer

Kasagi, S., Mizusawa, K., Murakami, N., Andoh, T., Furufuji, S., Kawamura, S., & Takahashi, A. (2015). Molecular and functional characterization of opsins in barfin flounder (Verasper moseri). Gene, 556(2), 182-191.

Katoh, K., Asimenos, G., & Toh, H. (2009). Multiple alignment of DNA sequences with MAFFT. In Bioinformatics for DNA sequence analysis (pp. 39-64). Humana Press.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., ... & Thierer, T. (2012). Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics, 28(12), 1647-1649.

Lamb, T. D. (2013). Evolution of phototransduction, vertebrate photoreceptors and retina. Progress in retinal and eye research, 36, 52-119.

Land MF, Nilsson D-E (2002) Animals eyes: Oxford University Press. 221 p.

Laver, C. R., & Taylor, J. S. (2011). RT-qPCR reveals opsin gene upregulation associated with age and sex in guppies (Poecilia reticulata)-a species with color-based sexual selection and 11 visual-opsin genes. BMC evolutionary biology, 11(1), 81.

Locket, A., & Crescitelli, F. (1977). Handbook of Sensory Physiology, Vol. VII/5: The Visual System of Vertebrates.

Locket, N. A. (1977). Adaptations to the deep-sea environment. In The visual system in vertebrates (pp. 67-192). Springer, Berlin, Heidelberg.

Locket, N. A. (1980). Variation of architecture with size in the multiple-bank retina of a deep-sea teleost, Chauliodus sloani. Proceedings of the Royal Society of London. Series B. Biological Sciences, 208(1171), 223-242.

Ma, J. X., Znoiko, S., Othersen, K. L., Ryan, J. C., Das, J., Isayama, T., ... & Cameron, D. A. (2001). A visual pigment expressed in both rod and cone photoreceptors. Neuron, 32(3), 451-461.

Madden, T. (2013). The BLAST sequence analysis tool. In The NCBI Handbook [Internet]. 2nd edition. National Center for Biotechnology Information (US).

Malinsky, M., Challis, R. J., Tyers, A. M., Schiffels, S., Terai, Y., Ngatunga, B. P., … Turner, G. F. (2015). Genomic islands of speciation separate cichlid ecomorphs in an East African crater lake. Science (New York, N.Y.), 350(6267), 1493–8. http://doi.org/10.1126/science.aac9927

Manousaki, T., Hull, P. M., Kusche, H., Machado-Schiaffino, G., Franchini, P., Harrod, C., ... & Meyer, A. (2013). Parsing parallel evolution: ecological divergence and differential gene expression in the adaptive radiations of thick-lipped M idas cichlid fishes from N icaragua. Molecular ecology, 22(3), 650-669.

Mears, A. J., Kondo, M., Swain, P. K., Takada, Y., Bush, R. A., Saunders, T. L., ... & Swaroop, A. (2001). Nrl is required for rod photoreceptor development. Nature genetics, 29(4), 447-452.

Morrow, J. M., Lazic, S., Fox, M. D., Kuo, C., Schott, R. K., Gutierrez, E. D. A., ... & Chang, B. S. (2017). A second visual rhodopsin gene, rh1-2, is expressed in zebrafish photoreceptors and found in other ray-finned fishes. Journal of Experimental Biology, 220(2), 294-303.

Munk, O. (1975). On the eyes of two foveate notosudid teleosts, Scopelosaurus hoedti and Ahliesaurus berryi. Vidensk Meddr. Dansk Naturh. Foren, 138, 87-125.

Munk, O. (1989). Duplex retina in the mesopelagic deep-sea teleost Lestidiops affinis (Ege, 1930). Acta Zoologica, 70(3), 143-149.

Munz, F. W. (1958). Photosensitive pigments from the retinae of certain deep-sea fishes. The Journal of physiology, 140(2), 220-235.

Musilova, Z., Cortesi, F., Matschiner, M., Davies, W. I., Patel, J. S., Stieb, S. M., ... & Mountford, J. K. (2019). Vision using multiple distinct rod opsins in deep-sea fishes. Science, 364(6440), 588-592.

Musilova, Z., Indermaur, A., Bitja-Nyom, A. R., Omelchenko, D., Kłodawska, M., Albergati, L., … Salzburger, W. (2019). Evolution of the visual sensory system in cichlid fishes from crater lake Barombi Mbo in Cameroon. *Molecular Ecology*, (August), 5010–5031. http://doi.org/10.1111/mec.15217

Nakayama, T. A., & Khorana, H. G. (1991). Mapping of the amino acids in membrane-embedded helices that interact with the retinal chromophore in bovine rhodopsin. Journal of Biological Chemistry, 266(7), 4269-4275.

Nicol, J. A. C., & Somiya, H. (1989). The eyes of fishes. Oxford University Press.

P**ointer, M. A., Carvalho, L. S., Cowing, J. A., Bowmaker, J. K., & Hunt, D. M. (2007)**. The visual pigments of a deep-sea teleost, the pearl eye Scopelarchus analis. Journal of Experimental Biology, 210(16), 2829-2835.

Pointer, M. A., Freedman, M. S., & Hunt, D. M. (2002). Molecular characterisation of the visual pigments in the deep-sea scopelarchid, Scopelarchus analis. Investigative Ophthalmology & Visual Science, 43(13), 1372-1372.

Protas, M., Conrad, M., Gross, J. B., Tabin, C., & Borowsky, R. (2007). Regressive evolution in the Mexican cave tetra, Astyanax mexicanus. Current biology, 17(5), 452-454.

Raymond, P. A. (1995). Development and morphological organization of photoreceptors. In Neurobiology and Clinical Aspects of the Outer Retina (pp. 1-23). Springer, Dordrecht.

Robinson, E., Jerrett, A., Black, S., & Davison, W. (2017). Ontogenetic shift in spectral sensitivity of Pacific snapper Chrysophrys auratus. New Zealand Natural Sciences, 42, 18-22.

Ronquist, F., Teslenko, M., Van Der Mark, P., Ayres, D. L., Darling, A., Höhna, S., ... & Huelsenbeck, J. P. (2012). MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic biology, 61(3), 539-542.

Sabatés, A., Bozzano, A., & Vallvey, I. (2003). Feeding pattern and the visual light environment in myctophid fish larvae. Journal of Fish Biology, 63(6), 1476-1490.

Schott, R. K., Bhattacharyya, N., & Chang, B. S. (2019). Evolutionary signatures of photoreceptor transmutation in geckos reveal potential adaptation and convergence with snakes. Evolution, 73(9), 1958-1971.

Schott, R. K., Müller, J., Yang, C. G., Bhattacharyya, N., Chan, N., Xu, M., ... & Chang, B. S. (2016). Evolutionary transformation of rod photoreceptors in the all-cone retina of a diurnal garter snake. Proceedings of the National Academy of Sciences, 113(2), 356-361.

Sernagor, E., Eglen, S., Harris, B., & Wong, R. (Eds.). (2006). Retinal development. Cambridge University Press.

Shen, Y. C., & Raymond, P. A. (2004). Zebrafish cone-rod (crx) homeobox gene promotes retinogenesis. Developmental biology, 269(1), 237-251.

Simoes, B. F., Sampaio, F. L., Loew, E. R., Sanders, K. L., Fisher, R. N., Hart, N. S., ... & Gower, D. J. (2016). Multiple rod–cone and cone–rod photoreceptor transmutations in snakes: evidence from visual opsin gene expression. Proceedings of the Royal Society B: Biological Sciences, 283(1823), 20152624.

Stieb, S.M., de Busserolles, F., Carleton, K.L., Cortesi, F., Chung, W.S., Dalton, B.E., Hammond, L.A. and Marshall, N.J., 2019. A detailed investigation of the visual system and

visual ecology of the Barrier Reef anemonefish, Amphiprion akindynos. *Scientific reports*, *9*(1), pp.1-14.

Sugawara, T., Terai, Y., Imai, H., Turner, G. F., Koblmüller, S., Sturmbauer, C., ... & Okada, N. (2005). Parallelism of amino acid changes at the RH1 affecting spectral sensitivity among deep-water cichlids from Lakes Tanganyika and Malawi. Proceedings of the National Academy of Sciences, 102(15), 5448-5453.

Takechi, M., & Kawamura, S. (2005). Temporal and spatial changes in the expression pattern of multiple red and green subtype opsin genes during zebrafish development. Journal of Experimental Biology, 208(7), 1337-1345.

Tettamanti, V., de Busserolles, F., Lecchini, D., Marshall, N.J. and Cortesi, F., 2019. Visual system development of the spotted unicornfish, Naso brevirostris (Acanthuridae). Journal of Experimental Biology, 222(24).

Turner, J. R., White, E. M., Collins, M. A., Partridge, J. C., & Douglas, R. H. (2009). Vision in lanternfish (Myctophidae): adaptations for viewing bioluminescence in the deep-sea. Deep Sea Research Part I: Oceanographic Research Papers, 56(6), 1003-1017.

Underwood, G. (1968). Some suggestions concerning vertebrate visual cells. Vision research, 8(4), 483-488.

Valen, R., Eilertsen, M., Edvardsen, R. B., Furmanek, T., Rønnestad, I., van der Meeren, T., ... & Helvik, J. V. (2016). The two-step development of a duplex retina involves distinct events of cone and rod neurogenesis and differentiation. Developmental biology, 416(2), 389-401.

Wagner, H. J., Fröhlich, E., Negishi, K., & Collin, S. P. (1998). The eyes of deep-sea fish II. Functional morphology of the retina. Progress in retinal and eye research, 17(4), 637-685.

Wangsa-Wirawan, N. D., & Linsenmeier, R. A. (2003). Retinal oxygen: fundamental and clinical aspects. Archives of ophthalmology, 121(4), 547-557.

Ward, R. D., Zemlak, T. S., Innes, B. H., Last, P. R., & Hebert, P. D. (2005). DNA barcoding Australia's fish species. Philosophical Transactions of the Royal Society B: Biological Sciences, 360(1462), 1847-1857.

Warrant, E. J., & Locket, N. A. (2004). Vision in the deep sea. Biological Reviews, 79(3), 671-712.

Warrant, E. J., Collin, S. P., & Locket, N. A. (2003). Eye design and vision in deep-sea fishes. In Sensory processing in aquatic environments (pp. 303-322). Springer, New York, NY.

Yokoyama, S. (2008). Evolution of dim-light and color vision pigments. Annu. Rev. Genomics Hum. Genet., 9, 259-282.

Yokoyama, S., & Jia, H. (2020). Origin and adaptation of green-sensitive (RH2) pigments in vertebrates. FEBS Open Bio, 10(5), 873-882.

Table 1: Samples used in the study and results of the opsin gene expression in the eyes or retina

species	order	stage	code	size	date of collection	raw reads	reads after bacteria filtering	RH1	RH2	SWS1	SWS2	accession number
Anoplogaster cornuta	Trachichthyiformes	Larva	39016	4mm		36,373,372	36,125,630	<0.01	0.97		0.02	
		Larva	39017	4mm		34,712,538	34,604,364	<0.01	0.97		0.02	
		Larva	4_23	9mm		41,592,840	41,519,070	0.02	0.96		0.02	
		Larva	4_22	11mm		75,109,270	74,990,214	0.1	0.89		<0.01	
		Larva	4_21			28,945,804	28,869,552	0.2	0.79		<0.01	
		Larva	261s03	12mm		48,458,044	48,457,472	0.48	0.51		<0.01	
		Adult	56H6			11,658,040	11,635,610	1				
Ceratoscopelus warmingii	Myctophiformes	Adult	300s03	63mm		49,078,974	44,114,639	0.66[RH1a]				
								0.01[RH1b]				
								0.33[RH1c]				
		Adult	S1			8,796,786	8,789,961	0.93[RH1a]				
								0.02[RH1b]				
								0.05[RH1c]				
		Adult	S2			8,655,248	8,649,342	0.90[RH1a]				
								0.05[RH1b]				
								0.05[RH1c]				
		Adult	S3			7,904,714	7,899,617	0.95[RH1a]				
								0.01[RH1b]				
								0.04[RH1c]				
Chauliodus sp.	Stomiiformes	Larva	67I2_2	11mm		34,445,252	34,371,394		1			
		Larva	67I1	17mm		20,701,686	20,683,764	<0.01	0.99			
		Juvenile	109C7	24mm		32,865,334	32,834,300	1				
		Juvenile	109A2	20mm		25,389,318	25,344,182	1				
		Juvenile	109D7			18,540,248	18,533,484	1				
		Adult	109B6	20mm		24,854,866	24,835,646	1				
Coccorella atlantica	Aulopiformes	Larva	109G8			26,376,620	26,359,060	0.01	0.99[RH2a]			
		Adult	56C7			36,794,070	36,790,854	0.67	0.33[RH2b]			
		Adult	297_7	68mm		43,948,534	40,511,279	0.99	0.01[RH2b]			
Grammastomias flagellibarba	Stomiiformes	Adult	56H8			9,775,520	9,742,864	1				
Gyrinomimus myersi	Beryciformes	Larva	S25			19,270,096	19,122,986	<0.01	0.35[RH2a]	0.07	<0.01	
									0.10[RH2b]			
									0.12[RH2c]			
									0.14[RH2d]			
									0.20[RH2e]			
Howella brodiei	Pempheriformes	Adult	56D8			56,905,264	43,936,178	0.20[RH1a]				
								0.80[RH1b]				
		Adult	56D9			61,909,674	61,590,270	0.07[RH1a]				
								0.93[RH1b]				
Hygophum reinhardtii	Myctophiformes	Larva	67I2_1	5mm		17,222,626	17,122,880	<0.01	0.99			
Idiacanthus fasciola	Stomiiformes	Larva	71C2			23,382,468	23,379,360		1			
		Larva	67D2			6,647,384	6,623,496		1			
		Larva	71C1			23,632,774	23,497,715		1			
		Larva	67I7	25mm		25,713,960	25,701,768		1			
		Larva	71B9			43,695,620	43,633,424		1			
		Larva	67B8			28,333,642	28,313,024		1			
		Larva	109A1	41mm		27,834,274	27,818,750		1			
		Adult	228s01			21,704,528	21,622,274	1				
		Adult	67F8			15,744,604	15,739,004	1				
		Adult	67B6			11,368,044	15,439,954	1				
Melamphaes sp.	Beryciformes	Juvenile	4_26			38,583,370	38,534,507	1				
		Juvenile	4_28			35,489,970	35,431,421	1				
Melanolagus bericoides	Argentiniformes	Larva	71H3			26,219,646	26,210,402	<0.01	0.99			
Pteraclis aesticola	Scombriformes	Larva	109H4			19,918,922	19,870,484	0.45	0.55			
Pollichthys mauli	Stomiiformes	Adult	109I2			55,767,498	54,875,747	1				
		Adult	109I3			68,655,686	67,082,709	1				
Scombrolabrax heterolepis	Scombriformes	Adult	,56E3			65,770,014	5,527,135	0.98	0.02			
		Adult	,56E4			60,643,652	60,472,479	0.91	0.09			
Scopelarchus sp.	Aulopifoformes	Larva	71B7			43,695,620	43,682,534		1[RH2a]			
		Larva	109H1			37,542,802	37,416,486		1[RH2a]			
		Larva	109B9	21mm		9,971,250	9,919,148		1[RH2a]			
		Adult	272_10	66mm		45,565,202	43,729,338	0.87[RH1a]	0.09[RH2b]			
								0.04[RH1b]				
Scopelogadus mizolepis	Beryciformes	Adult	,57E2			5,160,876	5,160,876	1				
		Adult	300s01			15,448,240	15,420,650	1				
Scopelosaurus hoedti	Aulopifoformes	Adult	297_9	69mm		43,948,534	42,248,053	0.86	0.14			
Vinciguerria poweriae	Stomiiformes	Larva	109H2			25,863,628	25,755,100		1			
		Larva	109C8	16mm		22,914,004	22,858,398		1			

Table 2: key-tuning amino acid sites in the rhodopsin RH1 gene

Species	Order	83	90	96	102	111	113	118	122	124	132	164	183	188	194	195	207	208	211	214	253	261	265	269	289	292	295	299	300	317	lmax	reference
bovine RH1		D	G	Y	Y	N	E	T	E	A	A	A	M	G	P	H	M	F	H	I	M	F	W	A	T	A	A	A	V	M	500 nm	Yokoyama 2008
ancestral teleost RH1		D	G	Y	Y		E	T	E	A	A	A	M		L	N	M	F	H	I	M	F	W	A	T	A	A	A	L	M		Musilova et al., 2019a
Melanolagus bericoides	Argentiniformes	.	.	V	F	.	.	.	.	S	.	.	.	.	R	A	.	.	.	T	.	Y	.	.	.	.	.	.	.	.		
Bathysaurus ferox	Aulopiformes	N	.	.	.	.	.	.	.	S	.	.	.	.	R	A	.	.	.	.	.	.	.	.	.	S	.	T	L	.	481 nm	Collin, S. P., & Marshall, N. J. (2003)
Bathysaurus mollis	Aulopiformes	N	.	.	.	.	.	.	.	S	.	.	.	.	R	A	.	.	.	.	.	.	.	T	.	S	.	T	L	.	479 nm	Collin, S. P., & Marshall, N. J. (2003)
Coccorella atlantica	Aulopiformes	N	.	.	.	.	.	.	M	.	.	.	.	.	R	A	.	.	.	C	.	.	.	.	.	.	S	.	I	.	480 nm‡	Douglas & Partridge (1996)
Scopelarchus sp. RH1a	Aulopiformes	N	.	.	.	.	.	.	.	.	.	.	.	.	R	A	.	.	.	C	.	.	.	.	.	S	S	S	I	.	486 nm	Pointer et al., 2007
Scopelarchus sp. RH1b	Aulopiformes	N	.	.	.	.	.	.	.	.	.	.	.	.	L	K	.	.	.	V	.	.	.	.	.	S	.	.	L	.	479 nm	Pointer et al., 2007
Scopelosaurus hoedti	Aulopiformes	.	.	.	.	.	.	.	.	.	.	.	.	.	R	A	.	.	.	.	.	.	.	.	.	S	S	.	I	.		
Gyrinomimus myersi	Beryciformes	N	.	.	.	.	.	.	.	.	.	.	.	.	R	A	.	.	.	.	.	.	.	.	.	S	.	.	I	.		
Melamphaes sp.	Beryciformes	.	.	.	.	.	.	.	Q	.	S	.	.	.	R	V	.	.	.	G	.	.	.	.	.	.	.	S	I	.		
Scopelogadus mizolepis	Beryciformes	.	.	.	.	.	.	.	Q	G	S	.	.	.	R	V	.	.	.	G	.	.	.	T	.	.	.	S	.	.	488 nm§	Douglas & Partridge (1996)
Lepisosteus oculatus	Lepisosteiformes	.	.	.	.	.	.	.	Q	S	.	.	.	.	L	K	.	.	.	L	.	Y	.	G	.	.	.	.	L	.		
Ceratoscopelus warmingii RH1a	Myctophiformes	.	.	.	.	T	.	.	Q	.	S	.	.	.	R	A	.	.	.	G	.	.	.	.	A	.	.	S	I	.	?468 nm†	Collin, S. P., & Marshall, N. J. (2003)
Ceratoscopelus warmingii RH1b	Myctophiformes	.	.	W	.	I	.	.	I	T	A	.	.	.	R	A	.	.	.	L	.	Y	.	.	.	.	.	.	I	.	prob. 488 nm†	Collin, S. P., & Marshall, N. J. (2003)
Ceratoscopelus warmingii RH1c	Myctophiformes	N	.	.	.	T		.	Q	A	S	S	.	.	N	V	.	.	Y	G	.	F	.	.	.	S	.	.	I	.	?468 nm†	Collin, S. P., & Marshall, N. J. (2003)
Hygophum reinhardtii	Myctophiformes	.	.	.	.	A	.	.	Q	.	.	.	.	.	R	A	.	.	.	G	.	.	.	.	.	.	.	.	I	.		
Howella brodiei RH1a	Pempheriformes	N	.	.	.	.	.	.	.	G	.	.	.	.	R	A	.	.	.	.	.	.	.	.	G	S	S	S	I	.		
Howella brodiei RH1b	Pempheriformes	N	.	.	.	.	.	.	Q	S	.	.	.	.	R	A	.	.	.	A	.	.	.	.	.	S	S	S	I	.		
Pteraclis aesticola	Scombriformes	.	.	.	.	.	.	.	Q	.	.	.	.	.	R	A	.	.	.	.	.	.	.	.	.	.	.	S	I	.		
Scombrolabrax heterolepis	Scombriformes	.	.	.	.	.	.	.	Q	.	.	.	.	.	R	A	.	.	.	.	.	.	.	S	.	S	.	S	I	.		
Chauliodus sp.	Stomiiformes	N	.	.	.	.	.	.	.	G	.	.	.	.	R	A	.	.	.	V	.	.	.	.	A	S	.	.	L	V	484 nm	Collin, S. P., & Marshall, N. J. (2003)
Grammatostomias flagellibarba	Stomiiformes	N	.	.	.	.	.	.	.	.	.	.	.	.	R	A	.	.	.	V	.	.	.	.	.	S	.	.	L	.	480 - 487 nm	Kenaley et al. (2013)
Idiacanthus fasciola	Stomiiformes	N	.	.	.	.	.	.	.	.	.	.	.	.	R	A	.	.	.	.	.	.	.	.	.	S	.	.	L	.	485 nm	Collin, S. P., & Marshall, N. J. (2003)
Pollichthys mauli	Stomiiformes	N	.	.	.	.	.	.	.	.	.	.	.	.	R	A	.	.	.	.	.	.	.	.	.	S	.	.	L	.		
Anoplogaster cornuta	Trachichthyiformes	N	.	.	.	.	.	.	.	.	.	.	.	.	R	A	.	S	.	.	.	.	.	.	S	S	.	S	I	.	485 nm	Collin, S. P., & Marshall, N. J. (2003)

† = two pigments reported without assignment to the gene; see also Figure 2
‡ = for *Evermannella balbo* ; sequence not available
§ = for *Scopelogadus beani* ; sequence not available

Table 3: key-tuning amino acid sites in the cone opsin RH2 gene

species	order	83	122	207	255	292	lmax	reference
Bovine RH1		D	E	M	I	A	500 nm	Yokoyama 2008
Ancestral teleost		D	Q	M	I	A	488 nm	Yokoyama & Yia (2020)
Melanolagus bericoides	Argentiniformes	G	Q	.	V	.		
Coccorella atlantica adult	Aulopiformes	G	Q	.	.	.		
Coccorella atlantica larva	Aulopiformes	G	Q	.	V	.		
Scopelarchus sp. adult	Aulopiformes	G	Q	I	C	.		
Scopelarchus sp. larva	Aulopiformes	G	Q	.	V	.	505 nm	Pointer et al. 2007
Scopelosaurus hoedti	Aulopiformes	G	Q	.	.	.		
Gyrinomimus myersi RH2a	Beryciformes	G	Q	.	F	.		
Gyrinomimus myersi RH2b	Beryciformes	G	Q	L	F	.		
Gyrinomimus myersi RH2c	Beryciformes	G	Q	L	F	.		
Gyrinomimus myersi RH2d	Beryciformes	G	Q	L	F	.		
Gyrinomimus myersi RH2e	Beryciformes	G	Q	L	F	.		
Lepisosteus platyrhincus	Lepisosteiformes	G	.	.	.	.		
Hygophum reinhardtii	Myctophiformes	G	Q	.	V	.		
Lepidopus fitchi RH2a	Scombriformes	G	.	.	V	.	496 nm	Yokoyama & Yia (2020)
Lepidopus fitchi RH2b	Scombriformes	G	Q	.	V	.		
Lepidopus fitchi RH2c	Scombriformes	G	Q	.	V	.	506 nm	Yokoyama & Yia (2020)
Lepidopus fitchi RH2d	Scombriformes	G	Q	.	V	.		
Pteraclis aesticola	Scombriformes	G	Q	L	.	.		
Scombrolabrax heterolepis	Scombriformes	G	Q	.	.	.		
Aristostomias scintillans	Stomiiformes	G	Q	L	V	.	468 nm	Yokoyama & Yia 2020
Chauliodus sp.	Stomiiformes	G	Q	.	F	.		
Grammastomias flagellibarba	Stomiiformes	G	Q	.	.	.		
Idiacanthus fasciola	Stomiiformes	G	Q	.	V	.		
Vinciguerria poweriae	Stomiiformes	G	Q	.	F	.		
Anoplogaster cornuta	Trachichthyiformes	G	Q	.	.	.		