

# 1 **Visual gene expression reveals a cone to rod developmental progression in** 2 **deep-sea fishes**

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14 **Keywords:** opsin, evolution, mesopelagic, adaptation, convergence, phototransduction, vision,  
15 gene expression, rhodopsin

16

## 17 **Abstract**

18 Vertebrates generally use cone cells in retina for colour vision and rod cells to see in the dim  
19 light. Many deep-sea fishes have only rod cells in the retina, while both rod and cone genes are  
20 still preserved in their genomes. As deep-sea fish larvae start their lives in the shallow, well-lit  
21 epipelagic zone, they have to cope with diverse environmental conditions during ontogeny.  
22 Using a comparative transcriptomic approach in 20 deep-sea fish species from eight teleost  
23 orders, we report on a developmental cone-to-rod switch. While adults mostly rely on rod opsin  
24 (*RH1*) for vision in the dim light, larvae almost exclusively express mid-wavelength-sensitive  
25 cone opsins (*RH2*) in their retinas. The phototransduction cascade genes follow a similar  
26 ontogenetic pattern of cone- followed by rod-specific gene expression in most orders, except  
27 for the pearleye and sabretooth (Aulopiformes), in which the cone cascade remains dominant  
28 throughout development. By inspection of whole genomes of five deep-sea species (four of  
29 them sequenced within this study: *Idiacanthus fasciola*, *Chauliodus sloani*; Stomiiformes;  
30 *Coccorella atlantica*, *Scopelarchus michaelsarsi*; Aulopiformes), we found that deep-sea fish  
31 possess mostly the rod RH1 opsin, and multiple copies of the green-sensitive RH2 opsin genes  
32 in their genomes, while other cone opsin classes have been lost. Our findings provide molecular  
33 support for a limited opsin gene repertoire and a conserved vertebrate pattern whereby cone

34 photoreceptors develop first and rod photoreceptors are added only at later developmental  
35 stages.

## 36 INTRODUCTION

37 Vision is a primary sense used by most vertebrates for navigation, predator avoidance,  
38 communication and to find food and shelter. At its initiation, vertebrate vision is enabled by  
39 cone (photopic, colour vision) and rod (scotopic, mostly colour blind) photoreceptors in the  
40 retina containing a light absorbing pigment that consists of an opsin protein covalently bound  
41 to a vitamin-A-derived chromophore (Lamb 2013). The absorbance of photons by the  
42 chromophore leads to a conformational change of the opsin protein, which initiates a  
43 photoreceptor-specific G-protein-coupled phototransduction cascade, propagating the signal to  
44 the brain (Downes & Gautam 1999, Larhammar et al. 2009, Lamb et al. 2019). It is thought  
45 that the development of the visual system follows a conserved molecular pattern whereby cone  
46 specific genes are activated first before the rod molecular pathway is initiated later during  
47 ontogeny (Mears et al. 2001, Shen & Raymond 2004, Sernagor et al. 2006). However, whether  
48 this is the case for all vertebrates and especially for those that have retinas that contain only  
49 rods as adults, remains unclear.

50 Changes in the light environment, ecology, and phylogenetic inertia are thought to be  
51 primary drivers for visual system diversity in vertebrates (Hunt et al. 2014). For example, most  
52 mesopelagic deep-sea fishes (200 – 1,000 m depth), either living strictly at depth or migrating  
53 to the shallows at night, have evolved visual systems that are sensitive to the dominant blue  
54 light (~ 470 – 490 nm) of their environment (Turner et al. 2009). Moreover, as the daylight and  
55 the bioluminescent light emitted by deep-sea critters are quickly dimmed with depth, deep-sea  
56 fish visual systems have evolved peculiar morphologies to maximise photon capture including  
57 barrel-eyes, reflective tapeta and the use of rod-dominated and in many cases rod-only retinas  
58 that might be stacked into multiple banks (reviewed in de Busserolles et al. 2020). However,  
59 most mesopelagic fishes start their lives in the shallow well-lit epipelagic zone (0 – 200 m  
60 depth) (Moser & Smith 1993, Sassa & Hirota 2013). Consequently, their visual systems must  
61 cope with a variety of light intensities and spectra throughout development.

62 Studies investigating the gene expression in the retina of deep-sea fishes are scarce and  
63 usually focus on a selected few species (Zhang et al. 2000, Douglas et al. 2016; de Busserolles  
64 et al. 2017, Musilova et al. 2019a, Byun et al. 2020). In adults, species with pure rod retinas  
65 tend to only express rod opsin(s) (Douglas et al. 2016, Musilova et al. 2019a), albeit two species  
66 of pearlsides (*Maurolicus* spp.) have been found to express cone-specific genes (i.e., cone  
67 transduction pathway and opsin genes) inside rod-looking cells (de Busserolles et al. 2017). It  
68 remains unknown whether deep-sea fishes that have a low proportion of cone photoreceptors  
69 as adults (e.g. Munk 1990, Collin et al. 1998, Bozanno et al. 2007, Pointer et al. 2007, Biagioni

70 et al. 2016) also express cone-specific genes at any stages of their lives or whether molecularly  
71 these fishes rely on the rod machinery alone. To investigate whether the retinal development  
72 in deep-sea fishes follows a similar cone-to-rod molecular pathway as found in other  
73 vertebrates or whether some species start their lives with rod pathway activated, we set out to  
74 sequence the retinal transcriptomes of 20 deep-sea fish species, including the larval stages in  
75 ten species, belonging to eight different teleost orders (Argentiniformes, Aulopiformes,  
76 Beryciformes, Myctophiformes, Pempheriformes, Scombriformes, Stomiiformes and  
77 Trachichthyiformes). We have further investigated the genomic repertoire in five selected  
78 species.

79

## 80 **RESULTS AND DISCUSSION**

81 **Opsin gene repertoire in the genome.** In teleost fishes, gene duplications and deletions  
82 followed by functional diversification have resulted in extant species having between 1-40  
83 visual opsin genes within their genomes (Musilova et al., 2019a; Musilova et al., 2021). These  
84 genes are defined by their phylogeny and their spectrum of maximal sensitivity ( $\lambda_{\max}$ ) and fall  
85 within five major classes, four cone opsins ('ultraviolet or UV sensitive' *SWS1*: 347–383 nm,  
86 'blue' *SWS2*: 397–482 nm, 'green' *RH2*: 452–537 nm and 'red' *LWS*: 501–573 nm) and one  
87 rod opsin ('blue-green' rhodopsin, *RH1* or Rho: 447–525 nm) (Carleton et al. 2020). We  
88 analyzed whole genomes of five deep-sea species (sawtail fish *Idiacanthus fasciola*, viperfish  
89 *Chauliodus sloani*; both Stomiiformes; sabretooth *Coccorella atlantica*, pearleye *Scopelarchus*  
90 *michaelsarsi*; both Aulopiformes; and fangtooth *Anoplogaster cornuta*; Trachichthyiformes).  
91 All species possess one or two copies of the rod opsin *RH1* gene, and one to seven copies of  
92 the *RH2* cone opsin (Fig. 1). All other cone opsin classes, i.e. the *SWS1*, *SWS2* (except for the  
93 fangtooth) and *LWS* are missing and have been putatively lost in evolution. This is in accord  
94 with the observation that the *LWS* gene abundance decreases with the habitat depth (Musilova  
95 et al., 2019a). Such limited genomic repertoire most likely represents an evolutionary response  
96 to the deep-sea scotopic environment where also the shortest (UV-violet) and longest (red)  
97 wavelengths of light get filtered out first in the water column (reviewed in Musilova et al.,  
98 2021, De Busserolles et al., 2020, and Carleton et al., 2020). The increased *RH2* diversity  
99 observed in the two aulopiform species, on the other hand, illustrates the versatility of this cone  
100 opsin class and confirms its dominance in various dimmer-light habitats (Musilova & Cortesi,  
101 2021). Here we confirm that *RH2* is undoubtedly the most important (and often the only) cone  
102 opsin gene present in deep-sea fish genomes.

103 **Visual opsin gene expression.** Transcriptomic sequencing of 20 deep-sea teleost species  
104 revealed that deep-sea fishes mainly express rod opsins and/or green-sensitive cone opsins  
105 (*RH2s*) in their retinas (Fig. 1, Table 1). While larvae mostly expressed *RH2*, adults and  
106 juveniles mostly expressed *RH1* and in a few cases a combination of both. We found none or  
107 very low expression of any of the other cone opsin genes: the red sensitive *LWS* was not  
108 expressed at all, the UV sensitive *SWS1* was only found in the larva of the whalefish,  
109 *Gyrinomimus* sp. (Beryciformes), and the blue/violet sensitive *SWS2* only in the larvae of the  
110 whalefish, and the fangtooth, *Anoplogaster cornuta* (Trachichthyiformes), (Fig. 1, Table 1).  
111 Differences in gene expression patterns are likely to be driven by ontogenetic transitions in  
112 light habitat from bright to dim environments and also by changes in ecological demands, as  
113 discussed in more detail below.

114 Similar to the opsin genes, we also detected ontogenetic differences in the expression  
115 of phototransduction cascade genes (Fig. 2). Here we focused on the comparison of five species  
116 from three teleost orders for which we had both larval and adult specimens available and found  
117 that the cone-specific genes were mostly expressed in the larval stages (e.g., cone transducin,  
118 *GNAT2*), while adults from three species mostly expressed rod-specific genes (e.g., rod  
119 transducin, *GNAT1*; Fig. 2b). Hence, at the molecular level, the visual systems of deep-sea  
120 fishes start out with a cone-based expression pattern. Moreover, in the fangtooth, where  
121 samples from various sized specimens were available, we found that the cone-specific  
122 expression was gradually replaced with the rod profile as the fish grew (Fig. 2c, Table S1).  
123 This is similar to the visual development in shallower living fishes (e.g., Atlantic cod (Valen  
124 et al. 2016), zebrafish (Sernagor et al. 2006)) and terrestrial vertebrates (e.g., mice (Mears et  
125 al. 2001), rhesus monkey (La Vail et al. 1991)), where cone photoreceptors are first to develop,  
126 followed by temporally and spatially distinct rods (Raymond 1995, Shen & Raymond 2004).  
127 The cone-to-rod developmental sequence is therefore likely to be shared across vertebrates,  
128 even in species that have pure rod retinas as adults.

129

130 **Ontogenetic shift in expression profiles and the transition phase.** The developmental  
131 changes in the visual system we uncovered are best explained by the different habitats larval  
132 and adult deep-sea fishes inhabit. In general, deep-sea fish larvae live in the shallow epipelagic  
133 zone (Moser & Smith 1993) where ambient light levels are sufficiently high to warrant a cone-  
134 based visual system. After metamorphosis, deep-sea fishes start to submerge deeper and take  
135 up a life at different depths in the mesopelagic or even bathypelagic (below 1,000 m depth)  
136 zone, where the sun- and moonlight is gradually replaced by bioluminescence as the main

137 source of light (Denton 1990). In this extremely dim environment, rods work at their best and  
138 cone photoreceptors would be obsolete for the most part at least. Rod-based vision is also  
139 favoured in those deep-sea species that exhibit diel vertical migrations to feed in the plankton  
140 rich surface layers at night (de Busserolles et al. 2020). In addition, we discovered that in some  
141 species there was a switch in the type of cone-based *RH2* opsin that was expressed (Fig. 1).  
142 For example, in Aulopiformes, the larvae expressed an *RH2* that is presumably sensitive to  
143 longer wavelengths of light compared to the *RH2* that was found in adults (Table 2). This  
144 clearly shows that larval and adult deep-sea fishes rely on different opsin expression profiles,  
145 which is similar to ontogenetic changes in opsin gene expression in diurnal shallow-water  
146 fishes such as freshwater cichlids (Carleton et al. 2016) and coral reef dottybacks (Cortesi et  
147 al. 2015, 2016) or between the freshwater and deep-sea maturation stages in eels (Zhang et al.,  
148 2000).

149 Our data furthermore suggests that the ontogenetic change in visual gene expression  
150 precedes morphological changes such as metamorphosis from larva to juvenile and also habitat  
151 transitions. For example, in the fangtooth, the larvae which were collected from the shallows  
152 (0 – 300 m) showed increasing amounts of *RH1* expression with growth, despite displaying  
153 larval phenotypes throughout (horns and small teeth; Fig. 1). A similar pattern of changing  
154 opsin gene expression ahead of metamorphosis has also been reported from shallow-water  
155 fishes such as European eels (Bowmaker et al. 2008), dottybacks (Cortesi et al. 2016) and  
156 surgeonfishes (Tettamanti et al. 2019). Interestingly, all our fangtooth larvae (incl. the smallest  
157 individual with a total length of 4 mm) already expressed a small amount of *RH1* (Fig. 2c).  
158 Whether fangtooth start their lives with a pure-cone retina or low-levels of rod opsin expression  
159 are normal even in pre-flexation larvae remains therefore unclear. In addition to the green-  
160 sensitive cone opsin *RH2*, the fangtooth larvae also expressed low levels of the blue-sensitive  
161 *SWS2*, potentially conferring dichromatic colour vision to the early life stages of this species  
162 (Fig. 1).

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164 **Photoreceptor cell identities.** Interestingly, two aulopiform species; the Atlantic sabretooth,  
165 *Coccorella atlantica*, and the Bigfin pearleye, *Scopelarchus michaelisarsis*, despite expressing  
166 mostly *RH1* as adults, retained a cone-dominated phototransduction cascade expression profile  
167 akin to the one found in the larval stages (Fig. 2, Table S1). This begs the question whether the  
168 photoreceptors they are using are of cone or rod nature. Initially described in snakes and geckos  
169 (Simoes et al. 2016, Schott et al. 2019) and recently also in a deep-sea fish (de Busserolles et  
170 al. 2017), it appears that the dichotomy of rods and cones is not always as clear cut as one

171 might think. For example, adult deep-sea pearlsides, *Maurolicus* spp. have a retina that  
172 expresses ~ 99% *RH2* and ~ 1% *RH1* with corresponding cone and rod phototransduction gene  
173 expressions. Their photoreceptors, however, are all rod-shaped and careful histological  
174 examination has shown that these consist of a tiny proportion of true rods and a majority of  
175 transmuted rod-like cones (de Busserolles et al. 2017). In the case of the pearlside, and also in  
176 geckos and snakes, the opsin and phototransduction genes correspond to each other making it  
177 possible to distinguish photoreceptor types at the molecular level. However, in the aulopiforms,  
178 high expression of rod opsin is seemingly mismatched with high levels of cone  
179 phototransduction gene expression (Fig. 2). In salamanders, the opposite pattern can be found  
180 whereby a cone opsin is combined with the rod phototransduction cascade inside a rod looking  
181 cone photoreceptor (Mariani 1986). Anatomically, the retina of *S. michaelsarsi* is composed of  
182 mostly rods with low numbers of cone cells (Collin et al. 1998), while the adult retina of  
183 *Evermanella balbo*, an evermannellid species related to *C. atlantica*, appears to consist of two  
184 differently looking rod populations (Wagner et al. 2019). It is therefore likely, that similar to  
185 what was found in pearlsides (de Busserolles et al. 2017), these fishes have a high proportion  
186 of transmuted rod-like cone photoreceptors that use *RH1* instead of a cone opsin as the visual  
187 pigment. Alternatively, a proportion of true rods might make use of the cone phototransduction  
188 cascade. Either way, combining more stable rod opsin in a rod-shaped cell with the cone-  
189 specific cascade is likely to increase sensitivity while also maintaining high transduction and  
190 recovery speeds of cells (Baylor 1987, Kawamura & Tachibanaki 2012, Luo et al. 2020).  
191 Histology, fluorescent in-situ hybridisation and ideally physiological recordings are needed to  
192 ultimately disentangle the identity of photoreceptor cells in aulopiform.

193

194 **Evolutionary history of deep-sea fish opsins.** While the majority of adult fishes relied on a  
195 single *RH1* copy, three species were found that expressed multiple *RH1* copies: The Warming's  
196 lanternfish, *Ceratoscopelus warmingii* (Myctophiformes), expressed three different *RH1*  
197 genes, and *S. michaelsarsi* and the basslet, *Howella brodiei* (Pempheformes), expressed two  
198 copies each. Larvae and a few adult deep-sea fishes mostly expressed a single *RH2* copy, except  
199 for the pearleyes, *Scopelarchus* spp., and the Reinhardt's lanternfish, *Hygophum reinhardtii*,  
200 which expressed three larval copies each, and the whalefish which expressed five larval copies  
201 (Fig. 1).

202 The *RH1* and *RH2* phylogenies revealed that most deep-sea fish visual opsins cluster  
203 together by species or order (Fig. 3). For example, in the whalefish all *RH2s* are clustered  
204 together suggesting that these genes are lineage or species-specific (Fig. 3b). However, there



205 were a few exceptions, suggesting more ancient duplication events. In *Scopelarchus* the two  
206 *RH1* copies are not in a sister relationship and result in different clusters, suggesting that these  
207 copies originated in the common ancestor of aulopiforms or perhaps even earlier (Fig. 3a).  
208 Also, the *RH2s* in aulopiforms (*Scopelarchus*, *Coccorella*) cluster by ontogenetic stage,  
209 making it likely that the developmental switch in gene expression was already present in the  
210 aulopiform ancestor (Fig. 3b).

211

212 **Molecular complexity of deep-sea fish visual systems.** The complexity of deep-sea fish  
213 visual systems at the molecular level varied quite substantially. For example, the three  
214 Stomiiformes species: The Ribbon sawtail fish, *Idiacanthus fasciola*, and two species of  
215 viperfish, *Chauliodus sloani* and *Ch. danae*, appeared to have a very basic visual set up; these  
216 fishes were found to express a single *RH2* cone opsin and a single *RH1* rod opsin as larvae and  
217 adults, respectively (Fig. 1). On the contrary, a number of the deep-sea fish orders examined  
218 here expressed more than two opsin genes. Adult lanternfishes and basslets have rod-only  
219 retinas but expressed multiple *RH1* copies that have functionally diversified (Fig. 3). Other  
220 species expressed both cone and rod opsins as adults (some aulopiform species and  
221 *Scombrobrax*), which is similar to the opsin gene expression profiles found in shallow-living  
222 nocturnal reef fishes (Cortesi et al. 2020).

223 The most complex visual system described here was found in *S. michaelisarsi*. In  
224 general, this species is known for its numerous morphological and anatomical adaptations to  
225 vision in the deep-sea, including having barrel eyes with a main and an accessory retina, rods  
226 that are organised in bundles, large ganglion cells and corneal lens pads (Collin et al., 1998).  
227 The two copies of *RH1* (*RH1a* and *RH1b*) it expressed showed high sequence diversity  
228 differing in 66 amino acids, three of which are key tuning sites likely to change the spectral  
229 sensitivity of the pigments via a shift in  $\lambda_{\max}$  (Fig. 3, Tables 2 and 3) (Yokoyama 2008,  
230 Musilova et al. 2019a, Yokoyama & Yia 2020). This supports the findings by Pointer et al.  
231 (2007) (Pointer et al. 2007) which using microscopetrophotometry (MSP) in another pearleye  
232 species, *S. analis*, found two rod photoreceptors with different absorption maxima at 479 and  
233 486 nm. The situation is less clear for the green-sensitive *RH2* opsin. While in *S. analis* cones  
234 have been found in the accessory and main retinas, in *S. michaelisarsi* cone photoreceptors  
235 appear restricted to the accessory retina alone (Collin et al. 1998). This is intriguing as it  
236 suggests substantial differences in visual systems even between closely related species from  
237 the same genus.

238



239 **The visual ecology of deep-sea fishes.** We found molecular support for deep-sea visual  
240 adaptations on multiple levels:

241 1) *Opsin gene diversity in the genome.* Retinal transcriptomes in the Stomiiformes pointed  
242 towards a simple visual system that expresses a single opsin gene at different developmental  
243 stages (*RH2* in larvae, *RH1* in adults) (Fig. 1). Besides *RH2*, searching for visual opsins in a  
244 newly sequenced (*I. fasciola* and *Ch. sloani*) and several published genomes (Musilova et al.,  
245 2019a), revealed that Stomiiformes are likely to have lost all other cone opsin gene families  
246 (Fig. 1). For *RH2*, they seem to only have a single or at most two gene copies, which is  
247 substantially less compared to other teleosts (Musilova et al. 2019a). The Stomiiform example,  
248 therefore, shows that a decrease in light intensity and the spectrum of light in the deep-sea may  
249 not only restrict gene expression at adult stages, but also lead to the loss of opsin genes  
250 altogether. Similarly, a loss in opsin and other vision-related genes (e.g. *otx5b*, *crx*) has  
251 previously been reported from shallow living fishes that are either nocturnal (Luehrmann et al.  
252 2019), live in murky waters (Liu et al. 2019), or inhabit caves or similarly dim environments  
253 (Huang et al. 2019, Musilova et al. 2019a).

254  
255 2) *Visual gene expression.* Previous work has found that the expression of the longest- (*LWS* -  
256 red) and shortest- (*SWS1* - UV) sensitive opsins is reduced or absent in deeper living coral reef  
257 fishes (Cortesi et al. 2020) and also in fishes inhabiting deep freshwater lakes (e.g., Hunt 1997,  
258 Sugawara et al. 2005, Musilova et al. 2019b), which is correlated with a loss of short- and long-  
259 wavelengths with depth. Accordingly, we found here that deep-sea fishes lack any *LWS*  
260 expression even in the shallow-living larval stages (Fig. 1), which is accompanied by the loss  
261 of *LWS* cone opsin in many deep-sea fish lineages (Musilova et al. 2019a). Similarly, *SWS1* is  
262 not expressed in any of the species studied, except for in the larval whalefish, and is also absent  
263 from many deep-sea fish genomes (Fig. 1) (Musilova et al. 2019a). However, shallow larval  
264 stages are likely to explain why all deep-sea fishes studied to date maintain at least some cone  
265 opsins in their genomes (Musilova et al. 2019a).

266 Most deep-sea fish larvae expressed a single *RH2* gene, but the larvae of some species  
267 (fangtooth, whalefish and lanternfish) expressed multiple cone opsin genes, likely providing  
268 them with similar visual systems to the larvae of shallow-living marine (Britt et al. 2001) and  
269 freshwater species (Carleton et al. 2016), possibly aiding them in detecting residual light,  
270 discriminating brightness and/or colours. Juvenile deep-sea fishes, on the other hand, showed  
271 rod-based expression profiles also found in the adult stages (Fig. 1). This shift in opsin gene  
272 expression correlates with developmental changes in ecology. As opposed to the adults which

273 are exposed to a narrow and dim light environment where food is scarce, larvae typically live  
274 in well-lit, broad spectrum shallow waters where food and predators are abundant (Moser &  
275 Smith 1993).

276

277 3) *Functional adaptation in key spectral tuning sites.* When multiple *RHI* copies were  
278 expressed, they often showed distinct differences in key amino acid sites that are likely to shift  
279 the spectral sensitivities of the pigments (Fig. 3 and Tables 2 and 3) (Yokoyama 2008,  
280 Musilova et al. 2019a). As such, the three different *RHIs* in *C. warmingii* differed in 15 key-  
281 tuning sites. Opsin gene expression together with spectral-sensitivity estimations revealed a  
282 dominant rod opsin copy (*RHI-1*), and a shorter- (*RHI-2*) and a longer-shifted (*RHI-3*) copy  
283 with lower transcript levels (Figs. 1 and 3). The MSP in *C. warmingii* found two distinct rod  
284 types with  $\lambda_{\max}$  values of 488 and 468 nm (Collin and Marshall 2003) corresponding most  
285 likely to the RH1a and RH1b, respectively. It is possible that multiple *RHI* copies are  
286 coexpressed within the same photoreceptor, something that has previously been reported for  
287 cone opsins in shallow-water marine (Savelli et al. 2018, Stieb et al. 2019) and freshwater  
288 fishes (Dalton et al. 2014, Torres-Dowdall et al. 2017). Coexpression could produce visual  
289 pigment mixtures that shift photoreceptor sensitivity and enhance visual contrast, aiding in  
290 predator-prey interactions or mate finding (Dalton et al. 2014). Alternatively, a third rod  
291 photoreceptor type might have been overlooked during MSP, which can occur especially if the  
292 cell-type is rare. While the function of having multiple rod opsins in *C. warmingii* remains to  
293 be investigated, several possible benefits for a multi-rod visual system have recently been  
294 proposed including that it might enable conventional or unusual colour vision in dim light, it  
295 might be used to increase visual sensitivity, or enhance object's contrast against a certain  
296 background (Musilova et al. 2019a).

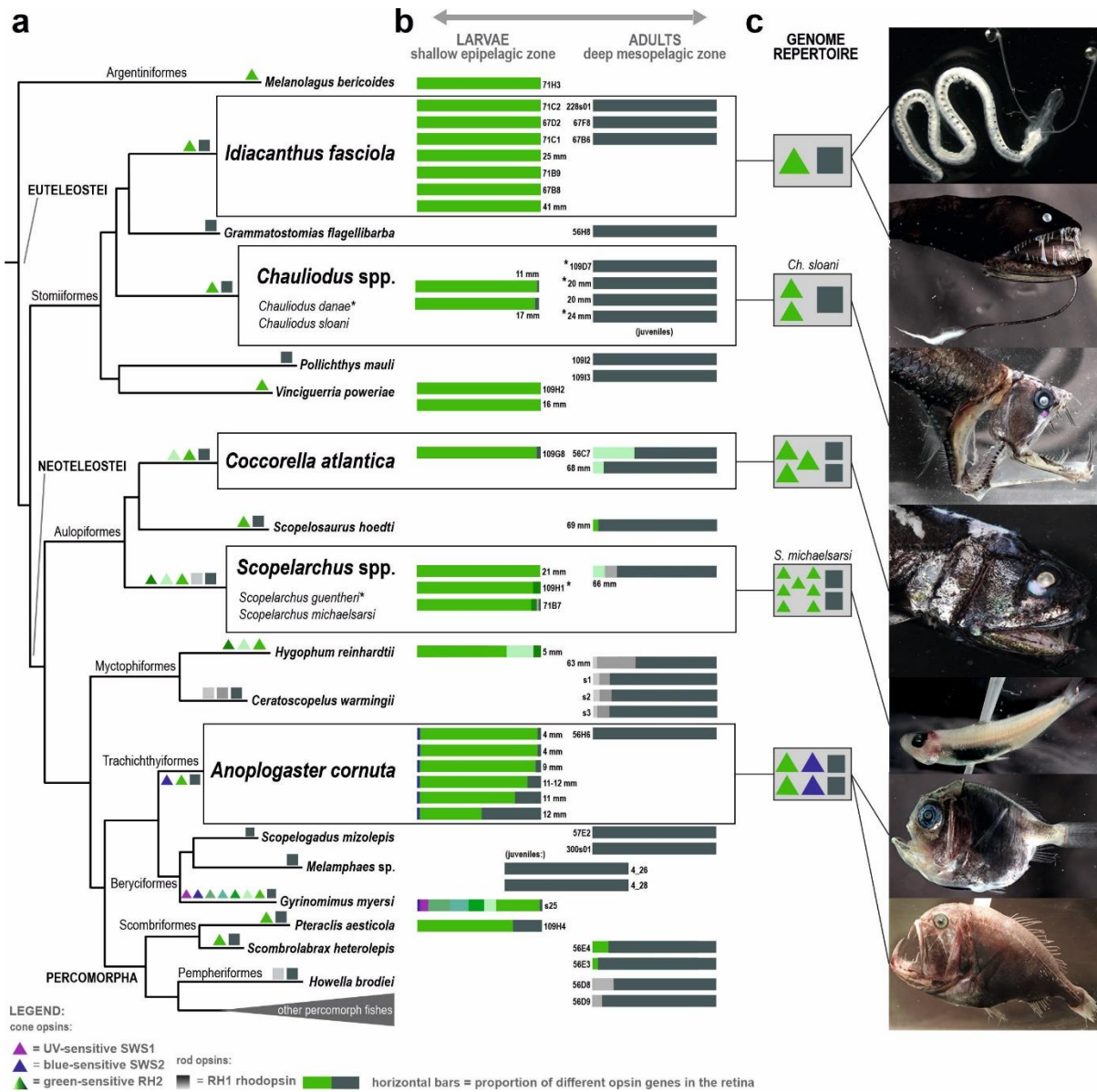
297 In *H. brodiei*, the second *RHI* copy (*RHI-2*) differed in two key tuning sites, E122Q (-15 nm)  
298 and G124S (-11 nm), known to cause major short-wavelength shifts in other fishes (Yokoyama  
299 2008). This supports the MSP measurements in its sister species, *Howella sherborni*, which  
300 found two different rod types with spectral sensitivities of 463 and 492 nm (Fig. 2) (Partridge  
301 et al. 1989).

302 Having multiple differently tuned rod photoreceptors, one centred on the prevailing light  
303 (bioluminescence and/or ambient light ~ 480 – 490 nm) and a second one that is offset from it  
304 (i.e. the offset pigment hypothesis; Lythgoe 1966), may be beneficial to break counter  
305 illumination - a way of active camouflage in mesopelagic organisms where ventral photophores  
306 emit bioluminescent light that matches the residual down-welling light (Denton et al. 1985).

307 Hence, revealing an individual's silhouette could help to distinguish prey and predators from  
308 the background lighting, or visually finding mates. Apart of lanternfishes with three (or more)  
309 and basslets with two rod opsins, or exceptional cases of tube-eye (six) and spinyfin (38), the  
310 majority of the deep-sea fishes however seems to have only one rod opsin (Musilova et al.,  
311 2019a).

## 312 313 **Conclusions**

314 So far, the development of deep-sea fish vision at the molecular level had not been studied and  
315 only limited morphological information was available. In this study we compared opsin and  
316 visual gene expression between 20 deep-sea fish species revealing a major change in expression  
317 between larval and adult stages. While deep-sea fish genomes contain both cone and rod opsin  
318 genes, larvae rely on the cone pathway and most adult fishes switch to a rod-dominated or rod-  
319 only visual system. The cone vs. rod-specific phototransduction cascade genes follow the  
320 opsins in some lineages, however, not in aulopiforms. We detected limited opsin gene  
321 repertoire in the genomes of five deep-sea fish species composed only of one rod (RH1) and  
322 one or two cone (RH2, SWS2) opsin gene classes. Interestingly, we have discovered lineage-  
323 specific opsin gene duplications, possibly allowing for increased visual sensitivity and certain  
324 kind of colour vision in the depth in some species. Our molecular results therefore support a  
325 conserved developmental progression in vertebrates whereby cones appear first in the retina  
326 and rod photoreceptors are added later during development.



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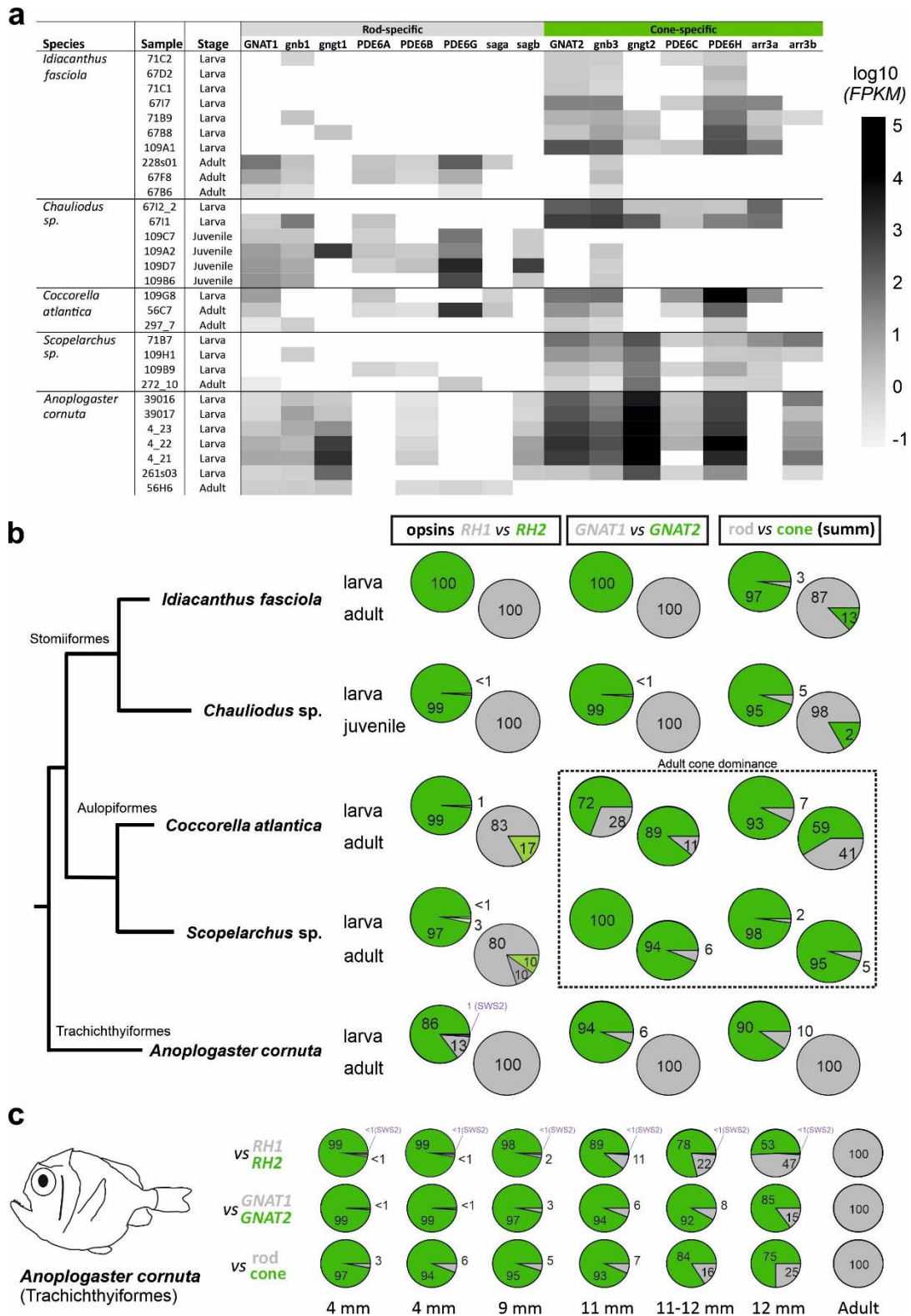
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**Fig. 1: Cone and rod opsin gene expression in larval and adult deep-sea fishes.** A) Transcriptomes were used to characterise the opsin gene expression in the retinas of 20 deep-sea fish species, belonging to eight different orders and mapped onto a simplified teleost phylogeny (topology after Betancur et al., 2017). Boxes highlight the five species for which both larval and adult samples were available. B) Proportional opsin gene expression for each individual (horizontal bar) at different developmental stages. Different colours correspond to cone (colours) or rod (shades of grey) opsin genes, depicted as the proportional expression over the total sum of visual opsins expressed. Different shades of the same colour represent multiple copies of the same gene family. Based on the opsin gene expression, 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. Juvenile specimens in two species had an adult expression profile. Note that some species expressed multiple RH1 copies (*Scopelarchus*, *Howella brodiei* and *Ceratoscopelus warmingii* adults) or multiple RH2 copies (*Gyrinomimus* sp. larva, *Hygophum reinhardtii* larva). Notably, adults and larvae of *Scopelarchus* sp. and *Coccorella atlantica* expressed different copies of RH2 (more details in Fig. 2). Details about the samples and expression levels are listed in Table 1. C) The genomic repertoire

342 *of the visual opsins is shown for five species* *Idiacanthus fasciola*, *Chauliodus sloani*, *Coccorella atlantica*,  
343 *Scopelarchus michaelsarsi* (*all this study*), and *Anoplogaster cornuta* (*Musilova et al., 2019a*). *The rod RH1 opsin*  
344 *and the cone RH2 opsin genes are present in all studied species in one or multiple (up to seven) copies. The SWS2*  
345 *opsin gene was found only in the fangtooth, and the SWS1 and LWS are missing from all five studied genomes.*  
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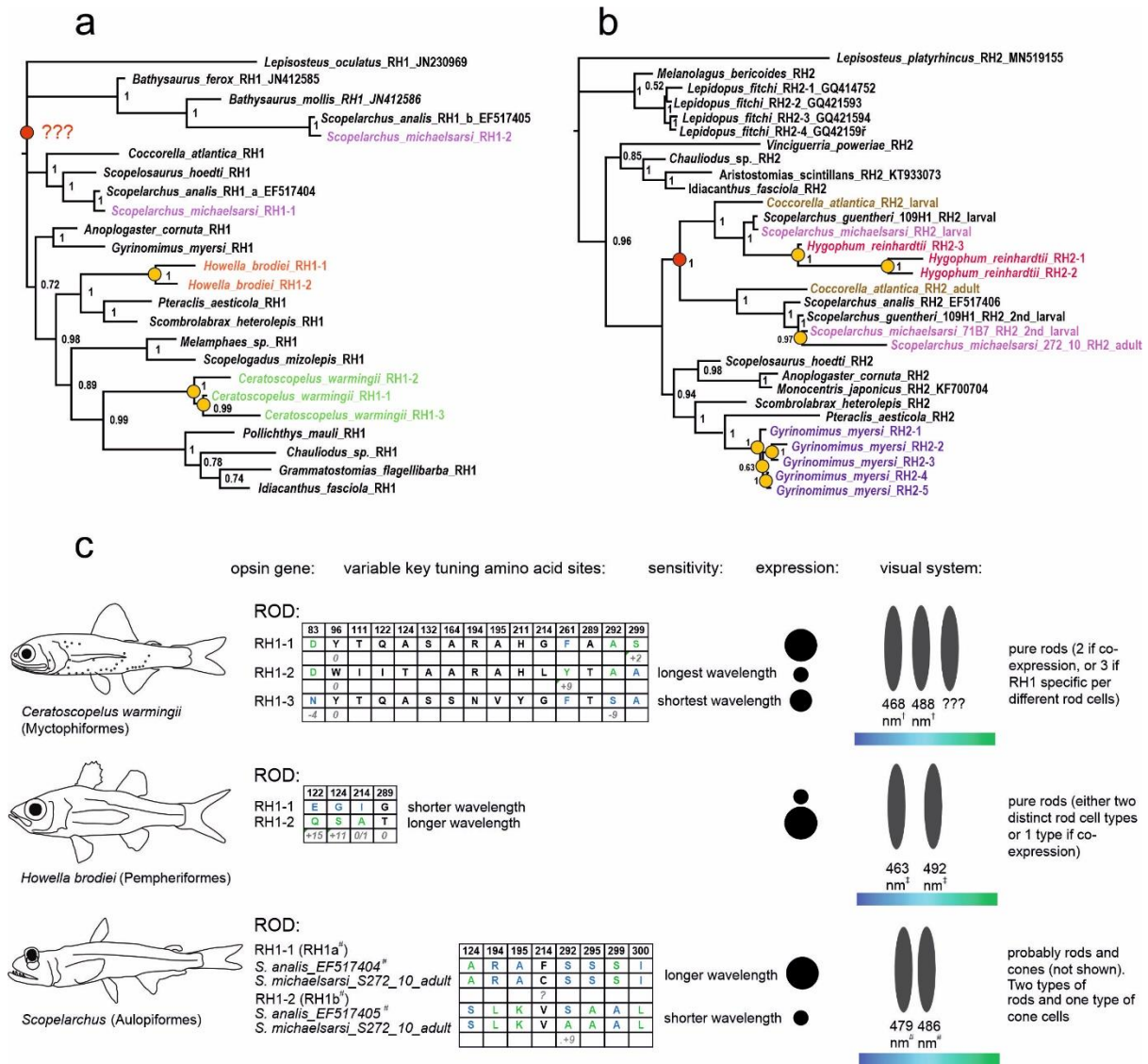
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349 **Fig. 2:** Phototransduction cascade gene expression in the retina of five deep-sea fish species. A) Heat map of the  
 350 expression of individual phototransduction cascade genes for each sample, based on normalised numbers of reads  
 351 (FPKM). B) Pie charts comparing mean values of relative expression of the opsin genes (rod *RH1* and cone *RH2*),  
 352 photoreceptor-specific cascade transducin genes (rod-type *GNAT1* and cone-type *GNAT2*), and all cascade genes  
 353 (photoreceptor-specific transducins, arrestins and phosphodiesterases) summarized. The black square highlights

354 *the two aulopiform species with the discordance between the opsin type (rod-specific) and phototransduction*  
355 *cascade genes (cone-specific) in adults. C) Focus on the common fangtooth (Anoplogaster cornuta) transitional*  
356 *phase shown as a sequence for six larval and one adult sample; size given as standard length (SL). Note that all*  
357 *fangtooth larvae expressed both RH1 and RH2, with an increasing proportion of RH1 to RH2 as the larvae*  
358 *increased in size. These individuals all had traits of larval phenotypes (dorsal and ventral horns and small teeth;*  
359 *Fig. 1) and were collected relatively shallow between 0-300 m using the plankton trawls.*  
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**Fig. 3:** Gene trees of the A) RH1 and B) RH2 opsin genes mined from the retinal transcriptomes of deep-sea fishes. Species with multiple copies are highlighted in colour. Additional gene sequences from public databases are listed with their GenBank accession numbers. Note the topology within Aulopiformes; the adult RH2s of *Coccorella atlantica* and *Scopelarchus* cluster together as do the major larval RH2s. Circles mark gene duplication events with red circles pinpointing (A) the ancestral duplication of RH1 impacting the *Scopelarchus* genus, and (B) the duplication of RH2 in the aulopiform ancestor (or at least the common ancestor of *Coccorella* and *Scopelarchus*). C) Predicted rod photoreceptor spectral sensitivities based on key spectral tuning site mutations in species with multiple rod opsins. Multiple rod opsins differed in three species, *Ceratoscopelus warmingii* (Myctophiformes), *Howella brodiei* (Pempheriformes) and *Scopelarchus michaelisarsis* (Aulopiformes),

## 372 MATERIALS AND METHODS

373 Specimens used in this study were collected in the Sargasso Sea during two multipurpose  
374 fishery surveys conducted by the German Thünen Institute of Fisheries Ecology onboard the  
375 research vessel *Walther Herwig III* in March to April in 2014 and in 2017. The sampling of  
376 adults occurred during both day and night at depths of 600 – 1'000 m using a mid-water pelagic  
377 trawl net (Engel Netze, Bremerhaven, Germany) with an opening of 30 m x 20 m, a length of  
378 145 m, and mesh sizes (knot to knot) from 90 cm decreasing stepwise to 40, 20, 10, 5, 4, 3, 2  
379 cm, with a 1.5-cm mesh in the 27-m-long codend. The larvae were mostly collected using an  
380 Isaacs-Kidd Midwater Trawl net (IKMT; 6.2 m<sup>2</sup> mouth-opening, 0.5 mm mesh size; Hydro-  
381 Bios Apparatebau GmbH) at depths of 0 - 300 m by double-oblique transect tows. Adult fish  
382 were flash-frozen at -80 °C upon arrival on board and their fin clip was stored in 96% ethanol.  
383 Larval samples were fixed in RNAlater™ (ThermoFisher) and stored at -80 °C until further  
384 use.

385

386 To sequence the whole genome of *Idiacanthus fasciola*, *Chauliodus sloani*, *Coccorella*  
387 *atlantica*, and *Scopelarchus michaelsarsi*, the genomic DNA was extracted from the fin clip  
388 using the DNeasy Blood and Tissue kit (Qiagen) following the enclosed protocol. The library  
389 preparation and genome sequencing on Illumina NovaSeq platform (150 bp PE and the yield  
390 over 20 Gb per genome) has been outsourced to the sequencing centre Novogene, Singapore  
391 (<https://en.novogene.com/>). To analyze the opsin gene repertoire, the raw genomic reads were  
392 mapped in Geneious software version 11.0.3 (Kearse et al. 2012) against the opsin references  
393 (single exons of all five opsin classes from the reference species: Nile tilapia, Round goby,  
394 Blind cavefish, Spotted gar), as well as against the genes found in the transcriptomes of each  
395 species. The parameters have been set to the Medium Sensitivity to capture all reads that match  
396 any visual opsin gene. The captured reads mapping to all exons were then remapped against  
397 one reference per exon and the species-specific consensus sequence has been generated. If  
398 present, multiple paralogous genes were disentangled manually and the consensus sequence  
399 has been exported for each variant (see below more details for the transcriptomic analysis). The  
400 obtained consensus sequences served as references for the second round of mapping, when all  
401 genomic reads were again mapped with the Low Sensitivity settings, and each reference has  
402 been then elongated by the overhanging sequence. This step has been repeated until the full  
403 gene region has been covered. In case of *Scopelarchus michaelsarsi*, we were not able to cover  
404 the full length of five out of seven RH2 genes and these are reported in two parts, one covering  
405 the exon 1 and 2, and one covering exons 3, 4 and 5. All genomic data are available in the

406 GenBank database (acc. no *tba*) and the opsin gene sequences are provided as Supplementary  
407 file 1.

408

409 Total RNA was extracted from the whole eyes using either the RNeasy micro or mini kit  
410 (Qiagen) and the extracted RNA concentration and integrity were subsequently verified on a  
411 2100 Bioanalyzer (Agilent). RNAseq libraries for 31 samples were constructed in-house from  
412 unfragmented total RNA using Illumina's NEBNext Ultra II Directional RNA library  
413 preparation kit, NEBNext Multiplex Oligos and the NEBNext Poly(A) mRNA Magnetic  
414 Isolation Module (New England Biolabs). Multiplexed libraries were sequenced on the  
415 Illumina HiSeq 2500 platform as 150 bp paired-end (PE) reads. Library construction and  
416 sequencing (150 bp PE) for an additional 10 samples was outsourced to Novogene, Singapore  
417 (<https://en.novogene.com/>). We additionally re-analyzed 11 retinal transcriptomes previously  
418 published in Musilová et al. (2019a). Together, then, our dataset comprised 52 samples of  
419 which, based on morphology, 25 were classified as larvae, 5 as juveniles and 22 as adults.  
420 Sample IDs, number of raw reads and further parameters are listed in Table 1.

421

422 The sequence data was quality-checked using FastQC (Andrews 2010). Opsin gene expression  
423 was then quantified using Geneious software version 11.0.3 (Kearse et al. 2012). For each  
424 sample we first mapped the reads against a general fish reference dataset comprising all visual  
425 opsin genes from the Nile tilapia, *Oreochromis niloticus* and the zebrafish, *Danio rerio*, with  
426 the Medium-sensitivity settings in Geneious. This enabled us to identify cone and rod opsin  
427 specific reads. If present, paralogous genes were subsequently disentangled following the  
428 methods in Musilova et al. 2019a and de Busserolles et al. 2017. Briefly, we created species-  
429 specific references of the expressed opsin genes and their several copies (Musilova et al. 2019a)  
430 and re-mapped the transcriptome reads with Medium-Low sensitivity to obtain copy-specific  
431 expression levels. If multiple opsin genes were found to be expressed, we report their  
432 proportional expression in relation to the total opsin gene expression (Fig. 1). We used the same  
433 pipeline to quantify expression of phototransduction cascade genes in five focal deep-sea  
434 species (Fig. 2, Tables S1).

435

436 To check for key amino-acid substitutions in *RH1* and *RH2* and potential shifts in its  
437 absorbance, we first translated the opsin coding sequences into amino acid sequences, and then  
438 aligned them with the bovine *RH1* (GenBank Acc.No: M12689). We have specifically focused  
439 on the positions identified as key-tuning sites in Yokoyama (2008) and Musilova et al. (2019a).

440 For details, see Tables 2 and 3. Unfortunately, we were not able to estimate the exact sensitivity  
441 shift in *C. warmingii* as only four of the amino acids that were substituted at the 15 key-tuning  
442 amino acid sites corresponded with previously tested cases (Yokoyama, 2008, Musilova et al.  
443 2019a). Out of the three copies, *RHI-2* has three out of four longer-shifting amino acid variants  
444 in these four sites and we assume is therefore red-shifted. *RHI-1* is most likely sensitive to 488  
445 nm, and *RHI-3*, being the shortest, to 468nm.

446

447 In order to properly estimate the  $\lambda_{\max}$ , we have further checked the samples for expression of  
448 the *Cyp27c1* gene, the factor assuming conversion of the A1 retinal to A2. Expression of  
449 *Cyp27c1* would assume that opsin protein binds to the A2 instead of A1, which would cause  
450 a maximum shift of ca 25-30 nm (Enright et al. 2015). The expression of this gene has not been  
451 detected suggesting only A1 retinal in the studied species of the deep-sea fishes (Table S2).

452

453 A dataset containing *RHI* opsin gene sequences from our transcriptomes, and additional *RHIs*  
454 obtained from GenBank (link to NCBI) were aligned using the MAFFT (Kato et al. 2009)  
455 plugin as implemented in Geneious and a phylogenetic tree was subsequently reconstructed  
456 using MrBayes v3.2.1 (Ronquist & Huelsenbeck 2003) (Fig. 2). Trees were produced using the  
457 Markov chain Monte Carlo analysis which ran for 1 million generations. Trees were sampled  
458 every 100 generations, and the printing frequency was 1000, discarding the first 25% of trees  
459 as burn-in. The evolutionary model chosen was GTR model with gamma-distributed rate  
460 variation across sites and a proportion of invariable sites. Posterior probabilities (PP) were  
461 calculated to evaluate statistical confidence at each node. We used the same approach with an  
462 *RH2*-specific reference dataset to reconstruct the phylogenetic relationship between the  
463 transcriptome-derived deep-sea *RH2* genes (Fig. 3).

464

#### 465 **Acknowledgements:**

466

467 We would like to express our thanks to both scientific and technical crew of the Walther Herwig  
468 III research cruises in 2014 and 2017. In addition, we thank Tina Blancke for help with the  
469 sample management, and Veronika Truhlářová for technical support and lab management. NL  
470 and ZM were supported by the Swiss National Science Foundation (PROMYS - 166550), ZM  
471 by the PRIMUS Research Programme (Charles University), the Czech Science Foundation (21-  
472 31712S) and the Basler Stiftung fuer Experimentelle Zoologie, and FC by an Australian  
473 Research Council (ARC) DECRA Fellowship (DE200100620).

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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	reads after		RH1	RH2	SWS1	SWS2	accession number
						raw reads	bacteria filtering					
<i>Anoplogaster cornuta</i>	Trachichthyiformes	Larva	39016	4mm		36,373,372	36,125,630	<0.01	0.99		<0.01	
		Larva	39017	4mm		34,712,538	34,604,364	<0.01	0.99		<0.01	
		Larva	4_23	9mm		41,592,840	41,519,070	0.02	0.98		<0.01	
		Larva	4_22	11mm		75,109,270	74,990,214	0.11	0.89		<0.01	
		Larva	4_21	11-12 mm		28,945,804	28,869,552	0.22	0.78		<0.01	
		Larva	261s03	12mm		48,458,044	48,457,472	0.47	0.53		<0.01	
<i>Ceratoscopelus warmingii</i>	Myctophiformes	Adult	56H6			11,658,040	11,635,610	1				
		Adult	300s03	63mm		49,078,974	44,114,639	0.66 <sup>RH1-1</sup>				
								0.01 <sup>RH1-2</sup>				
								0.33 <sup>RH1-3</sup>				
		Adult	S1			8,796,786	8,789,961	0.94 <sup>RH1-1</sup>				
								0.02 <sup>RH1-2</sup>				
						0.04 <sup>RH1-3</sup>						
		Adult	S2		8,655,248	8,649,342	0.93 <sup>RH1-1</sup>					
						0.02 <sup>RH1-2</sup>						
						0.05 <sup>RH1-3</sup>						
		Adult	S3		7,904,714	7,899,617	0.94 <sup>RH1-1</sup>					
						0.01 <sup>RH1-2</sup>						
						0.05 <sup>RH1-3</sup>						
<i>Chauliodus sloani</i>	Stomiiformes	Larva	67I2_2	11mm		34,445,252	34,371,394	0.01	0.99			
		Larva	67I1	17mm		20,701,686	20,683,764	<0.01	0.99			
<i>Chauliodus danae</i>	Stomiiformes	Juvenile	109B6	20mm		24,854,866	24,835,646	1				
		Juvenile	109C7	24mm		32,865,334	32,834,300	1				
<i>Coccorella atlantica</i>	Aulopiformes	Juvenile	109A2	20mm		25,389,318	25,344,182	1				
		Juvenile	109D7			18,540,248	18,533,484	1				
		Larva	109G8			26,376,620	26,359,060	0.01	0.99 <sup>RH2-1</sup>			
		Adult	56C7		36,794,070	36,790,854	0.69	0.31 <sup>RH2-2</sup>				
		Adult	297_7	68mm	43,948,534	40,511,279	0.96	0.04 <sup>RH2-3</sup>				
<i>Grammatostomias flagellibarba</i>	Stomiiformes	Adult	56H8		9,775,520	9,742,864	1					
<i>Gyrinomimus myersi</i>	Beryciformes	Larva	S25		19,270,096	19,122,986	<0.01	0.45 <sup>RH2-1</sup>	0.07	<0.01		
								0.13 <sup>RH2-2</sup>				
								0.08 <sup>RH2-3</sup>				
								0.11 <sup>RH2-4</sup>				
								0.15 <sup>RH2-5</sup>				
<i>Howella brodiei</i>	Pempheriformes	Adult	56D8		56,905,264	43,936,178	0.17 <sup>RH1-1</sup>					
							0.83 <sup>RH1-2</sup>					
		Adult	56D9		61,909,674	61,590,270	0.07 <sup>RH1-1</sup>					
							0.93 <sup>RH1-2</sup>					
<i>Hygophum reinhardtii</i>	Myctophiformes	Larva	67I2_1	5mm	17,222,626	17,122,880		0.26 <sup>RH2-1</sup>				
								0.68 <sup>RH2-2</sup>				
								0.06 <sup>RH2-3</sup>				
<i>Idiacanthus fasciola</i>	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					
<i>Melamphaes sp.</i>	Beryciformes	Juvenile	4_26		38,583,370	38,534,507	1					
		Juvenile	4_28		35,489,970	35,431,421	1					
<i>Melanolagus bericoides</i>	Argentiniformes	Larva	71H3		26,219,646	26,210,402		1				
<i>Pteraclis aesticola</i>	Scombriformes	Larva	109H4		19,918,922	19,870,484	0.22	0.78				
<i>Pollichthys mauli</i>	Stomiiformes	Adult	109I2		55,767,498	54,875,747	1					
		Adult	109I3		68,655,686	67,082,709	1					
<i>Scombrolabrax heterolepis</i>	Scombriformes	Adult	,56E3		65,770,014	5,527,135	0.98	0.02				
		Adult	,56E4		60,643,652	60,472,479	0.93	0.07				
<i>Scopelarchus guentheri</i>	Aulopiformes	Larva	109H1		37,542,802	37,416,486		0.96 <sup>RH2_larval</sup>				
								0.04 <sup>RH2_2nd_larval</sup>				
<i>Scopelarchus michaelisarsi</i>	Aulopiformes	Larva	109B9	21mm	9,971,250	9,919,148		1 <sup>RH2_larval</sup>				
		Larva	71B7		43,695,620	43,682,534	<0.01 <sup>RH1a</sup>	0.96 <sup>RH2_larval</sup>				
						<0.01 <sup>RH1b</sup>	0.03 <sup>RH2_2nd_larval</sup>					
		Adult	272_10	66mm	45,565,202	43,729,338	0.80 <sup>RH1a</sup>	0.10 <sup>RH2_adult</sup>				
						0.10 <sup>RH1b</sup>						
<i>Scopelogadus mizolepis</i>	Beryciformes	Adult	,57E2		5,160,876	5,160,876	1					
		Adult	300s01		15,448,240	15,420,650	1					
<i>Scopelosaurus hoedti</i>	Aulopiformes	Adult	297_9	69mm	43,948,534	42,248,053	0.98	0.02				
<i>Vinciguerria poweriae</i>	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 cone opsin RH2 gene**

species	order	83	122	207	255	292	lmax	ref.
Bovine RH1		D	E	M	I	A	500 nm	41
Ancestral teleost		D	Q	M	I	A	488 nm	42
<i>Melanolagus bericoides</i>	Argentiniformes	G	Q	.	V	.	505 nm	21
<i>Coccorella atlantica</i> adult	Aulopiformes	G	Q	.	.	.		
<i>Coccorella atlantica</i> larval	Aulopiformes	G	Q	.	V	.		
<i>Scopelarchus michaelsarsi</i> adult	Aulopiformes	?	Q	I	C	T		
<i>Scopelarchus guentheri</i> larval	Aulopiformes	G	Q	.	V	.		
<i>Scopelarchus michaelsarsi</i> larval	Aulopiformes	G	Q	.	V	.		
<i>Scopelarchus guentheri</i> 2nd larval	Aulopiformes	G	Q	.	C	.		
<i>Scopelarchus michaelsarsi</i> 2nd larval	Aulopiformes	G	Q	.	C	.		
<i>Scopelosaurus hoedti</i>	Aulopiformes	G	Q	.	.	.		
<i>Gyrinomimus myersi</i> RH2-1	Beryciformes	G	Q	.	F	.		
<i>Gyrinomimus myersi</i> RH2-2	Beryciformes	G	Q	L	F	.		
<i>Gyrinomimus myersi</i> RH2-3	Beryciformes	G	Q	L	F	.		
<i>Gyrinomimus myersi</i> RH2-4	Beryciformes	G	Q	L	F	.		
<i>Gyrinomimus myersi</i> RH2-5	Beryciformes	G	Q	L	F	.		
<i>Lepisosteus platyrhincus</i>	Lepisosteiformes	G	.	.	.	.		
<i>Hygophum reinhardtii</i> RH2-1	Myctophiformes	G	Q	.	V	.		
<i>Hygophum reinhardtii</i> RH2-2	Myctophiformes	G	Q	.	V	.		
<i>Hygophum reinhardtii</i> RH2-3	Myctophiformes	G	Q	.	V	.		
<i>Lepidopus fitchi</i> RH2-1	Scobriformes	G	.	.	V	.	496 nm	42
<i>Lepidopus fitchi</i> RH2-2	Scobriformes	G	Q	.	V	.		
<i>Lepidopus fitchi</i> RH2-3	Scobriformes	G	Q	.	V	.	506 nm	42
<i>Lepidopus fitchi</i> RH2-4	Scobriformes	G	Q	.	V	.		
<i>Pteraclis aesticola</i>	Scobriformes	G	Q	L	.	.		
<i>Scombrolabrax heterolepis</i>	Scobriformes	G	Q	.	.	.		
<i>Aristostomias scintillans</i>	Stomiiformes	G	Q	L	V	.	468 nm	42
<i>Chauliodus</i> sp.	Stomiiformes	G	Q	.	F	.		
<i>Grammastomias flagellibarba</i>	Stomiiformes	G	Q	.	.	.		
<i>Idiacanthus fasciola</i>	Stomiiformes	G	Q	.	V	.		
<i>Vinciguerrria poweriae</i>	Stomiiformes	G	Q	.	F	.		
<i>Anoplogaster cornuta</i>	Trachichthyiformes	G	Q	.	.	.		

Table 3: 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	λmax	ref.	
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	41	
ancestral teleost RH1		D	G	Y	Y	E	T	E	A	A	A	M	G	L	N	M	F	H	I	M	F	W	A	T	A	A	A	L	M		16		
<i>Bathysaurus ferox</i>	Aulopiformes	N	.	.	.	.	.	.	S	.	.	.	.	R	A	.	.	.	.	.	.	.	.	.	.	S	.	T	L	.	481 nm	50	
<i>Bathysaurus mollis</i>	Aulopiformes	N	.	.	.	.	.	.	S	.	.	.	.	R	A	.	.	.	.	.	.	.	.	.	T	.	S	.	T	L	.	479 nm	50
<i>Coccorella atlantica</i>	Aulopiformes	N	.	.	S	.	.	M	.	.	.	.	.	R	A	.	.	.	C	.	.	.	.	.	.	.	S	.	I	.	480 nm†	64	
<i>Scopelarchus</i> spp. RH1-1	Aulopiformes	N	.	.	.	.	.	.	.	.	.	.	.	R	A	.	.	.	C	.	.	.	.	.	.	S	S	S	I	.	486 nm	21	
<i>Scopelarchus</i> spp. RH1-2	Aulopiformes	N	.	.	.	.	.	.	S	.	.	.	.	L	K	.	.	.	V	.	.	.	.	.	.	S	.	L	.	479 nm	21		
<i>Scopelosaurus hoedti</i>	Aulopiformes	.	.	.	.	.	.	.	.	.	.	.	.	R	A	.	.	.	.	.	.	.	.	.	.	.	S	S	I	.			
<i>Gyrinomimus myersi</i>	Beryciformes	N	.	.	.	.	.	.	.	.	.	.	.	R	A	.	.	.	.	.	.	.	.	.	.	.	.	S	.	I	.		
<i>Melamphaes</i> sp.	Beryciformes	.	.	.	.	.	.	Q	.	S	.	.	.	R	V	.	.	.	G	.	.	.	.	.	.	.	S	.	S	I	.		
<i>Scopelogadus mizolepis</i>	Beryciformes	.	.	.	.	.	.	Q	G	S	.	.	.	R	V	.	.	.	G	.	.	.	.	.	T	.	.	S	.	.	488 nm <sup>§</sup>	64	
<i>Lepisosteus oculatus</i>	Lepisosteiformes	.	.	.	.	.	.	Q	S	.	.	.	.	L	K	.	.	.	L	.	Y	.	G	.	.	.	.	.	L	.			
<i>Ceratoscopelus warmingii</i> RH1-1	Myctophiformes	.	.	.	T	.	.	Q	S	.	.	.	.	R	A	.	.	.	G	.	.	.	.	.	.	.	.	S	I	.	2468 nm†	50	
<i>Ceratoscopelus warmingii</i> RH1-2	Myctophiformes	.	.	W	.	I	.	I	T	.	.	.	.	R	A	.	.	.	V	.	Y	.	.	.	.	.	.	.	I	.	prob. 488 nm†	50	
<i>Ceratoscopelus warmingii</i> RH1-3	Myctophiformes	.	.	.	A	.	.	Q	S	S	.	.	.	N	V	.	.	Y	G	.	.	.	.	.	.	.	S	.	I	.	2468 nm†	50	
<i>Hygophum reinhardtii</i>	Myctophiformes	.	.	.	A	.	.	Q	.	.	.	.	.	R	A	.	.	.	G	.	.	.	.	.	.	.	.	.	I	.			
<i>Howella brodiei</i> RH1-1	Pempheriformes	N	.	.	.	.	.	.	G	.	.	.	.	R	A	.	.	.	.	.	.	.	.	.	.	G	S	S	I	.			
<i>Howella brodiei</i> RH1-2	Pempheriformes	N	.	.	.	.	.	Q	S	.	.	.	.	R	A	.	.	.	A	.	.	.	.	.	.	.	S	S	S	I	.		
<i>Pteraclis aesticola</i>	Scombriformes	.	.	.	.	.	.	Q	.	.	.	.	.	R	A	.	.	.	.	.	.	.	.	.	.	.	.	.	S	I	.		
<i>Scambrolabrax heterolepis</i>	Scombriformes	.	.	.	.	.	.	Q	.	.	.	.	.	R	A	.	.	.	.	.	.	.	.	.	.	.	S	.	S	I	.		
<i>Chauliodus</i> spp.	Stomiiformes	N	.	.	.	.	.	G	.	.	.	.	.	R	A	.	.	.	V	.	.	.	.	.	A	S	.	.	L	V	484 nm	50	
<i>Grammatostomias flagellibarba</i>	Stomiiformes	N	.	.	.	.	.	.	.	.	.	.	.	R	A	.	.	.	V	.	.	.	.	.	.	.	S	.	L	.	480 - 487 nm	65	
<i>Idiacanthus fasciola</i>	Stomiiformes	N	.	.	.	.	.	.	.	.	.	.	.	R	A	.	.	.	.	.	.	.	.	.	.	.	S	.	L	.	485 nm	50	
<i>Pollichthys maui</i>	Stomiiformes	N	.	.	.	.	.	.	.	.	.	.	.	R	A	.	.	.	.	.	.	.	.	.	.	.	S	.	L	.			
<i>Anoplogaster cornuta</i>	Trachichthyiformes	N	.	.	.	.	.	.	.	.	.	.	.	R	A	.	.	.	.	.	.	.	.	.	.	T	S	.	S	I	.	485 nm	50

† = two pigments reported without assignment to the gene; see also Figure 3

‡ = for *Evermannella balbo*; sequence not available

§ = for *Scopelogadus beani*; sequence not available