Detailed Materials and Methods for the spectrophotometry analyses.

Retinal wholemount preparations were placed onto the stage of a modified Zeiss Universal microscope and the transmission of light (300-800 nm) through the retina was measured systematically every 0.5 mm or 1 mm (depending on the size of the retina) across the wholemount. Light produced by a 175 W Xenon Arc lamp (Spectral Products, Putnam, CT, USA) was delivered to the retina via a 200 μm diameter quartz fibre optic (Ocean Optics, Dunedin, FL, USA) and focused into a beam approximately 1 mm in diameter using a fused silica 74-UV collimating lens (Ocean Optics). Light transmitted through the retina was collected by a second lens situated immediately below the stage (which was covered with a Teflon diffuser to capture off-axis scattered light) and delivered *via* a 1000 μm diameter quartz fibre optic to a S2000 CCD (charge-coupled device) Spectrometer (Ocean Optics) connected to a Toshiba PC laptop running Windows 98.

At the start and periodically throughout, a 'dark' scan was made with all light blocked from entering the collecting lens connected to the spectroradiometer. This was done to correct for drift and electrical noise in the S2000. At the start of a row of sampling points, a baseline or 'reference' scan was made outside the retina, which was subtracted by the S2000 operating software (OOIbase32, Ocean Optics) from the sample transmission scan recorded at each sampling point within the retina along the row. Each scan (reference or sample) was the average of 50 individual scans (averaging performed by the S2000 acquisition software). Percentage transmission spectra were converted to decadic absorbance spectra offline and further analysed in Microsoft Excel 2007.

A 'corrected' absorbance spectrum was calculated by subtracting, from each sample spectrum, a mean reference spectrum made from five 'white' absorbance sample scans measured in the far periphery of the retina. Each of the corrected absorbance spectra were fitted with an 11-point (approx. equivalent to 3 nm) unweighted running average to smooth the data. To account for minor variations in baseline absorbance, the corrected absorbance at the wavelength of maximum absorbance of the yellow pigment ($\lambda YPmax$) for each sampling site was obtained by subtracting the absorbance at 750 nm (where absorbance by the yellow pigment is negligible) from the absorbance at the wavelength of $\lambda YPmax$ for that species. The $\lambda YPmax$ value varied between species and was defined as the wavelength of peak absorbance of the corrected sample scan with the highest absorbance in that species.

Table S1. List of primer sets used in this study along with their annealing temperature (Ann. temp.) and the expected size of PR product. bp = base pair.

Primer sets	Annealing temp. °C	PCR product (bp)
AOAS F1 + AOAS R2	45	535
AOAS F2 + AOAS R2	50	395
AOAS F1 + AOAS R1	50	363
3RACE1	55	635
3RACE2	55	325

Primer sequences (5' - 3')

AOAS F1	CGCGAGAGATACATNGTNRTNTGYAARCC
AOAS F2	ATTTTAGAAGGTCTGCCRGWSNTCNTGYGG
AOAS R1	ATTGGTCACCTCCTTYTCNGCYYTYTGNGT
AOAS R2	CCCGGAAGACGTAGATGANNGGRTTRWANA
3RACE1	CTCCTGGGTAATGGCGACCAC
3RACE2	GTCTGCTGGGTGCCGTACGCT

Figure S1. Normalised corrected absorbance spectra of the yellow pigment along with its maximum absorbance λ_{YPmax} and the full-width at half-maximum bandwidth (FWHM) in the lanternfish *Symbolophorus rufinus*. Measurements made from a paraformaldehyde-fixed retina (black line) and an unfixed frozen retina (grey line) show that chemical fixation had only a small effect on the shape of the absorbance spectrum.

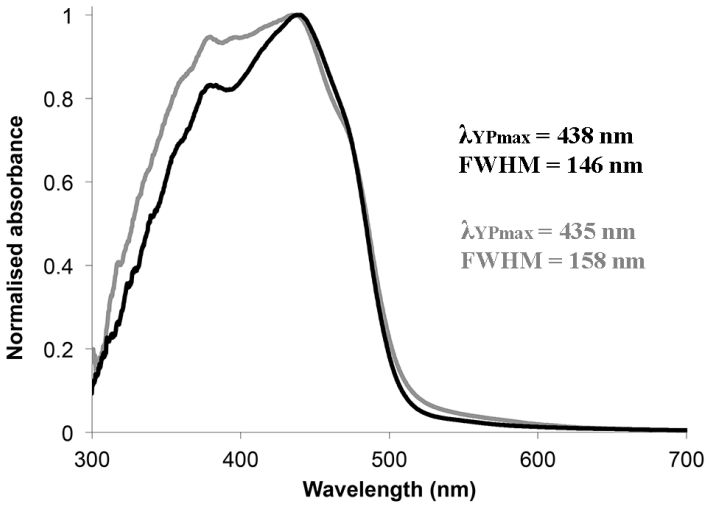

Figure S2. Phylogenetic trees of the two rod opsins found in the lanternfish *Symbolophorus evermanni*. The trees were constructed by (*a*) Neighbour-joining (Saitou and Nei 1987) and (*b*) Maximum Likelihood methods using opsin gene nucleotide sequences of the zebrafish, *Danio rerio*: *Rh1-A* (HM367063), *Rh1-B* (HM367062), *Rh2-1* (NM131253), *Rh2-2* (NM182891), *Rh2-3* (NM182892), *Rh2-4* (NM131254), *SWS1* (NM131319), *SWS2* (NM131192), *LWS1* (NM131175), *LWS2* (NM001002443). The bootstrap confidence values are shown for each branch. The scale bar if calibrated at 0.1 substitutions per site.

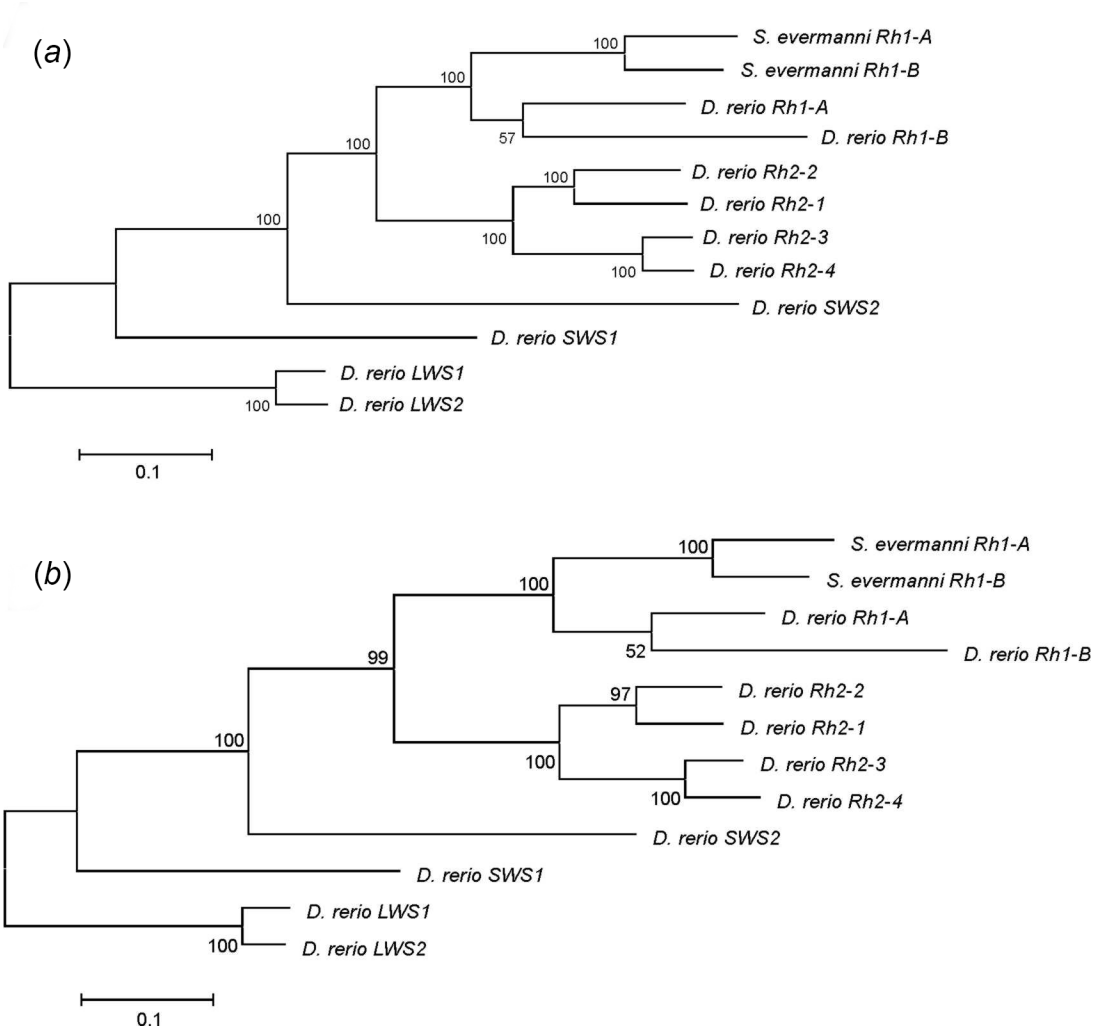

Figure S3. Amino acids sequences of the two rod opsins identified in *Symbolophorus evermanni*. The two sequences (*Rh1A* and *Rh1B*) were aligned with *Stenobrachium leucopsarus* rod opsin sequence (EU407251). Identical residues are indicated by a dot, missing data by a dash. The black arrws indicate the tuning sites identified in *S. evermanni*.