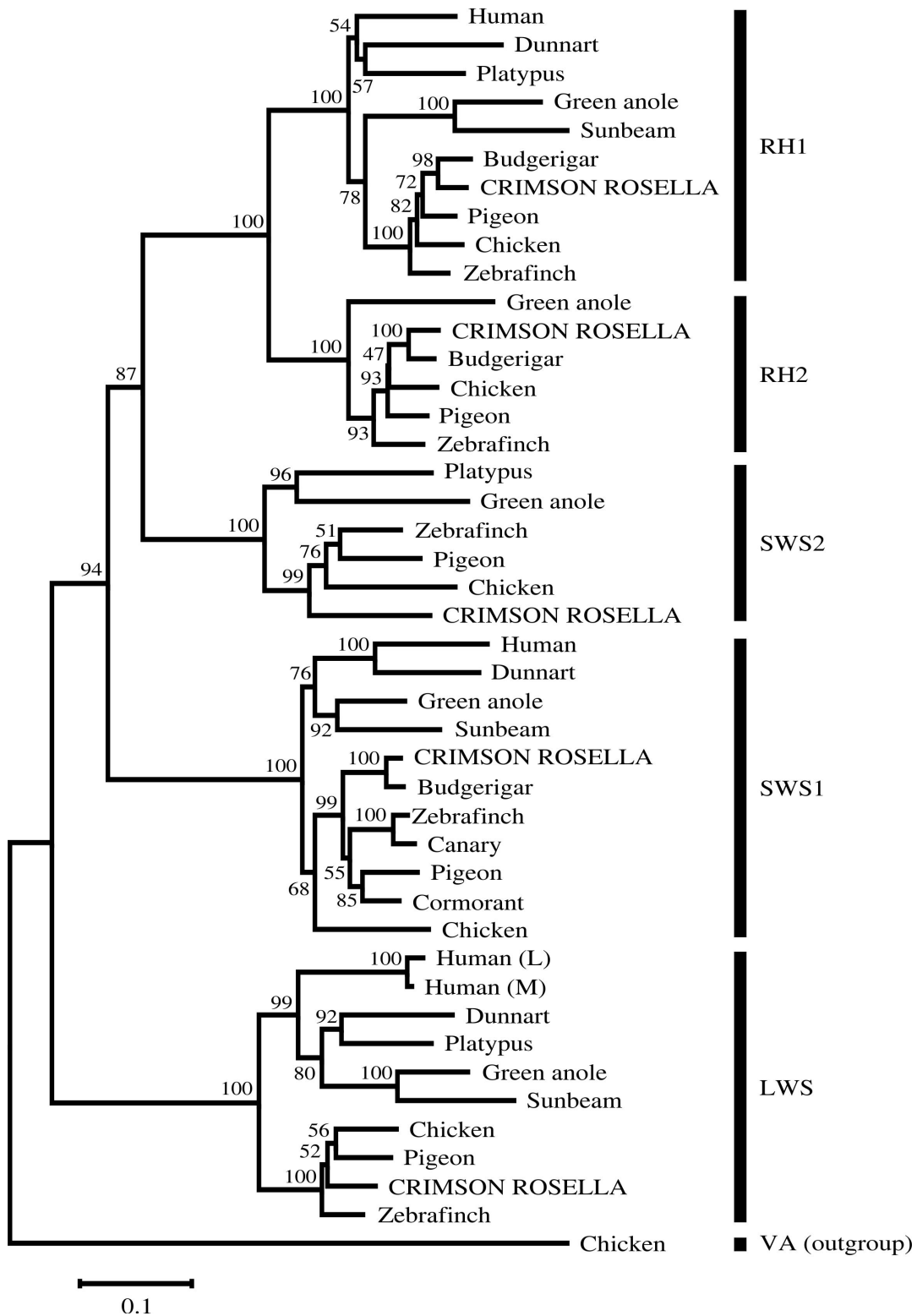


1 **Data Supplement Fig. S1.**



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3 **Data Supplement Fig. S1.** Phylogenetic analysis of *P. elegans* LWS, SWS1, SWS2, RH2
 4 and RH1 visual opsins (GenBank accession nos. KF134487-KF134493) compared to
 5 orthologues present in representative reptiles, birds and mammals, with chicken (*Gallus*

6 *gallus*) vertebrate ancient (VA) opsin (GenBank accession no. GQ280390) included as an
7 outgroup. The degree of support for internal branching is expressed as a percentage with the
8 scale bar indicating the number of nucleotide substitutions per site. The sequences used for
9 generating the tree are as follows: (1) RH1 opsin class: human (*Homo sapiens*), NM000539;
10 fat-tailed dunnart (*Sminthopsis crassicaudata*), AY159786; platypus (*Ornithorhynchus*
11 *anatinus*), EF050076; green anole (*Anolis carolinensis*), AOIRHODOPS; sunbeam snake
12 (*Xenopeltis unicolor*), FJ497233; budgerigar (*Melopsittacus undulatus*), AF021242; pigeon
13 (*Columba livia*), AH007730; chicken (*Gallus gallus*), NM001030606; and zebrafinch
14 (*Taeniopygia guttata*), NM001076695; (2) RH2 opsin class: green anole (*Anolis*
15 *carolinensis*), AH004781; budgerigar (*Melopsittacus undulatus*), AF021241; chicken (*Gallus*
16 *gallus*), M92038; pigeon (*Columba livia*), AH007731; and zebrafinch (*Taeniopygia guttata*),
17 NM001076696; (3) SWS2 opsin class; platypus (*Ornithorhynchus anatinus*), EF050077;
18 green anole (*Anolis carolinensis*), AF133907; zebrafinch (*Taeniopygia guttata*),
19 NM001076697; pigeon (*Columba livia*), AH007799; and chicken (*Gallus gallus*),
20 NM205517; (4) SWS1 opsin class: human (*Homo sapiens*), NM001708; fat-tailed dunnart
21 (*Sminthopsis crassicaudata*), AY442173; green anole (*Anolis carolinensis*), AH007736;
22 sunbeam snake (*Xenopeltis unicolor*), FJ497234; budgerigar (*Melopsittacus undulatus*),
23 Y11787; zebrafinch (*Taeniopygia guttata*), NM001076704; canary (*Serinus canaria*),
24 AJ277922; pigeon (*Columba livia*), AH007798; cormorant (*Phalacrocorax carbo*),
25 EF568933; and chicken (*Gallus gallus*), NM205438; (5) LWS opsin class: human (*Homo*
26 *sapiens*), NM020061 (L cone) and NM000513 (M cone); fat-tailed dunnart (*Sminthopsis*
27 *crassicaudata*), AY430816; platypus (*Ornithorhynchus anatinus*), EF050078; green anole
28 (*Anolis carolinensis*), ACU08131; sunbeam snake (*Xenopeltis unicolor*), FJ497235; chicken
29 (*Gallus gallus*), NM205440; pigeon (*Columba livia*), AH007800; and zebrafinch
30 (*Taeniopygia guttata*), NM001076702.

1 **DATA SUPPLEMENT**

2 **Data Supplement Table 1. Oligonucleotides used in 5'- and 3'-RACE**

Primer name	Sequence (5' to 3')
ROS_LWS_F1	TATCTCTGGTCCTTGGCCATCATCTCCTGG
ROS_LWS_F2	AACATCAAGTTCGATGGGAAGCTGGCGGTG
ROS_LWS_F3	TAATAAATCGTGCGGCCCCGACGTGTTTCAG
ROS_LWS_R1	TATATTATGGCCAAGGACCAGAGCGCGGTG
ROS_LWS_R2	ATATCTCCAGACCCAGGAGAAGACGTACCC
ROS_LWS_R3	TAATATATCGGGGCCGCACGAGGTCTTCAG
ROS_RH2_F1	TTCATCCACTTCATCATCCCAGTCGTGGTC
ROS_RH2_F2	TAAATCGTGGTGGCGTTCTGGATCTTCACC
ROS_RH2_R1	TTAAAATCGATGCCCATCATGGCGTGGCTC
ROS_RH2_R2	ATAAGGCGCCCGTAGGAGAAGAAAATCTCC
ROS_RH2_R3	TTTGTTGGTGAAGATCCAGAACGCCACCAC
ROS_RH1_F1	TTTCTTCTGCTACGGGAACCTGGTTTGCAC
ROS_RH1_F2	CCAGTGTCGCTTTCTACATCTTCACCAACC
ROS_RH1_R1	AATTAAGGAGAAGGCAACGCCCATGATGGC
ROS_RH1_R2	CTGATCATCAGCGGGATCATGAAGTGAACC
ROS_RH1_R3	TAGTCATGAAGATGGGCCCAAAGTCTGACC

31 **Data Supplement Table S2.**

Species	Common name	GenBank accession no:
<i>Homo sapiens</i>	Human	NM000539
<i>Sminthopsis crassicaudata</i>	Fat-tailed dunnart	AY159786
<i>Ornithorhynchus anatinus</i>	Platypus	EF050076
<i>Columba livia</i>	Pigeon	AH007730
<i>Melopsittacus undulatus</i>	Budgerigar	AF021242
<i>Gallus gallus</i>	Chicken	NM001030606
<i>Taeniopygia guttata</i>	Zebra finch	NM001076695
<i>Platyercus elegans</i>	Crimson rosella	KF134487
<i>Anolis carolinensis</i>	Green anole	AOIRHODOPS
<i>Xenopeltis unicolor</i>	Sunbeam snake	FJ497233
<i>Xenopus tropicalis</i>	Western clawed frog	NM001097334
<i>Danio rerio</i>	Zebrafish	HM367063
<i>Callorhynchus milii</i>	Elephant shark chimaera	EF565167
<i>Geotria australis</i>	Pouched lamprey	AY366493

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33 **Data Supplement Table S2.** Sequences used for generating the RH1 alignment in Figure 2
34 of the main article.

1 **Data Supplement Table S3. Spectral tuning of visual pigments in *P. elegans***

Pigment (gene/ λ_{\max})	Tuning site location	Ancestral tuning sites	Tuning site consensus in birds	Visual pigments in <i>P. elegans</i>			
				Tuning Site	Expected spectral peak (Exp; λ_{\max})	Observed spectral peak (Obs; λ_{\max})	Δ spectral peak (Exp-Obs)
LWS (ancestral λ_{\max} = 560 nm (Davies et al., 2007); average avian λ_{\max} = 563 nm ^{#1})	164/181/261/ 269/292	SHYTA	SHYTA	SHYTA	560 nm (ancestor) 563 nm (Aves)	567 nm	-7 nm -4 nm
SWS1 (ancestral λ_{\max} = 360 nm (Davies et al., 2007); average avian λ_{\max} = 365 nm [UV-sensitive pigments only] ^{#2})	46/49/52/86/ 90/93/114/118	FFTFSTAS	(L/V)(L/V)T(A/C)CTGA	VLTACTG A	360 nm (ancestor) 381 nm (modified from budgerigar (<i>Melopsittacus undulates</i>) and other vertebrates)	365 nm	-5 nm +16 nm

					365 nm (Aves)		0 nm
SWS2 (ancestral λ_{\max} = 440 nm (Davies et al., 2007); average avian λ_{\max} = 451 nm ^{#3})	46/49/52/93/164 /207/269	FIVTALA	(A/L)(A/L)(G/V)TGL(A/C/ S/T)	AAVTGLA	440 nm (ancestor) 451 nm (modified from pigeon (<i>Columba livia</i>) and other vertebrates; Aves)	440 nm	0 nm +11 nm
RH2 (ancestral λ_{\max} = 495 nm (Davies et al., 2007); average avian λ_{\max} = 505 nm ^{#4})	83/122/207/211/ 265/292/295	DEMHWAS	DQMHWAS	DQMHWAS	476 nm (modified from ancestral and bovine (<i>Bos taurus</i>) pigments) 505 nm (Aves)	509 nm	-33 nm -4 nm
RH1 (ancestral λ_{\max} = 500 nm (Davies et al., 2007); average avian λ_{\max} = 505 nm ^{#5})	83/122/207/211/ 265/292/295	DEMHWA A	DEMHWAA	DEMHWA A	500 nm (ancestor) 503 nm (Aves)	510 nm	-10 nm -7 nm

3 #1, average avian spectral peak ($\lambda_{\max} = 563 \pm 5$ nm) derived from LWS pigments of pigeon, *Columba livia* ($\lambda_{\max} = 558$ nm) (Kawamura et al.,
4 1999), chicken, *Gallus gallus* ($\lambda_{\max} = 571$ nm) (Okano et al., 1992), zebrafinch, *Taeniopygia guttata* ($\lambda_{\max} = 560$ nm) (Yokoyama et al., 2000)
5 and turkey, *Meleagris gallopavo* ($\lambda_{\max} = 564$ nm) (Hart et al., 1999); #2, average avian spectral peak ($\lambda_{\max} = 365 \pm 6$ nm) derived from UV-
6 sensitive SWS1 pigments of canary, *Serinus canaria* ($\lambda_{\max} = 366$ nm) (Das et al., 1999), budgerigar, *Melopsittacus undulates* ($\lambda_{\max} = 371$ nm)
7 (Wilkie et al., 1998) and zebrafinch, *Taeniopygia guttata* ($\lambda_{\max} = 359$ nm) (Yokoyama et al., 2000); #3, average avian spectral peak ($\lambda_{\max} = 451$
8 ± 8 nm) derived from SWS2 pigments of pigeon, *Columba livia* ($\lambda_{\max} = 448$ nm) (Kawamura et al., 1999), chicken, *Gallus gallus* ($\lambda_{\max} = 455$
9 nm) (Okano et al., 1992), zebrafinch, *Taeniopygia guttata* ($\lambda_{\max} = 441$ nm) (Yokoyama et al., 2000) and turkey, *Meleagris gallopavo* ($\lambda_{\max} = 460$
10 nm) (Hart et al., 1999); #4, average avian spectral peak ($\lambda_{\max} = 505 \pm 2$ nm) derived from RH2 pigments of pigeon, *Columba livia* ($\lambda_{\max} = 503$
11 nm) (Kawamura et al., 1999), chicken, *Gallus gallus* ($\lambda_{\max} = 508$ nm) (Okano et al., 1992), zebrafinch, *Taeniopygia guttata* ($\lambda_{\max} = 505$ nm)
12 (Yokoyama et al., 2000) and turkey, *Meleagris gallopavo* ($\lambda_{\max} = 505$ nm) (Hart et al., 1999); and #5, average avian spectral peak ($\lambda_{\max} = 503 \pm$
13 1 nm) derived from RH1 pigments of pigeon, *Columba livia* ($\lambda_{\max} = 502$ nm) (Kawamura et al., 1999), chicken, *Gallus gallus* ($\lambda_{\max} = 503$ nm)
14 (Okano et al., 1992), zebrafinch, *Taeniopygia guttata* ($\lambda_{\max} = 502$ nm) (Yokoyama et al., 2000) and turkey, *Meleagris gallopavo* ($\lambda_{\max} = 504$ nm)
15 (Hart et al., 1999).

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20 **Data Supplement References**

- 21 **Das, D., Wilkie, S. E., Hunt, D. M. and Bowmaker, J. K.** (1999). Visual pigments and oil droplets in the retina of a passerine bird, the canary *Serinus canaria*:
22 microspectrophotometry and opsin sequences. *Vision Res* **39**, 2801-2815.
- 23 **Davies, W. L., Cowing, J. A., Carvalho, L. S., Potter, I. C., Trezise, A. E., Hunt, D. M. and Collin, S. P.** (2007). Functional characterization, tuning, and
24 regulation of visual pigment gene expression in an anadromous lamprey. *FASEB J* **21**, 2713-2724.
- 25 **Hart, N. S., Partridge, J. C. and Cuthill, I. C.** (1999). Visual pigments, cone oil droplets, ocular media and predicted spectral sensitivity in the domestic turkey
26 (*Meleagris gallopavo*). *Vision Res* **39**, 3321-3328.
- 27 **Kawamura, S., Blow, N. S. and Yokoyama, S.** (1999). Genetic analyses of visual pigments of the pigeon (*Columba livia*). *Genetics* **153**, 1839-1850.
- 28 **Okano, T., Kojima, D., Fukada, Y., Shichida, Y. and Yoshizawa, T.** (1992). Primary structures of chicken cone visual pigments: vertebrate rhodopsins have
29 evolved out of cone visual pigments. *Proc Natl Acad Sci U S A* **89**, 5932-5936.
- 30 **Wilkie, S. E., Vissers, P. M., Das, D., Degrip, W. J., Bowmaker, J. K. and Hunt, D. M.** (1998). The molecular basis for UV vision in birds: spectral
31 characteristics, cDNA sequence and retinal localization of the UV-sensitive visual pigment of the budgerigar (*Melopsittacus undulatus*). *Biochem J* **330 (Pt**
32 **1)**, 541-547.
- 33 **Yokoyama, S., Blow, N. S. and Radlwimmer, F. B.** (2000). Molecular evolution of color vision of zebra finch. *Gene* **259**, 17-24.