Supplementary Information

Retinal transcriptome sequencing sheds light on the adaptation to nocturnal and diurnal lifestyles in raptors

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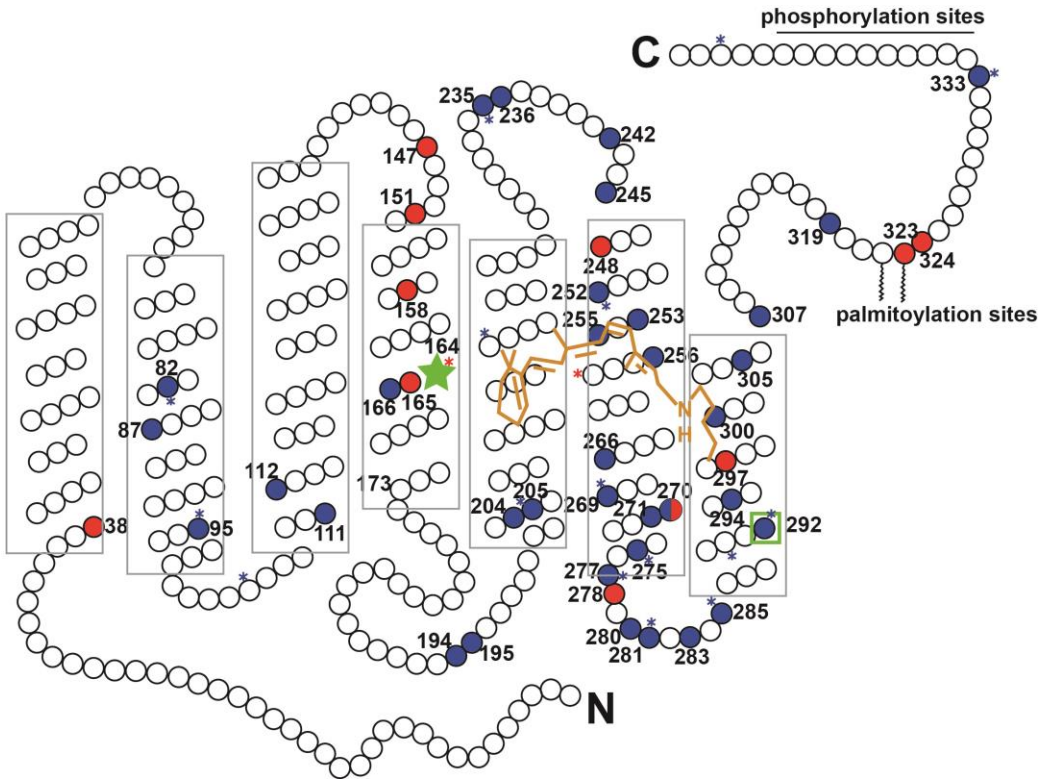
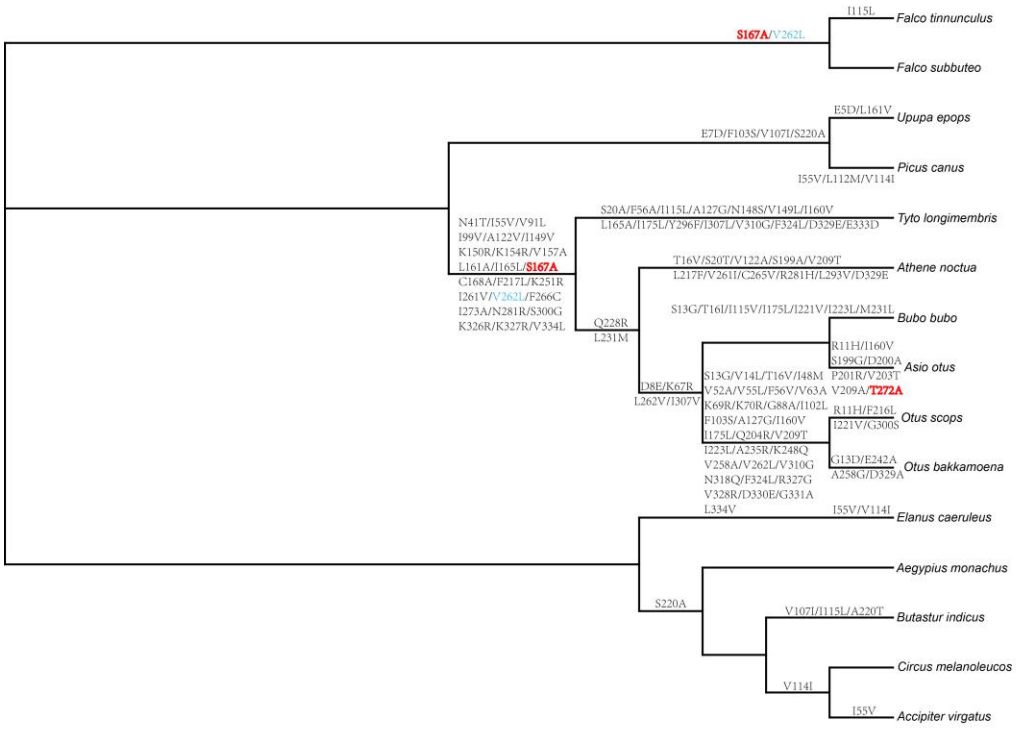
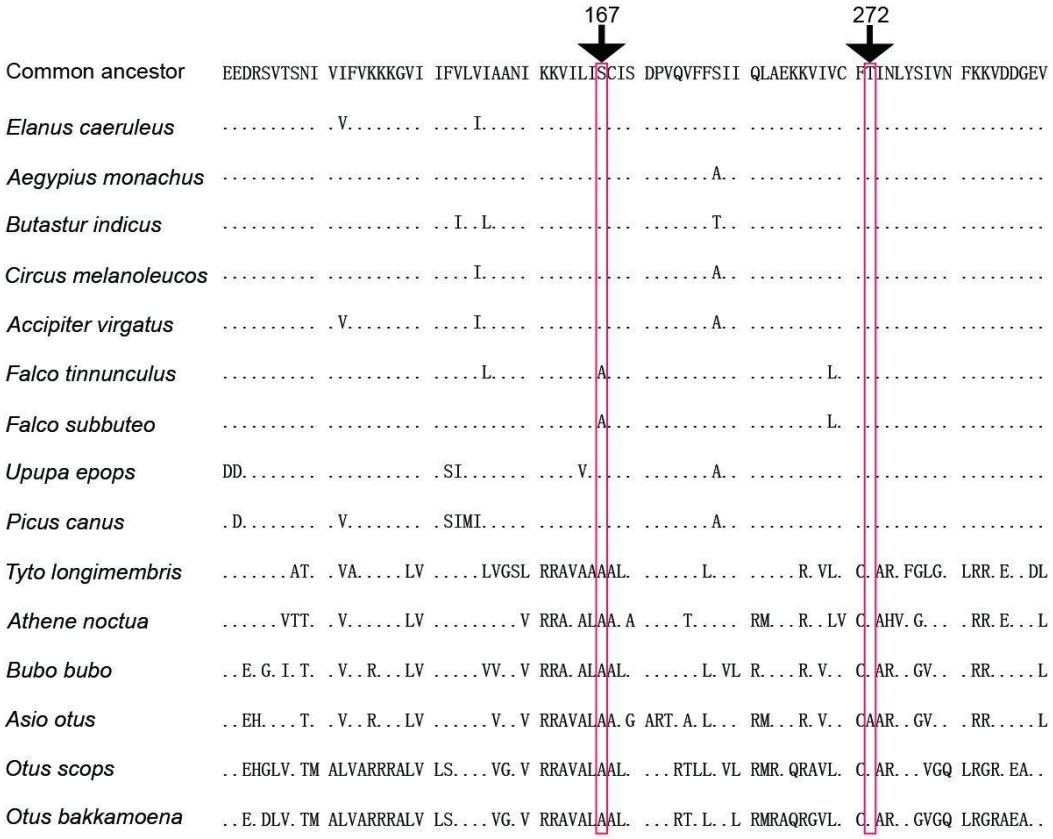


Supplementary Figure 1 The positively selected sites of *LWS* (red) and *SWS2* (blue) mapping on the secondary structure of bovine rhodopsin. The numbering is based on the bovine rhodopsin. The model is based on previous studies[16,59-60]. The spectral tuning sites of *LWS* and *SWS2* found in this study are shown in a green star and a green square, respectively. The 11-cis-retinal is shown in orange. Red * and blue * show amino acid sites of *LWS* and *SWS2* under parallel amino acid changes between owls and falcons, respectively. The grey rectangle shows the even transmembrane domains of rhodopsin.

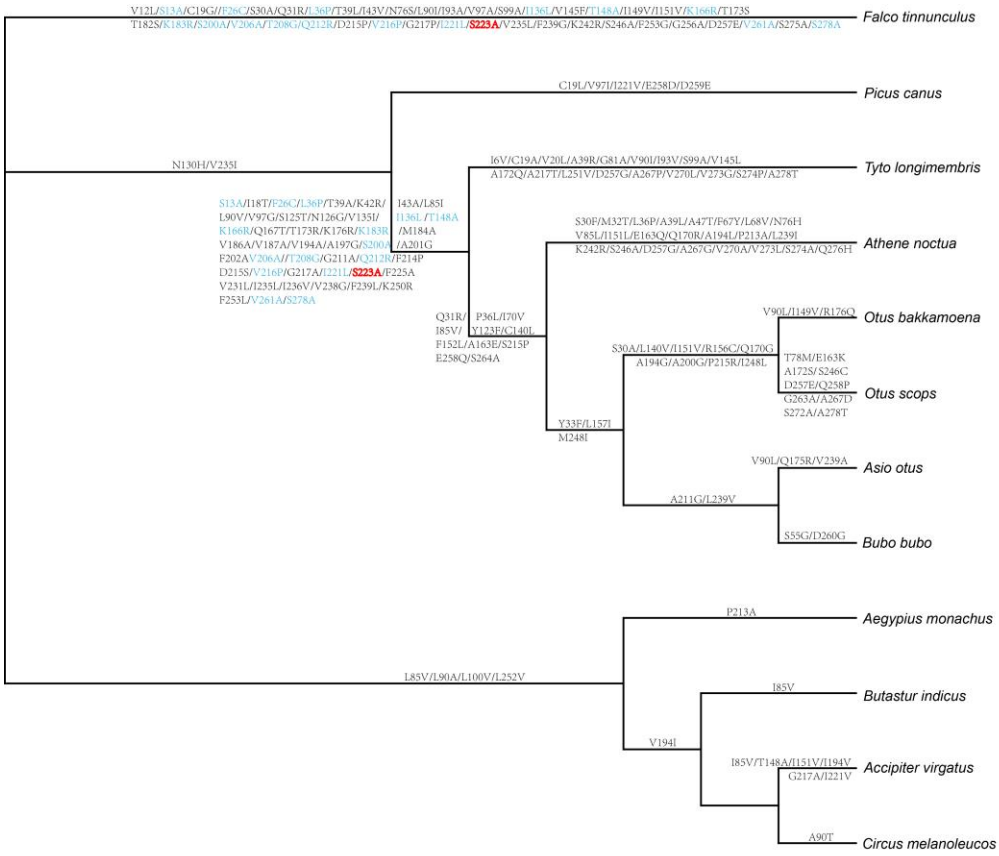


Supplementary Figure 2 Amino acid replacements of *LWS* along different branches. Phylogenetic relationships among species followed previous studies[1,49-51]. The critical amino acid replacements that are considered to be responsible for the spectral tuning are highlighted in red. Blue shows parallel amino acid changes between ancestral branches of owls and falcons.

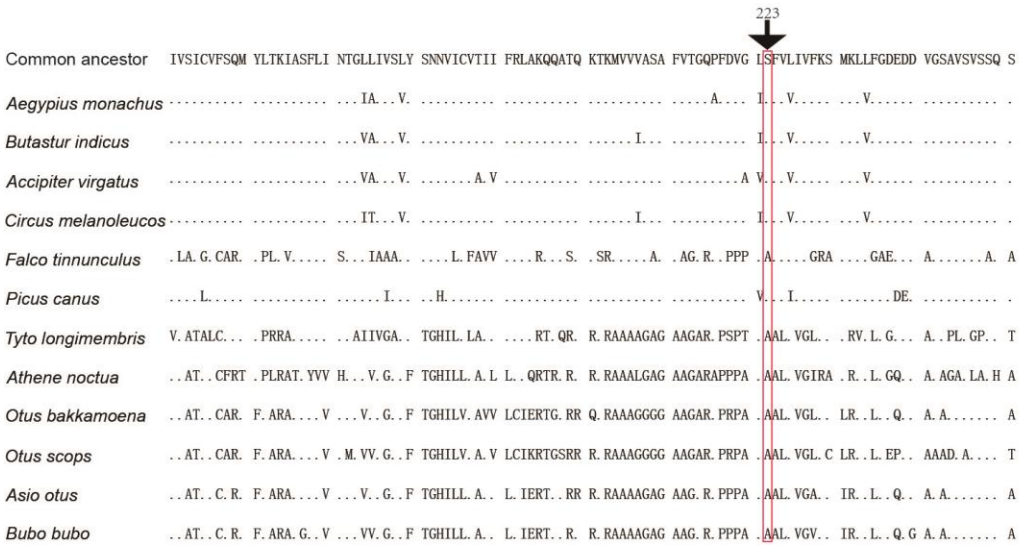


Supplementary Figure 3 Variable amino acid sites of *LWS* in 15 species and their common ancestor.

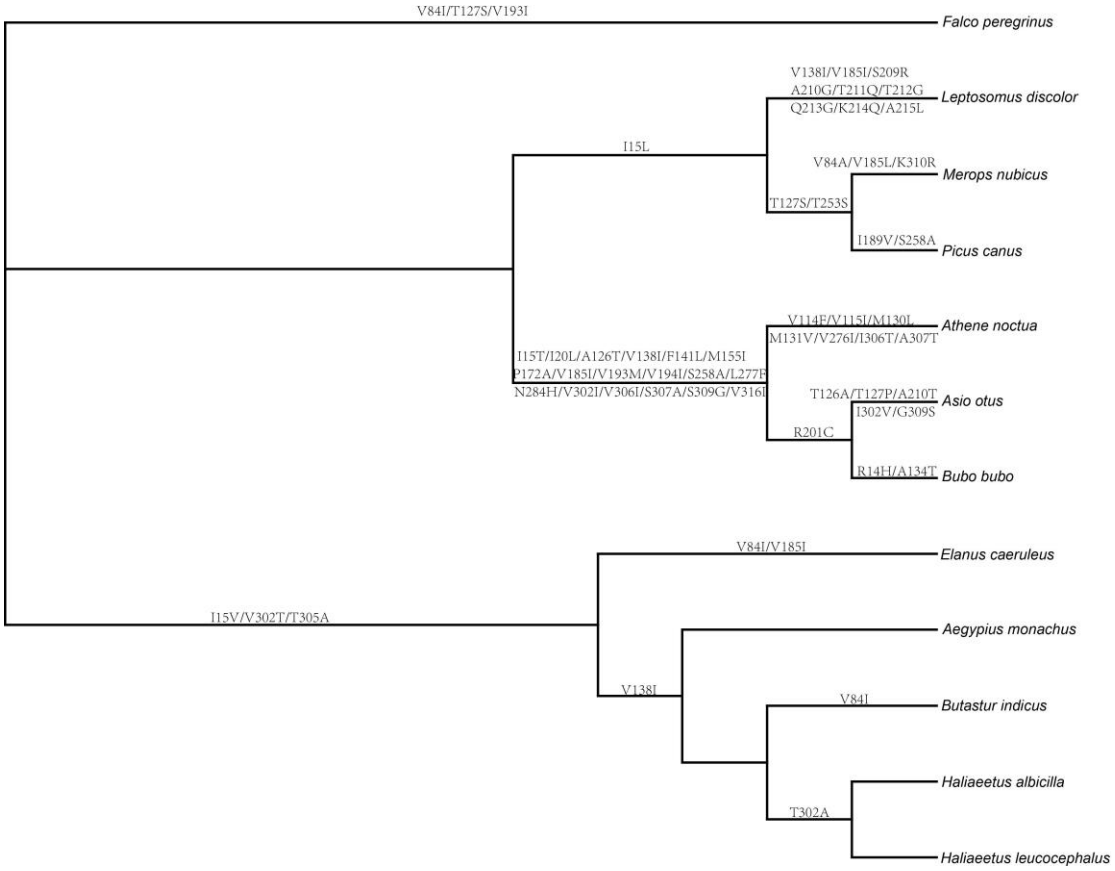
The dot shows identical amino acid to the common ancestor. Two amino acid sites associated with the *LWS* spectral turning are highlighted.



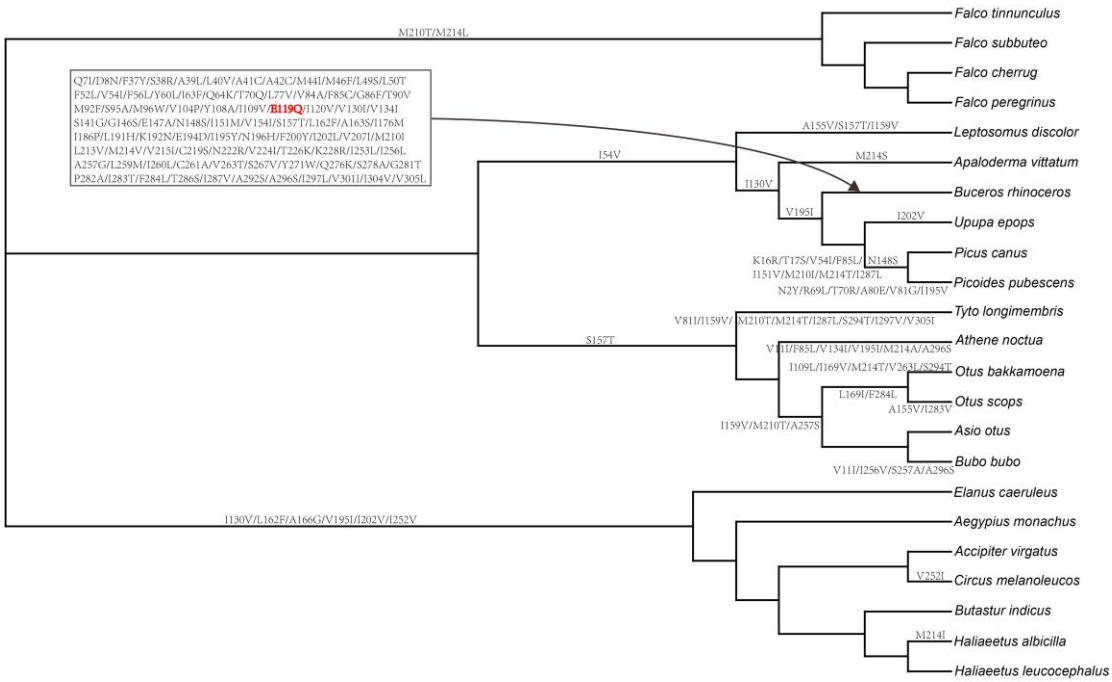
Supplementary Figure 4 Amino acid replacements of *SWS2* along different branches. Phylogenetic relationships among species followed previous studies[1,49-51]. The amino acid replacements associated with the *SWS2* spectral tuning are highlighted in red. Blue shows parallel amino acid changes between ancestral branches of owls and falcon branch.



Supplementary Figure 5 Variable amino acid sites of SWS2 in 12 species and their common ancestor. The dot shows identical amino acid to the common ancestor. The amino acid site associated with the *SWS2* spectral turning is highlighted.



Supplementary Figure 6 Amino acid replacements of *RH2* along different branches. Phylogenetic relationships among species followed previous studies[1,49-51]. No amino acid replacement associated with the *RH2* spectral turning is found.



Supplementary Figure 7 Amino acid replacements of *RH1* along different branches. Phylogenetic relationships among species followed previous studies[1,49-51]. The amino acid replacement associated with the *RH1* spectral tuning is highlighted in red. The amino acid replacement E119Q (corresponding to E122Q in bovine rhodopsin) along the branch of rhinoceros hornbill (*Buceros rhinoceros*) is considered to reduce λmax of *RH1* by 20 nm (Supplementary Table 5).

Supplementary Table 1 The basic statistics of the transcriptome sequencing for 15 species studied.

Species/Average	Raw reads (No.)	Clean reads (No.)	Clean bases (bp)	Unigene (No.)	Mean length (bp)	N50 (bp)
Falco tinnunculus	51627942	50327827	12670306819	181077	681	1271
Falco subbuteo	51265913	50094480	12608048272	196648	679	1208
Upupa epops	46289873	45071285	11340043000	128819	695	1437
Picus canus	59253971	58407841	14707622487	160139	640	1082
Tyto longimembris	47123024	44590406	11209515376	169590	620	992
Athene noctua	54430631	53735717	13532670717	162550	693	1333
Otus bakkamoena	67375596	67324756	6715503299	190941	714	1359
Otus scops	50255927	47780582	12020208310	162940	666	1216
Asio otus	44516155	43716816	11006115275	161612	693	1325
Bubo bubo	51964845	50656163	12747030463	160561	674	1255
Elanus caeruleus	53858822	52591133	13235187097	191698	673	1212
Aegypius monachus	49449600	47841645	12036588777	178557	653	1155
Accipiter virgatus	48522401	46686932	11743460419	168270	662	1204
Circus melanoleucos	49208081	48228181	12141863212	182491	649	1152
Butastur indicus	49568683	48503775	12210776879	165290	706	1423
Average	51647431	50370503	11994996027	170746	673	1242

Supplementary Table 2 Identity of coding sequence of *RH1* gene based on the transcriptome sequencing and cloning sequencing. The fragment lengths of each species obtained by cloning sequencing are shown.

Species	Length (bp)	Identity (%)
Falco tinnunculus	880	99.55
Falco subbuteo	713	99.72
Upupa epops	333	99.40
Picus canus	519	99.42
Tyto longimembris	519	99.23
Athene noctua	519	99.42
Otus bakkamoena	1053	99.92
Otus scops	1053	99.43
Asio otus	1053	99.72
Bubo bubo	1053	99.72
Elanus caeruleus	696	99.71
Aegypius monachus	880	99.66
Accipiter virgatus	882	99.55
Circus melanoleucos	1053	99.81
Butastur indicus	882	99.77

Supplementary Table 3 The 120 vision genes examined for the four bird taxa in this study. Dots show the available gene sequences and red crosses show the unavailable gene sequences.

Gene symbols	Strigiformes	Accipitriformes	Falconiformes	Coraciimorphae
ABCA4	•	•	•	•
ARR3	•	•	•	•
ATP8A2	•	•	•	•
ATP8B1	•	•	•	•
BBS4	•	•	•	•
BHLHE23	•	•	•	•
CACNA2D4	•	•	•	•
CACNB2	•	•	•	•
CACNB4	•	•	•	•
CCDC66	•	•	•	•
CHRNB2	•	•	•	•
CLN5	•	•	•	•
CLN6	•	•	•	•
CLN8	•	•	•	•
CNGA1	•	•	•	•
CNGA3	•	•	•	•
CNGB1	•	•	•	•
CNGB3	•	•	•	•
CNIH2	•	•	•	•
COL11A1	•	•	X	•
COL2A1	•	•	•	X
CRABP1	•	•	•	•
CRABP2	•	•	•	•
CRDS2	•	•	•	•
CRYBA1	•	•	•	•
CRYBB2	•	•	•	•
DMD	•	•	•	•
DNAJC19	•	•	•	•
EPAS1	•	•	•	•
EPHB2	•	•	•	X
EYS	•	•	•	X

GABRR2	•	•	•	•
GJA10	•	•	•	•
GJC1	•	•	•	•
GJD2	•	•	•	•
GLRA1	•	•	•	•
GLRB	•	•	•	•
GNAT1	•	•	•	•
GNAT2	•	•	•	•
GNB1	•	•	•	•
GNB3	•	•	•	•
GNB5	•	•	•	•
GNGT2	•	•	•	•
GPR98	•	•	•	•
GRK1	•	•	•	X
GRK7	•	•	•	•
GUCA1A	•	•	•	•
GUCA1B	•	•	•	•
GUCA1C	•	•	•	•
GUCY2D	•	•	•	•
GUCY2F	•	•	•	•
HBEGF	•	•	•	•
HCN1	•	•	X	•
ISL1	•	•	•	X
KCNA2	•	•	•	•
LAMB2	•	•	•	•
LAMC3	•	•	•	•
LUM	•	•	•	•
LWS	•	•	•	•
MYO5A	•	•	•	•
MYO7A	•	•	•	•
NAV2	•	•	•	•
NOB1	•	•	•	•
NR2E1	•	•	•	•
NRP1	•	•	•	•

NRP2	•	•	•	•
NTRK2	•	•	•	•
NXNL2	•	•	•	•
NYX	•	•	•	•
OPA1	•	•	•	•
OPN3	•	•	•	•
OPN4-1	•	•	•	•
OPN5	•	•	•	•
PCDH15	•	•	•	•
PDCL	•	•	•	•
PDE6B	•	•	•	•
PDE6C	•	•	•	•
PDE6D	•	•	•	•
PDE6G	•	•	•	•
PDE6H	•	•	•	•
PHOX2B	•	•	•	•
PLXNA4	•	•	•	•
POU4F3	•	•	•	•
PPT1	•	•	•	•
PRPH2	•	•	•	•
RBP4	•	•	•	•
RBP4B	•	•	•	•
RCVRN	•	•	•	•
RDH10	•	•	•	•
RDH8	•	•	•	X
REEP6	•	•	•	•
RGR	•	•	•	•
RGS9	•	•	•	•
RGS9BP	•	•	•	•
RH1	•	•	•	•
RH2	•	•	•	•
RPE65	•	•	•	•
RPGR	•	•	•	•
RRH	•	•	•	•

SAG	•	•	•	•
SALL1	•	•	•	•
SEMA3A	•	•	•	•
SEMA3F	•	•	•	•
SIX4	•	•	•	•
SLC1A3	•	•	•	•
SLC24A1	•	•	•	•
SLC24A2	•	•	•	•
SLITRK6	•	•	•	•
SWS1	X	•	•	•
SWS2	•	•	•	•
TFAP2A	•	•	•	•
THY1	•	•	•	•
TMEM126A	•	•	•	•
TRPM1	•	•	•	•
TULP1	•	•	•	•
VISININ	•	•	X	•
VSX1	•	•	•	•
VSX2	•	•	•	•
WFS1	•	•	•	•

Supplementary Table 5 Amino acid replacement of visual pigments (*LWS*, *SWS2*, *RH1*, *RH2*) and their effects on the wavelength shift of maximal absorption (Δλ). Amino acid site numbers are based on the bovine rhodopsin.

Visual pigment	Amino acid change	Δλ (nm)	Source
LWS			
	S164A	-7	(16)
	H181Y	-28	(16)
	Y261F	-8	(16)
	T269A	-15	(16)
	A292S	-27	(16)
	S164A & H181Y	+11	(16)
SWS2			
	S91P	+10	(63)
	T93L	-9	(63)
	T93V	-6	(63)
	A94S	+14	(63)
	S127C	+2	(63)
	L207I	-6	(63)
	C211S	+2	(63)
	F261Y	+5	(63)
	A269S	+3	(63)
	A269T	+5	(63)
	S292A	+8	(63)
RH1			
	D83N	-6	(62)
	N83D	+2	(62)
	G90S	-13	(62)
	E113D	+7	(62)
	T118A	-16	(62)
	E122Q	-20	(62)
	Q122E	+10	(62)

	I133F	blue-shift	(16)
	A164S	+2	(62)
	F261Y	+10	(62)
	Y261F	-8	(62)
	W265Y	-15	(62)
	A269T	+14	(62)
	A292S	-10	(62)
	S292A	+8	(62)
	Q122E & S292A	+26	(16)
RH2			
	F49A & L52M	−4	(61)
	T97A	−8	(61)
	D83N & M86T & T97A	−15	(61)
	Q122E	+13-16	(61)
	E122Q	-10	(16)
	S164A	−1	(61)
	F49S & Q122E & S164A	+15	(61)
	L207M	+6	(16)
	Q122E & L207M	+21	(16)
	S292A	+7	(61)
	Q122E & S292A	+17	(61)

Supplementary Table 6 Recombination analyses of the positively selected genes. The statistical significance for each of the breakpoints found is shown.

Genes	BPs	AICc	ΔAICc	Breakpoint(LHS *p-value*, RHS *p-value*)	Significance
ABCA4	2	32348.50	4.00	2748(0.43040, 0.00040), 4473(0.10400, 0.00040)	N.S,N.S
ARR3	2	7303.37	11.42	541(0.00040, 0.00040), 867(0.00040, 0.00040)	***,***
CCDC66	/	/	/	/	no recombination
CLN8	1	6974.88	20.84	654(0.02580, 0.00020)	**
CNGA1	1	11703.50	25.62	459(0.00220, 0.01180)	**
CNGB1	3	12449.40	4.12	93(0.00600, 0.20100), 351(0.24840, 1.00000), 510(0.76200, 0.08460)	N.S,N.S,N.S
CNGB3	1	9297.42	15.36	412(0.04540, 0.15760)	N.S
COL2A1	3	14413.60	0.73	132(0.06120, 0.35820), 1167(0.20820, 0.00060), 1320(0.00060, 1.00000)	N.S,N.S,N.S
GRK1	3	8812.24	3.23	117(1.00000, 0.61440) , 287(1.00000, 0.00060) , 453(0.01080, 1.00000)	N.S,N.S,N.S
GUCA1A	/	/	/	/	no recombination
GUCY2D	2	19351.50	16.78	449(0.14480, 0.09080), 690(0.00560, 0.60480)	N.S,N.S
GUCY2F	1	23712.70	9.24	185(0.0002, 0.0934)	*
LWS	3	7539.23	35.81	309(0.01440, 0.00060), 552(0.00060, 0.00060), 714(0.00060, 0.00180)	**,***,***
NXNL2	1	1576.88	2.06	240(0.07660, 0.54580)	N.S
PCDH15	5	37648.60	26.58	1797(0.30500, 0.00100), 2193(0.00100, 0.22900), 2541(0.00200, 0.16300)	N.S,N.S,N.S
				3456(1.00000, 0.00100), 4485(1.00000, 0.94100)	N.S, N.S
PDE6B	/	/	/	/	no recombination
PDE6C	1	14610.80	25.04	822(0.00340, 0.41540)	N.S
PDE6H	/	/	/	/	no recombination
RPGR	1	9033.37	36.56	435(0.00020, 0.00560)	***
SAG	1	5639.12	26.09	339(0.00020, 0.05800)	*
SLC24A1	2	9896.00	62.88	363(0.80240, 0.00040), 588(0.00040, 0.78600)	N.S,N.S
SWS2	3	6577.10	22.39	129(0.05160, 0.00060), 372(0.00060, 0.04620), 599(1.00000, 0.08820)	*,**, N.S

*P<0.1, **P<0.05,***P<0.01; N.S, not significant; BPs: number of breakpoints in the model; AICc: AIC-c score for the best model with this many BPs; ΔAICc: AIC-c improvement relative to the model with one fewer breakpoint; no recombination, no evidence of recombination was found.

Supplementary Table 7 Primer pairs used for amplifying exons 1-5 of *RH1* gene. The annealing temperatures (T) for amplicaiton of the different exons are also shown.

Exon	Primer (5'-3')	T (℃)	Source
Exon 1	Rho.1F: MGTGGTCYCCAACAAT	54.0	This study
	Rho.1R: RGTGYCATTTGYYACTTTC		This study
Exons 2-4	Rhod.2F: GAAATTGCTCTCTGGTCRCTGGTYGT	63.3	(64)
	Rhod.4R: AAAGAANGCYGGGATGGTCATGAAGA		(64)
Exons 4-5	Rho.4F: CACYACCCAGAAGGCAGAGAARGAA	60.9	This study
	Rho.5R: CTCAGCRAGTSAACAGAAAKGCGGA		This study

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