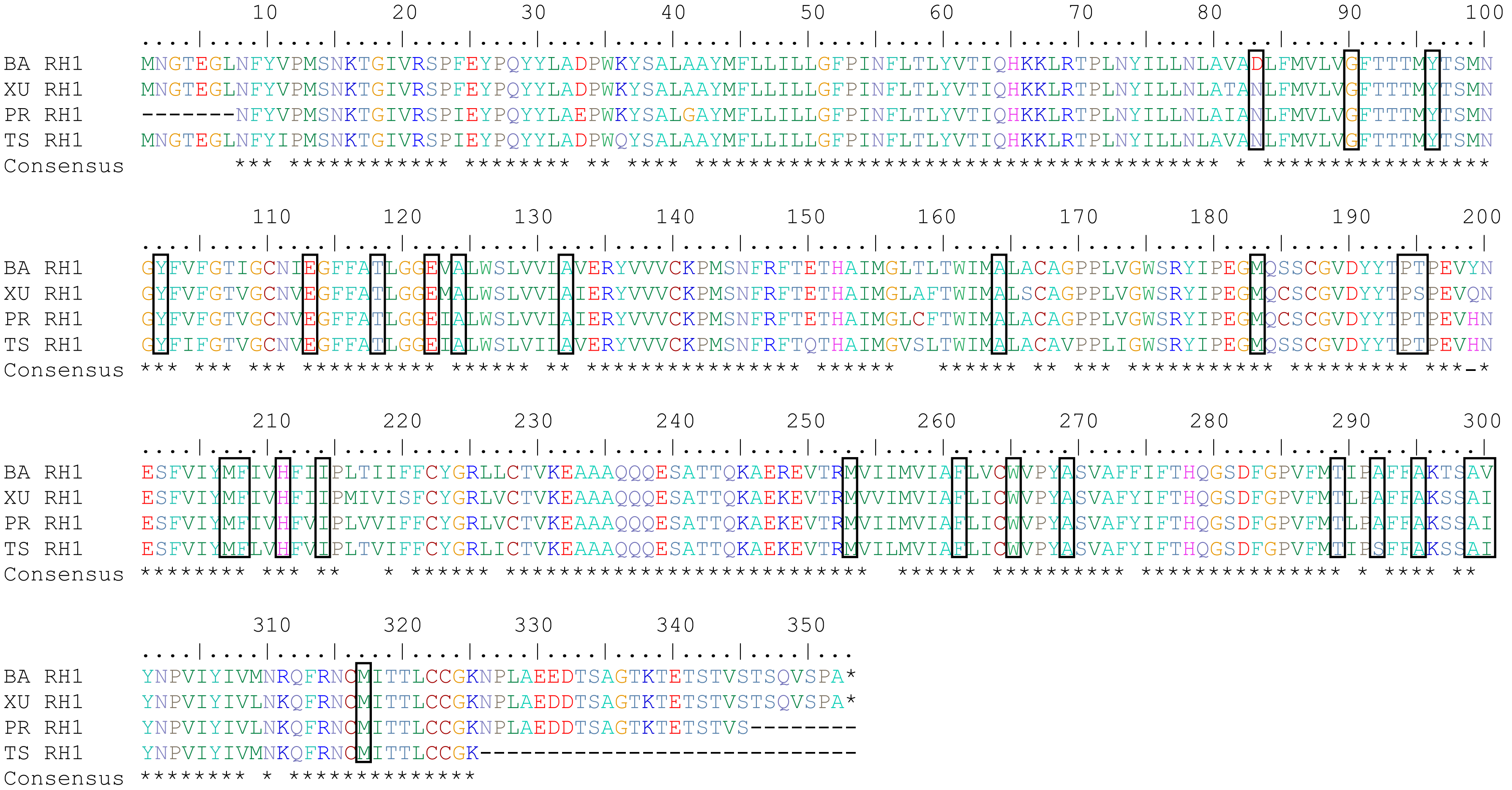
Supplementary Materials:



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                  10        20        30        40        50        60        70        80        90       100
          ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
BA RH1    MNGTEGLNFYVPMSNKTGIVRSPFEYPQYYLADPWKYSALAAYMFLLILLGFPINFLTLYVTIQHKKLRTPLNYILLNLAVADLFMVLVGFTTTMYTSMN
XU RH1    MNGTEGLNFYVPMSNKTGIVRSPFEYPQYYLADPWKYSALAAYMFLLILLGFPINFLTLYVTIQHKKLRTPLNYILLNLATANLFMVLVGFTTTMYTSMN
PR RH1    -------NFYVPMSNKTGIVRSPIEYPQYYLAEPWKYSALGAYMFLLILLGFPINFLTLYVTIQHKKLRTPLNYILLNLAIANLFMVLVGFTTTMYTSMN
TS RH1    MNGTEGLNFYIPMSNKTGIVRSPIEYPQYYLADPWQYSALAAYMFLLILLGFPINFLTLYVTIQHKKLRTPLNYILLNLAVANLFMVLVGFTTTMYTSMN
Consensus        *** ************ ******** ** **** *************************************** * *****************

                 110       120       130       140       150       160       170       180       190       200
          ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
BA RH1    GYFVFGTIGCNIEGFFATLGGEVALWSLVVLAVERYVVVCKPMSNFRFTETHAIMGLTLTWIMALACAGPPLVGWSRYIPEGMQSSCGVDYYTPTPEVYN
XU RH1    GYFVFGTVGCNVEGFFATLGGEMALWSLVVLAIERYVVVCKPMSNFRFTETHAIMGLAFTWIMALSCAGPPLVGWSRYIPEGMQCSCGVDYYTPSPEVQN
PR RH1    GYFVFGTVGCNVEGFFATLGGEIALWSLVVLAIERYVVVCKPMSNFRFTETHAIMGLCFTWIMALACAGPPLVGWSRYIPEGMQCSCGVDYYTPTPEVHN
TS RH1    GYFIFGTVGCNVEGFFATLGGEIALWSLVILAVERYVVVCKPMSNFRFTQTHAIMGVSLTWIMALACAVPPLIGWSRYIPEGMQSSCGVDYYTPTPEVHN
Consensus *** *** *** ********** ****** ** **************** ******   ****** ** *** *********** ********* ***_*

                 210       220       230       240       250       260       270       280       290       300
          ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
BA RH1    ESFVIYMFIVHFIIPLTIIFFCYGRLLCTVKEAAAQQQESATTQKAEREVTRMVIIMVIAFLVCWVPYASVAFFIFTHQGSDFGPVFMTIPAFFAKTSAV
XU RH1    ESFVIYMFIVHFIIPMIVISFCYGRLVCTVKEAAAQQQESATTQKAEKEVTRMVVIMVIAFLICWVPYASVAFYIFTHQGSDFGPVFMTLPAFFAKSSAI
PR RH1    ESFVIYMFIVHFVIPLVVIFFCYGRLVCTVKEAAAQQQESATTQKAEKEVTRMVIIMVIAFLICWVPYASVAFYIFTHQGSDFGPVFMTLPAFFAKSSAI
TS RH1    ESFVIYMFLVHFVIPLTVIFFCYGRLICTVKEAAAQQQESATTQKAEKEVTRMVILMVIAFLICWVPYASVAFYIFTHQGSDFGPVFMTIPSFFAKSSAI
Consensus ******** *** **   * ****** **************************   ****** ********** *************** * **** **

                 310       320       330       340       350
          ....|....|....|....|....|....|....|....|....|....|...
BA RH1    YNPVIYIVMNRQFRNCMITTLCCGKNPLAEEDTSAGTKTETSTVSTSQVSPA*
XU RH1    YNPVIYIVLNKQFRNCMITTLCCGKNPLAEDDTSAGTKTETSTVSTSQVSPA*
PR RH1    YNPVIYIVLNKQFRNCMITTLCCGKNPLAEDDTSAGTKTETSTVS--------
TS RH1    YNPVIYIVMNKQFRNCMITTLCCGK----------------------------
Consensus ******** * **************
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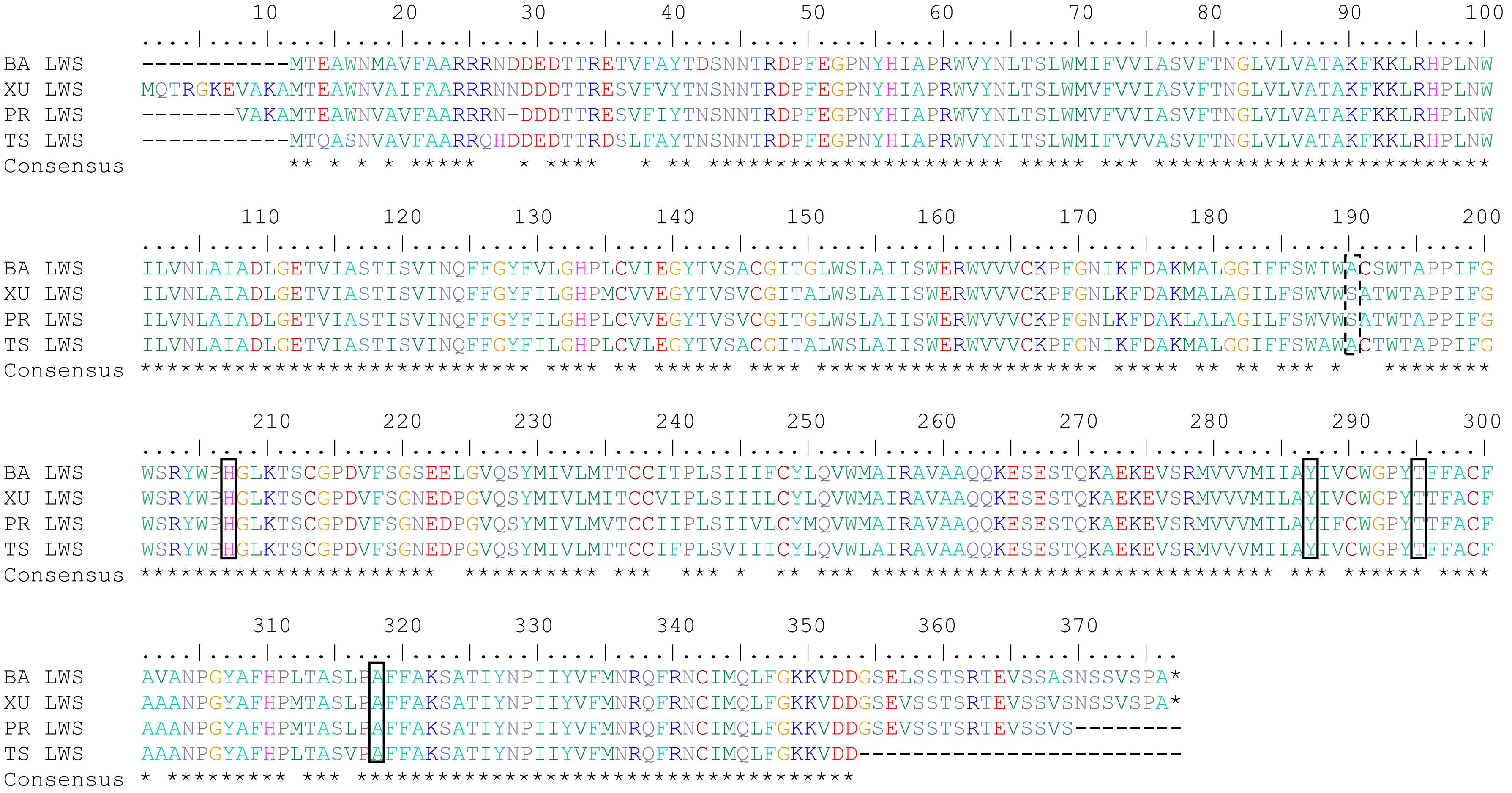
Figure S1: Codon-matched alignment of the complete amino acid sequence of RH1 expressed in the retina of *B. atrox* (BA), with RH1 sequences of *X. unicolor* (XU), *P. regius* (PR) and *T. sirtalis* (TS). Amino acids located at important spectral tuning sites (numbering based on comparison with bovine rhodopsin) are shown in boxes (Chan, Lee, & Sakmar, 1992; Davies, Collin, & Hunt, 2012; Davies et al., 2007; Hope et al., 1997; Hunt et al., 2001; Janz & Farrens, 2001; Sakmar, Franke, & Khorana, 1991; Yokoyama, 2000; Yokoyama, 2008; Yokoyama, Tada, & Yamato, 2007; Yokoyama et al., 2008; Yokoyama et al., 1999). Each residue is colour-coded based on their biochemical properties (e.g. charged vs hydrophobic), with asterisks denoting identical consensus residues between all snake opsin sequences. Dashes represent gaps that were inserted to maintain a high degree of sequence identity present between the different opsins classes.



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                  10        20        30        40        50        60        70        80        90       100
          ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
BA LWS    -----------MTEAWNMAVFAARRRNDDEDTTRETVFAYTDSNNTRDPFEGPNYHIAPRWVYNLTSLWMIFVVIASVFTNGLVLVATAKFKKLRHPLNW
XU LWS    MQTRGKEVAKAMTEAWNVAIFAARRRNNDDDTTRESVFVYTNSNNTRDPFEGPNYHIAPRWVYNLTSLWMVFVVIASVFTNGLVLVATAKFKKLRHPLNW
PR LWS    -------VAKAMTEAWNVAVFAARRRN-DDDTTRESVFIYTNSNNTRDPFEGPNYHIAPRWVYNLTSLWMVFVVIASVFTNGLVLVATAKFKKLRHPLNW
TS LWS    -----------MTQASNVAVFAARRQHDDEDTTRDSLFAYTNSNNTRDPFEGPNYHIAPRWVYNITSLWMIFVVVASVFTNGLVLVATAKFKKLRHPLNW
Consensus            ** * * * *****   * ****   * ** ********************** ***** *** *************************

                 110       120       130       140       150       160       170       180       190       200
          ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
BA LWS    ILVNLAIADLGETVIASTISVINQFFGYFVLGHPLCVIEGYTVSACGITGLWSLAIISWERWVVVCKPFGNIKFDAKMALGGIFFSWIWACSWTAPPIFG
XU LWS    ILVNLAIADLGETVIASTISVINQFFGYFILGHPMCVVEGYTVSVCGITALWSLAIISWERWVVVCKPFGNLKFDAKMALAGILFSWVWSATWTAPPIFG
PR LWS    ILVNLAIADLGETVIASTISVINQFFGYFILGHPLCVVEGYTVSVCGITGLWSLAIISWERWVVVCKPFGNLKFDAKLALAGILFSWVWSATWTAPPIFG
TS LWS    ILVNLAIADLGETVIASTISVINQFFGYFILGHPLCVLEGYTVSACGITALWSLAIISWERWVVVCKPFGNIKFDAKMALGGIFFSWAWACTWTAPPIFG
Consensus *****************************  **** ** ****** **** ********************* ***** ** ** *** *   ********

                 210       220       230       240       250       260       270       280       290       300
          ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
BA LWS    WSRYWPHGLKTSCGPDVFSGSEELGVQSYMIVLMTTCCITPLSIIIFCYLQVWMAIRAVAAQQKESESTQKAEKEVSRMVVVMIIAYIVCWGPYTFFACF
XU LWS    WSRYWPHGLKTSCGPDVFSGNEDPGVQSYMIVLMITCCVIPLSIIILCYLQVWMAIRAVAAQQKESESTQKAEKEVSRMVVVMILAYIVCWGPYTTFACF
PR LWS    WSRYWPHGLKTSCGPDVFSGNEDPGVQSYMIVLMVTCCIIPLSIIVLCYMQVWMAIRAVAAQQKESESTQKAEKEVSRMVVVMILAYIFCWGPYTTFACF
TS LWS    WSRYWPHGLKTSCGPDVFSGNEDPGVQSYMIVLMTTCCIFPLSVIIICYLQVWLAIRAVAAQQKESESTQKAEKEVSRMVVVMIIAYIVCWGPYTFFACF
Consensus **********************  ********** ***  *** *  ** *** ****************************** *** ****** ****

                 310       320       330       340       350       360       370
          ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|..
BA LWS    AVANPGYAFHPLTASLPAFFAKSATIYNPIIYVFMNRQFRNCIMQLFGKKVDDGSELSSTSRTEVSSASNSSVSPA*
XU LWS    AAANPGYAFHPMTASLPAFFAKSATIYNPIIYVFMNRQFRNCIMQLFGKKVDDGSEVSSTSRTEVSSVSNSSVSPA*
PR LWS    AAANPGYAFHPMTASLPAFFAKSATIYNPIIYVFMNRQFRNCIMQLFGKKVDDGSEVSSTSRTEVSSVS--------
TS LWS    AAANPGYAFHPLTASVPAFFAKSATIYNPIIYVFMNRQFRNCIMQLFGKKVDD------------------------
Consensus * ********* *** *************************************
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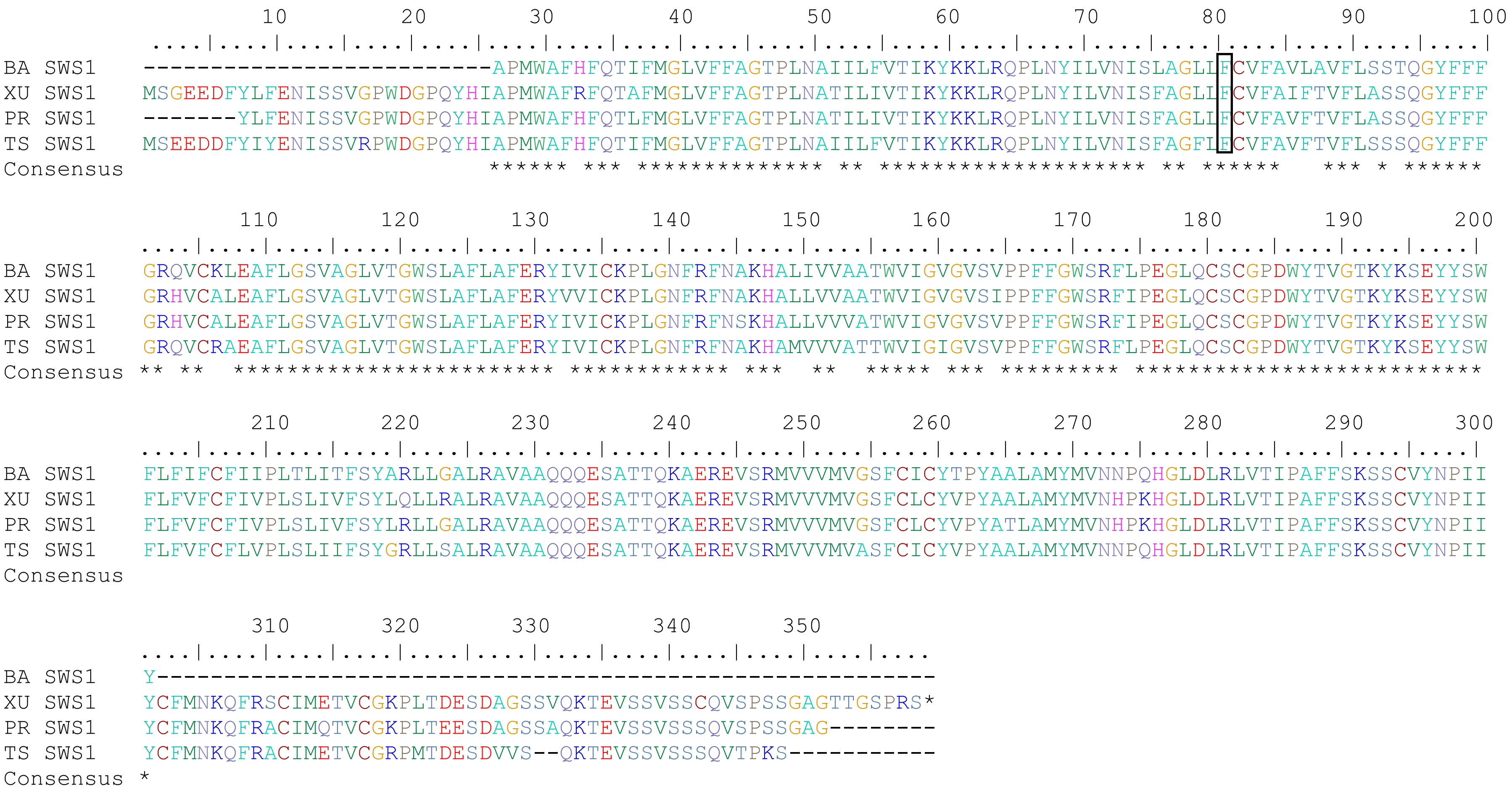
Figure S2: Codon-matched alignment of the complete amino acid sequence of LWS expressed in the retina of *B. atrox* (BA), with LWS sequences of *X. unicolor* (XU), *P. regius* (PR) and *T. sirtalis* (TS). Amino acids located at important spectral tuning sites (numbering based on comparison with bovine rhodopsin) are shown in boxes (Davies, Collin, & Hunt, 2012; Yokoyama, 2000; Yokoyama, Yang, & Starmer, 2008). The box with dotted lines indicates amino acid Ala164 that is important for spectral tuning in *B. atrox* and others snakes. Each residue is colour-coded based on their biochemical properties (e.g. charged vs hydrophobic), with asterisks denoting identical consensus residues between all snake opsin sequences and bovine RH1. Dashes represent gaps that were inserted to maintain a high degree of sequence identity present between the different opsins clases.



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                   10        20        30        40        50        60        70        80        90       100
           ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
BA SWS1    --------------------------APMWAFHFQTIFMGLVFFAGTPLNAIILFVTIKYKKLRQPLNYILVNISLAGLLFCVFAVLAVFLSSTQGYFFF
XU SWS1    MSGEEDFYLFENISSVGPWDGPQYHIAPMWAFRFQTAFMGLVFFAGTPLNATILIVTIKYKKLRQPLNYILVNISFAGLLFCVFAIFTVFLASSQGYFFF
PR SWS1    -------YLFENISSVGPWDGPQYHIAPMWAFHFQTLFMGLVFFAGTPLNATILIVTIKYKKLRQPLNYILVNISFAGLLFCVFAVFTVFLASSQGYFFF
TS SWS1    MSEEDDFYIYENISSVRPWDGPQYHIAPMWAFHFQTIFMGLVFFAGTPLNAIILFVTIKYKKLRQPLNYILVNISFAGFLFCVFAVFTVFLSSSQGYFFF
Consensus                            ****** *** ************** ** ******************** ** ******   *** * ******

                  110       120       130       140       150       160       170       180       190       200
           ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
BA SWS1    GRQVCKLEAFLGSVAGLVTGWSLAFLAFERYIVICKPLGNFRFNAKHALIVVAATWVIGVGVSVPPFFGWSRFLPEGLQCSCGPDWYTVGTKYKSEYYSW
XU SWS1    GRHVCALEAFLGSVAGLVTGWSLAFLAFERYVVICKPLGNFRFNAKHALLVVAATWVIGVGVSIPPFFGWSRFIPEGLQCSCGPDWYTVGTKYKSEYYSW
PR SWS1    GRHVCALEAFLGSVAGLVTGWSLAFLAFERYIVICKPLGNFRFNSKHALLVVVATWVIGVGVSVPPFFGWSRFIPEGLQCSCGPDWYTVGTKYKSEYYSW
TS SWS1    GRQVCRAEAFLGSVAGLVTGWSLAFLAFERYIVICKPLGNFRFNAKHAMVVVATTWVIGIGVSVPPFFGWSRFLPEGLQCSCGPDWYTVGTKYKSEYYSW
Consensus  ** **  ************************ ************ ***  **  ***** *** ********* **************************

                  210       220       230       240       250       260       270       280       290       300
           ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
BA SWS1    FLFIFCFIIPLTLITFSYARLLGALRAVAAQQQESATTQKAEREVSRMVVVMVGSFCICYTPYAALAMYMVNNPQHGLDLRLVTIPAFFSKSSCVYNPII
XU SWS1    FLFVFCFIVPLSLIVFSYLQLLRALRAVAAQQQESATTQKAEREVSRMVVVMVGSFCLCYVPYAALAMYMVNHPKHGLDLRLVTIPAFFSKSSCVYNPII
PR SWS1    FLFVFCFIVPLSLIVFSYLRLLGALRAVAAQQQESATTQKAEREVSRMVVVMVGSFCLCYVPYATLAMYMVNHPKHGLDLRLVTIPAFFSKSSCVYNPII
TS SWS1    FLFVFCFLVPLSLIIFSYGRLLSALRAVAAQQQESATTQKAEREVSRMVVVMVASFCICYVPYAALAMYMVNNPQHGLDLRLVTIPAFFSKSSCVYNPII
Consensus

                  310       320       330       340       350
           ....|....|....|....|....|....|....|....|....|....|....|....
BA SWS1    Y----------------------------------------------------------
XU SWS1    YCFMNKQFRSCIMETVCGKPLTDESDAGSSVQKTEVSSVSSCQVSPSSGAGTTGSPRS*
PR SWS1    YCFMNKQFRACIMQTVCGKPLTEESDAGSSAQKTEVSSVSSSQVSPSSGAG--------
TS SWS1    YCFMNKQFRACIMETVCGRPMTDESDVVS--QKTEVSSVSSSQVTPKS-----------
Consensus  *
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Figure S3: Codon-matched alignment of the complete amino acid sequence of SWS1 expressed in the retina of *B. atrox* (BA), with SWS1 sequences of *X. unicolor* (XU), *P. regius* (PR) and *T. sirtalis* (TS). Amino acids located at important spectral tuning sites (numbering based on comparison with bovine rhodopsin) are shown in boxes, where amino acid 86 (boxed) is known to determine photopigment spectral sensitivity (i.e. ultraviolet- or violet-sensitive; UVS or VS) (Cowing et al., 2002). In *B. atrox* a Phe86 is present making this photopigment UVS. Each residue is colour-coded based on their biochemical properties (e.g. charged vs hydrophobic), with asterisks denoting identical consensus residues between all snake opsin sequences and bovine RH1. Dashes represent gaps that were inserted to maintain a high degree of sequence identity present between the different opsins classes.

Primer	Sequence (**5**' to **3**')	**Gene**	Application
[a]**DIAPLMF1**	AAGCGTATTYAYTTAYACCRACASCAACAA	LWS	RT-PCR
[a]**DIAPLMF2**	AGTGTCATCAACCAGWTCTYBGGSTAYTTC	LWS	RT-PCR
[a]**DIAPLMR1**	CATCCTBGACACYTCCYTCTCVGCCTTCTG	LWS	RT-PCR
[a]**DIAPLMR2**	CATCATCCACTTTYTTSCCRAASAGCTGCA	LWS	RT-PCR
[a]**DIAPR1F1**	GTCAAAATTTCTAYRTBCCCWTKTCCAACA	RH1	RT-PCR
[a]**DIAPR1F2**	AATAGGATGCWRCWTYGARGGCTTCTTTGC	RH1	RT-PCR
[a]**DIAPR1R1**	ACAGTGCAGACAAGRYKYCCRTAGCAGAAG	RH1	RT-PCR
[a]**DIAPR1R2**	ATTCTTTCCACARCARAGRGTBRTGATCAT	RH1	RT-PCR
[a]**DIAPS1F1**	TCCCATGTCCGGAGAVGAVGABTTYTACCT	SWS1	RT-PCR
[a]**DIAPS1F2**	GGCCTTCGARCGHTACATYGTYATCTGCAA	SWS1	RT-PCR
[a]**DIAPS1R1**	CACCACSACCATSCGVGASACCTCCCGCTC	SWS1	RT-PCR
[a]**DIAPS1R2**	TTAGCTGGGGCYGACYTGRCTGGAGGACAC	SWS1	RT-PCR
[a]**DIAPS1F3**	GGGGCCBTTSGAYGGBCCCCARTAYCACAT	SWS1	RT-PCR
[b]**DIAPS1R4**	GCADSCNCGGAACTGYTTGTTCATGAAGCA	SWS1	RT-PCR
[a]**Oligo-dT-ANC**	GCGAGCACAGAATTAATACGACTCACTATAGGT$_{12}$VN	3'-polyA tail	RT-PCR
[a]**5'-RACE-ANC-F**	GGCCACGCGTCGACTAGTACGGGIIGGGIIGGGIIG	5'-polyC tail	RACE
[a]**5'-RACE-SRT-F**	GGCCACGCGTCGACTAGTAC	5'-UTR	RACE
[a]**3'-RACE-IN-R**	CGCGGATCCGAATTAATACGACTCACTATAGG	3'-UTR	RACE
[a]**3'-RACE-OUT-R**	GCGAGCACAGAATTAATACGACT	3'-UTR	RACE
[b]**LM-FA-REP**	GGAAGTRTCRAGRATGGTMGTGG	LWS	RACE
[b]**LM-FB-SN**	GGGGACCATATRCAKYTTTTGCC	LWS	RACE
[b]**LM-RA**	ATCRGCKAYHGCCAAGTTSACC	LWS	RACE
[b]**LM-RB**	CVACAAAGAYCATCCAGAGDGAAG	LWS	RACE
[b]**LM-RC**	ACTGGTTGATGACACTGATRGTGCTAGC	LWS	RACE
[b]**RH1-FA**	TGGGTCCCTTATGCCTCTGTGGC	RH1	RACE
[b]**RH1-FB**	CYGTCTTTATGACCATCCCRGCC	RH1	RACE
[b]**RH1-RA**	GGGCTTACAAACTACTAMRTATC	RH1	RACE
[b]**RH1-RC**	GACCATGAAAAGATCGGCTACTGCC	RH1	RACE

Table 1: Degenerate and gene-specific oligonucleotides. Primers used in nested-PCR and RACE reactions to generate sequences for rhodopsin-like-1 (RH1), short-wavelength-sensitive-1 (SWS1) and long-wavelength-sensitive (LWS) opsins from *B. atrox*. [a]Davies, Cowing et al. (2009); [b]This work.

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