

Overcoming the loss of blue sensitivity through opsin duplication in the largest animal group, beetles

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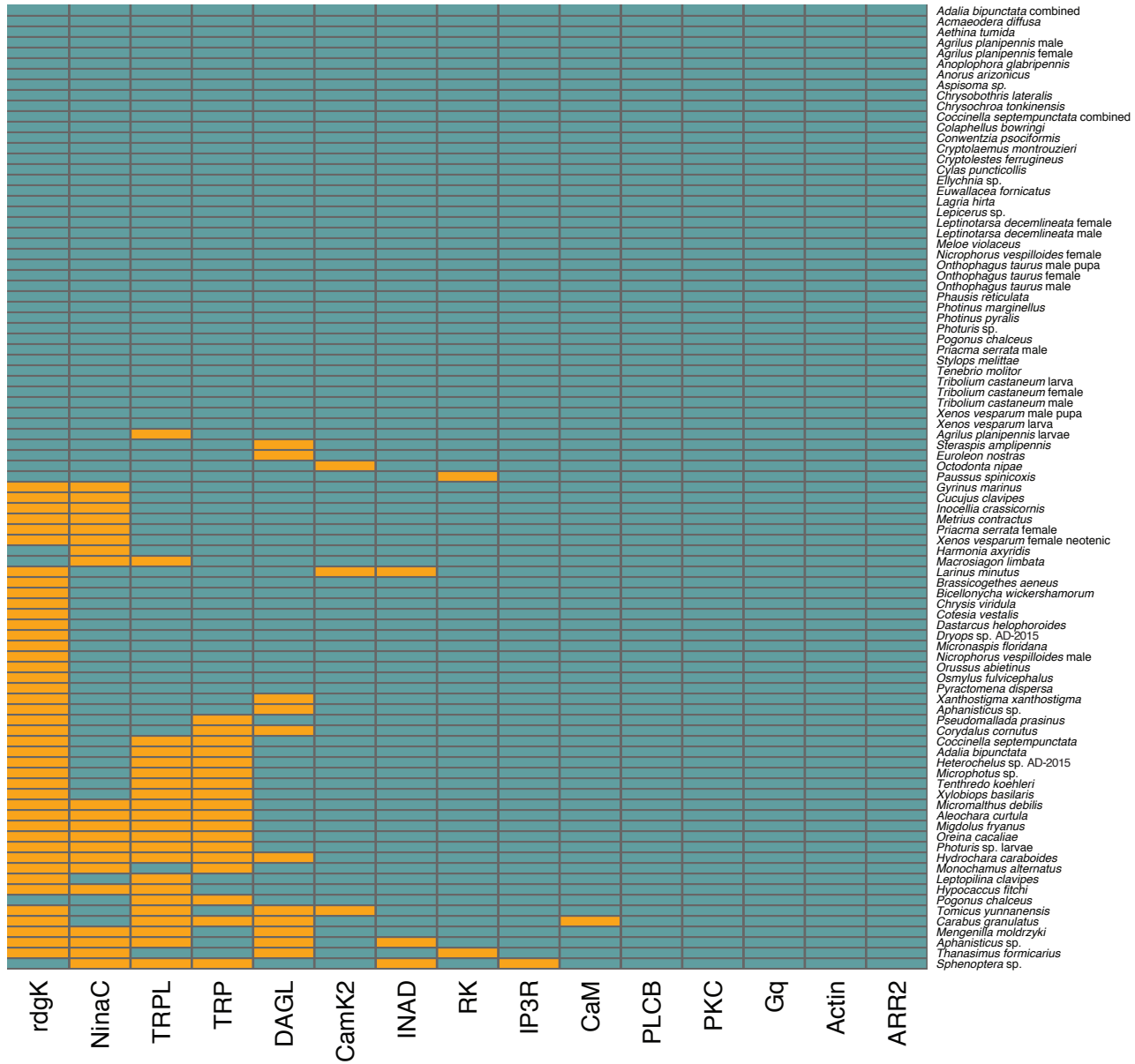
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Supplementary Data

a



b

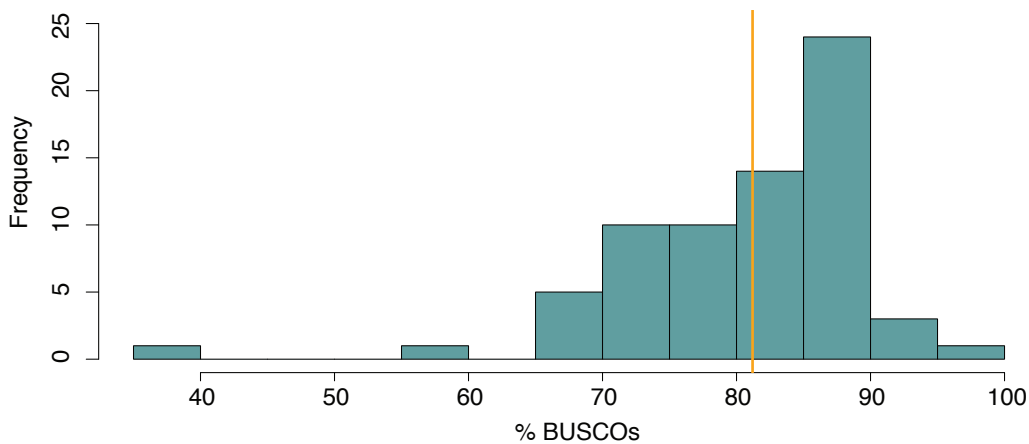


Figure S1. a: Heatmap showing the presence (blue) or absence (orange) of 15 insect phototransduction genes for each transcriptome assembly used in this study. Orthologs were detected at a conservative threshold of e^{-100} .

b: The distribution of BUSCOs (Benchmarking Universal Single-Copy Orthologs) across all whole-body samples and the mean value (orange line). Higher values denote a larger proportion of BUSCOs present and therefore indicate a more complete transcriptome.



Figure S2. Full maximum-likelihood species phylogeny, including poorly supported strepsipterans (*Xenos* sp., *Mengenilla* sp. and *Stylops* sp.), using 358 gene clusters from translated transcriptome assemblies. Node values indicate UFboot supports. Five hymenopteran species were used as an outgroup.

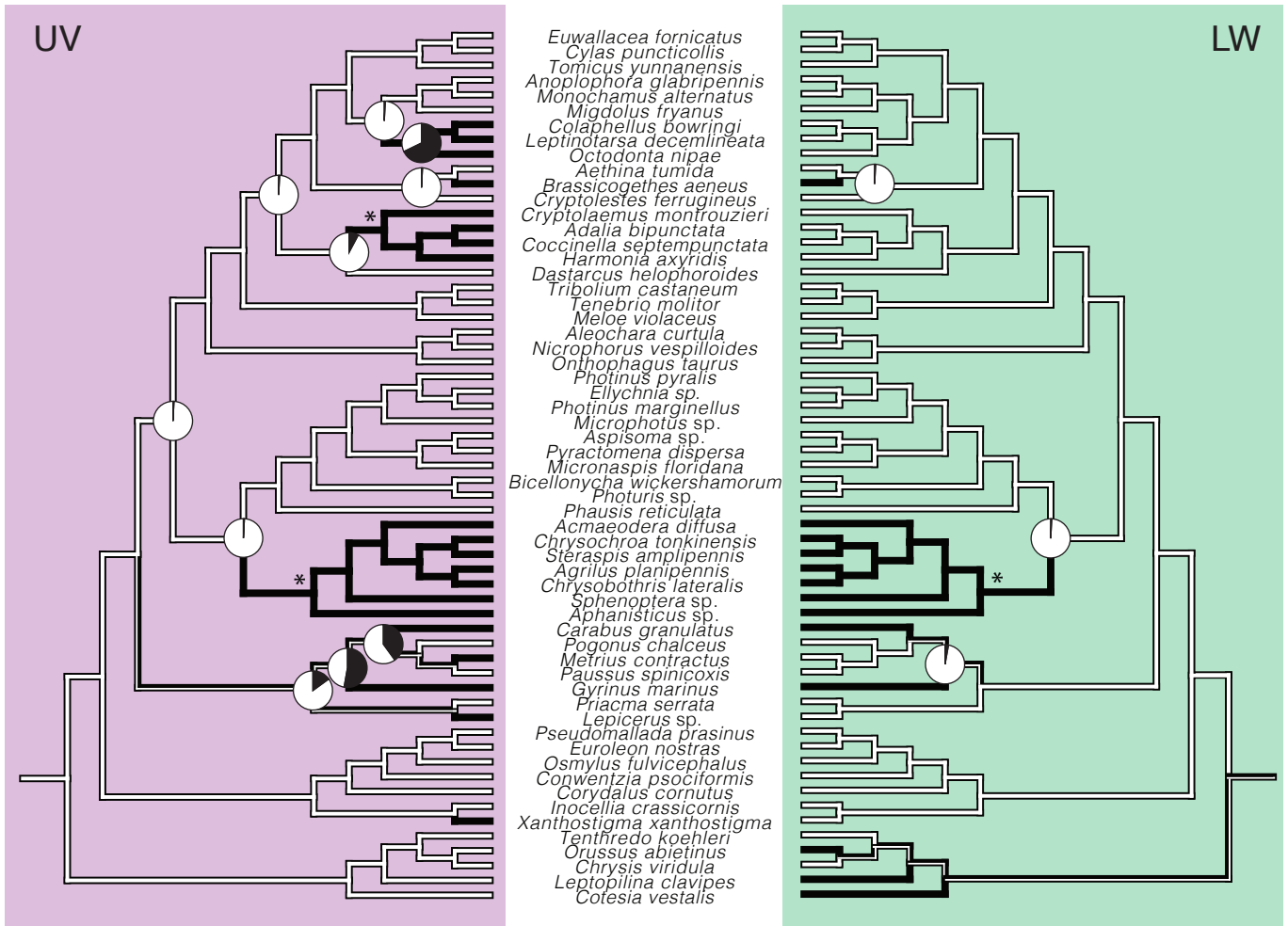


Figure S3. Ancestral state reconstruction for the presence or absence of UV (purple) and LW (green) opsin duplications for all species included in the species phylogeny. The probability that an opsin duplication is present is indicated on the topology (parsimony) and proportionally represented on nodes of interest (likelihood). Black and white indicate the presence or absence of a duplication, respectively. Asterisks denote a likelihood of 1.

Order	Species	Family	Superfamily/Suborder	UV	SW	LW
Coleoptera	<i>Carabus granulatus</i>	Carabidae	Adephaga	2	0	2
Coleoptera	<i>Metrius contractus</i>	Carabidae	Adephaga	2*	0	1
Coleoptera	<i>Paussus spinicoxis</i>	Carabidae	Adephaga	1	0	1
Coleoptera	<i>Pogonus chalceus</i>	Carabidae	Adephaga	1	0	1
Coleoptera	<i>Gyrinus marinus</i>	Gyrinidae	Adephaga	2	0	2
Coleoptera	<i>Micromalthus debilis</i>	Micromalthidae	Archostemata	1	0	1
Coleoptera	<i>Thanasimus formicarius</i>	Cleridae	Cleroidea	2	0	1
Coleoptera	<i>Xylobiops basilaris</i>	Bostrichidae	Bostrichoidea	1	0	1
Coleoptera	<i>Acmaeodera diffusa</i>	Buprestidae	Buprestoidea	2	0	2
Coleoptera	<i>Agrilus planipennis</i>	Buprestidae	Buprestoidea	2	0	2
Coleoptera	<i>Aphanisticus</i> sp.	Buprestidae	Buprestoidea	2	0	2
Coleoptera	<i>Chrysobothris lateralis</i>	Buprestidae	Buprestoidea	2	0	2
Coleoptera	<i>Chrysochroa tonkinensis</i>	Buprestidae	Buprestoidea	2	0	2
Coleoptera	<i>Sphenoptera</i> sp.	Buprestidae	Buprestoidea	2	0	2
Coleoptera	<i>Steraspis amplipennis</i>	Buprestidae	Buprestoidea	2	0	2
Coleoptera	<i>Dryops</i> sp. AD-2015	Dryopidae	Byrrhoidea	2	0	1
Coleoptera	<i>Anoplophora glabripennis</i>	Cerambycidae	Chrysomeloidea	1	0	1
Coleoptera	<i>Monochamus alternatus</i>	Cerambycidae	Chrysomeloidea	0	0	1
Coleoptera	<i>Colaphellus bowringi</i>	Chrysomelidae	Chrysomeloidea	2	0	1
Coleoptera	<i>Leptinotarsa decemlineata</i>	Chrysomelidae	Chrysomeloidea	2	0	1
Coleoptera	<i>Octodonta nipae</i>	Chrysomelidae	Chrysomeloidea	2	0	1
Coleoptera	<i>Oreina cacaliae</i>	Chrysomelidae	Chrysomeloidea	3*	0	1
Coleoptera	<i>Migdolus fryanus</i>	Vesperidae	Chrysomeloidea	2	0	1
Coleoptera	<i>Cryptolaemus montrouzieri</i>	Coccinellidae	Coccinelloidea	2	0	1
Coleoptera	<i>Dastarcus helophoroides</i>	Bothrideridae	Cucujoidea	0	0	1
Coleoptera	<i>Adalia bipunctata</i>	Coccinellidae	Cucujoidea	2	0	1
Coleoptera	<i>Coccinella septempunctata</i>	Coccinellidae	Cucujoidea	2	0	1
Coleoptera	<i>Harmonia axyridis</i>	Coccinellidae	Cucujoidea	2	0	1
Coleoptera	<i>Cryptolestes ferrugineus</i>	Laemophloeidae	Cucujoidea	1	0	1
Coleoptera	<i>Aethina tumida</i>	Nitidulidae	Cucujoidea	1	0	1
Coleoptera	<i>Brassicogethes aeneus</i>	Nitidulidae	Cucujoidea	3	0	2
Coleoptera	<i>Priacma serrata</i>	Cupedidae	Archostemata	1	0	1
Coleoptera	<i>Cylas puncticollis</i>	Curculionidae	Curculionoidea	1	0	1
Coleoptera	<i>Dendroctonus ponderosae</i>	Curculionidae	Curculionoidea	1	0	1
Coleoptera	<i>Hypothenemus hampei</i>	Curculionidae	Curculionoidea	1	0	1
Coleoptera	<i>Larinus minutus</i>	Curculionidae	Curculionoidea	1	0	3
Coleoptera	<i>Euwallacea fornicatus</i>	Scolytinae	Curculionoidea	1	0	1
Coleoptera	<i>Tomiscus yunnanensis</i>	Scolytinae	Curculionoidea	2*	0	1
Coleoptera	<i>Anorus arizonicus</i>	Dascillidae	Dascilloidea	1	0	1
Coleoptera	<i>Aspisoma</i> sp.	Lampyridae	Elateroidea	1	0	1
Coleoptera	<i>Bicellonycha wickershamorum</i>	Lampyridae	Elateroidea	1	0	1
Coleoptera	<i>Ellychnia</i> sp.	Lampyridae	Elateroidea	1	0	1
Coleoptera	<i>Micronaspis floridana</i>	Lampyridae	Elateroidea	1	0	1
Coleoptera	<i>Microphotus</i> sp.	Lampyridae	Elateroidea	1	0	0
Coleoptera	<i>Phausis reticulata</i>	Lampyridae	Elateroidea	1	0	1
Coleoptera	<i>Photinus marginellus</i>	Lampyridae	Elateroidea	1	0	1
Coleoptera	<i>Photinus pyralis</i>	Lampyridae	Elateroidea	1	0	1
Coleoptera	<i>Photuris</i> sp.	Lampyridae	Elateroidea	1	0	1
Coleoptera	<i>Pyractomena dispersa</i>	Lampyridae	Elateroidea	1	0	1
Coleoptera	<i>Hydrochara caraboides</i>	Hydrophilidae	Hydrophiloidea	1	0	1
Coleoptera	<i>Lepicerus</i> sp.	Lepiceridae	Myxophaga	2	0	1
Coleoptera	<i>Cucujus clavipes</i>	Cucujidae	Cucujoidea	1	0	1
Coleoptera	<i>Heterochelus</i> sp. AD-2015	Scarabaeidae	Scarabaeoidea	1	0	2
Coleoptera	<i>Onthophagus taurus</i>	Scarabaeidae	Scarabaeoidea	1	0	1
Coleoptera	<i>Nicrophorus vespilloides</i>	Silphidae	Staphylinoidea	1	0	1
Coleoptera	<i>Aleochara curtula</i>	Staphylinidae	Staphylinoidea	1	0	1
Coleoptera	<i>Meloe violaceus</i>	Meloidae	Tenebrionoidea	1	0	1
Coleoptera	<i>Macrosiagon limbatum</i>	Rhipiphoridae	Tenebrionoidea	1	0	1
Coleoptera	<i>Hypocaccus fitchi</i>	Histeridae	Histeroidea	1	0	1
Coleoptera	<i>Lagria hirta</i>	Tenebrionidae	Tenebrionoidea	1	0	1
Coleoptera	<i>Tenebrio molitor</i>	Tenebrionidae	Tenebrionoidea	0	0	1
Coleoptera	<i>Tribolium castaneum</i>	Tenebrionidae	Tenebrionoidea	1	0	1
Strepsiptera	<i>Mengenilla moldrzyki</i>	Mengenillidae	-	1	0	2
Strepsiptera	<i>Stylops melittae</i>	Stylopidae	-	1	0	1
Strepsiptera	<i>Xenos vesparum</i>	Xenidae	-	2	0	6
Raphidioptera	<i>Xanthostigma xanthostigma</i>	Raphidiidae	-	2	0	1
Raphidioptera	<i>Inocellia crassicornis</i>	Inocelliidae	Raphidiomorpha	1	0	1
Megaloptera	<i>Corydalus cornutus</i>	Corydalidae	-	1	0	1
Neuroptera	<i>Osmylus fulvicephalus</i>	Osmylidae	-	1	0	1
Neuroptera	<i>Pseudomallada prasinus</i>	Chrysopidae	Chrysopoidea	1	0	1
Neuroptera	<i>Conwentzia psociformis</i>	Coniopterygidae	Coniopterygoidea	1	0	1
Neuroptera	<i>Euroleon nostras</i>	Myrmeleontidae	Myrmeleontoidea	1	0	1
Hymenoptera	<i>Chrysis viridula</i>	Chrysididae	Chrysidioidea	1	1	1
Hymenoptera	<i>Leptopilina clavipes</i>	Figitidae	Cynipoidea	1	1	2
Hymenoptera	<i>Cotesia vestalis</i>	Braconidae	Ichneumonoidea	1	1	2
Hymenoptera	<i>Orussus abietinus</i>	Orussidae	Orussoidea	1	1	2
Hymenoptera	<i>Tenthredo koehleri</i>	Tenthredinidae	Tenthredinoidea	1	1	1

Table S2. Opsin copy number for all species sampled in this study. Asterisks indicate two opsin duplicates that do not overlap and therefore may represent a single opsin copy.

Opsin sequence	length (AAs)	Sequence identity (AA) (%)	Sequence identity (nucleotide) (%)	Sequence coverage (AA) (%)
<i>Acmaeodera diffusa</i> LW1	379	61	66	94
<i>Acmaeodera diffusa</i> LW2	378			
<i>Acmaeodera diffusa</i> UV1	377	63	65	91
<i>Acmaeodera diffusa</i> UV2	390			
<i>Adalia bipunctata</i> LW	375			
<i>Adalia bipunctata</i> UV1	391	84	77	96
<i>Adalia bipunctata</i> UV2	389			
<i>Aethina tumida</i> LW	380			
<i>Aethina tumida</i> UV	382			
<i>Agrilus planipennis</i> LW1	379	69	70	98
<i>Agrilus planipennis</i> LW2	380			
<i>Agrilus planipennis</i> LW3	327			
<i>Agrilus planipennis</i> UV1	372	72	69	91
<i>Agrilus planipennis</i> UV2	394			
<i>Aleochara curtula</i> LW	381			
<i>Aleochara curtula</i> UV	380			
<i>Anoplophora glabripennis</i> LW	377			
<i>Anoplophora glabripennis</i> UV	319			
<i>Anorus arizonicus</i> LW	378			
<i>Anorus arizonicus</i> UV	386			
<i>Aphanisticus</i> sp. LW1	269	69	67	74
<i>Aphanisticus</i> sp. LW2	381			
<i>Aphanisticus</i> sp. UV1	377	72	70	96
<i>Aphanisticus</i> sp. UV2	375			
<i>Aspisoma</i> sp. LW	380			
<i>Aspisoma</i> sp. UV	397			
<i>Bicellonycha wickershamorum</i> LW	380			
<i>Bicellonycha wickershamorum</i> UV	385			
<i>Brassicogethes aeneus</i> LW1	398	78	74	94
<i>Brassicogethes aeneus</i> LW2	381			
<i>Brassicogethes aeneus</i> UV1	380	82	74	79
<i>Brassicogethes aeneus</i> UV3	278			
<i>Brassicogethes aeneus</i> UV2	382			
<i>Carabus granulatus</i> LW1	364	100	99	53
<i>Carabus granulatus</i> LW2	137			
<i>Carabus granulatus</i> UV1	123	87	74	32
<i>Carabus granulatus</i> UV2	381			
<i>Chrysobothris lateralis</i> LW1	381	69	69	99
<i>Chrysobothris lateralis</i> LW2	380			
<i>Chrysobothris lateralis</i> UV1	375	68	70	97
<i>Chrysobothris lateralis</i> UV2	378			
<i>Chrysochroa tonkinensis</i> LW1	381	68	71	99
<i>Chrysochroa tonkinensis</i> LW2	380			
<i>Chrysochroa tonkinensis</i> UV1	385	74	70	96
<i>Chrysochroa tonkinensis</i> UV2	371			
<i>Coccinella septempunctata</i> LW	375			
<i>Coccinella septempunctata</i> UV1	396	83	78	98
<i>Coccinella septempunctata</i> UV2	389			
<i>Colaphellus bowringi</i> LW	375			
<i>Colaphellus bowringi</i> UV1	360	77	75	58
<i>Colaphellus bowringi</i> UV2	181			
<i>Conwentzia psociformis</i> LW	371			
<i>Conwentzia psociformis</i> UV	380			
<i>Corydalus cornutus</i> LW	376			
<i>Corydalus cornutus</i> UV	347			
<i>Cryptolaemus montrouzieri</i> LW	376			
<i>Cryptolaemus montrouzieri</i> UV1a	398	86	79	95
<i>Cryptolaemus montrouzieri</i> UV1b	376			
<i>Cryptolestes ferrugineus</i> LW	378			
<i>Cryptolestes ferrugineus</i> UV	236			
<i>Cucujus clavipes</i> LW	378			
<i>Cucujus clavipes</i> UV	391			
<i>Cylas puncticollis</i> LW	379			
<i>Cylas puncticollis</i> UV	374			
<i>Dastarcus helophoroides</i> LW	378			
<i>Dendroctonus ponderosae</i> LW	379			
<i>Dendroctonus ponderosae</i> UV	368			
<i>Dryops</i> sp. AD-2015 LW	382			
<i>Dryops</i> sp. AD-2015 UV1	181	81	75	55
<i>Dryops</i> sp. AD-2015 UV2	370			
<i>Ellychnia</i> sp. LW	379			
<i>Ellychnia</i> sp. UV	389			
<i>Euroleon nostras</i> LW	371			
<i>Euroleon nostras</i> UV	376			
<i>Euwallacea fornicatus</i> LW	378			
<i>Euwallacea fornicatus</i> UV	375			
<i>Gyrinus marinus</i> LW1	379	93	90	36
<i>Gyrinus marinus</i> LW2	104			
<i>Gyrinus marinus</i> UV1	387	89	85	73
<i>Gyrinus marinus</i> UV2	276			
<i>Harmonia axyridis</i> LW	361			
<i>Harmonia axyridis</i> UV1	393	81	75	73
<i>Harmonia axyridis</i> UV2	371			
<i>Heterochelus</i> sp. AD-2015 LW1	151	81	91	44
<i>Heterochelus</i> sp. AD-2015 LW2	376			
<i>Heterochelus</i> sp. AD-2015 UV	213			
<i>Hydrochara caraboides</i> LW	385			
<i>Hydrochara caraboides</i> UV	323			
<i>Hypocaccus fitchi</i> LW	381			
<i>Hypocaccus fitchi</i> UV	180			
<i>Hypothenemus hampei</i> LW	317			
<i>Hypothenemus hampei</i> UV	323			
<i>Inocellia crassicornis</i> LW	378			
<i>Inocellia crassicornis</i> UV	109			
<i>Largria hirta</i> LW	410			
<i>Largria hirta</i> UV	379			
<i>Larinus minutus</i> LW1	219	79	87	71
<i>Larinus minutus</i> LW3	379			
<i>Larinus minutus</i> LW2	149			
<i>Larinus minutus</i> UV	376			
<i>Lepicerus</i> sp. LW	375			
<i>Lepicerus</i> sp. UV1	188	95	88	49
<i>Lepicerus</i> sp. UV2	389			
<i>Leptinotarsa decemlineata</i> LW	410			
<i>Leptinotarsa decemlineata</i> UV1	379	74	73	96
<i>Leptinotarsa decemlineata</i> UV2	389			
<i>Macrosiagon limbatum</i> LW	377			
<i>Macrosiagon limbatum</i> UV	378			
<i>Meloe violaceus</i> LW	380			
<i>Meloe violaceus</i> UV	327			
<i>Mengenilla moldrzyki</i> LW1	243	66	68	66
<i>Mengenilla moldrzyki</i> LW2	383			
<i>Mengenilla moldrzyki</i> UV	135			
<i>Metrius contractus</i> LW	379			
<i>Metrius contractus</i> UV1	197	-	-	0
<i>Metrius contractus</i> UV2	138			
<i>Micromalthus debilis</i> LW	134			
<i>Micromalthus debilis</i> UV	379			
<i>Micronaspis floridana</i> LW	380			
<i>Micronaspis floridana</i> UV	394			
<i>Microphotus</i> sp. UV	389			
<i>Migdolus fryanus</i> LW	360			
<i>Monochamus alternatus</i> LW	378			
<i>Nicrophorus vespilloides</i> LW	380			
<i>Nicrophorus vespilloides</i> UV	388			
<i>Octodonta nipae</i> LW	380			
<i>Octodonta nipae</i> UV1	379	65	71	98
<i>Octodonta nipae</i> UV2	380			
<i>Onthophagus taurus</i> LW	393			
<i>Onthophagus taurus</i> UV	376			
<i>Oreina cacaliae</i> LW	377			
<i>Oreina cacaliae</i> UV1a	126	66	71	96
<i>Oreina cacaliae</i> UV2	184			
<i>Oreina cacaliae</i> UV1b	161			
<i>Osmylus fulvicephalus</i> LW	372			
<i>Osmylus fulvicephalus</i> UV	256			
<i>Paussus spinicoxis</i> LW	381			
<i>Paussus spinicoxis</i> UV	385			
<i>Phausis reticulata</i> LW	413			
<i>Phausis reticulata</i> UV	404			
<i>Photinus marginellus</i> LW	379			
<i>Photinus marginellus</i> UV	401			
<i>Photinus pyralis</i> LW	379			
<i>Photinus pyralis</i> UV	392			
<i>Photuris</i> sp. LW	381			
<i>Photuris</i> sp. UV	386			
<i>Pogonus chalceus</i> LW	383			
<i>Pogonus chalceus</i> UV	386			
<i>Priacma serrata</i> LW	378			
<i>Priacma serrata</i> UV	376			
<i>Pseudomallada prasinus</i> LW	372			
<i>Pseudomallada prasinus</i> UV	395			
<i>Pyraclomena dispersa</i> LW	380			
<i>Pyraclomena dispersa</i> UV	402			
<i>Sphenoptera</i> sp. LW1	386	71	70	96
<i>Sphenoptera</i> sp. LW2	380			
<i>Sphenoptera</i> sp. UV1	381	73	70	96
<i>Sphenoptera</i> sp. UV2	373			
<i>Steraspis amplipennis</i> LW1	381	70	70	99
<i>Steraspis amplipennis</i> LW2	380			
<i>Steraspis amplipennis</i> UV1	379	72	69	92
<i>Steraspis amplipennis</i> UV2	375			
<i>Stylops melittae</i> LW	237			
<i>Stylops melittae</i> UV	376			
<i>Tenebrio molitor</i> LW	386			
<i>Thanasimus formicarius</i> LW	359			
<i>Thanasimus formicarius</i> UV1	212	87	91	36
<i>Thanasimus formicarius</i> UV2	155			
<i>Tomicus yunnanensis</i> LW	379			
<i>Tomicus yunnanensis</i> UV	313			
<i>Tribolium castaneum</i> LW	379			
<i>Tribolium castaneum</i> UV	382			
<i>Xanthostigma xanthostigma</i> LW1	375	70	69	96
<i>Xanthostigma xanthostigma</i> LW2	377			
<i>Xanthostigma xanthostigma</i> UV1	381	87	82	95
<i>Xanthostigma xanthostigma</i> UV2	394			
<i>Xenos vesparum</i> LW1	369			
<i>Xenos vesparum</i> LW2	269			
<i>Xenos vesparum</i> LW3	208			
<i>Xenos vesparum</i> LW4	245			
<i>Xenos vesparum</i> LW5	124			
<i>Xenos vesparum</i> LW6	328			
<i>Xenos vesparum</i> UV1	374	96	99	62
<i>Xenos vesparum</i> UV2	182			
<i>Xylobiops basilaris</i> LW	385			
<i>Xylobiops basilaris</i> UV	381			

Table S3. Lengths of Neuropteroidea opsin protein sequences found in this study. Sequence identity and coverage is compared for opsin duplicates. Opsin sequences derived from RNA-seq data can be found on GenBank with accession numbers KY368182 - KY368379.

Clade	Family	Branch	Branch leading to	Site numbers (bovine)	Branch P-value	Test InL	Null InL	LRT
A	Chrysomelidae	A1	UV2	90 [†] , 91*	0.002	-189798.8	-189803.7	9.8
A	Chrysomelidae	A2	UV2	29*, 107**, 197***, 210** , 230***, 339*	0.000	-189796.8	-189806.7	19.7
A	Chrysomelidae	A3	UV2	136*, 151*	0.004	-189803.7	-189807.8	8.2
A	Chrysomelidae	A4	UV1	33**, 107*, 127*, 286***, 293***	0.000	-189797.1	-189803.7	13.2
A	Chrysomelidae	A5	UV1	205*	0.021	-189803.6	-189806.3	5.3
B	Coccinellidae	B1	UV1/UV2	14***, 31***, 34***, 158*, 159**, 173**, 317*, 333***, 341*	0.000	-189810.2	-189835.0	49.8
C	Buprestidae	C1	UV2	96*	0.046	-189803.4	-189805.4	4.0
C	Buprestidae	C2	UV2	37*, 105**, 108**, 112* , 164***	0.000	-189806.9	-189819.4	24.9
C	Buprestidae	C3	UV1	50*, 127*, 206***	0.000	-189814.8	-189821.2	12.7
C	Buprestidae	C4	UV1	22**, 23*	0.031	-189801.4	-189803.7	4.6
C	Buprestidae	C5	UV1	176*, 203*, 258*	0.000	-189797.3	-189803.7	12.9

Table S4. Branches and amino acid sites under positive selection in clades A (Chrysomelidae), B (Coccinellidae) and C (Buprestidae). Sites adjacent to chromophore binding sites are in bold. LRT = Likelihood Ratio Test, InL = log likelihood. Sites are numbered according to bovine rhodopsin. *** $P \leq 0.001$ ** $P \leq 0.01$, * $P \leq 0.05$. † Site 90 is included as it is close to significance ($P = 0.055$) and is an important site for shifts between UV and blue sensitivity in insect photopigments.

Clade A Chrysomelidae																			
Branch	A2	A4	A1	A1	A2	-	A4	A3	A3	A4	A4	A2	A5	A2	A2	A4	A4	A2	
UV clade	UV2	UV1	UV2	UV2	UV2	-	UV1	UV2	UV2	UV1	UV1	UV2	UV1	UV2	UV2	UV2	UV1	UV2	
AA site (bovine)	29	33	90	91	107	113	127	136	151	107	172	197	205	210	230	286	293	339	
<i>Colaphellus bowringi</i> UV1	V	I	K	S	H	F	T	Y	T	M	A	T	G	F	F	T	C	S	
<i>Leptinotarsa decemlineata</i> UV1	L	V	K	S	Y	F	A	Y	T	M	A	T	A	F	F	T	C	S	
<i>Octodonta nipae</i> UV1	A	P	K	S	I	F	L	F	T	T	L	T	G	F	F	G	I	N	
<i>Oreina cacaliae</i> UV1a	-	-	K	S	H	F	T	Y	T	M	T	-	-	-	-	-	-	-	
<i>Oreina cacaliae</i> UV1b	-	-	-	-	-	-	-	-	-	-	-	-	A	F	F	T	C	S	
<i>Colaphellus bowringi</i> UV2	-	-	-	-	W	Y	M	Y	T	A	M	D	G	T	F	-	-	-	
<i>Leptinotarsa decemlineata</i> UV2	Q	P	V	M	W	Y	M	F	P	A	M	D	G	M	F	G	L	L	
<i>Octodonta nipae</i> UV2	I	P	S	I	I	Y	M	Y	T	A	M	T	G	C	I	G	L	T	
<i>Oreina cacaliae</i> UV2	Q	P	V	M	W	Y	M	F	P	A	F	-	-	-	-	-	-	-	
Clade B Coccinellidae																			
Branch	B1	B1	B1	-	-	B1	B1	B1	B1	B1	B1								
UV clade	UV1/2	UV1/2	UV1/2	UV1/2	UV1/2	UV1/2	UV1/2	UV1/2	UV1/2	UV1/2	UV1/2								
AA site (bovine)	14	31	34	90	113	158	159	173	317	333	341								
<i>Adalia bipunctata</i> UV1	S	L	P	K	F	L	V	T	Q	V	E								
<i>Coccinella septempunctata</i> UV1	S	L	P	K	F	L	L	T	Q	V	E								
<i>Cryptolaemus montrouzieri</i> UV1	S	T	P	K	F	V	A	T	Q	T	Q								
<i>Harmonia axyridis</i> UV1	S	S	P	K	F	L	I	T	Q	V	E								
<i>Adalia bipunctata</i> UV2	S	M	P	K	Y	L	G	T	Q	V	-								
<i>Coccinella septempunctata</i> UV2	S	I	P	K	F	V	G	L	Q	V	-								
<i>Cryptolaemus montrouzieri</i> UV2	S	M	P	K	F	L	G	T	L	V	-								
<i>Harmonia axyridis</i> UV2	S	T	P	S	Y	L	G	I	Q	L	-								
Clade C Buprestidae																			
Branch	C1	C4	C4	C2	C3	-	C2	C2	C2	-	C3	C2	C5	C3	C5	C5			
UV clade	UV2	UV1	UV1	UV2	UV1	-	UV2	UV2	UV2	-	UV1	UV2	UV1	UV1	UV1	UV1			
AA site (bovine)	14	22	23	37	50	90	105	108	112	113	127	164	176	206	230	258			
<i>Acmaeodera diffusa</i> UV1	G	P	P	L	L	K	A	L	I	F	M	T	N	V	R	T			
<i>Agrilus planipennis</i> UV1	A	P	T	L	L	K	T	L	I	F	I	T	N	A	R	T			
<i>Aphanisticus</i> sp. UV1	S	P	P	L	L	K	T	I	I	F	I	T	N	A	Q	T			
<i>Chrysobothris lateralis</i> UV1	A	P	P	L	M	K	T	I	I	F	V	T	N	A	R	T			
<i>Chrysochroa tonkinensis</i> UV1	A	D	N	I	M	K	T	I	I	F	T	T	S	A	H	T			
<i>Sphenoptera</i> sp. UV1	A	P	P	L	M	K	T	I	I	F	A	T	N	A	R	T			
<i>Steraspis amplipennis</i> UV1	A	D	S	I	M	E	T	I	I	F	A	T	G	A	H	T			
<i>Acmaeodera diffusa</i> UV2	S	S	P	L	M	K	L	V	V	F	M	V	G	T	D	C			
<i>Agrilus planipennis</i> UV2	A	P	A	I	M	K	L	T	A	F	M	V	G	A	D	C			
<i>Aphanisticus</i> sp. UV2	P	P	A	I	M	K	L	T	T	F	M	V	G	A	N	C			
<i>Chrysobothris lateralis</i> UV2	Q	P	A	W	M	K	T	L	I	F	M	T	G	T	A	C			
<i>Chrysochroa tonkinensis</i> UV2	A	P	A	L	M	K	G	V	I	F	M	T	G	G	D	C			
<i>Sphenoptera</i> sp. UV2	R	P	A	L	M	K	T	L	I	F	M	T	G	S	S	C			
<i>Steraspis amplipennis</i> UV2	A	P	A	L	M	K	T	L	I	F	M	T	G	A	D	C			

Table S5. Amino acids present at sites under positive selection, for UV1 and UV2 opsins in clades A (Chrysomelidae), B (Cocclinellidae), and C (Buprestidae). Sites are numbered according to bovine rhodopsin. Sites 90 and 113 are included as they differ between UV1 and UV2 opsins but are invariant in other insect UV opsins.

Species	BioProject ID	Taxonomy ID	TSA accession #	Version
<i>Anorus arizonicus</i>	PRJNA286485	1661808	GDLJ00000000	GDLJ01000000
<i>Cucujus clavipes</i>	PRJNA286510	186067	GDNW00000000	GDNW01000000
<i>Dryops</i> sp. AD-2015	PRJNA286519	1661493	GDMY00000000	GDMY01000000
<i>Heterochelus</i> sp. AD-2015	PRJNA286531	1661495	GDNJ00000000	GDNJ01000000
<i>Hydrochara caraboides</i>	PRJNA286534	1208885	GDPR00000000	GDPR01000000
<i>Hypocaccus fitchi</i>	PRJNA286537	1661832	GDLI00000000	GDPQ01000000
<i>Lagria hirta</i>	PRJNA286540	296003	GDPQ00000000	GDLA01000000
<i>Larinus minutus</i>	PRJNA286543	1342029	GDLA00000000	GDPU01000000
<i>Macrosiagon limbatum</i>	PRJNA286549	1661804	GDPU00000000	GDOQ01000000
<i>Micromalthus debilis</i>	PRJNA286555	292448	GDOQ00000000	GDPL01000000
<i>Oreina cacaliae</i>	PRJNA286569	63706	GDPL00000000	GDPC01000000
<i>Thanasimus formicarius</i>	PRJNA286592	295689	GDPC00000000	GDLI01000000
<i>Xylobiops basilaris</i>	PRJNA286597	1661920	GDNM00000000	GDNM01000000

Table S6. 1KITE sample information (see www.1kite.org). All samples are derived from one project (accession PRJNA183205).

Opsin sequence	Accession #	Opsin sequence	Accession #	Opsin sequence	Accession #
<i>Allodessus bistrigatus</i> UV	KP219384	<i>Heliconius melpomene</i> LW	GU324702	<i>Nilaparvata lugens</i> LW	AB761147
<i>Allodessus bistrigatus</i> LW	KP219381	<i>Heliconius erato</i> SW	AY918906	<i>Nilaparvata lugens</i> UV1	AB761148
<i>Limbodessus palmulaoides</i> LW	KP219382	<i>Heliconius erato</i> LW	AY918907	<i>Nilaparvata lugens</i> UV2	AB761149
<i>Paroster nigroadumbratus</i> UV	KP219383	<i>Heliconius erato</i> UV1	AY918904	<i>Pieris rapae</i> blue	AB208675
<i>Paroster nigroadumbratus</i> LW	KP219380	<i>Heliconius erato</i> UV2	AY918905	<i>Pieris rapae</i> LW	AB177984
<i>Apis cerana</i> SW	AB355817	<i>Heliconius sapho</i> SW	GU324692	<i>Pieris rapae</i> UV	AB208673
<i>Apis cerana</i> LW	AB355818	<i>Heliconius sapho</i> LW	GU324705	<i>Pieris rapae</i> violet	AB208674
<i>Apis cerana</i> UV	AB355816	<i>Heliconius sapho</i> UV1	GQ451907	<i>Sogatella furcifera</i> LW	AB761150
<i>Apis mellifera</i> SW	BK005512	<i>Heliconius sapho</i> UV2	GQ451908	<i>Sogatella furcifera</i> UV1	AB761151
<i>Apis mellifera</i> LW1	BK005514	<i>Indolestes peregrinus</i> RhLWA1	LC009292	<i>Sogatella furcifera</i> UV2	AB761152
<i>Apis mellifera</i> LW2	BK005515	<i>Indolestes peregrinus</i> RhLWA2	LC009293	<i>Sympetrum frequens</i> RhLWA1	LC009060
<i>Apis mellifera</i> UV	BK005513	<i>Indolestes peregrinus</i> RhLWC1	LC009294	<i>Sympetrum frequens</i> RhLWA2	LC009061
<i>Ceratosolen solmsi</i> SW	JX422073	<i>Indolestes peregrinus</i> RhLWD1	LC009295	<i>Sympetrum frequens</i> RhLWB1	LC009062
<i>Ceratosolen solmsi</i> LW1	JX422114	<i>Indolestes peregrinus</i> RhLWD2	LC009296	<i>Sympetrum frequens</i> RhLWC1	LC009063
<i>Ceratosolen solmsi</i> LW2	JX422151	<i>Indolestes peregrinus</i> RhLWE1	LC009297	<i>Sympetrum frequens</i> RhLWD1	LC009064
<i>Ceratosolen solmsi</i> UV	JX422159	<i>Indolestes peregrinus</i> RhLWE2	LC009298	<i>Sympetrum frequens</i> RhLWE1	LC009065
<i>Danaus plexippus</i> SW	AY605544	<i>Indolestes peregrinus</i> RhLWF1	LC009299	<i>Sympetrum frequens</i> RhLWF1	LC009066
<i>Danaus plexippus</i> LW	AY605545	<i>Indolestes peregrinus</i> RhSWa1	LC009300	<i>Sympetrum frequens</i> RhLWF2	LC009067
<i>Danaus plexippus</i> UV	AY605546	<i>Indolestes peregrinus</i> RhSWb1	LC009301	<i>Sympetrum frequens</i> RhLWF3	LC009068
<i>Dianemobius nigrofasciatus</i> SW	AB291232	<i>Indolestes peregrinus</i> RhUV	LC009302	<i>Sympetrum frequens</i> RhLWF4	LC009069
<i>Dianemobius nigrofasciatus</i> LW	FJ232921	<i>Laodelphax striatella</i> LW	AB761153	<i>Sympetrum frequens</i> RhSWa1	LC009070
<i>Dianemobius nigrofasciatus</i> UV	AB458852	<i>Laodelphax striatella</i> UV1	AB761154	<i>Sympetrum frequens</i> RhSWb1	LC009071
<i>Drosophila melanogaster</i> Rh1	Flybase: CG4550	<i>Laodelphax striatella</i> UV2	AB761155	<i>Sympetrum frequens</i> RhSWc1	LC009072
<i>Drosophila melanogaster</i> Rh2	Flybase: CG16740	<i>Macroglossum stellatarum</i> SW	KF539426	<i>Sympetrum frequens</i> RhSWc2	LC009073
<i>Drosophila melanogaster</i> Rh3	Flybase: CG10888	<i>Macroglossum stellatarum</i> LW	KF539444	<i>Sympetrum frequens</i> RhSWc3	LC009074
<i>Drosophila melanogaster</i> Rh4	Flybase: CG9668	<i>Macroglossum stellatarum</i> UV	KF539456	<i>Sympetrum frequens</i> RhUV	LC009075
<i>Drosophila melanogaster</i> Rh5	Flybase: CG5279	<i>Manduca sexta</i> SW	AD001674	<i>Thermonectus marmoratus</i> UV2	EU921225
<i>Drosophila melanogaster</i> Rh6	Flybase: CG5192	<i>Manduca sexta</i> LW	L78080	<i>Thermonectus marmoratus</i> UV1	EU921226
<i>Gryllus bimaculatus</i> SW	HM363622	<i>Manduca sexta</i> UV	L78081	<i>Thermonectus marmoratus</i> LW	EU921227
<i>Gryllus bimaculatus</i> green1	HM363620	<i>Megoura viciae</i> LW	AF189714	OUTGROUP <i>Doryteuthis pealeii</i> rhodopsin	AY450853
<i>Gryllus bimaculatus</i> green2	HM363621	<i>Megoura viciae</i> UV	AF189715	OUTGROUP <i>Enteroctopus dofleini</i> rhodopsin	X07797
<i>Gryllus bimaculatus</i> UV	HM363623	<i>Nephotettix cincticeps</i> SW	AB761157	OUTGROUP <i>Loligo forbesii</i> rhodopsin	X56788
<i>Heliconius melpomene</i> UV1	GU324678	<i>Nephotettix cincticeps</i> LW	AB761156	OUTGROUP <i>Sepia officinalis</i> rhodopsin	AF000947
<i>Heliconius melpomene</i> UV2	GU324679	<i>Nephotettix cincticeps</i> UV	AB761158	OUTGROUP <i>Todarodes pacificus</i> rhodopsin	X70498
<i>Heliconius melpomene</i> SW	GU324689				

Table S7. Accession numbers for insect and cephalopod (outgroup) opsin sequences included in phylogenetic and selection analyses.