Metazoan opsin evolution reveals a simple route to animal vision

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Supplementary Online Materials

Supplementary Methods

Data mining and data set assembly. To identify homologues of sequences from GPCRdb in non-bilaterian metazoans we performed a series of blastP searches against the genomes of *Hydra magnipapillata*, *Nematostella vectensis* and *Trichoplax adherens.* These searches were seeded using the sequences we obtained from GPCRdb. To further enrich our data set of putative opsin homologues from non-bilaterian metazoans, we used our set of opsins to seed a series of blastP searches against the genome of the placozoan *Trichoplax adherens*, and against a large set of predicted GPCRs from the two available sponge genomes (that of the demosponge *Amphimedon queenslandica*, and that of the homoscleromorph *Oscarella carmela*). This data mining step was performed according to the following protocol: (I) each sequence in GPCRdb (a total of 42110 sequences) was used to seed a TblastN search of every scaffold of both sponge genomes. (II) Gene predictions were performed for all positive hits using Augustus (1), trained against the *Amphimedon quinslandica* genome. (III) Predicted genes from both sponges (a total of 13059 *Oscarella* sequences and 23858 *Amphimedon* sequences) were merged into a database that also included the entire proteome of the placozoans *Trichoplax adherans*. (IV) A series of blastP searches seeded using our set of 449 well-characterised opsins (see above) was performed against this database. All sequences with an e-value ≤ -20 were retained as representing putative opsin homologues. This procedure identified several putative opsin homologues from *Trichoplax*, one putative opsin homologue from *Oscarella*, but no putative opsin homologue from *Amphimedon.* Accordingly (V) a final BlastP analysis of the *Amphimedon* sequences was performed using, as a seed, the putative opsin homologue we identified in *Oscarella.* The two best hits from this final blastP search (E-values = $1e^{-08}$ and $1e^{-07}$) were added to our data set.

The complete set of sequences we identified included 625 GPCRs from across Metazoa, including 499 opsins. This dataset was subsampled to generate the data sets we used in our phylogenetic analyses.

Multiple sequence alignment and data set curation. To build our two master multiple sequence alignment (AOM and G&OM) we used Prank (2) with the +F option. The two master alignments were visualized and manually edited using Jalview (3) to eliminate gap-rich regions, as well as regions of dubious alignment quality.

In contrast to classical multiple sequence alignment software, Prank can distinguish insertions from deletions and has been suggested to have the potential to generate more realistic alignments. Indeed, our previous investigations shown that using Prank's alignments in phylogenetic studies based on single gene alignments, results in the recovery of more accurate phylogenetic trees (4). This suggests that Prank's alignments efficiently capture the phylogenetic signal single gene alignments.

Phylogenetic analyses. We used RAxML 7.2.6 (5) to estimate dataset specific GTR-matrices for our data sets. We used the AIC test to rank the fit to our data sets of the available empirical GTR matrices (like WAG, JTT and MtRev) and of our dataset specific GTR matrices. We further evaluated the difference between our GTR matrices and alternative, pre-computed, empirical GTR matrices (WAG and MtRev) by comparing their absolute substitution rates, and graphically displaying, for each amino acid, the difference (Δ-abs) between the GTR absolute substitution rate and the WAG or MtRev absolute substitution rate. In addition, we used 12-fold Bayesian Cross-validation as implemented in Phylobayes 3.2 (6), to evaluate whether any of the precomputed CAT-based models (7) would fit our data sets significantly better than a dataset specific GTR matrix. We thus compared the site-heterogeneous C20 + Γ, C30 + Γ, C40 + Γ, C50 + Γ, C60 + Γ, UL3+ Γ, WLSR5+ Γ (7), against GTR + Γ. Because of computational limitations the 12-fold Bayesian cross validation was only performed for the O&GM and O&O data sets (see main text). Results of the cross validation analyses showed (Tab. S4) that none of the pre-computed CAT-based models fit our data better than a data set specific GTR matrix.

All our analyses were performed under dataset specific GTR + Γ models in Phylobayes 3.2. For all analyses, two independent runs were performed and convergence was monitored using the maxdiff statistics calculated using the bpcomp program (see the Phylobayes manual). Analyses were considered to have converged when maxdiff dropped below 0.3 (see the Phylobayes manual). Results of the analyses of the O&O data sets where further confirmed by performing Maximum Likelihood (ML) analyses (under LG + Γ) in RAxML (5). Support for the nodes in the ML phylogeny were estimated using the bootstrap (108 replicates). ML analyses were performed under LG + Γ, rather then GTR + Γ, in n order to test also the sensitivity of our results to the use of less fitting models.

Posterior Predictive analysis (PPA; implemented in Phylobayes3.2) was used to evaluate whether our data sets contained compositionally heterogeneous sequences and to evaluate whether compositional heterogeneity could have affected our results.

The Approximately Unbiased (AU) test (8), implemented using RAxML under GTR + Γ, was used to evaluate whether our data set (O&O data set – see main text) allowed to statistically discriminating between our results and those of previously published studies (9-12).

We performed Bayesian (13) and ML–based ancestral character state reconstruction for the O&O data set and recovered the ancestral retinal-binding domain for two key, internal nodes. These nodes are the one identifying the last common ancestor of all the opsins (LOCA – see main text), and the one identifying the last common ancestor of all the eumetazoan opsins (LOCNA – see main text). Bayesian Ancestral character state reconstruction was performed using MrBayes3.2 (14) under the dataset specific GTR substitution matrix we derived from the O&O data set in RAxML. For the MrBayes analyses 2 runs of four chains were run until convergence and a burnin of 20% of the points in the chains was used. ML–based character state reconstruction was performed using PAML 4 (15) under GTR + Γ.

Supplementary Results

Comparison of our data-set specific matrices with available empirical GTR matrices.

To better illustrate how our data-set specific GTR matrices (e.g. Tab S2) differs from the existing empirical time reversible matrices, we present (Fig. S2) a comparison of the differences in absolute substitution rates between WAG – the matrix used in (9, 10), MtRev – the matrix used in (11), and one of our GTR matrices the O&O–GTR matrix, which was derived from the Opsins & Outgroups alignment (O&O alignment; see Methods).

Supplementary Discussion

Methodological problems with the results of Plachetzki and collaborators. The study of Plachetzki and collaborators (9) is, in part, methodologically flawed. This is because these authors did not include outgroups in their analyses as they "destabilize[d] the in-group topology". Instead, they used the AU test to select the branch where their unrooted (and outgroup-less) phylogeny should have been rooted. However, the time reversible model (WAG + G + I) that they used to estimate site-wise likelihood values for the AU test does not discriminate between the rooted resolutions of an unrooted tree. Accordingly, the differences between alternative rooting positions that they observed for a given unrooted topology must represent sampling and stochastic errors. Indeed, from a careful inspection of Plachetzki's et al. (9) Table 1, it is clear that their AU tests (as expected) only let them discriminate between the three unrooted topologies in Plachetzki's et al. (9) Figure 3, but not between the 15 rooted topologies reported in the same Figure. This invalidates the most important criterion used in (9) to select among alternative opsin phylogenies.

Supplementary References

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Table Supplementary 1: Further information is provided for sequences that were not obtained from NCBI or when an accession number is not from NCBI or Uniprot.

Sequence name in supplementary figures	Accession number	Further Information
001PapglRE_	AF077190_1	
002PieraRE_	BAD06459.1	
003DanplRE_	AAU07978.1	
004HelerRE_	AAY16540	
005PapglRE_	AAD29445.1	
006PapglRE_	AAD34220.1	
006RGRCafa_	XP_864762.1	
007ManseRE_	O02464.2	
007RGRBota_	NP_786969.1	
008BommoRE_	NP_001036882.1	
008RGRPatr_	XP_001154882.1	
009BommoRE_	NP_001036883.1	
009RGRHosa_	NP_002912.2	

010PapglRE_	AAD34224.1
010RGRDare_	NP_001017877.1
011ApomoRE_	AAT91645.1
011hypXetr_	NP_001016013.1
012LycruRE_	AAT91638.1
012RGRGaga_	NP_001026387.1
013ApomoRE_	AAT91644.1
013OpsBrbe	BAC76023.1
014PerBota	NP_001179153.1
014SchgrRE_	Q94741.1
015PerCafa	XP_853139.1
015SphspRE_	P35362.1
016OncniHE_	AAU95194.1
016PerHosa	NP_006574.1
017HomcoHE_	AAT01077.1
017PerRano	NP_001101196.1

018PerMumu	NP_033128.1
018TricaRE_	NP_001155991.1
019PerDare	NP_001004654.1
019ThemaRE_	ACH56536.1
020CamabRE_	Q17292.1
020PerGaga	NP_001073227.1
021CatboRE_	Q17296.1
021NeuDare_	NP_001186975.1
022AthroRE_	BAG32517.1
022NeuTagu_	XP_002189255.1
023ApimeRE_	Q17053.1
023NeuGaga_	NP_001124215.1
024NasviRE_	NP_001164379.1
024NeuMumu_	EDL23393
025AnogaRE_	XP_003435758.1
025NeuRano_	EDM18681.1

026AnogaRE_	XP_003435763.1
026NeuBota_	NP_001193009
027AnogaRE_	XP_322000.4
027NeuPatr_	XP_001146167.1
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029NeuCafa_	XP_003639476.1
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031CulpiRE_	XP_001862165.1
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042OpsCiin_	NP_001027727.1
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187LaukoBL_	EST assembled by Stuart Longhorn
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192RhoprRE_	EST assembled by Stuart Longhorn
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199RhoprR7_	CAA96518

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230EncMumu_	AAI05920.1	
231OPSBrbe_	BAC76022.1	
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Adre_AMI_AA2DB/	AA2DB	
All_PEP_A4IJ48_	A4IJ48	
All_PEP_NP_0010	NP_001027135	
Amq_15741g/58-2	_	Sequence assembled from the Amphimedon genome (see methods)
Amq_15747g/52-2	_	Sequence assembled from the Amphimedon genome

		(see methods)
Amq_7171g7/177-	_	Sequence assembled from the Amphimedon genome (see methods)
Ana_PEP_O55197/	O55197	
Ana_PEP_Q16581/	Q16581	
ANG_PEP_A2AUT5/	A2AUT5	
ANG_PEP_Q9WV26/	Q9WV26	
APJ_PEP_Q7SZP9/	Q7SZP9	
APJ_PEP_Q9JHG3/	Q9JHG3	
Bil_NP_00107065	NP_00107065	
Bil_Q8TDU6/1-12	Q8TDU6	
BOM_PEP_A9UJU7/	A9UJU7	
BOM_PEP_P35371/	P35371	
BRA_PEP_P48748/	P48748	
BRA_PEP_Q9BDQ5/	Q9BDQ5	

CAN_B1AZT0_117/	B1AZT0
CAN_P47936_/1-1	P47936
Cap_NP_00109170	NP_001091702
Cap_XP_00195801	XP_001958016
CCK1_PEP_A2VEY/	A2VEY5
CCK1_PEP_B2L04/	B2L044
CCR_PEP_32246_/	NP_001286
CCR_PEP_A9LS67/	A9LS67
Che_PEP_B0F9W3_	B0F9W3
Che_PEP_Q8BMP4_	Q8BMP4
CL_CN168_A	BAF95836.1
ClR_CN101S	BAF95825.1
ClR_CN102S	BAF95826.1
ClR_CN103S	BAF95828.1
ClR_CN104S	BAF95827.1
ClR_CN105S	BAF95842.1

ClR_CN106S	BAF95837.1
ClR_CN107S	BAF95836.1
ClR_CN108S	B332436.1
ClR_CN109S	BAF95838.1
ClR_CN110S	BAF95835.1
ClR_CN111S	BAF95834.1
ClR_CN112S	BAF95846.1
ClR_CN113S	AB332437.1
ClR_CN114S	BAF95846.1
ClR_CN115S	AB332436.1
ClR_CN116S	BAF95842.1
ClR_CN117S	BAF95841.1
ClR_CN118S	BAF95840.1
ClR_CN119S	BAF95838.1
ClR_CN120S	BAF95833.1
ClR_CN121S	BAF95832.1

ClR_CN122S	BAF95831.1
ClR_CN123S	BAF95830.1
ClR_CN124S	BAF95829.1
ClR_CN125S	BAF95828.1
ClR_CN126S	BAF95827.1
ClR_CN127S	BAF95826.1
ClR_CN128S	BAF95825.1
CR_CN100_K	BAG80696.1
Cys_B9UM22/1-15	B9UM22
Cys_Q2NNR5/1-15	Q2NNR5
DOP_AMI_5HTB1_/	5HTB1
DOP_AMI_DRD1_BO	NP_776467
EBV_B3DJU7/3-15	B3DJU7
EBV_NP_00110285	NP_001102856
Ecd_A7J264/1-17	A7J264
Ecd_NP_00107679	NP_001076793

End_PEP_A1Y2B8/	A1Y2B8
End_PEP_Q5KSU9/	Q5KSU9
fat_O14842/1-15	O14842
Fat_Q76EI6/1-15	Q76EI6
Fme_PEP_B6V697/	B6V697
Fme_PEP_C1BIM3/	C1BIM3
G151_NP_853664_	NP_853664
G151_Q7TSN6/7-1	Q7TSN6
Gal_PEP_A1XE40_	A1XE40
Gal_PEP_B6VCU2_	B6VCU2
GPR_PEP_Q17QD8_	Q17QD8
GRH_A1YZ03_/1-1	A1YZ03
GRH_A3DT36_/1-1	A3DT36
GRH_NP_00107680	NP_001076809
GRH_Q1JQR7_/1-1	Q1JQR7
His_AMI_P97292/	P97292

His_AMI_Q9H3N8/	Q9H3N8	
HM_CN131_2	XP_002163358.1	
HM_CN132_2	XP_002163327.1	
HM_CN133_2	XP_002163209.1	
HM_CN134_2	XP_002157157.1	
HM_CN170_U	XP_002160448.1	
HM_CNOK_CN	_	Plachetzki et al. (2010)
HM_CNOK_CV	_	Plachetzki et al. (2010)
HYM_389753/127-	XP_002155960.1	
HYM_389901_gi|2	XP_002160138.1	
HYM_397544/212-	XP_002169896.1	
HYM_398697_gi|2	XP_002170651.1	
Int_PEP_P55920/	P55920	
Int_PEP_Q2YEG2/	Q2YEG2	
Leu_C0HBJ9/1-16	C0HBJ9	
Leu_NP_062813/1	NP_062813	

LGR_B7ZMU5/10-1	B7ZMU5
LGR_Q9HBX8/62-1	Q9HBX8
Lys_B5B0C1_/1-1	B5B0C1
Lys_NP_00107501	NP_001075010
Lys_NP_00108763	NP_001087636
Lys_Q9BZJ6/1-17	Q9BZJ6
Lys_Q9PU17_/3-1	Q9PU17
MAS_B7ZP12/1-16	B7ZP12
MAS_P30554/1-15	P30554
MCR_PEP_B7T8C8/	B7T8C8
MCR_PEP_Q8TDT2/	Q8TDT2
Mel_PEP_A0N0W9/	A0N0W9
Mel_PEP_A8QXQ2/	A8QXQ2
MLT_B2Y4M8_/1-1	B2Y4M8
MLT_O88495_/1-1	O88495
MTR1C_XENL/7-16	P49219

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MUS_AMI_Q09388/	Q09388
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NEM_430210_jgi|	XP_001625478.1
NEM_432155_jgi|	XP_001641580.1
NEM_444070_jgi|	XP_001637517.1
NEM_444623_jgi|	XP_001635321.1
NEM_445570_jgi|	XP_001631194.1
NeT_PEP_Q538H6_	Q538H6
NeT_PEP_Q538H7_	Q538H7
Neu_PEP_C0J0A7_	C0J0A7
Neu_PEP_Q9TTQ9_	Q9TTQ9
Nme_PEP_A7XII4_	A7XII4
Nme_PEP_B1NPT7_	B1NPT7
NUC_B0BLU4_/7-1	B0BLU4
NUC_P28190_/1-1	P28190

NV_CN135_Suga0	FAA00413.1
NV_CN136Su	FAA00411.1
NV_CN136Suga20	43FAA00412.1
NV_CN137Suga08	FAA00410.1
NV_CN138Suga08	FAA00409.1
NV_CN139Suga08	FAA00408.1
NV_CN140Suga08	FAA00407.1
NV_CN141Suga08	FAA00406.1
NV_CN142Suga08	FAA00405.1
NV_CN143Suga08	FAA00404.1
NV_CN144Suga08	FAA00403.1
NV_CN145Suga08	XP_001632018.1
NV_CN146Su	FAA00401.1
NV_CN147Su	FAA00400.1
NV_CN148Suga08	FAA00399.1
NV_CN149Suga08	FAA00398.1

NV_CN150Suga08	FAA00397.1
NV_CN151Su	FAA00396.1
NV_CN152Su	FAA00394.1
NV_CN153Su	FAA00394.1
NV_CN154Su	FAA00393.1
NV_CN155Su	FAA00392.1
NV_CN156Su	XP_001622972.1
NV_CN157Su	FAA00390.1
NV_CN158Su	FAA00389.1
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NV_CN160Su	FAA00387.1
NV_CN161Su	FAA00386.1
NV_CN162Su	FAA00385.1
NV_CN163Su	FAA00384.1
NV_CN164Su	BR000656.1
Octo_AMI_A8E6P6	A8E6P6

Octo_AMI_Q93127	Q93127
OG10HelobP_	EST assembled by Stuart Longhorn
OG11HelobP_	EST assembled by Stuart Longhorn
OG12HelobP_	O15973
OG13HelobP_	P09241
OG14EupryP_	ACB05672.1
OG17SchmaP_	EST assembled by Stuart Longhorn
OG18SchimP_	XP_002581174.1
OG19SchmiP_	EST assembled by Stuart Longhorn
OG1LoligoP_	P24603.1
OG21HomspM_	AAI13559.1
OG22PanpaM_	XP_001135533.1
OG23BostaM_	NP_001179328

OG24MusmuM_	NP_001122071.1
OG25MondoM_	XP_001377369
OG26GalgaM_	NP_001038118.1
OG27TakruM_	EST assembled by Stuart Longhorn
OG28OrylaM_	EST assembled by Stuart Longhorn
OG29DanreM_	NP_001122233
OG2Sepia2P_	O16005.1
OG30DanreM_	XP_694492
OG31OrylaM_	EST assembled by Stuart Longhorn
OG34GalgaD_	NP_989956
OG35XenlaD_	NP_001079143.1
OG36TakruD_	EST assembled by Stuart Longhorn
OG37OrylaD_	EST assembled by

	Stuart Longhorn
OG38DanreD_	ADN39434
OG39TakruD_	EST assembled by Stuart Longhorn
OG3TodaroP_	P31356.2
OG40OrylaD_	EST assembled by Stuart Longhorn
OG41BraflM_	XP_002596283.1
OG42BraflM_	EST assembled by Stuart Longhorn
OG43AnogaT	XP_312502.2
OG44AnogaT	XP_312503
OG45ApimeT	NP_001035057.1
OG46RhoprT	EST assembled by Stuart Longhorn
OG47TricaT	EST assembled by Stuart Longhorn

OG48AcypiT	XP_001952294
OG49DappuT	EST assembled by Stuart Longhorn
OG4OctopuP_	P09241.1
OG50DappuT	EST assembled by Stuart Longhorn
OG5LottiaP_	EST assembled by Stuart Longhorn
OG6PatinoP_	O15973.1
OG7PlatynP_	CAC86665.1
OG8CapiteP_	EST assembled by Stuart Longhorn
OG9SchmaMP_	AAF73286.1
Olf_NP_00100020	NP_001000202
Olf_NP_00100038	NP_001000385
Olf_NP_00100135	NP_001001353
Olf_NP_00100182	NP_001001821

Opi_PEP_B3DK58_	B3DK58	Sequence from GPCRdb
Opi_PEPt_B3DH72	B3DH72	Sequence from GPCRdb
Ore_PEP_NP_0011	NP_001019755	
Ore_PEPt_NP_001	NP_001002933	
ORM_NP_732265/1	NP_732265	
ORM_NP_999614/1	NP_999614	
ORM_Q6PQA5_/3-1	Q6PQA5	Sequence from GPCRdb
ORM_Q7ZZC0_/3-1	Q7ZZC0	Sequence from GPCRdb
ORM_XP_538488/1	XP_538488	
Osc_348883g7549	_	Sequence assembled from the Oscarella genome (see methods)
Oth_A9XG45_/1-1	A9XG45	Sequence from GPCRdb
oth_Q7TMA4_/1-1	Q7TMA4	
oth_Q7TMA4_/125	Q7TMA4	
PC_CN130_O	NP_001000202	

PC_CN165Su	NP_001000385	
PC_CN166Su	NP_001001353	
Pla_C1BIQ7_51/1	C1BIQ7	Sequence from GPCRdb
Pla_NP_0010796/	NP_001079640	
Plos1_1_1-	XP_001622972	
Plos1_2_1-	FAA00390	
Plos1_3_2-	130042*	Plachetzki et al. 2007
Plos1_4_8-130	108738*	Plachetzki et al. 2007
PND_A0PJP9/7-17	A0PJP9	Sequence from GPCRdb
PND_NP_000944/1	NP_000944	
PND_NP_000951/1	NP_000951	
PND_NP_001051/1	NP_001051	
PNE_A2RT90_/2-1	A2RT90	
PNE_NP_00107638	NP_001076382	
PRK_PEP_NP_0673	NP_067356	
PRK_PEP_NP_6204	NP_620433	

Pro_PEP_B5XBJ7_	B5XBJ7	Sequence from GPCRdb
Pro_PEP_C0H8V6_	C0H8V6	Sequence from GPCRdb
Pro_PEP_NP_0011	b3xxm4	Sequence from GPCRdb
Pro_PEP_NP_9639	NP_963909	Sequence from GPCRdb
RDC_A8KBU0/1-15	A8KBU0	Sequence from GPCRdb
RDC_NP_00102560	A5YC96	Sequence from GPCRdb
Ser_AM_P28222_/	P28222	Sequence from GPCRdb
Ser_AMI_P46636/	P46636	Sequence from GPCRdb
Som_PEP_NP_0010	NP001005844	
Som_PEP_NP_0011	NP001077348	
SRE_A4KUT4/6-15	A4KUT4	Sequence from GPCRdb
SRE_A4KUT9/6-15	A4KUT9	Sequence from GPCRdb
Tac_PEP_P16177_	P16177	Sequence from GPCRdb
Tac_PEP_P51144_	P51144	Sequence from GPCRdb
Thy_A5A4L0/1-19	A5A4L0	Sequence from GPCRdb
Thy_B7ZQE5/1-17	B7ZQE5	Sequence from GPCRdb

Thy_O43193/1-19	O43193	Sequence from GPCRdb
Tra_429091jgi|T	XP_002112437.1	
Tra_429092jgi|T	XP_002112438.1	
Tra_430364jgi|T	XP_002113363.1	
Tra_433402jgi|T	XP_002108360.1	
Tra_434217jgi|T	XP_002109502.1	
Tra_435633jgi|T	XP_002114578.1	
Tra_435634jgi|T	XP_002114579.1	
Tra_435635jgi|T	XP_002114580.1	
Tra_435650jgi|T	XP_002114588.1	
Tra_435652jgi|T	XP_002114760.1	
Tra_435653jgi|T	XP_002114761.1	
Tra_435654jgi|T	XP_002114762.1	
Tra_435655jgi|T	XP_002114763.1	
Tra_435656jgi|T	XP_002114590.1	
Tra_435667jgi|T	XP_002114592.1	

Tra_435668jgi|T	XP_002114593.1	
Tra_435785jgi|T	XP_002114830.1	
Tra_436148jgi|T	XP_002115630.1	
Tra_436149jgi|T	XP_002115529.1	
TRA_523894/4-18	XP_002112613.1	
TRA_526691/4-16	XP_002107746.1	
TRA_526907/14-1	XP_002107856.1	
TRA_530235_jgi|	XP_002114830.1	
TRA_AMI_A5JMD0_	A5JMD0	Sequence from GPCRdb
TRA_AMI_P35412_	P35412	Sequence from GPCRdb
UPI0001560/4-18	upi000156058b	uniprot-reference
Uro_PEP_NP_0010	NP_001041476	
Uro_PEPt_NP_001	NP_001028066	
Vas_PEP_B0KWV9_	B0KWV9	Sequence from GPCRdb
Vas_PEP_B7PVR8_	B7PVR8	Sequence from GPCRdb
VIR_Q0PQ15/1-15	Q0PQ15	

VIR_Q2FA77/10-1	Q2FA77
VIR_Q6VFU4/2-15	Q6VFU4
VIR_Q6VFU7/2-14	Q6VFU7

Table Supplementary 2: Comparison of our O&O specific GTR Matrix (see Methods) against the WAG and mtREV matrices (see also main text and Fig. S2).

	WAG			GTR-O&O			mtrev		
Substitution	Rate	Frequency	Global Exchange Rate	Rate	Frequency	Global Exchange Rate	Rate	Frequency	Global Exchange Rate
A <-> R	0.551571	0.0866279	0.047781437	0.372470	0.086381	0.032174331	23.18	0.072	1.66896
A <-> N	0.509848	0.0866279	0.044167062	0.000017	0.086381	1.46848E-06	26.95	0.072	1.9404
A <-> D	0.738998	0.0866279	0.064017845	0.000020	0.086381	1.72762E-06	17.67	0.072	1.27224
A <-> C	1.02704	0.0866279	0.088970318	4.641280	0.086381	0.400918408	59.93	0.072	4.31496
A <-> Q	0.908598	0.0866279	0.078709937	0.977853	0.086381	0.08446792	1.9	0.072	0.1368
A <-> E	1.58285	0.0866279	0.137118972	3.679594	0.086381	0.317847009	9.77	0.072	0.70344
A <-> G	1.41672	0.0866279	0.122727478	12.167335	0.086381	1.051026565	120.71	0.072	8.69112
A <-> H	0.316954	0.0866279	0.027457059	0.950991	0.086381	0.082147554	13.9	0.072	1.0008
A <-> I	0.193335	0.0866279	0.016748205	0.000010	0.086381	8.6381E-07	96.49	0.072	6.94728
A <-> L	0.397915	0.0866279	0.034470541	2.024126	0.086381	0.174846028	25.46	0.072	1.83312
A <-> K	0.906265	0.0866279	0.078507834	0.815173	0.086381	0.070415459	8.36	0.072	0.60192
A <-> M	0.893496	0.0866279	0.077401682	2.787964	0.086381	0.240827118	141.88	0.072	10.21536
A <-> F	0.210494	0.0866279	0.018234653	1.852747	0.086381	0.160042139	6.37	0.072	0.45864

A <-> P	1.43855	0.0866279	0.124618566	3.358082	0.086381	0.290074481	54.31	0.072	3.91032
A <-> S	3.37079	0.0866279	0.292004459	20.448209	0.086381	1.766336742	387.86	0.072	27.92592
A <-> T	2.12111	0.0866279	0.183747305	11.897744	0.086381	1.027739024	480.72	0.072	34.61184
A <-> W	0.113133	0.0866279	0.009800474	0.450487	0.086381	0.038913518	1.9	0.072	0.1368
A <-> Y	0.240735	0.0866279	0.020854368	0.360413	0.086381	0.031132835	6.48	0.072	0.46656
A <-> V	2.00601	0.0866279	0.173776434	11.920004	0.086381	1.029661866	195.06	0.072	14.04432
R <-> N	0.635346	0.043972	0.027937434	2.169673	0.032432	0.070366835	13.24	0.019	0.25156
R <-> D	0.147304	0.043972	0.006477251	0.000017	0.032432	5.51344E-07	1.9	0.019	0.0361
R <-> C	0.528191	0.043972	0.023225615	1.170041	0.032432	0.03794677	103.33	0.019	1.96327
R <-> Q	3.0355	0.043972	0.133477006	22.282424	0.032432	0.722663575	220.99	0.019	4.19881
R <-> E	0.439157	0.043972	0.019310612	1.223350	0.032432	0.039675687	1.9	0.019	0.0361
R <-> G	0.584665	0.043972	0.025708889	2.100493	0.032432	0.068123189	23.03	0.019	0.43757
R <-> H	2.13715	0.043972	0.09397476	23.663289	0.032432	0.767447789	165.23	0.019	3.13937
R <-> I	0.186979	0.043972	0.008221841	0.803347	0.032432	0.02605415	1.9	0.019	0.0361
R <-> L	0.497671	0.043972	0.021883589	0.427901	0.032432	0.013877685	15.58	0.019	0.29602
R <-> K	5.35142	0.043972	0.23531264	40.425046	0.032432	1.311065092	141.4	0.019	2.6866
R <-> M	0.683162	0.043972	0.030039999	2.139456	0.032432	0.069386837	1.9	0.019	0.0361
R <-> F	0.102711	0.043972	0.004516408	0.000017	0.032432	5.51344E-07	4.69	0.019	0.08911
R <-> P	0.679489	0.043972	0.02987849	2.000515	0.032432	0.064880702	23.64	0.019	0.44916

R <-> S	1.22419	0.043972	0.053830083	2.560823	0.032432	0.083052612	6.04	0.019	0.11476
R <-> T	0.554413	0.043972	0.024378648	1.173869	0.032432	0.038070919	2.08	0.019	0.03952
R <-> W	1.16392	0.043972	0.05117989	0.935704	0.032432	0.030346752	21.95	0.019	0.41705
R <-> Y	0.381533	0.043972	0.016776769	1.162188	0.032432	0.037692081	1.9	0.019	0.0361
R <-> V	0.251849	0.043972	0.011074304	0.053303	0.032432	0.001728723	7.64	0.019	0.14516
N <-> D	5.42942	0.0390894	0.21223277	27.690006	0.033128	0.917314519	794.38	0.039	30.98082
N <-> C	0.265256	0.0390894	0.010368698	0.347347	0.033128	0.011506911	58.94	0.039	2.29866
N <-> Q	1.54364	0.0390894	0.060339961	6.320799	0.033128	0.209395429	173.56	0.039	6.76884
N <-> E	0.947198	0.0390894	0.037025402	3.176395	0.033128	0.105227614	63.05	0.039	2.45895
N <-> G	1.12556	0.0390894	0.043997465	2.943707	0.033128	0.097519125	53.3	0.039	2.0787
N <-> H	3.95629	0.0390894	0.154649002	27.610338	0.033128	0.914675277	496.13	0.039	19.34907
N <-> I	0.554236	0.0390894	0.021664753	0.978221	0.033128	0.032406505	27.1	0.039	1.0569
N <-> L	0.131528	0.0390894	0.005141351	0.054517	0.033128	0.001806039	15.16	0.039	0.59124
N <-> K	3.01201	0.0390894	0.117737664	5.958845	0.033128	0.197404617	608.7	0.039	23.7393
N <-> M	0.198221	0.0390894	0.00774834	1.006164	0.033128	0.033332201	65.41	0.039	2.55099
N <-> F	0.0961621	0.0390894	0.003758919	0.437898	0.033128	0.014506685	15.2	0.039	0.5928
N <-> P	0.195081	0.0390894	0.007625599	0.120693	0.033128	0.003998318	73.31	0.039	2.85909
N <-> S	3.97423	0.0390894	0.155350266	12.921326	0.033128	0.428057688	494.39	0.039	19.28121
N <-> T	2.03006	0.0390894	0.079353827	5.945131	0.033128	0.1969503	238.46	0.039	9.29994
N <-> W	0.0719167	0.0390894	0.002811181	0.588512	0.033128	0.019496226	10.68	0.039	0.41652
N <-> Y	1.086	0.0390894	0.042451088	2.568378	0.033128	0.085085226	191.36	0.039	7.46304
N <-> V	0.196246	0.0390894	0.007671138	0.000017	0.033128	5.63176E-07	1.9	0.039	0.0741

D <-> C	0.0302949	0.0570451	0.001728176	0.000017	0.017614	2.99438E-07	1.9	0.019	0.0361
D <-> Q	0.616783	0.0570451	0.035184448	26.277467	0.017614	0.462851304	55.28	0.019	1.05032
D <-> E	6.17416	0.0570451	0.352205575	24.935950	0.017614	0.439221823	583.55	0.019	11.08745
D <-> G	0.865584	0.0570451	0.049377326	6.865523	0.017614	0.120929322	56.77	0.019	1.07863
D <-> H	0.930676	0.0570451	0.053090505	8.900523	0.017614	0.156773812	113.99	0.019	2.16581
D <-> I	0.039437	0.0570451	0.002249688	0.121543	0.017614	0.002140858	4.34	0.019	0.08246
D <-> L	0.0848047	0.0570451	0.004837693	0.121480	0.017614	0.002139749	1.9	0.019	0.0361
D <-> K	0.479855	0.0570451	0.027373376	1.950613	0.017614	0.034358097	2.31	0.019	0.04389
D <-> M	0.103754	0.0570451	0.005918657	0.433017	0.017614	0.007627161	1.9	0.019	0.0361
D <-> F	0.0467304	0.0570451	0.00266574	0.000017	0.017614	2.99438E-07	4.98	0.019	0.09462
D <-> P	0.423984	0.0570451	0.02418621	1.696885	0.017614	0.029888932	13.43	0.019	0.25517
D <-> S	1.07176	0.0570451	0.061138656	2.537370	0.017614	0.044693235	69.02	0.019	1.31138
D <-> T	0.374866	0.0570451	0.021384268	0.077584	0.017614	0.001366565	28.01	0.019	0.53219
D <-> W	0.129767	0.0570451	0.007402571	0.000017	0.017614	2.99438E-07	19.86	0.019	0.37734
D <-> Y	0.325711	0.0570451	0.018580217	1.295757	0.017614	0.022823464	21.21	0.019	0.40299
D <-> V	0.152335	0.0570451	0.008689965	0.000017	0.017614	2.99438E-07	1.9	0.019	0.0361
C <-> Q	0.0988179	0.0193078	0.001907956	0.000017	0.035297	6.00049E-07	75.24	0.006	0.45144
C <-> E	0.021352	0.0193078	0.00041226	0.000017	0.035297	6.00049E-07	1.9	0.006	0.0114
C <-> G	0.306674	0.0193078	0.0059212	4.163803	0.035297	0.146969754	30.71	0.006	0.18426
C <-> H	0.248972	0.0193078	0.004807102	2.234821	0.035297	0.078882477	141.49	0.006	0.84894
C <-> I	0.170135	0.0193078	0.003284933	2.660553	0.035297	0.093909539	62.73	0.006	0.37638
C <-> L	0.384287	0.0193078	0.007419737	1.033351	0.035297	0.03647419	25.65	0.006	0.1539
C <-> K	0.0740339	0.0193078	0.001429432	0.000017	0.035297	6.00049E-07	1.9	0.006	0.0114

C <-> M	0.390482	0.0193078	0.007539348	1.374144	0.035297	0.048503161	6.18	0.006	0.03708
C <-> F	0.39802	0.0193078	0.007684891	4.337499	0.035297	0.153100702	70.8	0.006	0.4248
C <-> P	0.109404	0.0193078	0.002112351	0.000017	0.035297	6.00049E-07	31.26	0.006	0.18756
C <-> S	1.40766	0.0193078	0.027178818	11.039776	0.035297	0.389670973	277.05	0.006	1.6623
C <-> T	0.512984	0.0193078	0.009904592	4.559475	0.035297	0.160935789	179.97	0.006	1.07982
C <-> W	0.71707	0.0193078	0.013845044	1.540762	0.035297	0.054384276	33.6	0.006	0.2016
C <-> Y	0.543833	0.0193078	0.010500219	1.824575	0.035297	0.064402024	254.77	0.006	1.52862
C <-> V	1.00214	0.0193078	0.019349119	5.561946	0.035297	0.196320008	1.9	0.006	0.0114
Q <-> E	5.46947	0.0367281	0.200883241	35.682262	0.014995	0.535055519	313.56	0.025	7.839
Q <-> G	0.330052	0.0367281	0.012122183	4.111555	0.014995	0.061652767	6.75	0.025	0.16875
Q <-> H	4.29411	0.0367281	0.157714501	51.242334	0.014995	0.768378798	582.4	0.025	14.56
Q <-> I	0.113917	0.0367281	0.004183955	0.000017	0.014995	2.54915E-07	8.34	0.025	0.2085
Q <-> L	0.869489	0.0367281	0.031934679	0.819682	0.014995	0.012291132	39.7	0.025	0.9925
Q <-> K	3.8949	0.0367281	0.143052277	25.379126	0.014995	0.380559994	465.58	0.025	11.6395
Q <-> M	1.54526	0.0367281	0.056754464	4.755573	0.014995	0.071309817	47.37	0.025	1.18425
Q <-> F	0.0999208	0.0367281	0.003669901	0.090739	0.014995	0.001360631	19.11	0.025	0.47775
Q <-> P	0.933372	0.0367281	0.03428098	2.633334	0.014995	0.039486843	137.29	0.025	3.43225
Q <-> S	1.02887	0.0367281	0.03778844	4.048879	0.014995	0.060712941	54.11	0.025	1.35275
Q <-> T	0.857928	0.0367281	0.031510065	4.002151	0.014995	0.060012254	94.93	0.025	2.37325
Q <-> W	0.215737	0.0367281	0.00792361	0.546469	0.014995	0.008194303	1.9	0.025	0.0475
Q <-> Y	0.22771	0.0367281	0.008363356	0.180949	0.014995	0.00271333	38.82	0.025	0.9705
Q <-> V	0.301281	0.0367281	0.011065479	2.285398	0.014995	0.034269543	19	0.025	0.475
E <-> G	0.567717	0.0580589	0.032961025	2.182333	0.019375	0.042282702	28.28	0.024	0.67872

E <-> H	0.570025	0.0580589	0.033095024	6.273177	0.019375	0.121542804	49.12	0.024	1.17888
E <-> I	0.127395	0.0580589	0.007396414	0.411639	0.019375	0.007975506	3.31	0.024	0.07944
E <-> L	0.154263	0.0580589	0.00895634	0.000017	0.019375	3.29375E-07	1.9	0.024	0.0456
E <-> K	2.58443	0.0580589	0.150049163	5.413575	0.019375	0.104888016	313.86	0.024	7.53264
E <-> M	0.315124	0.0580589	0.018295753	2.270348	0.019375	0.043987993	1.9	0.024	0.0456
E <-> F	0.0811339	0.0580589	0.004710545	0.000017	0.019375	3.29375E-07	2.67	0.024	0.06408
E <-> P	0.682355	0.0580589	0.039616781	1.168452	0.019375	0.022638758	12.83	0.024	0.30792
E <-> S	0.704939	0.0580589	0.040927983	1.591212	0.019375	0.030829733	54.71	0.024	1.31304
E <-> T	0.822765	0.0580589	0.047768831	1.425746	0.019375	0.027623829	14.82	0.024	0.35568
E <-> W	0.156557	0.0580589	0.009089527	1.520005	0.019375	0.029450097	1.9	0.024	0.0456
E <-> Y	0.196303	0.0580589	0.011397136	0.808523	0.019375	0.015665133	13.12	0.024	0.31488
E <-> V	0.588731	0.0580589	0.034181074	0.998776	0.019375	0.019351285	21.14	0.024	0.50736
G <-> H	0.24941	0.0832518	0.020763831	0.000017	0.052284	8.88828E-07	1.9	0.056	0.1064
G <-> I	0.0304501	0.0832518	0.002535026	0.000017	0.052284	8.88828E-07	5.98	0.056	0.33488
G <-> L	0.0613037	0.0832518	0.005103643	1.801299	0.052284	0.094179117	2.41	0.056	0.13496
G <-> K	0.373558	0.0832518	0.031099376	0.752240	0.052284	0.039330116	22.73	0.056	1.27288
G <-> M	0.1741	0.0832518	0.014494138	0.764959	0.052284	0.039995116	1.9	0.056	0.1064
G <-> F	0.049931	0.0832518	0.004156846	0.747196	0.052284	0.039066396	1.9	0.056	0.1064
G <-> P	0.24357	0.0832518	0.020277641	0.395595	0.052284	0.020683289	1.9	0.056	0.1064
G <-> S	1.34182	0.0832518	0.11170893	4.378353	0.052284	0.228917808	125.93	0.056	7.05208
G <-> T	0.225833	0.0832518	0.018801004	1.784214	0.052284	0.093285845	11.17	0.056	0.62552
G <-> W	0.336983	0.0832518	0.028054441	0.965397	0.052284	0.050474817	10.92	0.056	0.61152
G <-> Y	0.103604	0.0832518	0.008625219	0.158693	0.052284	0.008297105	3.21	0.056	0.17976

G <-> V	0.187247	0.0832518	0.01558865	0.104953	0.052284	0.005487363	2.53	0.056	0.14168
H <-> I	0.13819	0.0244313	0.003376161	0.000017	0.013890	2.3613E-07	12.26	0.028	0.34328
H <-> L	0.499462	0.0244313	0.012202506	1.435801	0.013890	0.019943276	11.49	0.028	0.32172
H <-> K	0.890432	0.0244313	0.021754411	3.369225	0.013890	0.046798535	127.67	0.028	3.57476
H <-> M	0.404141	0.0244313	0.00987369	0.000017	0.013890	2.3613E-07	11.97	0.028	0.33516
H <-> F	0.679371	0.0244313	0.016597917	0.858364	0.013890	0.011922676	48.16	0.028	1.34848
H <-> P	0.696198	0.0244313	0.017009022	1.746018	0.013890	0.02425219	60.97	0.028	1.70716
H <-> S	0.740169	0.0244313	0.018083291	2.147079	0.013890	0.029822927	77.46	0.028	2.16888
H <-> T	0.473307	0.0244313	0.011563505	4.880825	0.013890	0.067794659	44.78	0.028	1.25384
H <-> W	0.262569	0.0244313	0.006414902	1.283476	0.013890	0.017827482	7.08	0.028	0.19824
H <-> Y	3.87344	0.0244313	0.094633175	18.232165	0.013890	0.253244772	670.14	0.028	18.76392
H <-> V	0.118358	0.0244313	0.00289164	0.538296	0.013890	0.007476931	1.9	0.028	0.0532
I <-> L	3.17097	0.048466	0.153684232	11.649315	0.104227	1.214173155	329.09	0.088	28.95992
I <-> K	0.323832	0.048466	0.015694842	0.000017	0.104227	1.77186E-06	19.57	0.088	1.72216
I <-> M	4.25746	0.048466	0.206342056	6.933572	0.104227	0.722665409	517.98	0.088	45.58224
I <-> F	1.05947	0.048466	0.051348273	2.657569	0.104227	0.276990444	84.67	0.088	7.45096
I <-> P	0.0999288	0.048466	0.004843149	0.342153	0.104227	0.035661581	20.63	0.088	1.81544
I <-> S	0.31944	0.048466	0.015481979	0.054045	0.104227	0.005632948	47.7	0.088	4.1976
I <-> T	1.45816	0.048466	0.070671183	3.274730	0.104227	0.341315284	368.43	0.088	32.42184
I <-> W	0.212483	0.048466	0.010298201	0.282943	0.104227	0.0294903	1.9	0.088	0.1672
I <-> Y	0.42017	0.048466	0.020363959	0.505561	0.104227	0.052693106	25.01	0.088	2.20088
I <-> V	7.8213	0.048466	0.379067126	25.958726	0.104227	2.705600135	1222.94	0.088	107.61872
L <-> K	0.257555	0.086209	0.022203559	0.000017	0.107297	1.82405E-06	14.88	0.169	2.51472

L <-> M	4.85402	0.086209	0.41846021	17.561533	0.107297	1.884299806	537.53	0.169	90.84257
L <-> F	2.11517	0.086209	0.182346691	9.513020	0.107297	1.020718507	216.06	0.169	36.51414
L <-> P	0.415844	0.086209	0.035849495	0.802655	0.107297	0.086122474	40.1	0.169	6.7769
L <-> S	0.344739	0.086209	0.029719604	0.363560	0.107297	0.039008897	73.61	0.169	12.44009
L <-> T	0.326622	0.086209	0.028157756	3.291709	0.107297	0.353190501	126.4	0.169	21.3616
L <-> W	0.665309	0.086209	0.057355624	1.506436	0.107297	0.161636063	32.44	0.169	5.48236
L <-> Y	0.398618	0.086209	0.034364459	0.504313	0.107297	0.054111272	44.15	0.169	7.46135
L <-> V	1.80034	0.086209	0.155205511	8.364695	0.107297	0.897506679	91.67	0.169	15.49223
K <-> M	0.934276	0.0620286	0.057951832	1.350050	0.037180	0.050194859	91.37	0.023	2.10151
K <-> F	0.088836	0.0620286	0.005510373	0.303948	0.037180	0.011300787	6.44	0.023	0.14812
K <-> P	0.556896	0.0620286	0.034543479	0.312545	0.037180	0.011620423	50.1	0.023	1.1523
K <-> S	0.96713	0.0620286	0.05998972	0.000017	0.037180	6.3206E-07	105.79	0.023	2.43317
K <-> T	1.38698	0.0620286	0.086032428	2.916861	0.037180	0.108448892	136.33	0.023	3.13559
K <-> W	0.137505	0.0620286	0.008529243	0.999162	0.037180	0.037148843	24	0.023	0.552
K <-> Y	0.133264	0.0620286	0.008266179	0.086881	0.037180	0.003230236	51.17	0.023	1.17691
K <-> V	0.305434	0.0620286	0.018945643	0.000017	0.037180	6.3206E-07	1.9	0.023	0.0437
M <-> F	1.19063	0.0195027	0.0232205	3.250092	0.036443	0.118443103	90.82	0.054	4.90428
M <-> P	0.171329	0.0195027	0.003341378	0.610211	0.036443	0.022237919	18.84	0.054	1.01736
M <-> S	0.493905	0.0195027	0.009632481	1.344576	0.036443	0.049000383	111.16	0.054	6.00264
M <-> T	1.51612	0.0195027	0.029568434	7.636555	0.036443	0.278298974	528.17	0.054	28.52118
M <-> W	0.515706	0.0195027	0.010057659	3.380240	0.036443	0.123186086	21.71	0.054	1.17234
M <-> Y	0.428437	0.0195027	0.008355678	0.506100	0.036443	0.018443802	39.96	0.054	2.15784
M <-> V	2.05845	0.0195027	0.040145333	6.104372	0.036443	0.222461629	387.54	0.054	20.92716

F <-> P	0.161444	0.0384319	0.0062046	0.130004	0.078440	0.010197514	17.31	0.061	1.05591
F <-> S	0.545931	0.0384319	0.020981166	1.505887	0.078440	0.118121776	64.29	0.061	3.92169
F <-> T	0.171903	0.0384319	0.006606559	1.890992	0.078440	0.148329412	33.85	0.061	2.06485
F <-> W	1.52964	0.0384319	0.058786972	5.505100	0.078440	0.431820044	7.84	0.061	0.47824
F <-> Y	6.45428	0.0384319	0.248050244	11.740286	0.078440	0.920908034	465.58	0.061	28.40038
F <-> V	0.649892	0.0384319	0.024976584	3.423294	0.078440	0.268523181	6.35	0.061	0.38735
P <-> S	1.61328	0.0457631	0.073828694	1.796229	0.042829	0.076930692	169.9	0.054	9.1746
P <-> T	0.795384	0.0457631	0.036399238	1.927018	0.042829	0.082532254	128.22	0.054	6.92388
P <-> W	0.139405	0.0457631	0.006379605	0.421593	0.042829	0.018056407	4.21	0.054	0.22734
P <-> Y	0.216046	0.0457631	0.009886935	0.520846	0.042829	0.022307313	16.21	0.054	0.87534
P <-> V	0.314887	0.0457631	0.014410205	0.075829	0.042829	0.00324768	8.23	0.054	0.44442
S <-> T	4.37802	0.0695179	0.304350757	22.576963	0.063663	1.437317195	597.21	0.072	42.99912
S <-> W	0.523742	0.0695179	0.036409444	0.000017	0.063663	1.08227E-06	38.58	0.072	2.77776
S <-> Y	0.786993	0.0695179	0.054710101	0.576053	0.063663	0.036673262	64.92	0.072	4.67424
S <-> V	0.232739	0.0695179	0.016179527	0.817618	0.063663	0.052052015	1.9	0.072	0.1368
T <-> W	0.110864	0.0610127	0.006764112	0.451270	0.058465	0.026383501	9.99	0.086	0.85914
T <-> Y	0.291148	0.0610127	0.017763726	0.408951	0.058465	0.02390932	38.73	0.086	3.33078
T <-> V	1.38823	0.0610127	0.084699661	7.891488	0.058465	0.461375846	204.54	0.086	17.59044
W <-> Y	2.48539	0.0143859	0.035754572	6.765614	0.021135	0.142991252	26.25	0.029	0.76125
W <-> V	0.365369	0.0143859	0.005256162	0.063870	0.021135	0.001349892	5.37	0.029	0.15573
Y <-> V	0.31473	0.0352742	0.011101849	1.000000	0.054331	0.054331	1.9	0.033	0.0627

Supplementary Table S3: Model selection. **:** This analysis illustrate that data set specific GTR+Γ models fits each of our data set better than precomputed GTR mode

	Data set	Model	log-likelihood	AIC
Part A. AIC tests	GPCR & Opsin Master alignment	GTR+Γ	-78625.11026	157672.2205
		LGF+Γ	-79573.94395	159149.8879
		WAGF+Γ	-79929.86067	159861.7231
	Opsins & Outgroups alignment	GTR+Γ	-33759.69164	67941.38327
		LGF+Γ	-34197.46653	68393.93306
		WAGF+Γ	-34414.51953	68831.03907
	All Opsin Master alignment	GTR+Γ	-78875.35627	158172.7125
		LGF+Γ	-79821.76843	159625.5369
		WAGF+Γ	-80417.84551	160837.691

Supplementary Table S4: Bayesian cross validation. This analysis was performed to compare the GTR+Γ models against precomputed site heterogeneous (CAT) models. The All Opsin Master alignment was not tested because of computational limitations. Note: In the cross validation a negative value implies that the reference model (GTR+Γ) is better than the tested model. Only in the case of O&O one of the heterogeneous models (C50) performs marginally better than GTR. However, for all considered models (including C50) the standard deviation around the cross validation scores is too large to claim that one of the two models fits the data better. As none of the precomputed empirical CAT models was found to fit the data significantly better than GTR+Γ, these models were not used to analyse the data.

	Data set	Compared Models	Mean Score	Stdev (+/-)
Part B. Cross Validation	G&OM	C20 versus GTR	-458.095	1412.83
		C30 versus GTR	-263.715	1271.08
		C40 versus GTR	-868.692	1100.33
		C50 versus GTR	-394.801	1335.45
		C60 versus GTR	-615.158	1212.5
		UL3 versus GTR	-344.796	1110.3
		WLSR5 versus GTR	-575.617	1480.18
	O&O	C20 versus GTR	-440.047	543.972
		C30 versus GTR	-149.996	769.156
		C40 versus GTR	-390.47	373.572
		C50 versus GTR	107.343	608.496
		C60 versus GTR	-135.062	470.151
		UL3 versus GTR	-151.482	587.579
		WLSR5 versus GTR	-209.752	482.316

Supplementary Table S5: Posterior predictive analysis for compositional homogeneity. Stars identify compositional heterogeneous sequences.

taxon	p-value	z-score
Plos1_4_8-130	0.778	-0.895
NV_CN144Suga08	0.677	-0.525
NV_CN139Suga08	0.429	0.004
NV_CN145Suga08	0.402	0.098
NV_CN135_Suga0	0.543	-0.205
NV_CN143Suga08	0.59	-0.314
NV_CN138Suga08	0.597	-0.412
NV_CN136Suga20	0.503	-0.166
* NV_CN140Suga08	0.046	1.94
* NV_CN141Suga08	0.006	3.95
* NV_CN148Suga08	0.006	3.927
NV_CN149Suga08	0.127	1.301
NV_CN150Suga08	0.12	1.383
NV_CN142Suga08	0.181	0.871
NV_CN137Suga08	0.771	-0.76
OG43AnogaT	0.832	-0.962
038InsCuqu	0.657	-0.536
OG44AnogaT	0.63	-0.434
OG46RhoprT	0.147	0.836
OG47TricaT	0.073	1.598
OG48AcypiT	0.59	-0.365
OG45ApimeT	0.067	1.519
OG49DappuT	0.892	-1.062
OG50DappuT	0.308	0.351

014PerBota	0.489	-0.149
015PerCafa	0.557	-0.331
016PerHosa	0.328	0.299
017PerRano	0.57	-0.356
018PerMumu	0.926	-1.339
019PerDare	0.563	-0.333
020PerGaga	0.12	1.181
CR_CN100_K	0.234	0.585
ClR_CN101S	0.214	0.532
ClR_CN128S	0.234	0.519
ClR_CN102S	0.255	0.507
ClR_CN127S	0.255	0.497
* ClR_CN103S	0.013	3.685
* ClR_CN125S	0.013	3.632
ClR_CN104S	0.174	0.834
ClR_CN126S	0.181	0.825
ClR_CN105S	0.308	0.347
ClR_CN116S	0.308	0.343
ClR_CN106S	0.073	1.602
ClR_CN107S	0.08	1.628
CL_CN168_A	0.073	1.54
ClR_CN108S	0.08	1.544
ClR_CN115S	0.08	1.548
* ClR_CN113S	0.02	2.599
ClR_CN109S	0.993	-1.773
ClR_CN114S	0.986	-1.611
ClR_CN112S	0.523	-0.238
ClR_CN119S	0.993	-1.735
ClR_CN117S	0.61	-0.452

ClR_CN118S	0.812	-0.778
ClR_CN110S	0.651	-0.523
ClR_CN111S	0.624	-0.424
ClR_CN124S	0.456	-0.132
ClR_CN123S	0.348	0.208
ClR_CN120S	0.785	-0.812
ClR_CN121S	0.932	-1.169
ClR_CN122S	0.395	0.042
HM_CN131_2	0.093	1.511
HM_CN132_2	0.107	1.234
HM_CNOK_CN	0.389	0.109
HM_CN133_2	0.08	1.632
PC_CN165Su	0.073	1.365
* PC_CN130_O	0	3.418
* HM_CN170_U	0	3.394
* HM_CNOK_CV	0.04	1.807
PC_CN166Su	0.087	1.704
* HM_CN134_2	0.046	1.888
NV_CN147Su	0.228	0.628
NV_CN157Su	0.214	0.695
Plos1_2_1-	0.194	0.798
NV_CN155Su	0.55	-0.309
Plos1_3_2-	0.51	-0.112
NV_CN156Su	0.825	-1.005
Plos1_1_1-	0.838	-0.996
NV_CN154Su	0.563	-0.189
NV_CN152Su	0.302	0.246
NV_CN153Su	0.281	0.244
* NV_CN159Su	0.013	2.603

NV_CN158Su	0.174	0.847
NV_CN160Su	0.201	0.681
NV_CN164Su	0.248	0.547
* NV_CN161Su	0.02	2.215
* NV_CN162Su	0.006	3.318
* NV_CN163Su	0.006	3.299
NV_CN136Su	0.087	1.309
NV_CN151Su	0.382	0.17
NV_CN146Su	0.053	1.765
* 231OPSBrbe_	0.046	1.979
232TMTTagu_	0.107	1.311
233TMTDare_	0.429	-0.001
234TMTTaru_	0.114	1.263
227EncHepu_	0.295	0.392
001PapglRE_	0.838	-1.021
002PieraRE_	0.778	-0.886
003DanplRE_	0.59	-0.399
004HelerRE_	0.912	-1.055
013ApomoRE_	1	-1.727
005PapglRE_	0.604	-0.323
006PapglRE_	0.302	0.482
007ManseRE_	0.758	-0.666
009BommoRE_	0.637	-0.37
008BommoRE_	0.536	-0.221
010PapglRE_	0.926	-1.15
011ApomoRE_	0.442	-0.036
012LycruRE_	0.161	1.063
014SchgrRE_	0.671	-0.531
186LocmiRE_	0.697	-0.666

015SphspRE_	0.899	-1.039
033DianiRE_	0.771	-0.837
192RhoprRE_	0.657	-0.395
018TricaRE_	0.993	-1.946
179LuccrRE_	0.912	-1.127
019ThemaRE_	0.872	-1.03
016OncniHE_	0.912	-1.239
017HomcoHE_	0.932	-1.249
020CamabRE_	0.677	-0.368
021CatboRE_	0.617	-0.347
022AthroRE_	0.959	-1.303
023ApimeRE_	0.677	-0.526
024NasviRE_	0.704	-0.543
185PedhuRE_	0.369	0.3
025AnogaRE_	0.463	-0.11
026AnogaRE_	0.469	-0.115
028AedaeRE_	0.395	0.072
029AedaeRE_	0.637	-0.412
030CulpiRE_	0.657	-0.533
031CulpiRE_	0.671	-0.603
027AnogaRE_	0.711	-0.739
032AedaeRE_	0.791	-0.898
035CulpiRE_	0.973	-1.402
034AedaeRE_	0.899	-1.082
037CulpiRE_	0.879	-1.09
036CulpiRE_	0.355	0.254
038CulpiRE_	0.758	-0.782
045AnogaRE_	0.563	-0.257
039AcypiRE_	0.637	-0.48

040MegviRE_	0.691	-0.645
041AnogaRE_	0.738	-0.674
042CulpiRE_	0.369	0.223
043AedaeRE_	0.187	0.813
044ApimeOC_	0.919	-1.099
190NasviOC_	0.496	-0.102
076CalviOC_	0.161	0.936
078DromeOC_	0.375	0.252
079BacdoOC_	0.617	-0.47
077DromeOC_	0.604	-0.459
069LimpoLW_	0.409	0.048
070LimpoLW_	0.51	-0.218
071PlepaLW_	0.778	-0.789
072HasadLW_	0.543	-0.21
073IxoscLW_	0.228	0.728
074PlepaLW_	0.624	-0.433
075HasadLW_	0.677	-0.565
048PromiL1_	0.617	-0.449
049ProclL1_	0.597	-0.461
050OrcauL1_	0.463	-0.045
051CamluL1_	0.724	-0.652
052CamshL1_	0.832	-0.908
055NeoamL1_	0.557	-0.309
058PetciL1_	0.214	0.73
056HolcoL1_	0.496	-0.205
057LitvaL1_	0.704	-0.595
060EupsuL1_	0.248	0.62
061ArcgrL1_	0.557	-0.202
063GampuL1_	0.865	-1.066

064GampuL1_	0.986	-1.821
194EurpuL1_	0.228	0.725
195NeoinL1_	0.489	-0.156
196NeoinL1_	0.355	0.376
197NeoinL1_	0.604	-0.356
198NeoinL1_	0.624	-0.4
053NeooeL1_	0.932	-1.37
054NeooeL1_	0.885	-1.103
204NeooeL1_	0.892	-1.146
206NeooeL1_	0.302	0.466
* 207NeooeL1_	0.033	2.105
205NeooeL1_	0.973	-1.567
059HomgaL1_	0.624	-0.431
065ParbaL1_	0.986	-1.46
066CasknL1_	0.771	-0.747
067MysdiL1_	0.738	-0.676
068HemanL1_	0.214	0.563
062NeooeL1_	0.832	-0.93
201NeooeL1_	0.61	-0.454
102PetciL1_	0.154	1.05
202NeooeL1_	0.718	-0.612
203NeooeL1_	0.342	0.127
080DappuM1_	0.785	-0.83
081DappuM1_	0.892	-1.154
082DappuM1_	0.872	-1.064
083DappuM1_	0.604	-0.329
084DappuM1_	0.342	0.246
085DappuM1_	0.845	-0.978
086DappuM1_	0.993	-1.726

087DappuM1_	0.939	-1.21
088DappuM1_	0.953	-1.29
089DappuM1_	0.953	-1.228
163BrakuM1_	0.939	-1.325
166BrakuM1_	0.852	-1.003
168BrakuM1_	0.892	-1.037
167BrakuM1_	0.812	-0.944
165BrakuM1_	1	-1.742
174TrigrM1_	0.335	0.277
171TriloM1_	0.919	-1.354
175TrigrM1_	0.926	-1.342
173TrigrM1_	0.449	-0.05
090DappuM2_	0.684	-0.499
091DappuM2_	0.436	0.069
092DappuM2_	0.644	-0.457
093DappuM2_	0.798	-0.858
094DappuM2_	0.597	-0.584
095DappuM2_	0.429	0.068
096DappuM2_	0.617	-0.519
172TriloM2_	0.892	-1.026
176TrigrM2_	0.959	-1.318
097PorpeM2_	0.147	0.978
098HemsaM2_	0.778	-0.814
178UcavoM2_	0.818	-0.842
099HemsaM2_	0.63	-0.431
177UcavoM2_	0.469	-0.107
100PetciM2_	0.577	-0.269
101PetciM2_	0.577	-0.322
193EurpuM2_	0.926	-1.347

208LimpoMW_	0.496	-0.158
103AedaeUV_	0.697	-0.646
104CulpiUV_	0.932	-1.237
105AnogaUV_	0.53	-0.311
106TricaUV_	0.664	-0.55
180LuccrUV_	0.912	-1.321
* 181ThemaUV_	0.04	2.312
182ThemaUV_	0.899	-1.226
108ApimeUV_	0.476	-0.041
109BomimUV_	0.697	-0.591
110CatboUV_	0.664	-0.465
111CamabUV_	0.724	-0.63
112NasviUV_	0.899	-1.161
107DianiUV_	0.852	-0.914
189LaukoUV_	0.993	-1.627
191RhoprUV_	0.476	-0.169
113CalviUV_	0.167	0.895
114DromeUV_	0.053	1.742
115DromeUV_	0.879	-1.035
116ApomoUV_	0.724	-0.672
117LycruUV_	0.657	-0.533
118DanplUV_	0.926	-1.315
119HelerUV_	0.859	-1.048
120PieraUV_	0.718	-0.675
121ManseUV_	1	-1.728
123BommoUV_	0.812	-0.89
122PapglUV_	0.919	-1.212
145AcypiUV_	0.859	-1.051
146MegviUV_	0.859	-1.019

147AcypiUV_	0.61	-0.318
183PedhuUV_	0.375	0.128
144BrakuU1_	0.845	-0.994
148TriloU1_	0.651	-0.53
149TrigrU1_	0.724	-0.693
151DappuU1_	0.939	-1.08
150DappuU1_	0.872	-0.998
124ManseBL_	0.671	-0.576
133BommoBL_	0.711	-0.752
125PapglBL_	0.624	-0.404
126DanplBL_	0.859	-0.984
127HelerBL_	0.979	-1.569
128ApomoBL_	0.744	-0.662
129LycruBL_	0.241	0.415
130LycruBL_	0.859	-1.028
131PieraBL_	0.53	-0.196
132PieraBL_	0.389	0.064
134CulpiBL_	0.926	-1.201
135CulpiBL_	0.557	-0.219
136AnogaBL_	0.718	-0.599
137AedaeBL_	0.919	-1.28
140ApimeBL_	0.308	0.323
141NasviBL_	0.711	-0.599
138SchgrBL_	0.355	0.042
139DianiBL_	0.785	-0.754
187LaukoBL_	0.221	0.558
152PlepaUW_	0.348	0.217
153HasadUW_	0.711	-0.721
154AedaeR7_	0.563	-0.376

155CulpiR7_	0.744	-0.779
156AnogaR7_	0.107	1.348
157DromeR7_	0.355	0.2
158BommoR7_	0.087	1.478
159AcypiR7_	0.812	-0.848
160AcypiR7_	0.778	-0.734
184PedhuR7_	0.818	-1.02
199RhoprR7_	0.724	-0.765
* 161DappuR7_	0	3.501
162DappuR7_	0.825	-0.862
* 200IxoscR7_	0	7.35
OG1LoligoP_	0.241	0.527
OG2Sepia2P_	0.295	0.422
OG3TodaroP_	0.255	0.63
OG14EupryP_	0.281	0.494
OG4OctopuP_	0.147	1.188
OG5LottiaP_	0.644	-0.521
OG6PatinoP_	0.174	0.788
OG7PlatynP_	0.389	0.191
OG8CapiteP_	0.147	1.171
OG10HelobP_	0.798	-0.945
OG11HelobP_	0.885	-1.106
OG12HelobP_	0.691	-0.591
OG13HelobP_	0.885	-1.082
OG9SchmaMP_	0.738	-0.64
OG19SchmiP_	0.234	0.813
OG17SchmaP_	0.389	0.185
OG18SchimP_	0.456	-0.042
OG41BraflM_	1	-1.679

OG42BraflM_	1	-1.79
237MelFeca_	0.483	-0.04
OG23BostaM_	0.563	-0.277
OG21HomspM_	0.342	0.265
OG22PanpaM_	0.328	0.265
OG24MusmuM_	0.845	-0.96
OG25MondoM_	0.248	0.619
OG26GalgaM_	0.51	-0.203
OG27TakruM_	0.577	-0.359
OG28OrylaM_	0.161	0.945
OG29DanreM_	0.604	-0.37
OG30DanreM_	0.375	0.075
OG31OrylaM_	0.295	0.337
OG34GalgaD_	0.147	0.844
OG35XenlaD_	0.067	1.535
OG36TakruD_	0.308	0.403
OG37OrylaD_	0.476	-0.156
OG38DanreD_	0.154	0.939
OG39TakruD_	0.067	1.905
OG40OrylaD_	0.174	1.106
021NeuDare_	0.194	0.731
022NeuTagu_	0.395	0.096
023NeuGaga_	0.248	0.67
024NeuMumu_	0.604	-0.39
025NeuRano_	0.704	-0.612
026NeuBota_	0.55	-0.197
027NeuPatr_	0.671	-0.496
028NeuHosa_	0.671	-0.472
029NeuCafa_	0.617	-0.313

030NeuEqca_	0.637	-0.35
031NeuOran_	0.677	-0.46
032NeuModo_	0.704	-0.569
226OpsPldu_	0.248	0.636
228EncDare_	0.241	0.616
229EncHosa_	0.08	1.636
230EncMumu_	0.08	1.294
* 006RGRCafa_	0.006	4.086
* 007RGRBota_	0.006	4.131
* 008RGRPatr_	0.04	2.036
* 009RGRHosa_	0.02	2.202
010RGRDare_	0.724	-0.725
011hypXetr_	0.442	0.123
012RGRGaga_	0.583	-0.389
040ParUtst_	0.241	0.57
041ParXetr_	0.214	0.632
042OpsCiin_	0.261	0.573
047PinPema_	0.053	1.662
048VAoXela_	0.959	-1.552
049VAoGaga_	1	-1.796
052VAoOrla_	0.51	-0.126
053VAoCyca_	0.194	0.712
054VAoDare_	0.456	-0.069
055LWSCami_	0.322	0.248
057OpsPhma_	0.563	-0.346
058LWSLeja_	0.872	-1.031
059LWSGeau_	0.791	-0.747
117SWSLoaf_	0.12	1.103
* 118SWSPtpu_	0.04	2.361

120UVoCaau_	0.442	-0.015
121UVoCyca_	0.241	0.51
122SWSDare_	0.268	0.468
125SWSSasa_	0.463	-0.041
126SWSOrla_	0.208	0.603
128SWSPore_	0.181	0.733
130GreDare_	0.06	1.707
131GreCaau_	0.255	0.576
132GreCyca_	0.295	0.354
135SWSOrla_	0.563	-0.209
136RH2Orla_	0.073	1.61
137GreTeni_	0.268	0.491
138GreTaru_	0.12	1.394
142OpsPhma_	0.214	0.736
143RH2Lach_	0.852	-0.955
144RH2Nefo_	0.496	-0.23
145RH2Anca_	0.798	-0.809
146RH2Tagu_	0.63	-0.448
147GreAnpl_	0.255	0.466
* 148RHOGaga_	0.046	1.646
143RH2Geau_	0.12	1.183
149RodSasa_	0.315	0.382
151RodTaru_	0.496	-0.168
150ExoDare_	0.268	0.573
153RhAGeau_	0.181	0.825
* 154RHOPema_	0.046	1.816
155RHOLeja_	0.14	1.251
156RHOLeja_	0.134	1.28
159RHOLeer_	0.114	1.162

160RHOScca_	0.208	0.735
161RHOCami_	0.08	1.709
162RHOPyre_	0.208	0.715
163RHOAnpl_	0.181	0.802
164RHOGaga_	0.181	0.809
165RHOTagu_	0.355	0.161
* 177OpsCypy_	0.033	2.169
166RHOOran_	0.335	0.275
167RHOBota_	0.14	0.961
168RHOCapo_	0.308	0.471
170RHOOrcu_	0.489	-0.143
171RHOHosa_	0.577	-0.313
172RHORano_	0.194	0.674
173RHOMumu_	0.268	0.555
175RHOFeca_	0.268	0.492
176RHOCafa_	0.409	0.11
174RHOMylu_	0.402	0.125
169RHOSusc_	0.59	-0.283
158RHOSasa_	0.597	-0.476
193RHOTaru_	0.422	-0.02
194RHOPore_	0.275	0.403
195RHOOrla_	0.187	0.968
196RH1Anan_	0.187	0.713
* 199RHOCyca_	0.04	2.15
200RHODare_	0.1	1.391
201BluGeau_	0.953	-1.348
210BluCyca_	0.979	-1.585
211SWSDare_	0.791	-0.83
212SWSSasa_	0.241	0.533

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  215SWSLugo_        0.463   -0.048
  224SWSOrla_        0.067   1.623
  217BluTaru_        0.255   0.463
* 218uppTeni_        0.04    2.131
  219SWSOrla_        0.127   0.987
  220SWSLugo_        0.469   -0.148
  202OpsCypy_        0.919   -1.181
  203OpsRaca_        0.979   -1.475
  204SWSNefo_        0.275   0.471
  205BluGaga_        0.114   1.125
  208SWSTagu_        0.201   0.781
  209SWSAnca_        0.073   1.6
  206SWSOran_        0.812   -0.884
  207SWSTaac_        0.516   -0.162
* 043uppTeni_        0.006   2.774
* 044ParLeja_        0.013   3.36
  045ParIcpu_        0.248   0.538
  046ParOnmy_        0.08    1.649

global test:
succeeded
observed   : 0.0240564
mean pred  : 0.0183843
p-value    : 0.154362
z-score    : 1.05464
```

Figure Captions:

Figure Supplementary 1: Alternative hypotheses of opsin relationships. (A) The phylogeny of (9). In this tree the cnidarian–specific opsins form two groups. One represent the sister group of the C–opsins and includes only one sequence. The second represent the sister group of the R– plus Go/RGR–opsins. This phylogeny can be explained with two duplications only. The first duplication happened in the stem neuralian lineage and separated the C–opsin lineage from the Cnidopsin, plus R, plus Go/RGR lineage. The second duplication happened in the stem bilaterian lineage and separated the R– from the Go/RGR–opsins. **(B)** The phylogeny of (12). In this hypothesis the cnidarian specific opsins are split into three groups. These represent the sister group of the C–opsins, of the R–opsins and of all the other opsins. In (12) these cnidarian–specific opsins were referred to as: Group 1, Group 2 and Group 3, respectively (see Figure 1b). To explain the opsin distribution in Fig. 1b three duplications and two deletions are necessary. The first duplication separates the Group 3 opsins from all the other opsins. The other two duplications separate the C– plus Go/RGR–opsins from the C–opsins, and the C– from the Go/RGR–opsins, respectively. The first of the two deletions affected the Bilateria that loose the Group 3 opsins. The second deletion affected Cnidaria that loose their Go/RGR opsin paralog. **(C)** The phylogeny of (10). This phylogeny implies that the Cnidopsins are the Cnidarian orthologue of the bilaterian opsins, and can be explained with two duplications in the stem bilaterian lineage only. The first of these duplications separated the C–opsins from the R plus Go/RGR lineage. The second separated the R–opsins from the Go/RGR–opsins. **(D)** The phylogeny of (11). This phylogeny can be explained with two duplications and one or two deletions. The first duplication separated the C–opsins from the R plus Go/RGR lineage and happened in the stem eumetazoan lineage. The second duplication separated the R–opsins from the Go/RGR–opsins. The two deletions happened in the Cnidarian lineage and caused the loss of the R and Go/RGR paralogues. If the duplication separating the R–opsins from the Go/RGR opsins happened in the stem bilaterian lineage then only one deletion (of the R–opsin plus Go/RGR–opsin orthologue) happened in the cnidarian lineage. Note reference numbers point to the SI reference list (supplementary references) not to the main text reference list.

Figure Supplementary 2. (A) A plot of the difference (Δ-abs), for each substitution in Table S2, between the GTR-O&O and WAG global exchange rates. A value of zero means that the rate is the same in both matrices. A positive value means that the GTR-O&O global exchange rate is higher then the WAG global exchange rate. A negative value means that the GTR-O&O global exchange rate is lower than the WAG global exchange rate. **(B)** A plot of the difference (Δ-abs), for each substitution in Table S2, between the GTR-O&O and mtRev global exchange rates. A value of zero means that the rate is the same in both matrices. A positive value means that the GTR-O&O global exchange rate is higher then the mtRev global exchange rate. A negative value means that the GTR-O&O global exchange rate is lower than the mtRev global exchange rate. On the X-axis: amino acid substitutions (ordered with reference to their Δ-abs – from smaller to big). On the Y-axis Δ-abs values.

Figure Supplementary 3. The unrooted Bayesian phylogeny of the opsin family (obtained using a GTR + Γ model). Groups in this tree are coloured as in Fig 1a. Support values are posterior probabilities. See main text and supplementary methods for details.

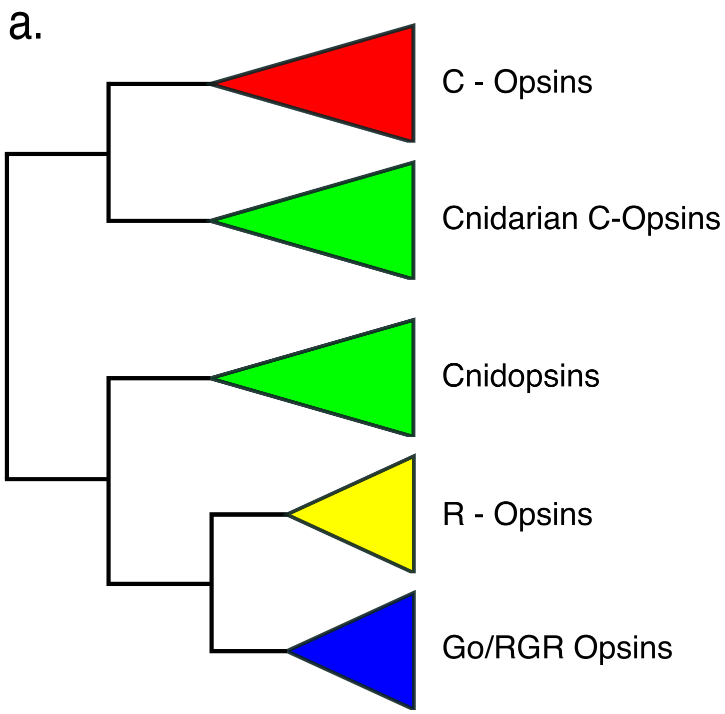
Figure Supplementary 4. The rooted Bayesian phylogeny of the G&OM dataset obtained using a GTR + Γ model. Sequences from *Amphimedon* and *Oscarella* are coloured in light blue. All other relevant sequences in this tree are coloured as in Fig 1b. Support values are posterior probabilities. See main text and supplementary methods for details.

Figure Supplementary 5. The rooted Bayesian phylogeny of the O&O dataset obtained using a GTR + Γ model. Groups in this tree are coloured as in Fig 1c. Support values are posterior probabilities. See main text and supplementary methods for details.

Figure Supplementary 6. The Maximum likelihood phylogeny of the O&O dataset obtained using a LG + Γ model. 108 bootstrap replicates were performed. Groups in this tree are coloured as in Fig 1c.

Figure Supplementary 7. Logos representing the results of the Bayesian ancestral character state reconstructions for the RBD. **(A)** The RBD of the LOCA. **(B)** The RBD of the LOCNA. Dimensions of the letters in the logos represent the posterior probability of observing a given amino acid at that alignment position. Ancestral character state reconstruction was performed under GTR + Γ on the O&O data set. In red is the position 296.

Feuda et al. Fig.S1



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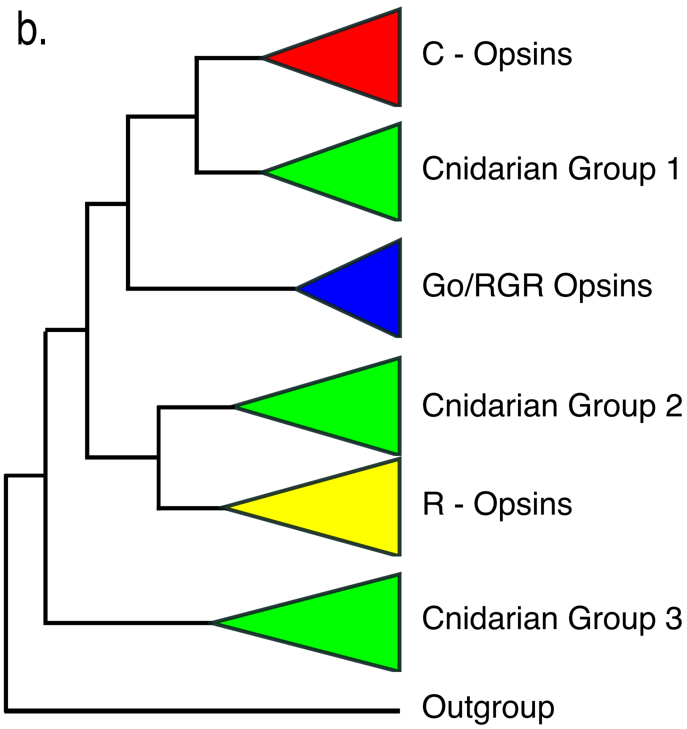

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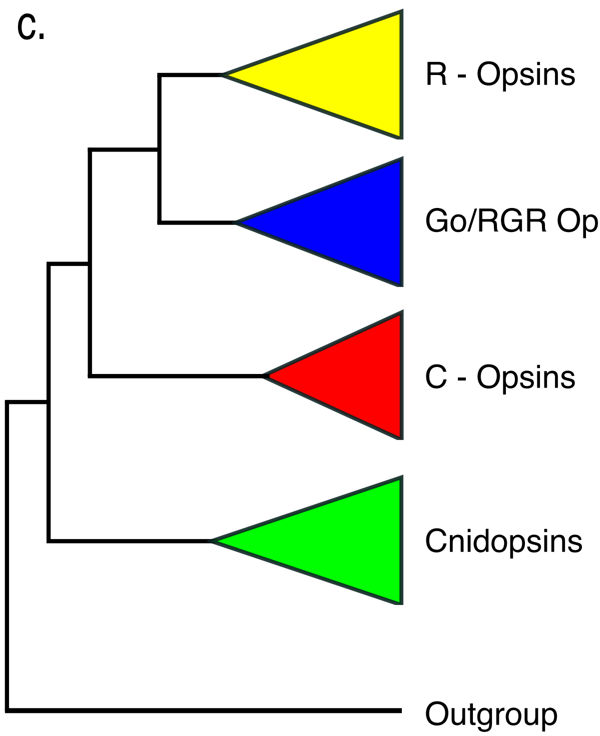

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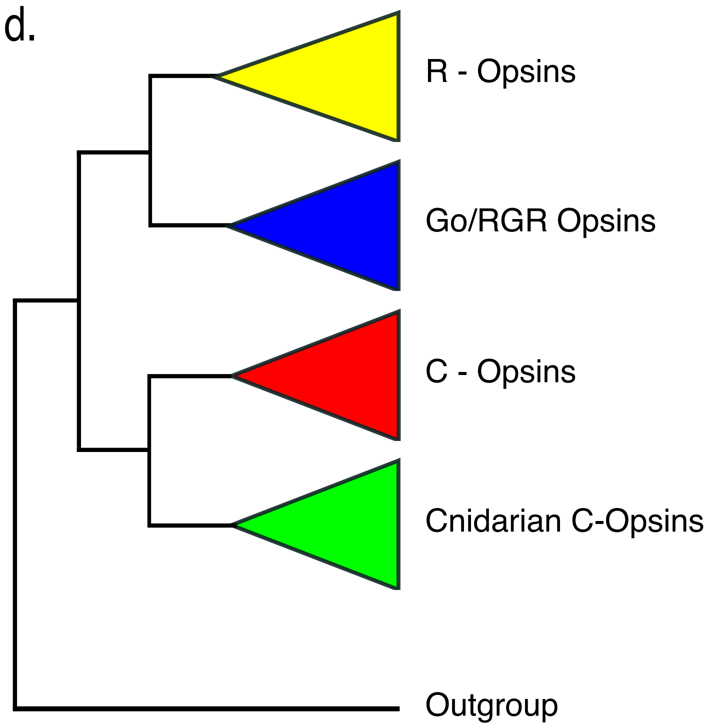

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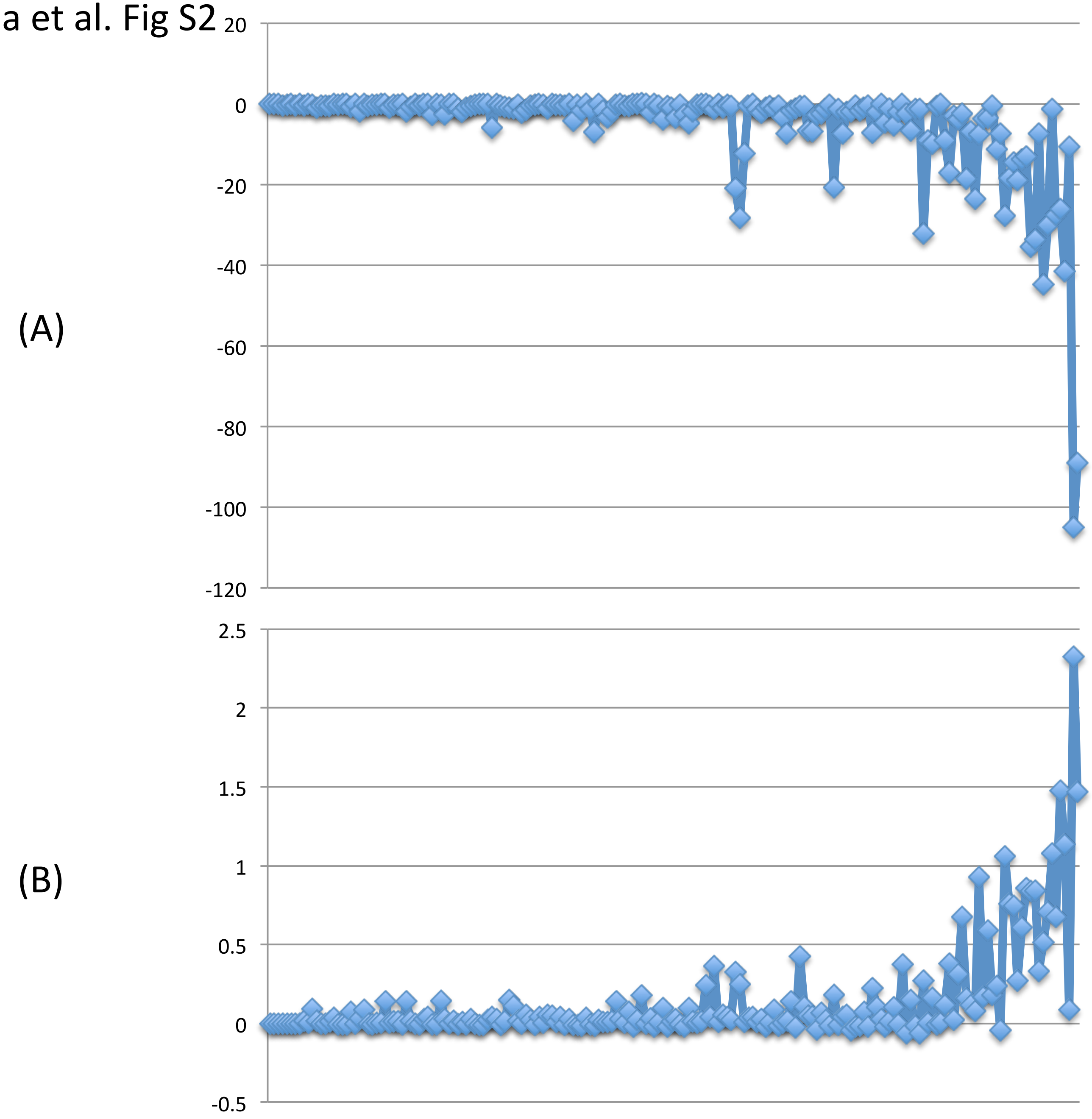
Feuda et al. Fig S2



Feuda et al. Fig S3

Feuda et al. Fig S4

Feuda et al. Fig. S5

Feuda et al. Figure S6

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