

Supplementary Material

Analysis of the Opsin Repertoire in the Tardigrade

***Hypsibius dujardini* Provides Insights into the Evolution of Opsin Genes in Panarthropoda**

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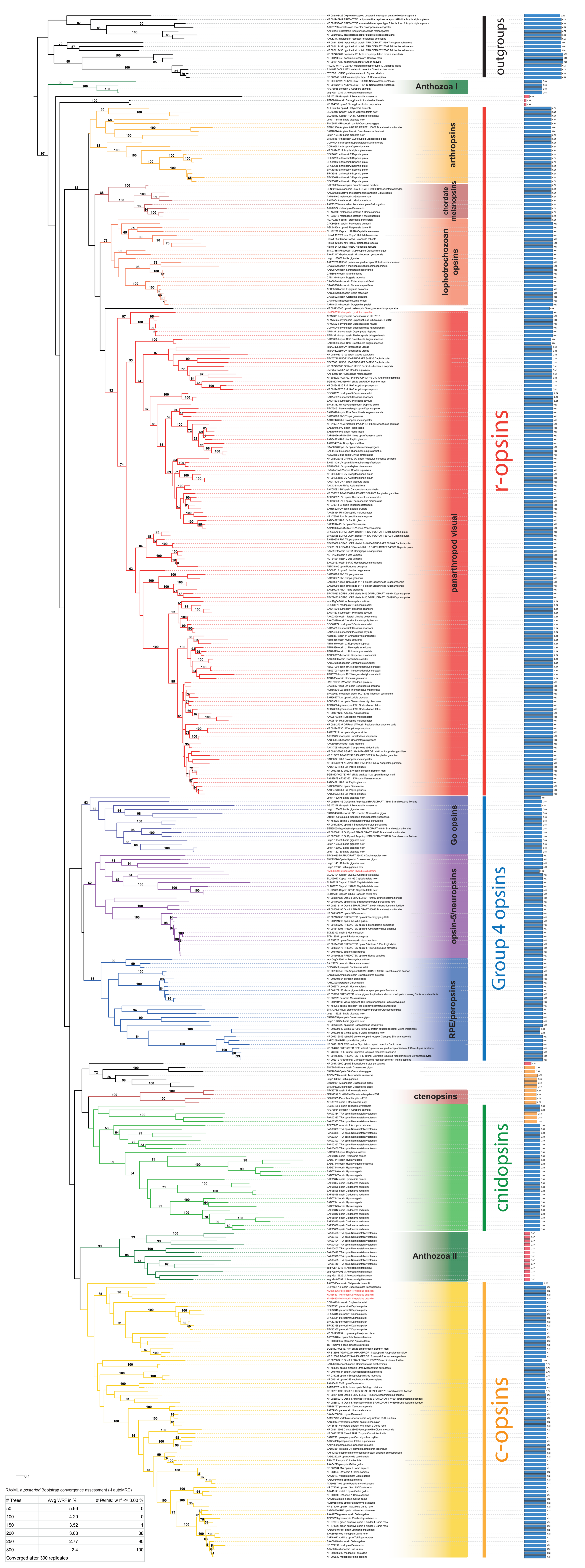


Figure S1. Maximum likelihood tree under a dataset-specific GTR+G model of amino acid sequence evolution of 401 opsins and opsin related proteins and the placement of the opsin genes of the tardigrade *Hypsibius dujardini* (highlighted in red). Bootstrap values (from 1,000 pseudoreplicates) > 50% are given at the nodes. According to the bootstrap convergence assessment, a sufficient number of replicates has been generated (table in lower left corner). Group 4 opsins are highlighted in blue. Group 5 opsins are highlighted in yellow. Group 6 opsins are highlighted in orange. Group 7 opsins are highlighted in green. Group 8 opsins are highlighted in purple. Group 9 opsins are highlighted in red. Group 10 opsins are highlighted in light blue. Group 11 opsins are highlighted in dark red. Group 12 opsins are highlighted in orange. Group 13 opsins are highlighted in green. Group 14 opsins are highlighted in purple. Group 15 opsins are highlighted in yellow. Group 16 opsins are highlighted in light blue. Group 17 opsins are highlighted in dark red. Group 18 opsins are highlighted in orange. Group 19 opsins are highlighted in green. Group 20 opsins are highlighted in purple. Group 21 opsins are highlighted in yellow. Group 22 opsins are highlighted in light blue. Group 23 opsins are highlighted in dark red. Group 24 opsins are highlighted in orange. Group 25 opsins are highlighted in green. Group 26 opsins are highlighted in purple. Group 27 opsins are highlighted in yellow. Group 28 opsins are highlighted in light blue. Group 29 opsins are highlighted in dark red. Group 30 opsins are highlighted in orange. Group 31 opsins are highlighted in green. Group 32 opsins are highlighted in purple. Group 33 opsins are highlighted in yellow. Group 34 opsins are highlighted in light blue. Group 35 opsins are highlighted in dark red. Group 36 opsins are highlighted in orange. Group 37 opsins are highlighted in green. Group 38 opsins are highlighted in purple. Group 39 opsins are highlighted in yellow. Group 40 opsins are highlighted in light blue. Group 41 opsins are highlighted in dark red. Group 42 opsins are highlighted in orange. Group 43 opsins are highlighted in green. Group 44 opsins are highlighted in purple. Group 45 opsins are highlighted in yellow. Group 46 opsins are highlighted in light blue. Group 47 opsins are highlighted in dark red. Group 48 opsins are highlighted in orange. Group 49 opsins are highlighted in green. Group 50 opsins are highlighted in purple. Group 51 opsins are highlighted in yellow. Group 52 opsins are highlighted in light blue. Group 53 opsins are highlighted in dark red. Group 54 opsins are highlighted in orange. Group 55 opsins are highlighted in green. Group 56 opsins are highlighted in purple. Group 57 opsins are highlighted in yellow. Group 58 opsins are highlighted in light blue. Group 59 opsins are highlighted in dark red. Group 60 opsins are highlighted in orange. Group 61 opsins are highlighted in green. Group 62 opsins are highlighted in purple. Group 63 opsins are highlighted in yellow. Group 64 opsins are highlighted in light blue. Group 65 opsins are highlighted in dark red. Group 66 opsins are highlighted in orange. Group 67 opsins are highlighted in green. Group 68 opsins are highlighted in purple. Group 69 opsins are highlighted in yellow. Group 70 opsins are highlighted in light blue. Group 71 opsins are highlighted in dark red. Group 72 opsins are highlighted in orange. Group 73 opsins are highlighted in green. Group 74 opsins are highlighted in purple. Group 75 opsins are highlighted in yellow. Group 76 opsins are highlighted in light blue. Group 77 opsins are highlighted in dark red. Group 78 opsins are highlighted in orange. Group 79 opsins are highlighted in green. Group 80 opsins are highlighted in purple. Group 81 opsins are highlighted in yellow. Group 82 opsins are highlighted in light blue. Group 83 opsins are highlighted in dark red. Group 84 opsins are highlighted in orange. Group 85 opsins are highlighted in green. Group 86 opsins are highlighted in purple. Group 87 opsins are highlighted in yellow. Group 88 opsins are highlighted in light blue. Group 89 opsins are highlighted in dark red. Group 90 opsins are highlighted in orange. Group 91 opsins are highlighted in green. Group 92 opsins are highlighted in purple. Group 93 opsins are highlighted in yellow. Group 94 opsins are highlighted in light blue. Group 95 opsins are highlighted in dark red. Group 96 opsins are highlighted in orange. Group 97 opsins are highlighted in green. Group 98 opsins are highlighted in purple. Group 99 opsins are highlighted in yellow. Group 100 opsins are highlighted in light blue.

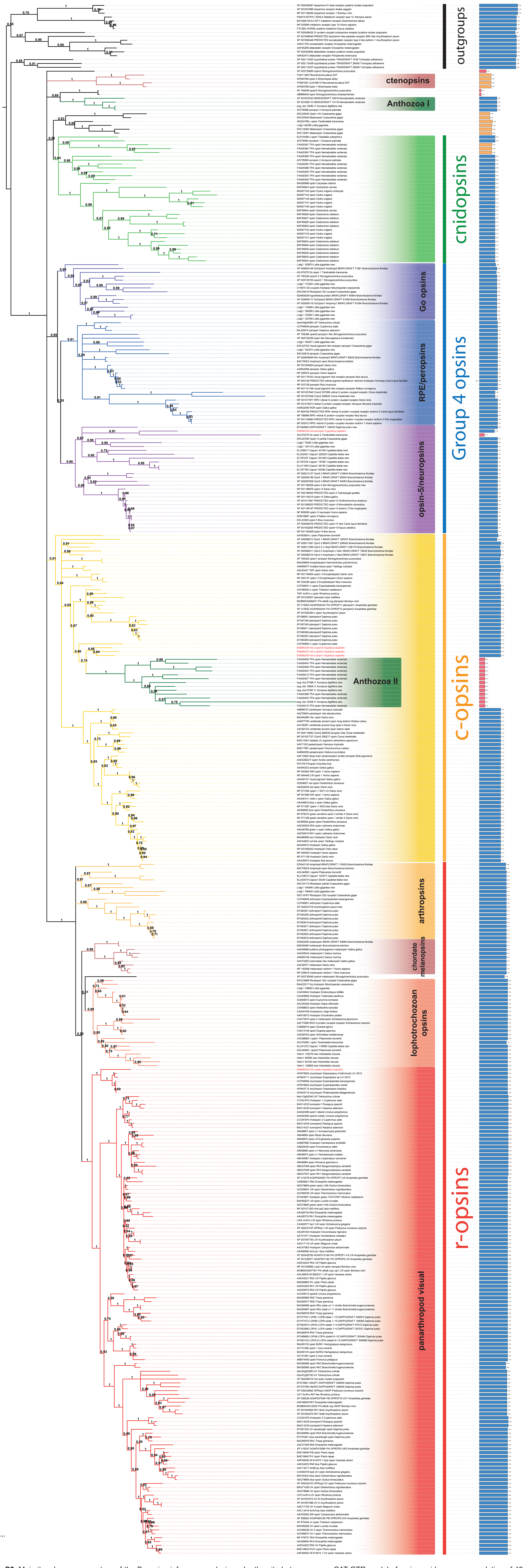


Figure S2. Majority-rule consensus tree of the Bayesian inference analysis under the site-heterogeneous CAT-GTR model of amino acid sequence evolution of 401 opsins and opsin related proteins and the placement of the opsin genes of the tardigrade *Hypsibius avaraimi* (highlighted in red). Posterior probabilities > 0.70 are given at the nodes. G-protein-coupled receptor sequences other than opsins were used as outgroups. Bars on the right indicate the leaf stability indices derived from 1,000 ML bootstrapping trees (red: LS ≤ 0.50, orange: 0.60 ≤ LS < 0.70, blue: LS > 0.70). Scale bar indicates the number of substitutions per site.

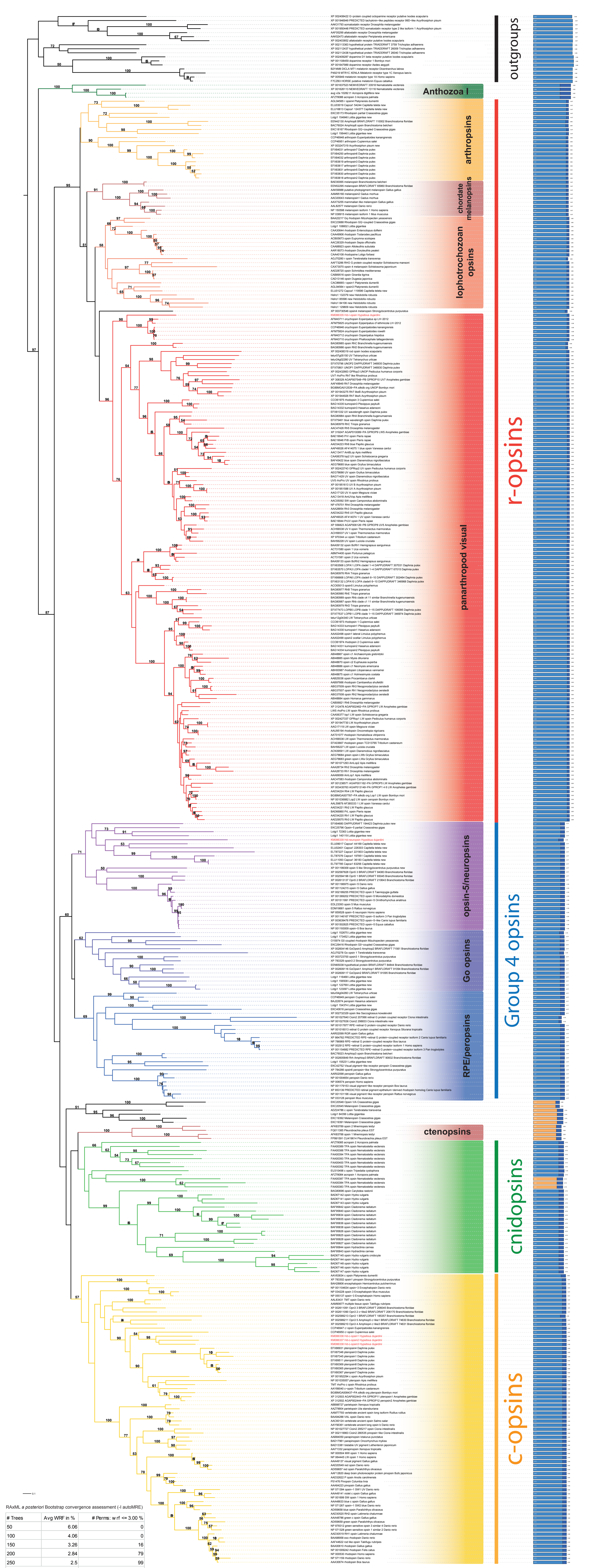


Figure S3. Maximum likelihood tree under a dataset-specific GTR+G model of amino acid sequence evolution of the pruned dataset LS50 (excluded taxa with leaf stability ≤ 0.50) and the maximum likelihood tree of the tardigrade *Hypsibius djardini* (highlighted in red). Bootstrap values (from 1,000 pseudoreplicates) > 50% are given at the nodes. According to the bootstrap convergence assessment, a sufficient number of replicates has been generated (table in lower left corner). G-protein-coupled receptor sequences other than opsins were used as outgroups. Bars on the right indicate the leaf stability indices derived from 1,000 ML bootstrapping trees on the full dataset (orange: $LS \leq 0.60$, light blue: $LS > 0.60$) and from 1,000 ML bootstrapping trees after pruning of taxa with $LS \leq 0.50$ (dark blue). Scale bar indicates the number of substitutions per site.

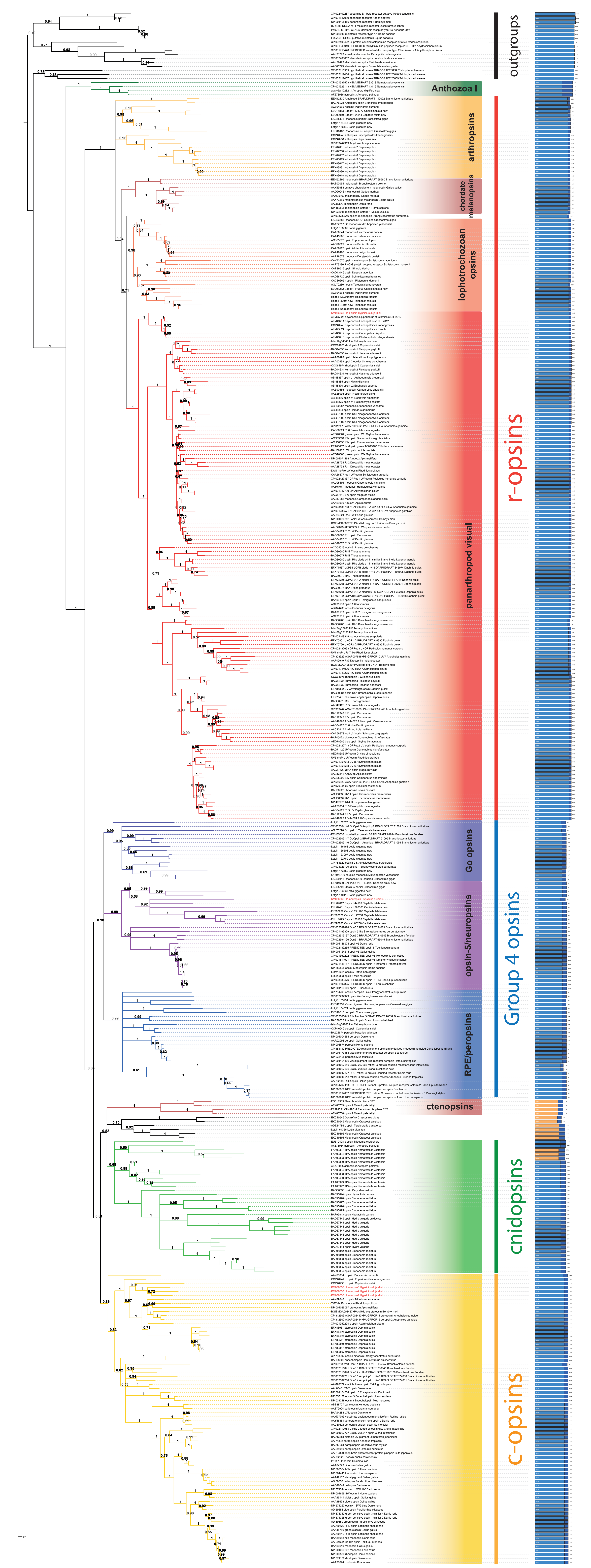


Figure S4. Majority-rule consensus tree of the Bayesian inference analysis under the site-heterogeneous CAT-GTR model of amino acid sequence evolution of the pruned dataset LS50 (excluded taxa with leaf stability ≤ 0.50) and the placement of the opsin genes of the tardigrade *Hypsibius dujardini* (highlighted in red). Posterior probabilities > 0.70 are given at the nodes. G-protein-coupled receptor sequences other than opsins were used as outgroups. Bars on the right indicate the leaf stability indices derived from 1,000 ML bootstrapping trees on the full dataset (orange: $LS \leq 0.60$, light blue: $LS > 0.60$) and from 1,000 ML bootstrapping trees after pruning of taxa with $LS \leq 0.50$ (dark blue). Scale bar indicates the number of substitutions per site.

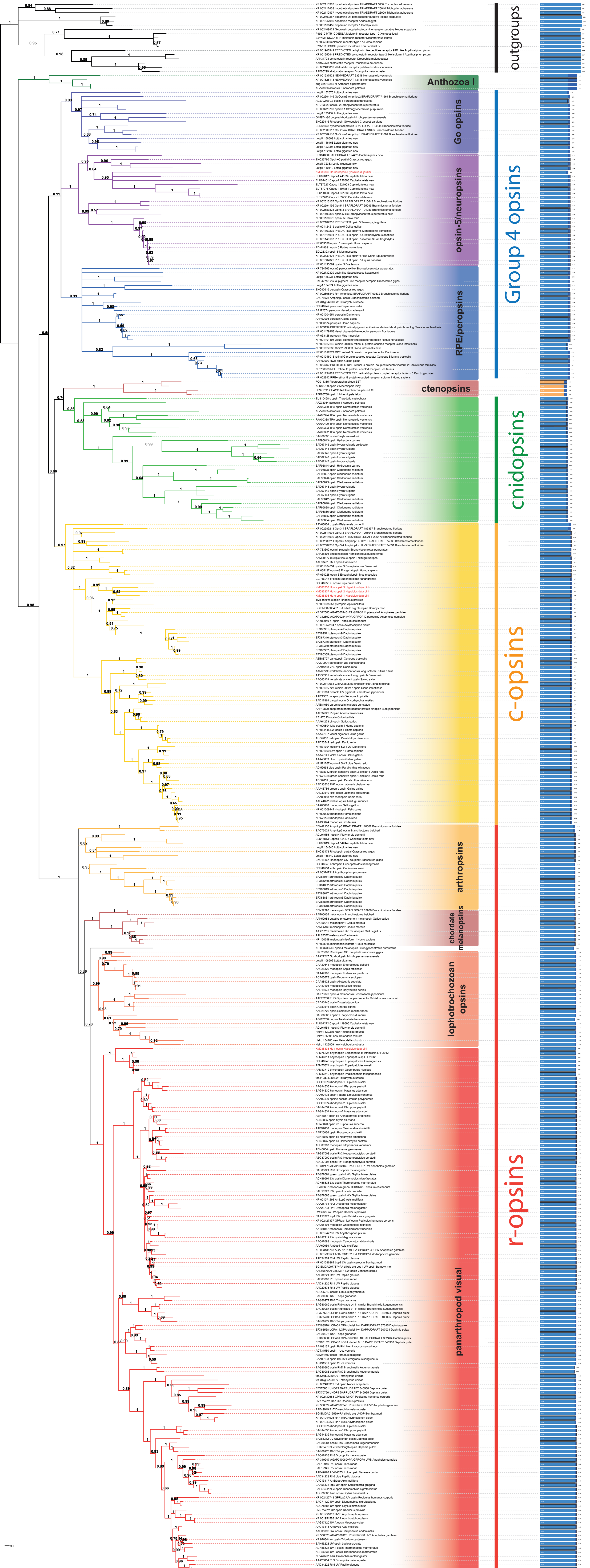


Figure S6. Majority-rule consensus of the Bayesian analysis under the site-heterogeneous CAT-GTR model of amino acid sequence evolution of the protein dataset LS60 (excluded taxa with leaf stability ≤ 0.60) and the placement of the opsin genes of the tardigrade *Hypsibius dujardini* (highlighted in red). Posterior probabilities > 0.70 are given at the nodes. G-protein-coupled receptor sequences other than opsins were used as outgroups. Bars on the right indicate the leaf stability indices derived from 1,000 ML bootstrapping trees on the full dataset (orange: $LS \leq 0.60$, light blue: $LS > 0.60$) and from 1,000 ML bootstrapping trees after pruning of taxa with $LS \leq 0.60$ (dark blue). Scale bar indicates the number of substitutions per site.

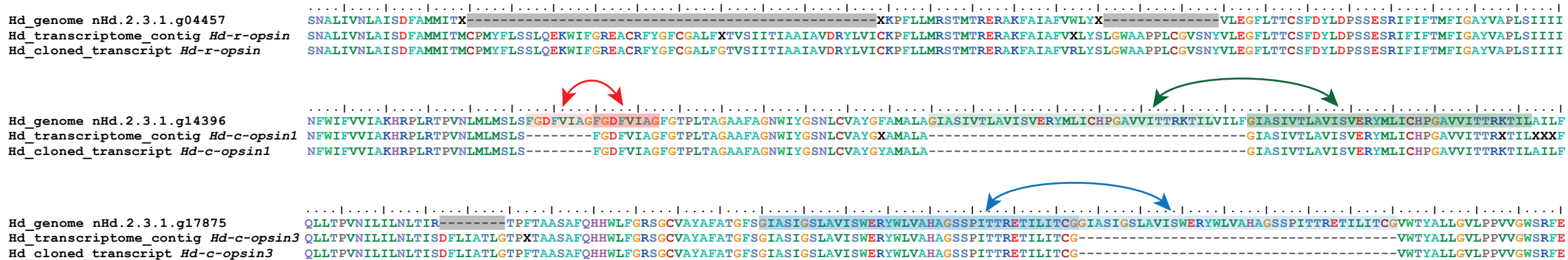


Figure S7. Three examples for inconsistencies between our cloned transcripts and the predicted transcripts from the genome of *Hypsibius dujardini*. Sequences from the genome are on top of each alignment. Apparently exonic regions are either missing (grey boxes) or duplicated (colored boxes). Arrows indicate corresponding repeats. This suggests that either the genomic contigs and/or scaffolds were assembled incorrectly or, if the assembly was correct and the introns indeed contain such repeated exonic sequences, the actual splice sites were predicted incorrectly during the gene annotation process.

A

		good			acceptable												bad													
		1000	2000	3000	4000	5000	6000	7000	8000	9000	10000	11000	12000	13000	14000	15000	16000	17000	18000	19000	20000	21000	22000	23000	24000	25000	26000	27000	28000	29000
<i>bpcomp</i>	burn-in																													
	maxdiff	0.227586	0.246071	0.254815	0.254615	0.2684	0.291667	0.326957	0.347273	0.350476	0.3475	0.351053	0.348889	0.330588	0.31375	0.27	0.269286	0.294615	0.289167	0.299091	0.303	0.371111	0.3875	0.335714	0.345	0.414	0.5175	0.686667	1	1
	meandiff	0.0104511	0.0108685	0.0110605	0.0111366	0.0112798	0.0115507	0.0116361	0.0119561	0.0122573	0.0122976	0.0127027	0.0124553	0.0122332	0.0127888	0.013156	0.0133335	0.0138974	0.0141828	0.0145487	0.0152713	0.0161897	0.0163555	0.0172672	0.0179059	0.0199917	0.0223186	0.0260841	0.0271345	0.0344127
<i>tracecomp</i>	loglik																													
	effsize	14	16	17	18	20	20	20	21	21	23	28	28	37	45	49	66	75	135	114	128	4505	786	314	78	53	149	104	1013	24
	rel_diff	0.0237819	0.0320142	0.0073386	0.010538	0.0255308	0.0146867	0.0177809	0.0200572	0.0251359	0.0398688	0.0831141	0.108848	0.144892	0.132515	0.120286	0.121249	0.107498	0.0988783	0.0604895	0.0355846	0.0364018	0.0749745	0.136808	0.239009	0.436755	0.664787	0.582707	0.658607	0.872734
length	effsize	16	16	18	20	24	27	28	27	23	18	16	15	15	16	20	26	82	190	512	167	204	139	45	24	11	12	14	17	31
	rel_diff	0.59494	0.555494	0.489466	0.413552	0.329719	0.243072	0.164827	0.0597252	0.0200417	0.105142	0.104941	0.106852	0.0383206	0.0699563	0.207181	0.23078	0.216472	0.203566	0.0686059	0.149484	0.24305	0.249832	0.219007	0.312343	0.512441	0.514247	0.758884	0.993322	2.30998
alpha	effsize	53	41	35	33	33	32	28	24	22	21	22	24	35	90	163	588	550	290	170	104	144	55	64	34	18	15	15	22	16
	rel_diff	0.12267	0.108048	0.092388	0.0560424	0.0010899	0.0729768	0.137979	0.205846	0.230726	0.252406	0.220637	0.205472	0.148581	0.0638998	0.052673	0.0374147	0.0596116	0.0648472	0.136254	0.211651	0.353363	0.47467	0.494006	0.509218	0.579304	0.63402	0.650666	1.34864	1.90301
Nmode	effsize	228	302	249	229	194	164	174	160	133	120	102	98	71	64	60	50	43	38	30	29	26	21	17	15	13	30	105	45	70
	rel_diff	0.062392	0.0322627	0.004876	0.0203364	0.0218474	0.0029722	0.0386522	0.053204	0.0345625	0.0645703	0.102909	0.107747	0.107272	0.066812	0.0823712	0.0863115	0.0905955	0.0554393	0.0074636	0.120675	0.219382	0.20732	0.110389	0.0598444	0.134983	0.0737169	0.131529	0.254452	0.294139
statent	effsize	177	143	126	111	96	76	68	56	50	52	57	61	74	81	111	99	80	85	84	99	91	103	117	69	92	54	42	42	47
	rel_diff	0.0069321	0.0131955	0.0254085	0.030594	0.0267037	0.0139304	0.0058153	0.0238568	0.0254698	0.0061962	0.0470645	0.0866865	0.142818	0.180217	0.251579	0.349704	0.375596	0.364787	0.307754	0.201912	0.174011	0.0816814	0.0017654	0.0092998	0.0859938	0.26313	0.165437	0.402805	0.914176
statalpha	effsize	165	173	180	181	181	187	186	172	187	173	172	161	165	217	205	177	143	107	100	97	121	443	199	125	62	52	106	158	165
	rel_diff	0.198715	0.229603	0.24966	0.27	0.287918	0.309947	0.330729	0.329187	0.351968	0.360207	0.374682	0.432911	0.473093	0.509202	0.497247	0.495631	0.52686	0.553188	0.588172	0.621136	0.599971	0.537581	0.430173	0.373435	0.195902	0.0963556	0.156394		
rrent	effsize	101	107	118	123	122	119	114	112	121	151	163	135	106	70	72	109	176	337	214	365	441	498	360	247	126	159	141	34	
	rel_diff	0.0373999	0.0194528	0.0050169	0.0300272	0.0468288	0.0668202	0.0783548	0.0877798	0.117076	0.161445	0.179888	0.175834	0.173662	0.176637	0.140455	0.166859	0.173895	0.153114	0.127956	0.171687	0.115893	0.0349035	0.0278022	0.0607422	0.132688	0.174942	0.0008221	0.204277	0.140034
rrmean	effsize	9665	7438	4845	6332	6396	5523	4294	3953	3596	3682	2737	3884	3541	2946	895	2116	4004	2953	3608	5646	4426	2519	2457	3489	1399	2127	690	299	326
	rel_diff	0.0409238	0.0346827	0.0326419	0.0365511	0.0346441	0.0350336	0.0385501	0.0421432	0.0299041	0.0386215	0.040789	0.0241635	0.0207875	0.0342367	0.050845	0.0428012	0.0305661	0.0438794	0.0713107	0.0848356	0.0725597	0.103969	0.0754991	0.0490341	0.0222611	0.0662515	0.0643522	0.0017475	0.0854575

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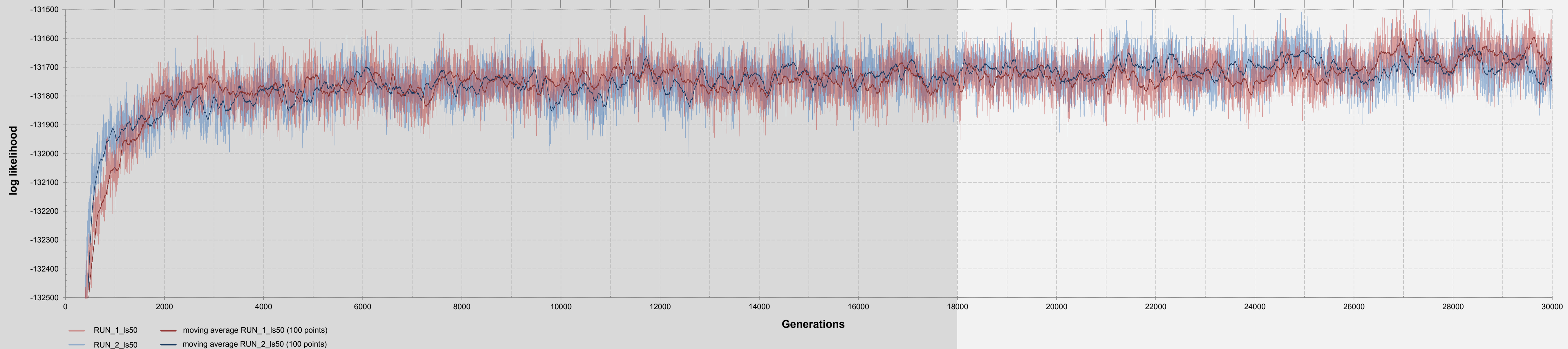


Figure S10. Assessment of chain convergence for two independently run Markov chains for 30,000 generations each on the pruned dataset LS50 (excluded taxa with leaf stability ≤ 0.50) using PhyloBayes MPI v1.4f. (A) Obtained summary statistics from the *bpcomp* and *tracecomp* programs of the PhyloBayes package, in dependency on the burn-in. Color coding and assessment for convergence following the guidelines of the PhyloBayes manual (*bpcomp*: maxdiff < 0.1 : good run, maxdiff < 0.3 : acceptable run, $0.3 < \text{maxdiff} < 1$: not converged; *tracecomp*: maxdiff < 0.1 and minimum effective size > 300 : good run, maxdiff < 0.3 and minimum effective size > 50 : acceptable run). According to this, we decided to discard the first 18,000 trees. (B) Traces of the log-likelihood scores for both chains. The moving average over 100 points is highlighted with a bold line.

A

		good			acceptable										bad															
		1000	2000	3000	4000	5000	6000	7000	8000	9000	10000	11000	12000	13000	14000	15000	16000	17000	18000	19000	20000	21000	22000	23000	24000	25000	26000	27000	28000	29000
<i>bpcomp</i>	burn-in																													
	maxdiff	0.273103	0.286071	0.282963	0.273462	0.2528	0.255833	0.276957	0.308182	0.295238	0.301	0.302105	0.32	0.298235	0.28125	0.261333	0.259286	0.262308	0.285833	0.343636	0.419	0.393333	0.38375	0.395714	0.416667	0.548	0.635	0.63	0.695	0.61
	meandiff	0.0104208	0.010345	0.0103392	0.0100695	0.0099627	0.0101575	0.0103081	0.0107091	0.0107733	0.0107825	0.0110704	0.0111804	0.0114978	0.0116109	0.0118667	0.0126462	0.013061	0.013533	0.0143955	0.0146153	0.0148385	0.0142961	0.0146444	0.014532	0.0162662	0.0178122	0.0190185	0.023924	0.030145
<i>tracecomp</i>	loglik																													
	effsize	24	51	114	112	79	67	75	93	114	149	232	201	211	147	129	122	106	104	82	69	72	81	122	164	112	109	142	94	51
	rel_diff	0.184214	0.166074	0.188046	0.168244	0.16248	0.145989	0.0898268	0.0568856	0.019022	0.0286931	0.0787531	0.0891936	0.149111	0.140452	0.163347	0.175663	0.219911	0.311178	0.348097	0.342912	0.279317	0.210993	0.120165	0.0413889	0.0752186	0.0512024	0.0255121	0.0269953	0.145787
length	effsize	33	51	67	99	113	214	409	424	418	354	282	213	143	124	127	107	102	65	54	124	186	138	96	185	296	198	158	189	35
	rel_diff	0.164156	0.144661	0.127026	0.103693	0.0369844	0.0138335	0.0048496	0.0234175	0.036614	0.0374792	0.0441695	0.0593014	0.0756194	0.0815619	0.0588745	0.0459217	0.0608338	0.0093364	0.0590438	0.0055116	0.168803	0.300597	0.333378	0.43381	0.552443	0.615486	0.71837	0.581245	0.49805
alpha	effsize	239	279	216	250	355	336	312	294	334	600	599	733	463	957	922	944	909	607	643	568	622	481	242	309	212	218	101	200	37
	rel_diff	0.0282	0.0502729	0.0547847	0.0831849	0.136464	0.183251	0.185696	0.224711	0.204626	0.186102	0.181723	0.16157	0.188199	0.178641	0.163819	0.157872	0.164795	0.161847	0.132846	0.128554	0.235925	0.237459	0.248436	0.20398	0.184695	0.0751227	0.073071	0.0324482	0.149687
Nmode	effsize	113	160	117	113	128	194	273	248	293	305	320	285	288	302	240	173	154	138	103	152	176	296	76	704	152	156	140	51	17
	rel_diff	0.597363	0.613999	0.635983	0.620903	0.602961	0.547335	0.485377	0.504773	0.483825	0.469604	0.451241	0.433558	0.383747	0.377108	0.382375	0.384283	0.370176	0.320008	0.277053	0.269519	0.298231	0.252598	0.198666	0.386053	0.448938	0.28502	0.218981	0.151693	0.453415
statent	effsize	180	184	197	239	207	150	113	145	159	141	151	154	140	107	98	79	95	139	163	159	129	86	121	124	89	58	83	82	405
	rel_diff	0.0467482	0.0688978	0.0860931	0.129296	0.15785	0.15259	0.165663	0.186465	0.174445	0.152293	0.1438	0.107092	0.109953	0.150786	0.126217	0.108946	0.0492713	0.0196057	0.0270609	0.028367	0.0721641	0.11059	0.190879	0.349064	0.398221	0.309814	0.372268	0.197466	0.223842
statalpha	effsize	113	166	168	207	254	248	214	209	202	185	188	184	221	268	392	643	521	804	1274	1784	967	544	376	243	192	228	130	151	331
	rel_diff	0.0033636	0.0314027	0.0215591	0.0393071	0.0657847	0.0487873	0.0242059	0.0184423	0.0183207	0.0157257	0.0045091	0.0168283	0.0456366	0.0584488	0.0979399	0.127749	0.151585	0.180957	0.224757	0.273623	0.247258	0.233284	0.254626	0.211922	0.196949	0.234193	0.251174	0.230008	0.36315
rrent	effsize	151	237	248	239	233	301	331	496	420	305	269	254	191	145	135	143	126	120	108	145	159	149	304	245	2581	273	182	91	23
	rel_diff	0.402483	0.408422	0.407051	0.402304	0.426692	0.394689	0.375511	0.357681	0.356412	0.365496	0.348028	0.31745	0.278419	0.312449	0.299781	0.277383	0.253492	0.239342	0.266522	0.260839	0.225059	0.146989	0.0363829	0.0176078	0.127489	0.0923387	0.0983439	0.0127663	0.110126
rrmean	effsize	4718	3434	3354	2947	4303	5325	5432	3573	4055	4324	3673	3173	2621	2083	1705	1760	1597	1227	923	2800	2419	2676	2380	2241	2954	1975	1350	387	668
	rel_diff	0.0170578	0.0243998	0.0223627	0.0215951	0.0096179	0.0040289	0.0099774	0.0159983	0.0092217	0.0015021	0.0011631	0.0001285	0.0020291	0.0174882	0.020949	0.008826	0.0143158	0.0136938	0.0001887	0.0044228	0.0421577	0.0682639	0.0872941	0.0834978	0.0882393	0.0825816	0.0548292	0.0433859	0.0288808

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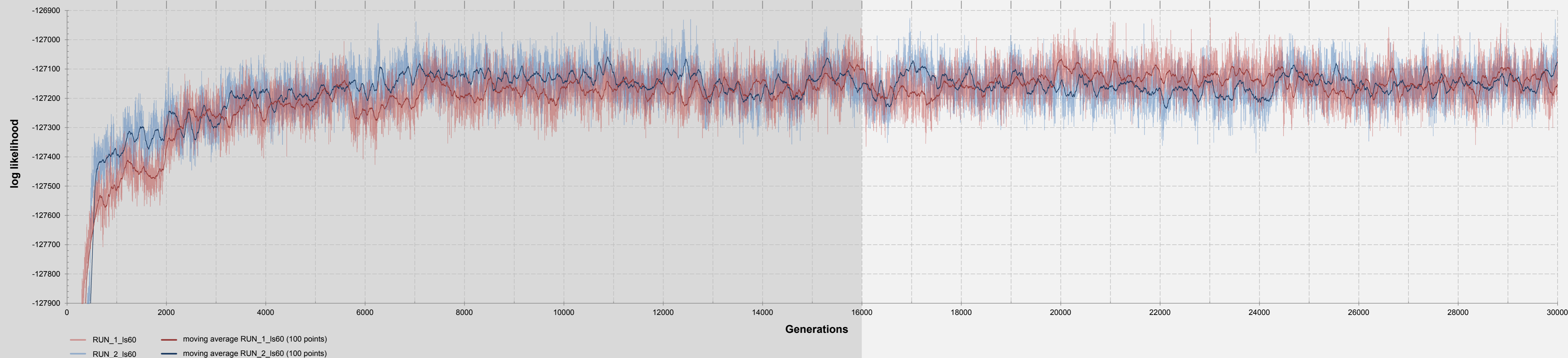


Figure S11. Assessment of chain convergence for two independently run Markov chains for 30,000 generations each on the pruned dataset LS60 (excluded taxa with leaf stability ≤ 0.60) using PhyloBayes MPI v1.4f. (A) Obtained summary statistics from the *bpcomp* and *tracecomp* programs of the PhyloBayes package, in dependency on the burn-in. Color coding and assessment for convergence following the guidelines of the PhyloBayes manual (*bpcomp*: maxdiff < 0.1 : good run, maxdiff < 0.3 : acceptable run, $0.3 < \text{maxdiff} < 1$: not converged; *tracecomp*: maxdiff < 0.1 and minimum effective size > 300 : good run, maxdiff < 0.3 and minimum effective size > 50 : acceptable run). According to this, we decided to discard the first 16,000 trees. (B) Traces of the log-likelihood scores for both chains. The moving average over 100 points is highlighted with a bold line.

Table S1. Summary statistics for transcriptome library assemblies using different quality filters and software packages. Filter15: reads with more than five bases below a quality score of 15 were removed; Filter25: reads with less than 20 bases in a row with a quality score of at least 25 were removed; Filter30: reads with less than 20 bases in a row with a quality score of at least 30 were removed. CLC: CLC Genomics Workbench v5.1; IDBA iso1: IDBA-Tran v1.1.0 allowing to retain one isoforms of a transcript; IDBA iso3: IDBA-Tran v1.1.0 allowing to retain three isoforms of a transcript.

Filter	Assembler	# reads	Giga-bases	% reads F15	# matched reads	% matched reads	# contigs	mean length	max length	total length	N50
Filter15	CLC	68,214,238	5.18	100.0	53,993,335	79.2	33,530	791	14,105	26,518,984	1,404
	IDBA iso1	68,214,238	5.18	100.0	39,404,978	57.8	29,288	1,186	20,533	34,741,313	1,858
	IDBA iso3	68,214,238	5.18	100.0	39,323,507	57.6	44,711	1,512	20,749	67,632,493	2,194
Filter25	CLC	42,382,860	3.22	62.1	33,519,856	79.1	31,386	781	14,315	24,506,359	1,354
	IDBA iso1	42,382,860	3.22	62.1	28,467,726	67.2	26,137	1,187	15,936	31,038,801	1,817
	IDBA iso3	42,382,860	3.22	62.1	28,369,455	66.9	40,516	1,496	15,936	60,614,950	2,151
Filter30	CLC	4,590,646	0.34	6.7	3,565,661	77.7	19,400	445	5,578	8,628,864	457
	IDBA iso1	4,590,646	0.34	6.7	2,706,624	59.0	12,908	646	6,267	8,348,433	710
	IDBA iso3	4,590,646	0.34	6.7	2,639,346	57.5	16,976	720	7,348	12,238,326	843

Table S2. GenBank accession numbers of opsin bait sequences used as BLAST queries.

Accession	Type	Name/Organism
AFM75824.1	r-opsin	onychopsin <i>Euperipatoides rowelli</i>
AAA02499.1	r-opsin	opsin <i>Limulus polyphemus</i>
AAA69069.1	r-opsin	long-wavelength rhodopsin <i>Apis mellifera</i>
BAG14332.1	r-opsin	kumopsin3 <i>Hasarius adansoni</i>
AAC26329.1	r-opsin	rhodopsin <i>Sepia officinalis</i>
CAC86665.1	r-opsin	rhabdomeric opsin <i>Platynereis dumerilii</i>
EFX83617.1	r-opsin	arthropsin1 <i>Daphnia pulex</i>
NP_150598.1	r-opsin	melanopsin isoform 1 <i>Homo sapiens</i>
AAV63834.1	c-opsin	ciliary opsin <i>Platynereis dumerilii</i>
AAA30674.1	c-opsin	rhodopsin <i>Bos taurus</i>
NP_001138950.1	c-opsin	c-opsin <i>Tribolium castaneum</i>
EFX86931.1	c-opsin	pteropsin4 <i>Daphnia pulex</i>
BAC76021.1	c-opsin	Amphiop4 <i>Branchiostoma belcheri</i>
BAJ22674.1	Group4 opsin	peropsin <i>Hasarius adansoni</i>
BAC76019.1	Group4 opsin	Amphiop1 <i>Branchiostoma belcheri</i>
FAA00384.1	“cnidopsin”	TPA: opsin <i>Nematostella vectensis</i>

Table S3. Bayesian cross-validation test. A 10-fold cross-validation with 10 replicates for each chosen model was performed. The cross-validation log-likelihood scores per model were averaged over the 10 replicates and compared with the reference model (LG). The site-heterogeneous CAT-GTR model fitted the data best in 8 out of the 10 replicates and was therefore used for Bayesian inference analyses. Negative values imply that the reference model (LG) fitted the data better than the tested model.

models compared	mean score +/- stdev	#times model is best
C20LG versus LG	-99.08 +/- 43.68	0
C30LG versus LG	-71.84 +/- 42.59	0
C40LG versus LG	-22.32 +/- 46.65	0
WLSR5LG versus LG	-19.12 +/- 21.10	0
C50LG versus LG	-11.39 +/- 40.74	0
LG versus LG	0 +/- 0	0
C20GTR versus LG	6.51 +/- 60.19	0
GTR versus LG	28.2 +/- 27.06	0
C60LG versus LG	28.7 +/- 43.24	0
WLSR5GTR versus LG	29.56 +/- 26.38	0
C30GTR versus LG	29.58 +/- 56.52	0
C40GTR versus LG	34.29 +/- 67.87	0
C50GTR versus LG	54.39 +/- 52.22	0
C60GTR versus LG	90.97 +/- 50.12	0
CATLG versus LG	291.69 +/- 36.69	2
CATGTR versus LG	308.15 +/- 36.85	8