

Figure S1. *In situ* hybridization of *rh1* in 3 dpf zebrafish embryos. Retinas from 3 dpf embryos were stained with the same 700 bp *rh1* probe used in all other experiments in this study both (A) with and (B) without the addition of full-length *rh1-2* blocking RNA, present at double the concentration of the *rh1* probe. No significant difference in staining was detected.

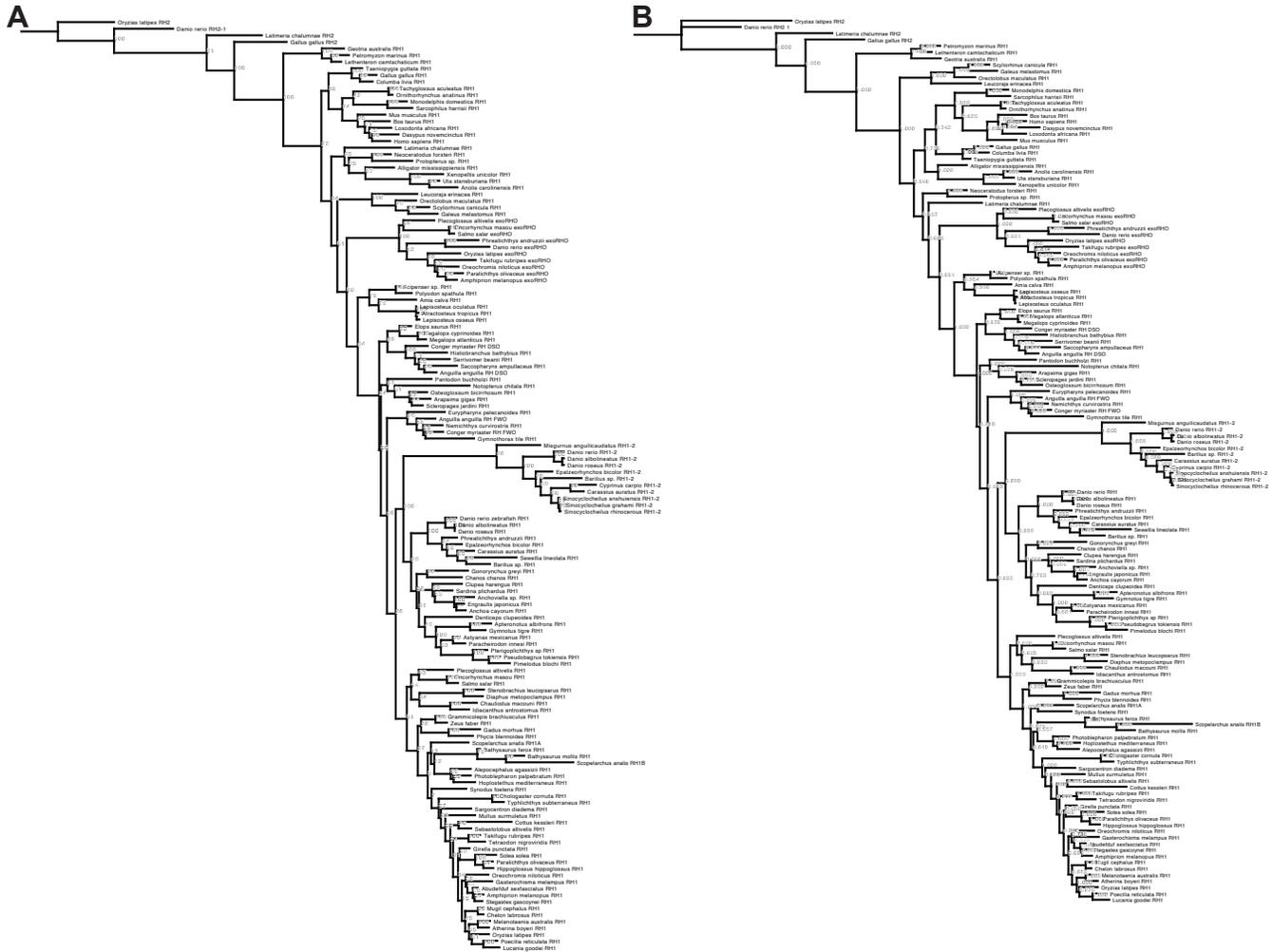


Figure S2. Detailed maximum-likelihood (A) and Bayesian phylogeny (B) of vertebrate rhodopsin genes. In both cases, high support was recovered for the placement of the *rh1-2* gene family as the sister group to Ostarioclupeomorpha. Several *rh2* opsin sequences were used as outgroups.

Table S1. List of sequences used in the phylogenetic and molecular evolutionary analyses of *rh1* genes.

Species Name	Common Name ¹	Gene	Accession/GI Number
<i>Amphiprion melanopus</i>	Fire clownfish	<i>exorh</i>	HM107820.1
<i>Danio rerio</i>	Zebrafish	<i>exorh</i>	71534272
<i>Oncorhynchus masou</i>	Masu salmon	<i>exorh</i>	478430872
<i>Oreochromis niloticus</i>	Nile tilapia	<i>exorh</i>	XM003438995.1
<i>Oryzias latipes</i>	Japanese medaka	<i>exorh</i>	XM004070540.1
<i>Paralichthys olivaceus</i>	Olive flounder	<i>exorh</i>	HM107825.1
<i>Phreatichthys andruzzii</i>	Cavefish	<i>exorh</i>	GQ404491.1
<i>Plecoglossus altivelis</i>	Ayu	<i>exorh</i>	28201134
<i>Salmo salar</i>	Atlantic salmon	<i>exorh</i>	185133057
<i>Takifugu rubripes</i>	Pufferfish	<i>exorh</i>	76362825
<i>Anguilla anguilla</i>	European eel	<i>rh1dso</i>	L78008.1
<i>Conger myriaster</i>	Whitespotted conger	<i>rh1dso</i>	12583666
<i>Anguilla anguilla</i>	European eel	<i>rh1fwo</i>	L78007.1
<i>Conger myriaster</i>	Whitespotted conger	<i>rh1fwo</i>	12583664
<i>Abudefduf sexfasciatus</i>	Scissortail sergeant	<i>rh1</i>	HQ286548.1
<i>Acipenser sp.</i>	Sturgeon	<i>rh1</i>	AF137206.1
<i>Alepocephalus agassizii</i>	Slickhead	<i>rh1</i>	JN544545.1
<i>Alligator mississippiensis</i>	American alligator	<i>rh1</i>	U23802.1
<i>Amia calva</i>	Bowfin	<i>rh1</i>	AF137208.1
<i>Amphiprion melanopus</i>	Fire clownfish	<i>rh1</i>	HM107824.1
<i>Anchoa cayorum</i>	Key anchovy marine	<i>rh1</i>	This study
<i>Anchoviella sp.</i>	Freshwater anchovy	<i>rh1</i>	This study
<i>Anolis carolinensis</i>	Carolina anole	<i>rh1</i>	XM003224879.1
<i>Apteronotus albifrons</i>	Black ghost knifefish	<i>rh1</i>	JN230983.1
<i>Arapaima gigas</i>	Arapaima	<i>rh1</i>	JN230972.1
<i>Astyanax mexicanus</i>	Mexican tetra	<i>rh1</i>	U12328.1
<i>Atherina boyeri</i>	Big-scale sand smelt	<i>rh1</i>	Y18676.1
<i>Atractosteus tropicus</i>	Tropical gar	<i>rh1</i>	JN230970.1
<i>Barilius sp.</i>	Barilius	<i>rh1</i>	This study
<i>Bathysaurus ferox</i>	Deepsea lizardfish	<i>rh1</i>	JN412585.1
<i>Bathysaurus mollis</i>	Highfin lizardfish	<i>rh1</i>	JN412586.1
<i>Bos taurus</i>	Cow	<i>rh1</i>	NM001014890.1
<i>Carassius auratus</i>	Golfish	<i>rh1</i>	L11863.1
<i>Chanos chanos</i>	Milfish	<i>rh1</i>	JN230981.1
<i>Chauliodus macouni</i>	Pacific viperfish	<i>rh1</i>	EU407250.1
<i>Chelon labrosus</i>	Thicklip grey mullet	<i>rh1</i>	Y18669.1
<i>Chologaster cornuta</i>	Swampfish	<i>rh1</i>	HQ729684.1
<i>Clupea harengus</i>	Atlantic herring	<i>rh1</i>	XM_012826141.1
<i>Columba livia</i>	Pigeon	<i>rh1</i>	4887218-4887219
<i>Cottus kessleri</i>	Kesslers sculpin	<i>rh1</i>	L42953.1
<i>Danio albolineatus</i>	Pearl danio	<i>rh1</i>	JQ614122.1

<i>Danio rerio</i>	Zebrafish	<i>rh1</i>	18859316:
<i>Danio roseus</i>	Rose danio	<i>rh1</i>	JQ614148.1
<i>Dasypus novemcinctus</i>	Armadillo	<i>rh1</i>	XM004477246.1
<i>Denticeps clupeoides</i>	Denticle herring	<i>rh1</i>	JN230976.1
<i>Diaphus metopoclampus</i>	Spothead lantern fish	<i>rh1</i>	JN544536.1
<i>Elops saurus</i>	Ladyfish	<i>rh1</i>	JN230971.1
<i>Engraulis japonicus</i>	Japanese anchovy	<i>rh1</i>	AB731902.1
<i>Epalzeorhynchus bicolor</i>	Redtailed black shark	<i>rh1</i>	HQ286332.1
<i>Eurypharynx pelecyanoides</i>	Pelican eel	<i>rh1</i>	JN544544.1
<i>Gadus morhua</i>	Atlantic cod	<i>rh1</i>	AF385832.1
<i>Galeus melastomus</i>	Blackmouth catshark	<i>rh1</i>	Y17586.1
<i>Gallus gallus</i>	Chicken	<i>rh1</i>	NM001030606.1
<i>Gasterochisma melampus</i>	Butterfly kingfish	<i>rh1</i>	DQ882021.1
<i>Geotria australis</i>	Pouched lamprey	<i>rh1</i>	AY366493.1
<i>Girella punctata</i>	Largescale blackfish	<i>rh1</i>	AB158262.3
<i>Gnorhynchus greyi</i>	Beaked salmon	<i>rh1</i>	EU409632
<i>Grammicolepis brachiusculus</i>	Thorny tinsselfish	<i>rh1</i>	EU637964.1
<i>Gymnothorax tile</i>	Moray eel	<i>rh1</i>	This study
<i>Gymnotus tigre</i>	Tiger knifefish	<i>rh1</i>	This study
<i>Hippoglossus hippoglossus</i>	Atlantic halibut	<i>rh1</i>	AF156265.1
<i>Histiobranchus bathybius</i>	Deepwater arrowtooth eel	<i>rh1</i>	JN544542.1
<i>Homo sapiens</i>	Human	<i>rh1</i>	169808383
<i>Hoplostethus mediterraneus</i>	Mediterranean slimehead	<i>rh1</i>	JN412583.1
<i>Idiacanthus antrostomus</i>	Pacific blackdragon	<i>rh1</i>	EU407249.1
<i>Latimeria chalumnae</i>	Coelacanth	<i>rh1</i>	4836673-4836677
<i>Lepisosteus oculatus</i>	Spotted gar	<i>rh1</i>	This study
<i>Lepisosteus osseus</i>	Longnose gar	<i>rh1</i>	AF137207.1
<i>Lethenteron camtschaticum</i>	Artic lamprey	<i>rh1</i>	46917273
<i>Leucoraja erinacea</i>	Little skate	<i>rh1</i>	U81514.1
<i>Loxodonta africana</i>	Elephant	<i>rh1</i>	344275992
<i>Lucania goodei</i>	Bluefin killifish	<i>rh1</i>	AY296738.1
<i>Megalops atlanticus</i>	Atlantic tarpon	<i>rh1</i>	AY158050
<i>Megalops cyprinoides</i>	Indo-Pacific tarpon	<i>rh1</i>	This study
<i>Melanotaenia australis</i>	Western rainbowfish	<i>rh1</i>	FJ940704.1
<i>Monodelphis domestica</i>	Short-tailed opossum	<i>rh1</i>	XM001366188.1
<i>Mugil cephalus</i>	Flathead mullet	<i>rh1</i>	4210736
<i>Mullus surmuletus</i>	Striped red mullet	<i>rh1</i>	4210732
<i>Mus musculus</i>	Mouse	<i>rh1</i>	NM_145383.1
<i>Nemichthys curvirostris</i>	Boxer snipe eel	<i>rh1</i>	This study
<i>Neoceratodus forsteri</i>	Lungfish	<i>rh1</i>	EF526295.1
<i>Notopterus chitala</i>	Clown knifefish	<i>rh1</i>	This study
<i>Oncorhynchus masou</i>	Masu salmon	<i>rh1</i>	478430870
<i>Orectolobus maculatus</i>	Spotted wobbegong	<i>rh1</i>	JX534163.1
<i>Oreochromis niloticus</i>	Niles tilapia	<i>rh1</i>	348502996
<i>Ornithorhynchus anatinus</i>	Platypus	<i>rh1</i>	NM001127627.1

<i>Oryzias latipes</i>	Japanese medaka	<i>rh1</i>	66796119
<i>Osteoglossum bicirrhosum</i>	Silver arowana	<i>rh1</i>	This study
<i>Pantodon buchholzi</i>	Freshwater butterflyfish	<i>rh1</i>	AF137210.1
<i>Paracheirodon innesi</i>	Neon tetra	<i>rh1</i>	84095053
<i>Paralichthys olivaceus</i>	Olive flounder	<i>rh1</i>	HQ413772.1
<i>Petromyzon marinus</i>	Sea lamprey	<i>rh1</i>	1513320-1513324
<i>Photoblepharon palpebratum</i>	Eyelightfish	<i>rh1</i>	EU637993.1
<i>Phreatichthys andruzzii</i>	Cavefish	<i>rh1</i>	JQ413240.1
<i>Phycis blennoides</i>	Greater forkbeard	<i>rh1</i>	JN412579.1
<i>Pimelodus blochii</i>	Blochs catfish	<i>rh1</i>	This study
<i>Plecoglossus altivelis</i>	Ayu	<i>rh1</i>	19912833
<i>Poecilia reticulata</i>	Guppy	<i>rh1</i>	DQ912024.1
<i>Polyodon spathula</i>	American paddlefish	<i>rh1</i>	AF369050.1
<i>Protopterus</i> sp.	African lungfish	<i>rh1</i>	AF369054.1
<i>Pseudobagrus tokiensis</i>	Balgrid	<i>rh1</i>	FJ197075.1
<i>Pterigoplichthys</i> sp.	Armored catfish	<i>rh1</i>	This study
<i>Saccopharynx ampullaceus</i>	Gulper eel	<i>rh1</i>	This study
<i>Salmo salar</i>	Atlantic salmon	<i>rh1</i>	185133085
<i>Sarcophilus harrisi</i>	Tasmanian devil	<i>rh1</i>	395516643
<i>Sardina pilchardus</i>	Sardine	<i>rh1</i>	Y18677.1
<i>Sargocentron diadema</i>	Crown squirrelfish	<i>rh1</i>	U57537.1
<i>Scleropages jardini</i>	Gulf saratoga	<i>rh1</i>	This study
<i>Scyliorhinus canicula</i>	Small-spotted catshark	<i>rh1</i>	3256019
<i>Sebastolobus altivelis</i>	Longspine thornyhead	<i>rh1</i>	DQ490124.1
<i>Serrivomer beanii</i>	Sawtooth eel	<i>rh1</i>	This study
<i>Sewellia lineolata</i>	Tiger hillstream loach	<i>rh1</i>	This study
<i>Solea solea</i>	Common sole	<i>rh1</i>	4210866
<i>Stegastes gascoynei</i>	Coral Sea gregory damselfish	<i>rh1</i>	HQ286557.1
<i>Stenobranchius leucopsarus</i>	Northern lampfish	<i>rh1</i>	EU407251.1
<i>Synodus foetens</i>	Inshore lizardfish	<i>rh1</i>	JN231001.1
<i>Tachyglossus aculeatus</i>	Echidna	<i>rh1</i>	398018454
<i>Taeniopygia guttata</i>	Zebra finch	<i>rh1</i>	115529257
<i>Takifugu rubripes</i>	Pufferfish	<i>rh1</i>	118344635
<i>Tetraodon nigroviridis</i>	Green spotted puffer	<i>rh1</i>	AJ293018.1
<i>Typhlichthys subterraneus</i>	Southern cavefish	<i>rh1</i>	HQ729699.1
<i>Uta stansburiana</i>	Side-blotched lizard	<i>rh1</i>	DQ100323.1
<i>Xenopeltis unicolor</i>	Sunbeam snake	<i>rh1</i>	FJ497233.1
<i>Zeus faber</i>	John dory	<i>rh1</i>	Y14484.1
<i>Barilius</i> sp.	Barilius	<i>rh1-2</i>	This study
<i>Carassius auratus</i>	Goldfish	<i>rh1-2</i>	This study
<i>Cyprinus carpio</i>	Common carp	<i>rh1-2</i>	Extracted from genome
<i>Danio albolineatus</i>	Pearl danio	<i>rh1-2</i>	HQ286328
<i>Danio rerio</i>	Zebrafish	<i>rh1-2</i>	HQ286326
<i>Danio roseus</i>	Rose danio	<i>rh1-2</i>	HQ286327
<i>Epalzeorhynchus bicolor</i>	Redtailed black shark	<i>rh1-2</i>	HQ286329

<i>Misgurnus anguilicaudatus</i>	Dojo loach	<i>rh1-2</i>	This study
<i>Sinocyclocheilus anshuiensis</i>	Tibetan cavefish	<i>rh1-2</i>	XM_016497364.1
<i>Sinocyclocheilus grahami</i>	Golden-line barbel	<i>rh1-2</i>	XM_016233062.1
<i>Sinocyclocheilus rhinoceros</i>	Tibetan cavefish	<i>rh1-2</i>	XM_016524871.1
<i>Scopelarchus analis</i>	Pearleye	<i>rh1A</i>	EF517404.1
<i>Scopelarchus analis</i>	Pearleye	<i>rh1B</i>	EF517405.1
<i>Gallus gallus</i>	Chicken	<i>rh2</i>	45382766
<i>Latimeria chalumnae</i>	Coelacanth	<i>rh2</i>	4836680-4836684
<i>Oryzias latipes</i>	Japanese medaka	<i>rh2</i>	86198067
<i>Danio rerio</i>	Zebrafish	<i>rh2-1</i>	42476236

Table S2. Results of random sites (PAML) analyses on subsets of the vertebrate *rh1* gene tree.

Tree ¹	Model and Partition	np	ln L	κ	Parameters ²			Null	LRT	df	p
					ω_0/p	ω_1/q	ω_2/ω_p				
Vert	M0	281	-49103.07	1.90	0.07			n/a			
	M1a	282	-48490.32	1.99	0.06 (92.7%)	1 (7.3%)		M0	1225.50	1	0.0000
	M2a	284	-48490.32	1.99	0.06 (92.7%)	1 (4.2%)	1 (3.1%)	M1a	0.00	2	1.0000
	M3	285	-47211.50	1.87	0.01 (54.8%)	0.11 (35.5%)	0.37 (9.7%)	M0	3783.13	4	0.0000
	M7	282	-47137.43	1.87	0.383	3.014		n/a			
	M8a	283	-47124.15	1.88	0.420	4.196	1 (0.8%)	n/a			
	M8	284	-47124.15	1.88	0.420	4.196	1 (0.8%)	M7 M8a	26.56 0.00	2 1	0.0000 1.0000
Acti	M0	221	-35771.32	1.99	0.08			n/a			
	M1a	222	-35181.81	2.10	0.06 (92.3%)	1 (7.7%)		M0	1179.02	1	0.0000
	M2a	224	-35181.81	2.10	0.06 (92.3%)	1 (3.6%)	1 (4.1%)	M1a	0.00	2	1.0000
	M3	225	-34324.43	1.97	0.01 (56.5%)	0.12 (33.9%)	0.41 (9.6%)	M0	2893.79	4	0.0000
	M7	222	-34262.33	1.97	0.32	2.35		n/a			
	M8a	223	-34245.81	1.97	0.36	3.27	1 (0.8%)	n/a			
	M8	224	-34245.81	1.97	0.36	3.27	1 (0.8%)	M7 M8a	33.04 0.00	2 1	0.0000 0.9984
<i>rh1-2</i>	M0	21	-3541.57	2.48	0.09			n/a			
	M1a	22	-3500.77	2.63	0.03 (89.4%)	1 (10.6%)		M0	81.60	1	0.0000
	M2a	24	-3500.77	2.63	0.03 (89.4%)	1 (7.9%)	1 (2.7%)	M1a	0.00	2	1.0000
	M3	25	-3488.86	2.46	0.00 (72.5%)	0.32 (26.9%)	3.08 (0.6%)	M0	105.41	4	0.0000
	M7	22	-3491.58	2.50	0.13	1.10		n/a			
	M8a	23	-3491.00	2.50	0.16	1.70	1 (2%)	n/a			
	M8	24	-3489.65	2.49	0.15	1.41	3.29 (0.5%)	M7 M8a	3.87 2.70	2 1	0.1442 0.1004

¹The full vertebrate *rh1* (Vert) and gene trees pruned to contain only actinopterygian rhodopsins (Acti) and only *rh1-2*.

² ω values of each site class are shown for models M0-M3 (ω_0 – ω_2) with the proportion of each site class in parentheses. For M7-M8, the shape parameters, p and q, which describe the beta distribution are listed. In addition, the ω value for the positively selected site class (ω_p , with the proportion of sites in parentheses) is shown for M8a (where ω_p is constrained to equal one) and M8.

Abbreviations—**np**, number of parameters; **lnL**, ln Likelihood; **κ** , transition/transversion ratio; **LRT**, likelihood ratio test statistic; **df**, degrees of freedom; **p**, p-value; **n/a**, not applicable.

Table S3. Results of branch and branch-site (PAML) analyses with the branch leading to the *rh1-2* clade placed into the foreground.

Partition	Model	np	ln L	κ	Parameters ¹				LRT	df	p
					ω_0	ω_1	ω_{2a}	ω_{2b}			
M0	Null	221	-35771.32	1.99	0.08						
Branch	Alt	222	-35770.90	1.99	B: 0.09 F: 0.06				0.84	1	0.3607
Branch-site	Null	223	-35175.73	2.10	B: 0.06 F: 0.06 (85.4%)	B: 1 F: 1 (7.2%)	B: 0.06 F: 1 (6.8%)	B: 1 F: 1 (0.6%)			
Branch-site	Alt	224	-35174.10	2.10	B: 0.06 F: 0.06 (88.1%)	B: 1 F: 1 (7.5%)	B: 0.06 F: 31.22 (4%)	B: 1 F: 31.22 (0.4%)	3.25	1	0.0713

¹ ω values of each site class are shown with the proportion of each site class in parentheses. B and F refer to the background and foreground partitions.

Abbreviations—**np**, number of parameters; **ln L**, ln Likelihood; κ , transition/transversion ratio; **LRT**, likelihood ratio test statistic; **df**, degrees of freedom; **p**, *p*-value; **n/a**, not applicable.

Table S4. Results of branch, branch-site, and clade model C (PAML) analyses with the *rh1-2* clade placed into the foreground.

Partition	Model	np	ln L	κ	Parameters ¹				LRT	df	p
					ω_0	ω_1	ω_{2a}/ω_d	ω_{2b}			
M0	Null	221	-35771.32	1.99	0.08						
Branch	Alt	222	-35771.23	1.99	B: 0.08 F: 0.07				0.18	1	0.6735
Branch-site	Null	223	-35150.11	2.10	B: 0.06 F: 0.06 (85.8%)	B: 1 F: 1 (7.2%)	B: 0.06 F: 1 (6.5%)	B: 1 F: 1 (0.5%)			
Branch-site	Alt	224	-35150.11	2.10	B: 0.06 F: 0.06 (85.8%)	B: 1 F: 1 (7.2%)	B: 0.06 F: 1 (6.5%)	B: 1 F: 1 (0.5%)	0.00	1	0.9901
M2a_rel	Null	224	-34416.38	2.03	0.01 (60.8%)	1 (3.1%)	0.16 (36.1%)				
CmC	Alt	225	-34416.36	2.03	B: 0.01 F: 0.01 (60.8%)	B: 1 F: 1 (3.1%)	B: 0.16 F: 0.16 (36.1%)		0.04	1	0.8437

¹ ω values of each site class are shown with the proportion of each site class in parentheses. B and F refer to the background and foreground partitions.

Abbreviations—**np**, number of parameters; **ln L**, ln Likelihood; κ , transition/transversion ratio; **LRT**, likelihood ratio test statistic; **df**, degrees of freedom; **p**, *p*-value; **n/a**, not applicable