Supplementary information

Relaxed selective constraints drove functional modifications in peripheral photoreception of the cavefish *P. andruzzii* and provide insight into the time of cave colonization

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Supplementary Tables

Supplementary Table 1. Ensembl or GenBank IDs of the sequences used in the molecular evolution analysis

	Melanopsin, *opn4m2*	**Rhodopsin, *rho***
Danio rerio	ENSDARG00000007553	ENSDARG00000002193
Cyprinus carpio	LN590693.1 (Region 5073779..5074962)	U02475.1
Rutilus rutilus	AY226847.1	
Carassius auratus		L11863.1
Astyanax mexicanus	XM_007229310.2	U12328.1
Clupea harengus	XM_012825665.1	XM_012826141.1
Ictalurus punctatus	FJ839438.1	
Esox lucius	XM_010895490.1	XM_010902101.2
Plecoglossus altivelis		AB074484.1

Supplementary Table 2. Melanopsin (A) and rhodopsin (B) variable positions in *P.andruzzi* and *G. barreimiae* individuals of this study (position 1 = A from the ATG starting codon). Amino acid changes are indicated as interspecific differences, not as ancestral vs derived

A																
	1	1	2	2	2	4	4	4	5	6	6	6	7	7	**8**[a]	**8 -8**
	0	7	0	4	9	2	6	8	5	0	1	3	3	3	**1**	**8 -8**
	7	2	6	6	1	0	8	4	2	5	4	1	2	5	**5**	**6 -8**
PA_GQ404489.1 (Cavallari et al. 2011)	A	G	T	T	C	C	C	T	G	A	T	C	T	T	TAAGGTGTATGAACGCATGCAGAACGAATGGAAGATGCCGAAAGTCGCCCTCCTGGTCATTGTGCTTTTTG**TAA**	
PA07	.	.	.	.	.	.	.	.	.	.	.	.	.	.	TAAGGTGTATGAACGCATGCAGAACGAATGGAAGATGCCGAAAGTCGCCCTCCTGGTCATTGTGCTTTTTG**TAA**	
PA11	.	.	.	.	.	.	.	.	G/T	.	.	.	.	.	TAAGGTGTATGAACGCATGCAGAACGAATGGAAGATGCCGAAAGTCGCCCTCCTGGTCATTGTGCTTTTTG**TAA**	
Gbar215 (surface)	G	G/A	C	C	C/T	T	T	C	.	G	C	.	A	C	ATAAGGTGTATGAACGCATGCAGAACGAATGGAAGATGGCGAAAGTCGCCCTCCTGGTCATTGTGCTTTTTGTA	
Gbar216 (surface)	G	.	C	C	.	T	T	C	.	G	C	.	A	C	ATAAGGTGTATGAACGCATGCAGAACGAATGGAAGATGGCGAAAGTCGCCCTCCTGGTCATTGTGCTTTTTGTA	
Gbar223	G	.	C	C	.	.	T	C	.	G	C	.	A	C	ATAAGGTGTATGAACGCATGCAGAACGAATGGAAGATGGCGAAAGTCGCCCTCCTGGTCATTGTGCTTTTTGTA	
Gbar224	G	.	C	C	.	.	T	C	.	G	C	.	A	C	ATAAGGTGTATGAACGCATGCAGAACGAATGGAAGATGGCGAAAGTCGCCCTCCTGGTCATTGTGCTTTTTGTA	
Gbar284 (surface)	G	.	C	C	.	.	T	C	.	G	C	T	A	C	ATAAGGTGTATGAACGCATGCAGAACGAATGGAAGATGGCGAAAGTCGCCCTCCTGGTCATTGTGCTTTTTGTA	
nonsynonymous	H36R		F69S					C162R		D202G		L211F			H272L	**296: stop codon in *P. andruzzii***
		D58N									V205A					

																					1	*1*	*1*
	1	1	2	2	2	4	4	4	5	6	6	6	7	7	**8**[a]	*8*	*9*	*9*	*9*	*9*	*0*	*0*	*1*
	0	7	0	4	9	2	6	8	5	0	1	3	3	3	**1**	*5*	*0*	*0*	*1*	*3*	*2*	*3*	*1*
	7	2	6	6	1	0	8	4	2	5	4	1	2	5	**5**	*3*	*3*	*9*	*0*	*6*	*8*	*2*	*3*
PA_GQ404489.1 (Cavallari et al. 2011)	A	G	T	T	C	C	C	T	G	A	T	C	T	T	**-**	*C*	*C*	*T*	*G*	*G*	*A*	*T*	*T*
PA07	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**-**	*.*	*T*	*C*	*.*	*.*	*.*	*.*	*.*
PA11	.	.	.	.	.	.	.	.	G/T	.	.	.	.	.	**-**	*.*	*T*	*C*	*.*	*.*	*.*	*.*	*.*
Gbar215 (surface)	G	G/A	C	C	C/T	T	T	C	.	G	C	.	A	C	**A**	*G*	*T*	*C*	*A*	*C*	*C*	*C*	*C*
Gbar216 (surface)	G	.	C	C	.	T	T	C	.	G	C	.	A	C	**A**	*G*	*T*	*C*	*A*	*C*	*C*	*C*	*C*
Gbar223	G	.	C	C	.	.	T	C	.	G	C	.	A	C	**A**	*G*	*T*	*C*	*A*	*C*	*C*	*C*	*C*
Gbar224	G	.	C	C	.	.	T	C	.	G	C	.	A	C	**A**	*G*	*T*	*C*	*A*	*C*	*C*	*C*	*C*
Gbar284 (surface)	G	.	C	C	.	.	T	C	.	G	C	T	A	C	**A**	*G*	*T*	*C*	*A*	*C*	*C*	*C*	*C*
nonsynonymous	H36R		F69S					C162R		D202G		L211F				*P285A*			*V304M*		*K343T*		
		D58N									V205A												

[a] deletion causing a frameshift leading to a premature stop codon after 14 amino acids (protein position 296)

in italics: alignment obtained when the frame is restored by shifting one base in *P. andruzzii* sequences as if the deletion did not occur

B																															
																						1	1	1	1	1	1	1	1	1	1
			1	1	1	3	3	3	4	4	4	5	6	7	7	7	8	8	8	9	9	0	0	0	0	0	0	0	0	0	0
	3	5	1	2	7	2	2	4	1	2	7	0	4	3	4	7	1	4	6	1	3	0	3	4	4	4	4	4	4	4	4
	0	5	2	3	4	0	5	8	0	6	2	4	8	8	2	4	1	2	0	1	2	2	9	0	1	2	3	4	5	6	7
PA_JQ413240 (Tarttelin et al. 2012)	T	G	G	G	T	A	G	T	C	C	A	T	A	A	T	A	A	G	G	T	G	T	G	T	G	T	C	G	T	C	C
PA07	.	.	.	.	.	.	.	C	.	.	.	.	.	.	C	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.
PA11	.	.	A	.	.	.	A	C	.	T	.	.	.	.	C	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.
Gbar215 (surface)	C	A	.	A	C	G	.	C	T	.	G	C	C	C	C	G	G	C	T	G	A	C	-	-	-	-	-	-	-	-	-
Gbar216 (surface)	C	A	.	A	C	G	.	C	T	.	G	C	C	C	C	G	G	C	T	G	A	C	-	-	-	-	-	-	-	-	-
Gbar223	C	A	.	A	C	G	.	C	T	.	G	C	C	C	C	G	G	C	T	G	A	C	-	-	-	-	-	-	-	-	-
Gbar224	C	A	.	A	C	G	.	C	T	.	G	C	C	C	C	G	G	C	T	G	A	C	-	-	-	-	-	-	-	-	-
Gbar284 (surface)	C	A	.	A	C	G	.	C	T	.	G	C	C	C	C	G	G	C	T	G	A	C	-	-	-	-	-	-	-	-	-
nonsynonymous		I19V	A38T			H107R	G109S		T137M		T158A				C248R		M271V		C287F		R311K					SSV 347,348,349					
																		S281T		F304C											

Supplementary Table 3. Fossil constraints used in the Phylobayes analysis. Each constraint calibrates the age of the last common ancestor of the two species reported in the table.

Species 1	Species 2	age (MYA)	reference
Danio rerio	*Squalius albus*	55.8 (maximum)	[a] calib. (i)
Tinca tinca	*Leuciscus leuciscus*	18.0 (maximum)	[a] calib. (ii)
Hypophthalmichthys molitrix	*Aristichthys nobilis*	1.81 (minimum)	[a] calib. (iii)
Chitala ornata	*Gymnarchus niloticus*	99.6 (minimum)	[b] calib. (5)
Cromeria nilotica	*Chanos chanos*	133.9 (minimum)	[b] calib. (6)
Ameiurus natalis	*Cranoglanis bouderius*	59.0 (minimum)	[b] calib. (7)
Ictiobus bubalus	*Hypentelium nigricans*	49.4 (minimum)	[b] calib. (8)
Novumbra hubbsi	*Esox americanus*	76.4 (minimum)	[b] calib. (9)
Coregonus clupeaformis	*Salvelinus alpinus*	51.8 (minimum)	[b] calib. (10)
Aphredoderus sayanus	*Polymixia japonica*	93.6 (minimum)	[b] calib. (11)
Zeus faber	*Stylephorus chordatus*	70.6 (minimum)	[b] calib. (14)
Monocentris japonica	*Barbourisia rufa*	93.6 (minimum)	[b] calib. (17)
Seriola dumerili	*Trachinotus carolinus*	55.8 (minimum)	[b] calib. (23)
Echeneis naucrates	*Rachycentron canadum*	30.1 (minimum)	[b] calib. (24)
Luvarus imperialis	*Zanclus cornutus*	55.8 (minimum)	[b] calib. (25)
Scatophagus argus	*Siganus spinus*	55.8 (minimum)	[b] calib. (26)
Heteromycteris japonicus	*Samariscus latus*	40.4 (minimum)	[b] calib. (28)
Chelmon rostratus	*Gazza minuta*	30.1 (minimum)	[b] calib. (30)
Diodon holocanthus	*Tetraodon miurus*	50.0 (minimum)	[b] calib. (33)
Ambloplites rupestris	*Archoplites interruptus*	15.5 (minimum)	[b] calib. (36)

[a] Wang et al. 2012

[b] Near et al. 2012

MYA: million years ago

Supplementary Table 4. Species, family and GenBank accession numbers of the *cytb* sequences included in the dataset analyzed with Phylobayes.

Species	Family	GenBank ID
Phreatichthys andruzzii	Cyprinidae	present study
Garra barreimiae	Cyprinidae	present study
Garra rufa	Cyprinidae	AF180857.1
Garra waterloti	Cyprinidae	JX074289.1
Garra lamta	Cyprinidae	JX074239.1
Garra nasuta	Cyprinidae	JX074295.1
Iranocypris typhlops	Cyprinidae	KF896299.1
Danio rerio	Cyprinidae	NC_002333.2
Opsariichthys uncirostris	Cyprinidae	AF308437.1
Tinca tinca	Cyprinidae	Y10451.1
Leuciscus leuciscus	Cyprinidae	AY509823.1
Hypophthalmichthys molitrix	Cyprinidae	AF051866.1
Aristichthys nobilis	Cyprinidae	AF051855.1
Ctenopharyngodon idella	Cyprinidae	AF051860.1
Pseudorasbora parva	Cyprinidae	AF051873.1
Barbus barbus	Cyprinidae	Y10450.1
Squalius cephalus	Cyprinidae	EU856046.1
Squalius albus	Cyprinidae	AY549460.1
Achondrostoma occidentale	Cyprinidae	KF529123.1
Achondrostoma oligolepis	Cyprinidae	KF529120.1
Pseudochondrostoma willkommii	Cyprinidae	KF529129.1
Pseudochondrostoma polylepis	Cyprinidae	KF529127.1
Scardinius acarnicus	Cyprinidae	AF090775.1
Scardinius erythrophthalmus	Cyprinidae	AY509848.1
Leuciscus idus	Cyprinidae	HM560099.1
Phoxinus phoxinus	Cyprinidae	Y10448.1
Naso lituratus	Acanthuridae	AB276964.1
Oryzias latipes	Adrianichthyidae	NC_004387.1

Alepocephalus agassizii	Alepocephalidae	NC_013564.1
Phenacogrammus interruptus	Alestidae	AB018998.1
Amia calva	Amiidae	AB018999.1
Anguilla rostrata	Anguillidae	AB021767.1
Antennarius striatus	Antennariidae	AB282828.1
Aphredoderus sayanus	Aphredoderidae	NC_004372.1
Apteronotus albifrons	Apteronotidae	NC_004692.1
Heterotis niloticus	Arapaimidae	AB035240.1
Arapaima gigas	Arapaimidae	AB035241.1
Argentina silus	Argentinidae	EU492323.1
Ateleopus japonicus	Ateleopodidae	NC_003178.1
Barbourisia rufa	Barbourisiidae	NC_012046.1
Rheocles wrightae	Bedotiidae	KC133646.1
Beryx decadactylus	Berycidae	DQ197929.1
Caranx crysos	Carangidae	EF392575.1
Seriola dumerili	Carangidae	EU036499.1
Trachinotus carolinus	Carangidae	NC_024184.1
Hypentelium nigricans	Catostomidae	AF454909.1
Ictiobus bubalus	Catostomidae	JF799443.1
Micropterus salmoides	Centrarchidae	AF479273.1
Lepomis macrochirus	Centrarchidae	NC_015984.2
Ambloplites rupestris	Centrarchidae	AY115978.1
Archoplites interruptus	Centrarchidae	AY225665.1
Macroramphosus scolopax	Centriscidae	NC_010265.1
Aeoliscus strigatus	Centriscidae	NC_010270.1
Centropomus undecimalis	Centropomidae	AF240739.1
Chelmon rostratus	Chaetodontidae	NC_025953.1
Chanos chanos	Chanidae	NC_004693.1
Astyanax mexicanus	Characidae	FJ439346.1
Paratilapia polleni	Cichlidae	NC_011170.1
Alosa pseudoharengus	Clupeidae	NC_009576.1

Heteroconger hassi	Congridae	NC_013629.1
Coryphaena hippurus	Coryphaenidae	DQ197936.1
Cranoglanis bouderius	Cranoglanididae	NC_008280.1
Denticeps clupeoides	Denticiptidae	NC_007889.1
Diodon holocanthus	Diodontidae	NC_009866.1
Echeneis naucrates	Echeneidae	KF021242.1
Elassoma zonatum	Elassomatidae	NC_011388.1
Elops saurus	Elopidae	NC_005803.1
Esox lucius	Esocidae	HM177470.1
Esox americanus	Esocidae	AY497436.1
Fistularia petimba	Fistulariidae	NC_024190.1
Brachygalaxias bullocki	Galaxiidae	JQ697797.1
Galaxias maculatus	Galaxiidae	NC_004594.1
Gasterosteus aculeatus	Gasterosteidae	NC_003174.1
Gasterosteus wheatlandi	Gasterosteidae	NC_011570.1
Lepidogobius lepidus	Gobiidae	KF415602.1
Gonorynchus greyi	Gonorynchidae	NC_004702.1
Gymnarchus niloticus	Gymnarchidae	NC_012707.1
Electrophorus electricus	Gymnotidae	AP011978.1
Aldrovandia affinis	Halosauridae	NC_005801.1
Helostoma temminckii	Helostomatidae	NC_022728.1
Hiodon alosoides	Hiodontidae	NC_005145.1
Hiodon tergisus	Hiodontidae	NC_015082.1
Hypoptychus dybowskii	Hypoptychidae	NC_004400.1
Ameiurus natalis	Ictaluridae	AF484158.1
Cromeria nilotica	Kneriidae	AP011560.1
Kurtus gulliveri	Kurtidae	NC_022477.1
Lampris guttatus	Lampridae	JF931966.1
Lates niloticus	Latidae	AB117106.1
Gazza minuta	Leiognathidae	NC_026232.1
Lophius americanus	Lophiidae	NC_004380.1

Lota lota	Lotidae	DQ174052.1
Luvarus imperialis	Luvaridae	NC_009851.1
Megalops atlanticus	Megalopidae	NC_005804.1
Mene maculata	Menidae	AB355909.1
Nansenia ardesiaca	Microstomatidae	NC_004596.1
Ranzania laevis	Molidae	NC_007887.1
Masturus lanceolatus	Molidae	NC_005837.1
Mola mola	Molidae	NC_005836.1
Monocentris japonica	Monocentridae	NC_004392.1
Gnathonemus petersii	Mormyridae	NC_012717.2
Morone chrysops	Moronidae	AF240745.1
Gymnoscopelus nicholsi	Myctophidae	AP012250.1
Serrivomer beanii	Nemichthyidae	NC_013627.1
Scopelengys tristis	Neoscopelidae	AP012228.1
Xenomystus nigri	Notopteridae	NC_012715.1
Chitala ornata	Notopteridae	AB035243.1
Dissostichus eleginoides	Nototheniidae	NC_018135.1
Mallotus villosus	Osmeridae	NC_015244.1
Osmerus mordax	Osmeridae	AB114911.1
Betta splendens	Osphronemidae	AB571120.1
Maccullochella peelii	Percichthyidae	AP014532.1
Etheostoma atripinne	Percidae	AF288444.1
Plecoglossus altivelis	Plecoglossidae	NC_002734.2
Gambusia affinis	Poeciliidae	NC_004388.1
Polymixia japonica	Polymixiidae	NC_002648.1
Chromis cyanea	Pomacentridae	AY208529.1
Psettodes erumei	Psettodidae	NC_020032.1
Rachycentron canadum	Rachycentridae	AB292793.1
Stokellia anisodon	Retropinnidae	JN232590.1
Retropinna semoni	Retropinnidae	JN232588.1
Rondeletia loricata	Rondeletiidae	NC_003186.1

Neosalanx jordani	Salangidae	EU656132.1
Coregonus clupeaformis	Salmonidae	JX960776.1
Thymallus brevirostris	Salmonidae	JX960865.1
Salvelinus alpinus	Salmonidae	JX960845.1
Parahucho perryi	Salmonidae	JX262005.1
Samariscus latus	Samaridae	NC_024263.1
Scatophagus argus	Scatophagidae	KF361860.1
Aplodinotus grunniens	Sciaenidae	AY225662.1
Sarda sarda	Scombridae	EU036483.1
Sebastolobus alascanus	Sebastidae	DQ678500.1
Sebastes fasciatus	Sebastidae	DQ678444.1
Cephalopholis argus	Serranidae	NC_022142.1
Siganus vulpinus	Siganidae	NC_025588.1
Siganus spinus	Siganidae	AB276821.1
Heteromycteris japonicus	Soleidae	NC_024921.1
Sphyraena barracuda	Sphyraenidae	NC_022484.1
Stylephorus chordatus	Stylephoridae	NC_009948.1
Monopterus albus	Synbranchidae	NC_003192.1
Tetraodon miurus	Tetraodontidae	NC_015361.1
Triacanthus biaculeatus	Triacanthidae	NC_009863.1
Triacanthodes anomalus	Triacanthodidae	NC_009861.1
Assurger anzac	Trichiuridae	NC_022494.1
Umbra limi	Umbridae	AY497458.1
Novumbra hubbsi	Umbridae	NC_022455.1
Xiphias gladius	Xiphiidae	DQ198018.1
Zanclus cornutus	Zanclidae	NC_009852.1
Zeus faber	Zeidae	DQ198019.1

Supplementary Table 5. Divergence time estimation between *P. andruzzii* and *G. barreimiae* using Phylobayes (mean and 95% HPD).	
1 - CAT model and Birth Death	13.4 MYA (7.4-21.3 MYA)
2 - GTR model and Birth Death	14.2 MYA (8.4-20.8 MYA)
3 - CAT model and Uniform	17.6 MYA (10.6-26.7 MYA)

Supplementary Table 6. Predicted phenotypic effect of species-specific amino acid changes of opn4m2 (A) and rho (B) observed comparing the cyprinid species used for the analysis of natural selection (see Figure 1A and 1B). The predicted effect of the amino acid changes common to the two cavefish is also shown. Grey background: portion of the gene 3' of the premature stop codon.

a) opn4m2, melanopsin

	Aminoacid	Aminoacid	PANTHER	SIFT database			
	position	change	$P_{deleterious}$	UniRef90	UniProt-SwissProt	UniProt-TrEMBL	overall
Phreatichthys	69	S > F	**0.55**	tolerated	tolerated	tolerated	tolerated
andruzzii	162	R > C	**1.00**	**deleterious**	**deleterious**	**deleterious**	**deleterious**
	202	G > D	**0.93**	**deleterious**	**deleterious**	**deleterious**	**deleterious**
	205	A > V	0.29	**deleterious**	tolerated	**deleterious**	mild
	285	A > P	**0.94**	**deleterious**	**deleterious**	**deleterious**	**deleterious**
	343	T > K	0.42	**deleterious**	**deleterious**	**deleterious**	**deleterious**
Garra barreimiae	36	H > R	NA	tolerated	deleterious[a]	deleterious[a]	mild
	304	V > M	0.24	**deleterious**	**deleterious**	**deleterious**	**deleterious**
shared by	22	G > S	NA	tolerated	deleterious[a]	deleterious[a]	mild
P. andruzzii	39	V > I	NA	tolerated	tolerated	tolerated	tolerated
and	175	R > Q	0.18	tolerated	tolerated	tolerated	tolerated
G. barreimiae	188	V > L	0.35	tolerated	tolerated	tolerated	tolerated
	313	Y > F	0.27	**deleterious**	tolerated	tolerated	tolerated
	349	V > T	0.17	tolerated	tolerated	tolerated	tolerated
Cyprinus carpio	21	A > T	NA	tolerated	tolerated	deleterious[a]	tolerated
	136	P > S	0.14	tolerated	tolerated	tolerated	tolerated
	160	A > C	0.33	**deleterious**	**deleterious**	**deleterious**	**deleterious**
	171	A > T	0.26	tolerated	**deleterious**	**deleterious**	mild
	183	A > V	0.10	tolerated	tolerated	tolerated	tolerated
	224	P > S	0.34	tolerated	tolerated	tolerated	tolerated
	360	P > L	0.08	tolerated	tolerated	tolerated	tolerated
	376	G > S	0.09	tolerated	tolerated	tolerated	tolerated
Rutilus rutilus	17	I > N	NA	tolerated	deleterious[a]	deleterious[a]	mild
	30	R > K	NA	tolerated	tolerated	tolerated	tolerated
	69	S > A	0.16	tolerated	tolerated	tolerated	tolerated
	131	V > I	0.09	tolerated	tolerated	tolerated	tolerated
	136	P > I	0.39	**deleterious**	tolerated	tolerated	tolerated
	175	R > N	0.20	tolerated	tolerated	tolerated	tolerated
	246	G > V	0.12	tolerated	tolerated	tolerated	tolerated
	250	F > V	0.08	tolerated	tolerated	tolerated	tolerated

	259	A > M	0.45	**deleterious**	**deleterious**	**deleterious**	**deleterious**
	272	H > Q	NA	tolerated	tolerated	tolerated	tolerated
	287	V > I	0.09	tolerated	tolerated	tolerated	tolerated
	360	P > T	0.08	tolerated	tolerated	tolerated	tolerated
	368	D > E	0.10	tolerated	tolerated	tolerated	tolerated
	386	T > S	0.11	tolerated	tolerated	tolerated	tolerated
	408	T > K	NA	tolerated	tolerated	**deleterious**	tolerated
Danio rerio	49	E > D	0.05	tolerated	tolerated	tolerated	tolerated
	86	I > V	0.10	tolerated	tolerated	tolerated	tolerated
	103	V > I	0.22	tolerated	tolerated	tolerated	tolerated
	112	F > L	0.29	tolerated	tolerated	tolerated	tolerated
	115	L > V	0.12	tolerated	tolerated	tolerated	tolerated
	174	G > S	0.12	tolerated	tolerated	**deleterious**	tolerated
	186	A > V	0.14	tolerated	tolerated	tolerated	tolerated
	235	I > V	0.13	tolerated	tolerated	tolerated	tolerated
	251	G > A	0.27	tolerated	tolerated	tolerated	tolerated
	255	A > T	0.21	tolerated	tolerated	tolerated	tolerated
	290	L > V	0.10	tolerated	tolerated	tolerated	tolerated
	296	V > I	0.14	tolerated	tolerated	tolerated	tolerated
	316	L > F	0.09	tolerated	tolerated	tolerated	tolerated
	395	I > M	NA	tolerated	tolerated	tolerated	tolerated

b) rhodopsin

	Aminoacid	Aminoacid	PANTHER	SIFT database			
	position	change	$P_{deleterious}$	UniRef90	UniProt-SwissProt	UniProt-TrEMBL	
Phreatichthys	107	R > H	0.21	tolerated	tolerated	tolerated	tolerated
andruzzii	137	M > T	0.17	**deleterious**	**deleterious**	deleterious[a]	**deleterious**
	158	A > T	0.28	**deleterious**	**deleterious**	deleterious[a]	**deleterious**
	248	R > C	**0.71**	**deleterious**	**deleterious**	deleterious[a]	**deleterious**
	271	V > M	**0.57**	tolerated	tolerated	deleterious[a]	mild
	287	F > C	**0.83**	**deleterious**	**deleterious**	deleterious[a]	**deleterious**
	304	C > F	0.11	**deleterious**	**deleterious**	deleterious[a]	**deleterious**
	311	K > R	0.13	tolerated	tolerated	tolerated	tolerated
Garra barreimiae	281	S > T	0.22	tolerated	tolerated	tolerated	tolerated
shared by	8	A > N	0.13	tolerated	tolerated	tolerated	tolerated
P. andruzzii	22	S > D	NA	tolerated	tolerated	deleterious[a]	tolerated
and	49	I > L	0.08	tolerated	tolerated	tolerated	tolerated
G. barreimiae	263	I > V	0.09	tolerated	tolerated	tolerated	tolerated
	266	L > V	0.08	tolerated	tolerated	tolerated	tolerated
	278	H > N	0.13	tolerated	tolerated	tolerated	tolerated

Cyprinus carpio	21	R > K	NA	tolerated	tolerated	deleterious[a]	tolerated
	54	V > I	0.08	tolerated	tolerated	tolerated	tolerated
	108	L > I	0.13	tolerated	tolerated	tolerated	tolerated
	209	I > L	0.16	tolerated	tolerated	tolerated	tolerated
	232	E > D	0.23	tolerated	tolerated	tolerated	tolerated
	297	T > S	0.14	tolerated	tolerated	tolerated	tolerated
Carassius auratus	7	P > D	0.36	tolerated	tolerated	tolerated	tolerated
	108	L > V	0.12	tolerated	tolerated	tolerated	tolerated
	112	L > P	**0.73**	**deleterious**	**deleterious**	deleterious[a]	**deleterious**
	195	T > P	NA	tolerated	tolerated	deleterious[a]	tolerated
	196	P > Q	0.14	tolerated	tolerated	deleterious[a]	tolerated
	197	G > A	0.14	tolerated	tolerated	deleterious[a]	tolerated
	237	Q > H	**0.97**	**deleterious**	**deleterious**	deleterious[a]	**deleterious**
	238	Q > E	**0.55**	tolerated	**deleterious**	tolerated	mild
Danio rerio	39	C > L	0.49	tolerated	tolerated	tolerated	tolerated
	82	S > A	0.47	tolerated	tolerated	tolerated	tolerated
	133	F > I	0.49	tolerated	tolerated	tolerated	tolerated
	198	Y > V	0.54	tolerated	tolerated	tolerated	tolerated
	213	I > F	0.08	tolerated	tolerated	tolerated	tolerated
	260	G > A	0.30	tolerated	tolerated	tolerated	tolerated
	270	S > G	0.12	tolerated	tolerated	tolerated	tolerated

[a] the prediction may be biased by the close phylogenetic relationship between the species of the multiple alignment