

Table S5 A. Inventory of polypeptide ligand genes in the MRCA of tetrapods and teleosts, human, and *T. nigroviridis*. Based on syntenic mapping, the 118 *T.nigroviridis* ligand genes are sub-divided as WGD-derived (WGD), singleton (S), undetermined (UD), and teleost-specific (T).

Receptor class	MRCA		Human	<i>Tetraodon nigroviridis</i>					
	No. of nGPCRs with a polypeptide ligand	No. of ligand genes	No. of human ligand genes	Total No. of ligand genes	WGD	UD	S	T	Ortholog found in <i>D. rerio</i> or <i>T. rubripes</i>
A5	26	31	37	41	8	8	23	2	0
A6	6	6	6	6	0	0	6	0	0
A7	36	24	57	46	4	19	14	8	1
B	13	15	18	25	6	10	9	0	0
Total	81	76	118	118	18	37	52	10	1

Table S5 B. List of polypeptide ligand genes in the MRCA of tetrapods and teleosts, and the derived ligand genes in human and *T. nigroviridis*. Whole genome duplication (WGD); unknown duplication(UD); singleton(S).

Ancestral count	Receptor Class	Ancestral ligand	Human ligand gene ID	<i>T.nigroviridis</i>				
				Ligand gene identifier	Origin of homologs	WGD	UD	S
1	A5	AVP - vasopressin	551	CAG09578	S	0	0	1
2	A5	OXT - oxytocin-neurophysin	5020	CAG09583	S	0	0	1
3	A5	CCK - cholecystokinin	885	CAF99479 CAG12884	WGD	1	0	0
						1	0	0
4	A5	CG α - glycoprotein hormones, alpha subunit	1081	CAG10482	S	0	0	1
5	A5	EDN1 - endothelin 1	1906	chrUn_md:105180240-344 chrUn_md:125929085-177	UD	0	1	0
						0	1	0
6	A5	EDN2 - endothelin 2	1907	CAG06007	S	0	0	1
7	A5	EDN3 - endothelin 3	1908	CAG07564	S	0	0	1
8	A5	FSH β - follicle-stimulating hormone	2488	CAF99923	S	0	0	1
9	A5	GAST - gastrin	2520	BAC44896	S	0	0	1
10	A5	GNRH1 - gonadotrophin-releasing hormone 1	2796	BAE45689	S	0	0	1
11	A5	GNRH2 - gonadotrophin-releasing hormone 2	2797	BAE45691	S	0	0	1
12	A5	GPB β 5 - glycoprotein hormone beta 5	122876	CAG03758 CAG05739	UD	0	1	0
						0	1	0
13	A5	GRP - gastrin-releasing peptide	2922	chr4:3430570-1166	S	0	0	1
14	A5	HCRT - orexin A and orexin B	3060	CAG02825	S	0	0	1
15	A5	INSL5 - insulin-like 5	10022	chr1:15664564-5453 chr15:3444219-81	WGD	1	0	0
						1	0	0
16	A5	RLN3 - relaxin 3, INSL3	117579/3640	CAG06046 chr3:8563693-833	WGD	1	0	0
						1	0	0
17	A5	RLN/RLN2/INSL4/INSL6	6013/6019/3641/11172	chr4:1081257-741	S	0	0	1
18	A5	LH β - luteinizing hormone, β subunit	3972	CAG00338	S	0	0	1
19	A5	NMB - neuromedin B	4828	chr5:2458757-852 chr13:2188886-963	WGD	1	0	0
						1	0	0
20	A5	NPFF, NPAF	8620	CAG07075	S	0	0	1
21	A5	NPY - neuropeptide Y	4852	CAG11987 CAG11107	UD	0	1	0
						0	1	0
22	A5	PYY, PPY	5697/5539	CAG02076 chr2:4330403-1166	UD	0	1	0
						0	1	0
23	A5	P518 - RF-Amide P518	347148	GSCT00026421001	S	0	0	1
24	A5	PrRP - prolactin-releasing peptide	51052	chr6:1313515-86	S	0	0	1

Ancestral count	Receptor Class	Ancestral ligand	Human ligand gene ID	<i>T.nigroviridis</i>				
				Ligand gene identifier	Origin of homologs	WGD	UD	S
25	A5	PROK1 - prokineticin 1 (EG-VEGF)	84432	CAG07489	S	0	0	1
26	A5	PROK2 - prokineticin 2	60675	CAF96920	S	0	0	1
27	A5	RFRP - RFamide-related peptide	64111	CAF99426	S	0	0	1
28	A5	TAC1 - substance P/tachykinin1	6863	CAG11662	S	0	0	1
29	A5	TAC3 - neurokinin B /tachykinin 3	6866	CAG07431	S	0	0	1
30	A5	TAC4 - tachykinin 4	255061	CAG04412	S	0	0	1
31	A5	TSHβ - thyroid-stimulating hormone	7252	CAG11806	S	0	0	1
Subtotal			36			8	8	23
1	A6	GHRL - ghrelin	51738	chr16:8282093-8282194	S	0	0	1
2	A6	MLN - motilin	4295	chr11:774453-775188	S	0	0	1
3	A6	NMS - neuromedin S	129521	chr2:17978277-659	S	0	0	1
4	A6	NMU - neuromedin U	10874	chr1_rnd:2147854-9206	S	0	0	1
5	A6	NTS - neuromedin N and neurotensin	4922	chr13:11758087-185	S	0	0	1
6	A6	TRH - thyrotropin-releasing hormone	7200	CAG11330	S	0	0	1
Subtotal			6			0	0	6
1	A7	AGT - angiotensin	183	CAG10094	S	0	0	1
2	A7	APLN - apelin	8862	CAG08364	S	0	0	1
3	A7	CORT - cortistatin	1325	CAG07556	S	0	0	1
4	A7	GAL - galanin	51083	chrUn_rnd:85620427-1348	S	0	0	1
5	A7	KISS1	3814	SCAF14526	S	0	0	1
6	A7	KNG1 - bradykinin	3827	CAF96097	S	0	0	1
7	A7	NPB - neuropeptide B	256933	CAG00214 CAG06031	WGD	1 1	0 0	0 0
8	A7	PDYN - dynorphin A	5173	<i>D.rei</i>	S	0	0	1
9	A7	PENK - proenkephalin	5179	CAG11635	S	0	0	1
10	A7	POMC - proopiomelanocortin	5443	Un_rnd:112416896-112417622 14:4949877-4950632	UD	0 0	1 1	0 0
11	A7	MCH - melanin-concentrating hormone	5367	CAF93560 chr19:990239-877	UD	0 0	1 1	0 0
12	A7	NOC - nociceptin/orphanin FQ	5368	CAG12991 CAG03094 chrUn_rnd:79082359-535	UD	0 0 0	1 1 1	0 0 0
13	A7	SST - somatostatin	6750	CAG07675 CAG02176	UD	0 0	1 1	0 0
14	A7	UTS2 - urotensin II	10911	CAG04941	S	0	0	1
15	A7	UTS2D - urotensin related peptide	257313	chr9:8502656-967	S	0	0	1
16	A7	CCL20	6364	chr15:4409609-10309	S	0	0	1

Ancestral count	Receptor Class	Ancestral ligand	Human ligand gene ID	<i>T.nigroviridis</i>				
				Ligand gene identifier	Origin of homologs	WGD	UD	S
17	A7	CCL22	6367	SCAF2108	S	0	0	1
18	A7	CCL24	6369	chr18:7004875-6283	S	0	0	1
19	A7	CCL21/25	6366/6370	chr15:5048784-9029	UD	0	1	0
				CAG07167	WGD	1	0	0
				CAG07971	WGD	1	0	0
20	A7	CCL27/28	10850/56477	chr15:5046891-5047156	UD	0	1	0
				chr15:4114798-956	UD	0	1	0
				CAF90799	UD	0	1	0
				chr2:17691812-907	UD	0	1	0
				CAG09577	UD	0	1	0
21	A7	CXCL8	3576	CAG11181	UD	0	1	0
				CAF90539	UD	0	1	0
22	A7	CXCL9/10/11	4283/3627/6373	GSCT00006672001	S	0	0	1
23	A7	CXCL12	6387	CAG09382	UD	0	1	0
				chr18:2280936-96670	UD	0	1	0
24	A7	CXCL14	9547	CAF90863	S	0	0	1
Subtotal			28			4	19	15
1	B	ADM - adrenomedullin	133	CAF99639	WGD	1	0	0
				CAG05606	WGD	1	0	0
2	B	IMD - intermedin	79924	chr15:7151047-646	UD	0	1	0
				chr13:3271568-697	UD	0	1	0
3	B	CALCA, CALCB- calcitonin; calcitonin gene-related peptide	796/797	CAF99659	WGD	1	0	0
				chr5: 6718835-9962	WGD	1	0	0
4	B	IAPP - amylin	3375	chrUn_md:66411282-990	UD	0	1	0
				CAF89835	S	0	0	1
5	B	CRH - corticotropin releasing factor	1392	chr6:2514921-5169	S	0	0	1
6	B	GCG - glucagon, GLP1, GLP2	2641	CAG04974	WGD	1	0	0
				CAG09338 (AA740-end)	WGD	1	0	0
7	B	GHRH - growth hormone-releasing hormone	2691	CAF96902	S	0	0	1
8	B	ADCYAP1 - adenylate cyclase activating polypeptide 1	116	CAG12289	UD	0	1	0
				GSTENT00031777001	UD	0	1	0
				CAG10213	UD	0	1	0
9	B	PTH parathyroid hormone	5741	chrUn_md:153387022-120	S	0	0	1
10	B	PTH LH - parathyroid hormone-like hormone	5744	CAG08679	UD	0	1	0
				CAF88940=CAF87848	UD	0	1	0
				chrUn_md:12100351-452	UD	0	1	0
11	B	TIP39	113091	CAG12455	S	0	0	1
12	B	UCN - urocortin	7349	chr14:9250391-492	S	0	0	1
13	B	UCN2 - urocortin 2	90226	chr11:1019834-998	S	0	0	1
14	B	UCN3 - urocortin 3	114131	CAG07278	S	0	0	1
15	B	VIP - vasoactive intestinal polypeptide	7432	CAF97050	S	0	0	1
Subtotal			16			6	10	9
Ligand genes not present in <i>T. nigroviridis</i>								
1	A5	NPS - neuropeptide S	594857					

Ancestral count	Receptor Class	Ancestral ligand	Human ligand gene ID	<i>T.nigroviridis</i>				
				Ligand gene identifier	Origin of homologs	WGD	UD	S
2	A7	GALP - galanin-like peptide	85569					
3	A7	NPW - neuropeptide W	283869					
4	A7	CCL1	6346					
5	A7	CCL2	6347					
6	A7	CCL3	6348					
7	A7	CCL4	6351					
8	A7	CCL5	6352					
9	A7	CCL7	6354					
10	A7	CCL8	6355					
11	A7	CCL11	6356					
12	A7	CCL13	6357					
13	A7	CCL14	6358					
14	A7	CCL15	6359					
15	A7	CCL16	6360					
16	A7	CCL17	6361					
17	A7	CCL18	6362					
18	A7	CCL19	6363					
19	A7	CCL23	6368					
20	A7	CCL26	10344					
21	A7	CXCL1	2919					
22	A7	CXCL2	2920					
23	A7	CXCL3	2921					
24	A7	CXCL4=PF4V	5197					
25	A7	CXCL5	6374					
26	A7	CXCL6	6372					
27	A7	CXCL7=PPBP	5473					
28	A7	CXCL13	10563					
29	A7	CXCL15	337989					
30	A7	CXCL16	58191					
31	B	SCT - secretin	6343					
32	B	GIP - gastric inhibitory polypeptide	2695					

Teleost-specific genes

1	A5	EDN-like	1000001	chr8:5583520-870				
2	A5	GNRH-like	1000002	BAE45693				
1	A7	CCL-like	1000003	CAG07793				
2				CAG07792				
3				chr2:17775947-17776174				
4				CAF98923				
5				chr12:3595953-6120				
6				chr12:3597953-8132				
7	A7	CXCL-like	1000004	CAF95728				
8				CAG09635				

Table S5 C. List of polypeptide ligands with WGD-derived duplicates in *T. nigroviridis* as well as their cognate receptors. Cognate receptors with WGD-derived duplicates in *T. nigroviridis* are shown in bold letters.

	Receptor class	Ligand gene symbol	Ligand description	Cognate receptors	Human ligand gene ID	<i>T. nigroviridis</i> ligand gene identifier
Coevolved with a pair of WGD-derived nGPCRs	A5	NMB	Neuromedin B	GRPR , <i>NMBR</i>	4828	chr5:2458757-852 chr13:2188886-963
	A5	INSL5	Insulin-like 5	RLN3R2	10022	chr1:15664564-5453 chr15:3444219-81
	A7	RLN3	Relaxin-3	RLN3R1 , <i>LGR7</i>	117579	CAG06046 chr3:8563693-833
	B	ADM	Adrenomedullin	CRLR	133	CAF99639 CAG05606
	B	CALCA	Calcitonin; calcitonin gene-related peptide	CRLR , <i>CR</i>	796	CAF99659 chr5: 6718835-9962
Not coevolved with a pair of WGD-derived nGPCRs	A5	CCK	Cholecystokinin	<i>CCKAR</i>	885	CAF99479 CAG12884
	A7	NPB	Neuropeptide B	<i>GPR7</i> , <i>GPR8</i>	256933	CAG00214 CAG06031
	A7	CCL25	CCL25	<i>CCR9</i>	6370	CAG07167 CAG07971
	B	GCG	Glucagon, GLP1, GLP2	GCGR , <i>GLP1R</i> , <i>GLP2R</i>	2641	CAG04974 CAG09338 (amino acid 740-end)