

Supplementary Information for

Chemokine *ccl33* is a key regulator of teleost fish barbel development

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This PDF file includes:

Figs. S1 to S5
Tables S1 to S8
References for SI reference citations

Other supplementary materials for this manuscript include the following:

Datasets S1 to S2

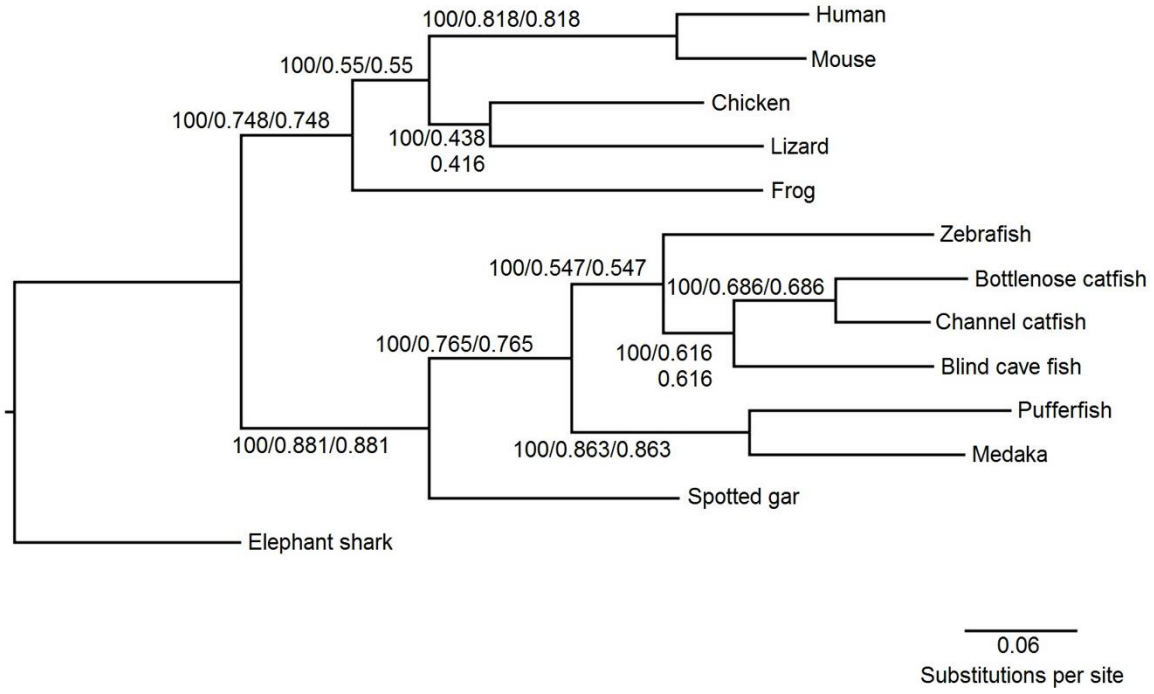


Fig. S1. Phylogenetic tree from bottlenose catfish and 12 representative species.

A: Channel catfish



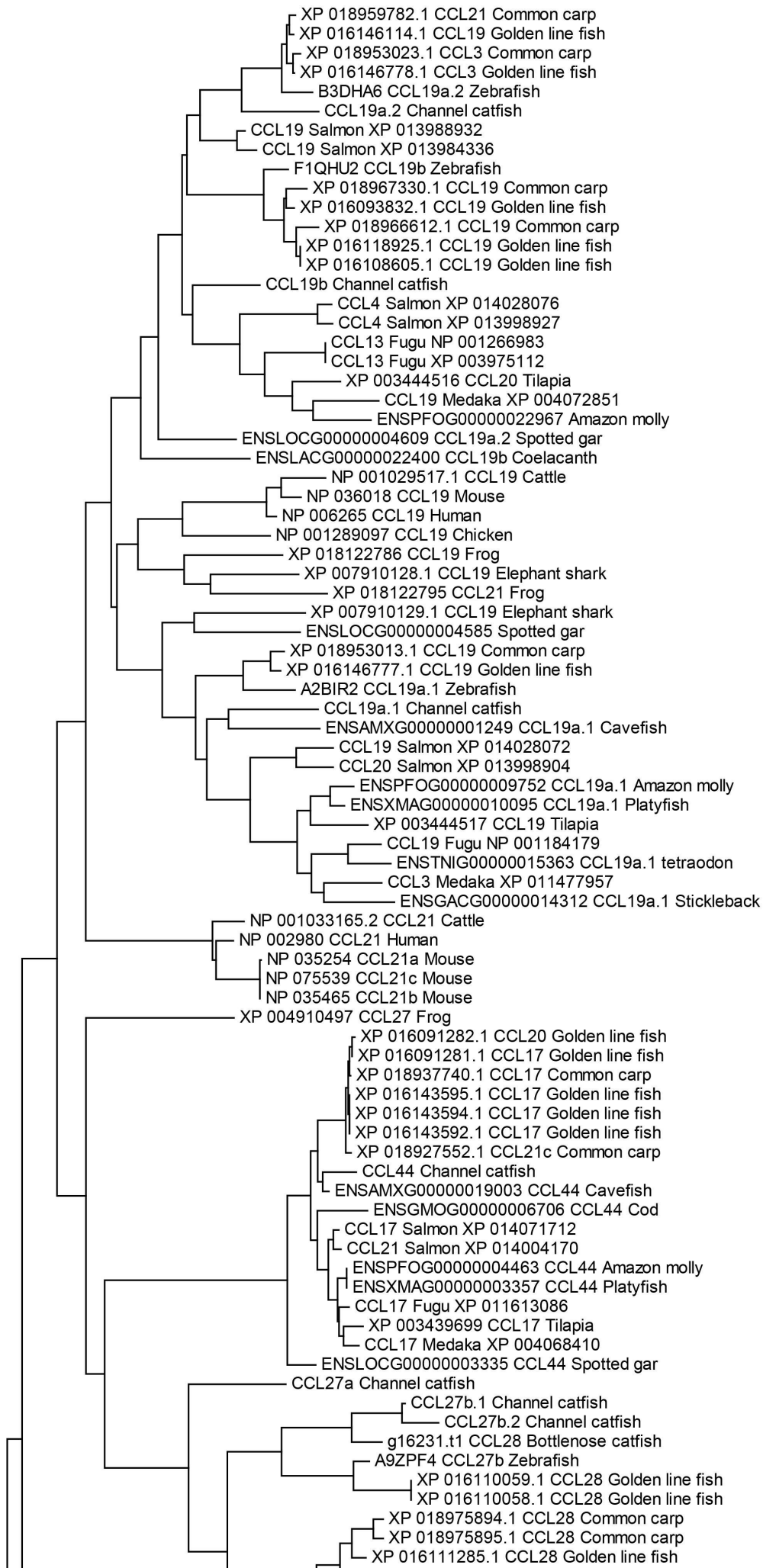
B: Bottlenose catfish

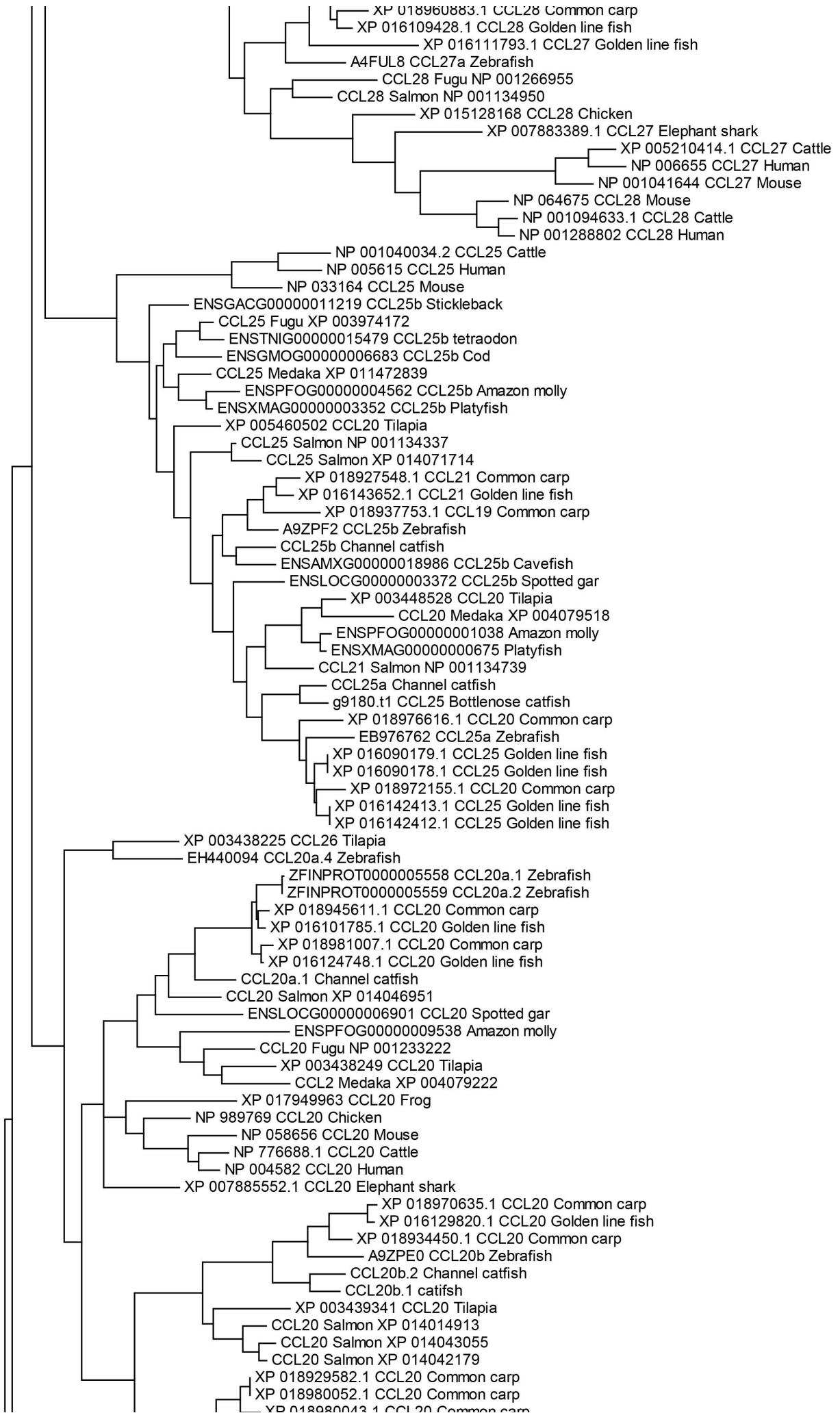


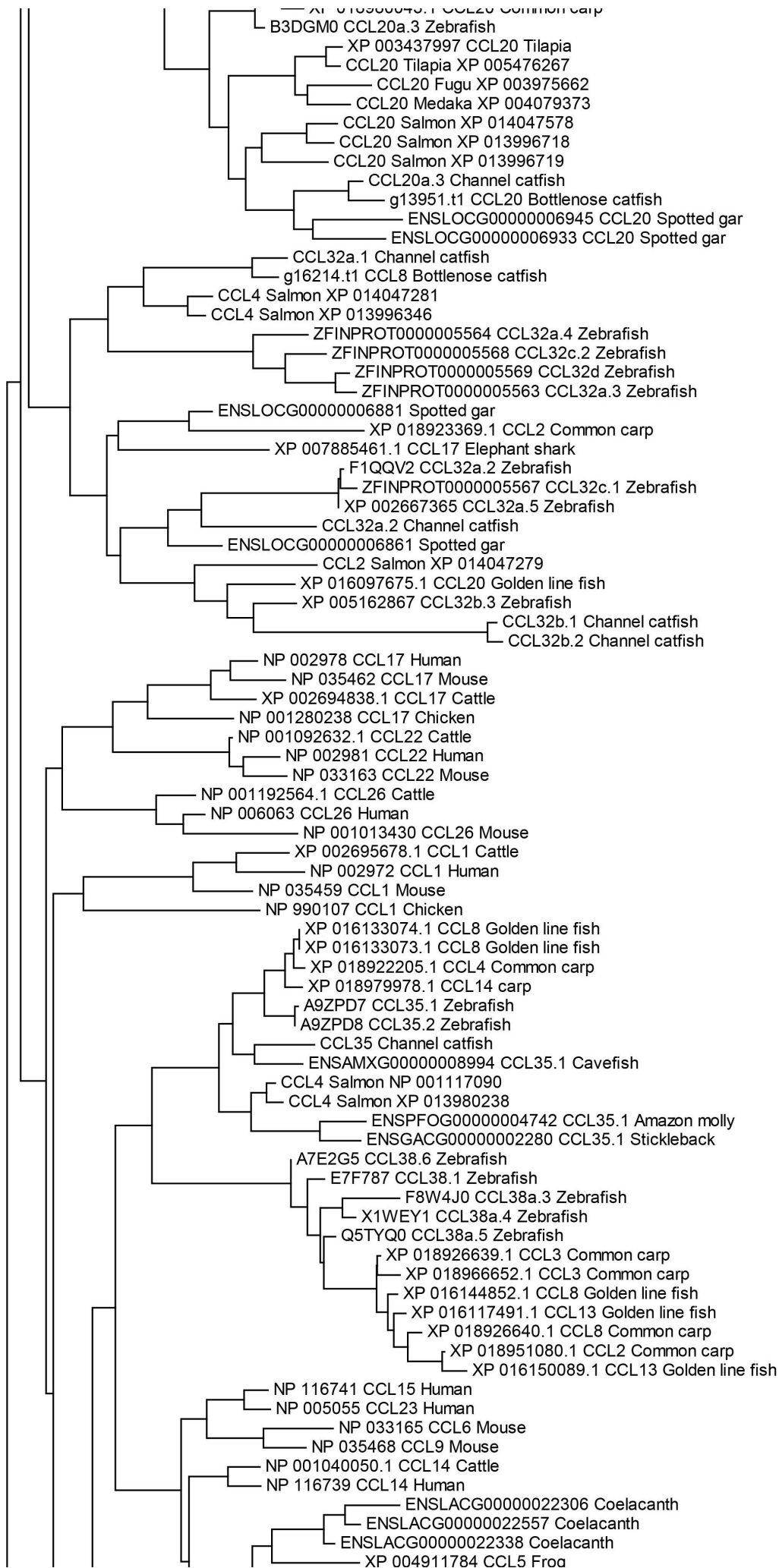
C: Details of ossified barbel of Bottlenose catfish

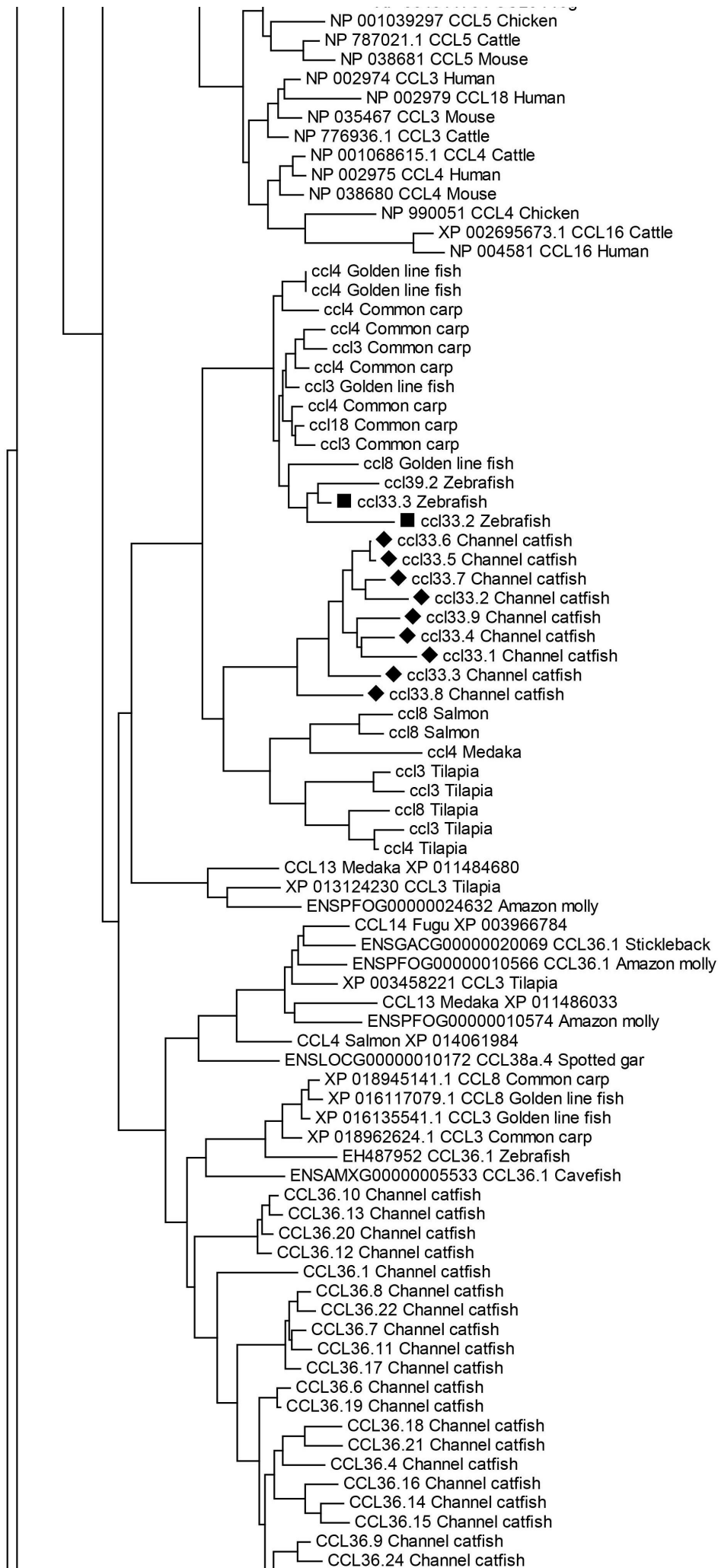


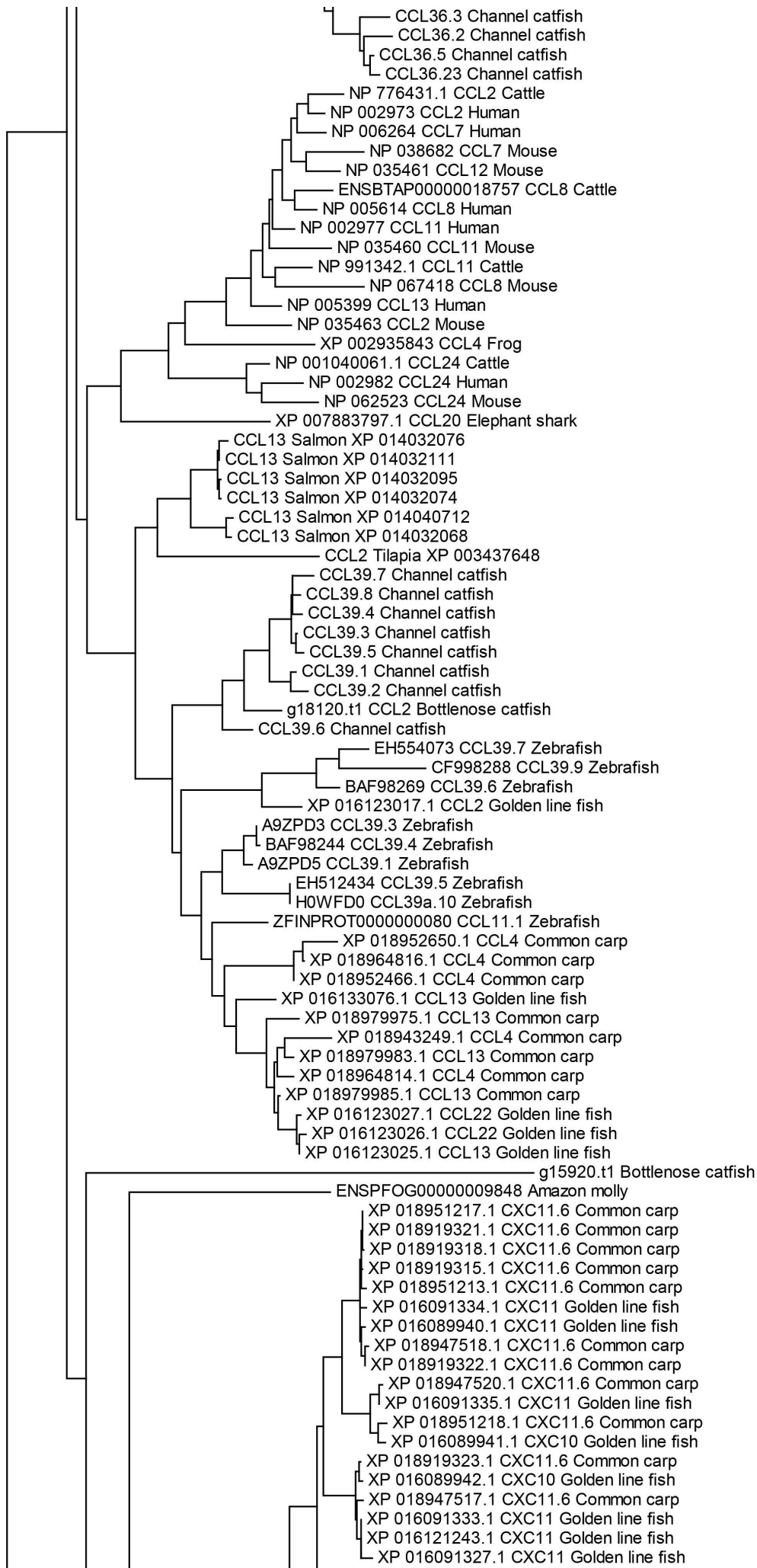
Fig. S2. Barbel features of channel catfish and bottlenose catfish.

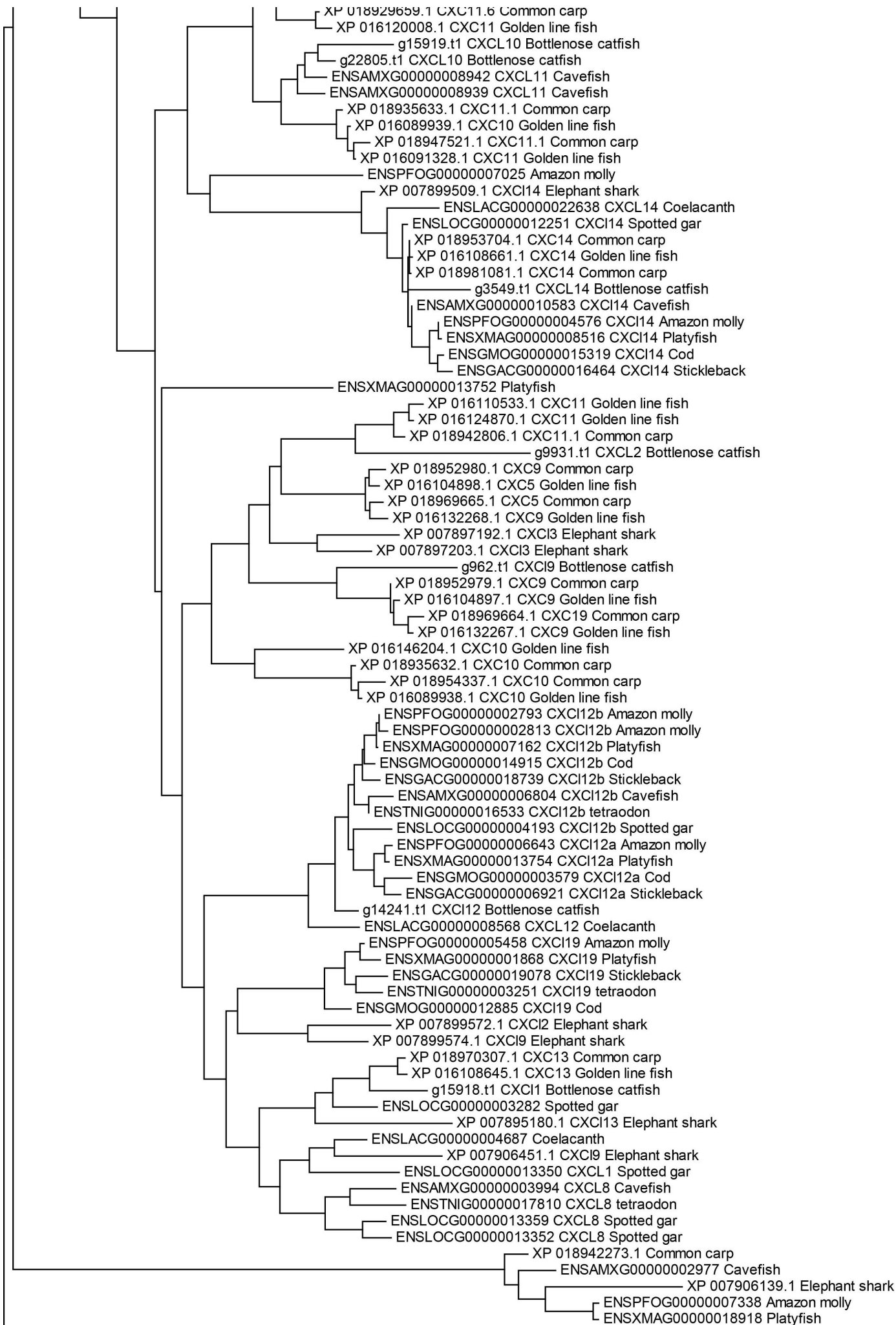












— CCI 34.1 Channel catfish

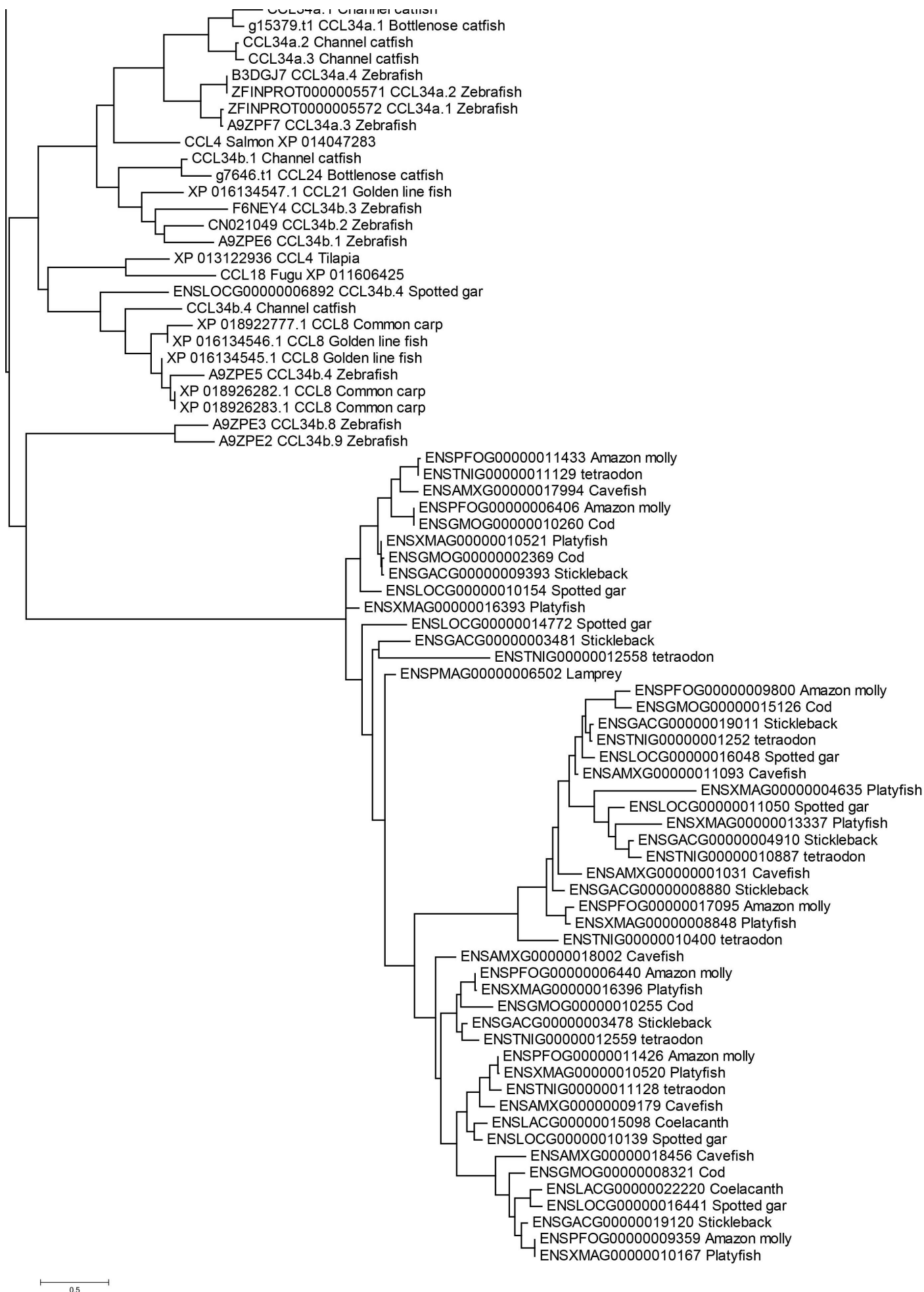


Fig. S3. Phylogenetic analysis of CC and CXC chemokine ligands from different species.

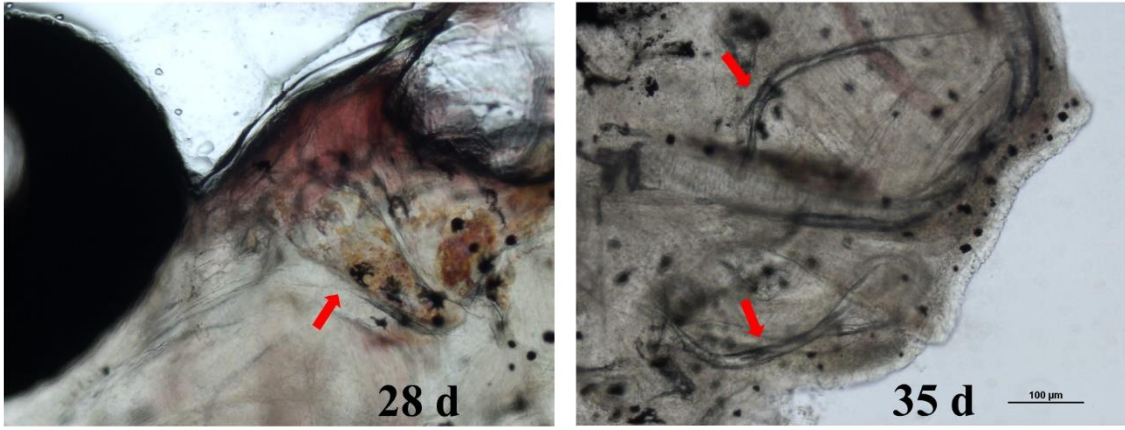


Fig. S4. Early barbel morphology of zebrafish. The red arrow indicates the barbel bud.

(A) CCL33.2

Wild type

AAGG**TGGTGGTTTTGGCTCTCGTGG**TGATGG**CGGTGGTGGCAGGGGCAAGGG**TAGTTGTGGT**GGACATCCATGGGCAGGGCGG**ACTGCTCA

Mutant, Phenotype: Short barbel, Genotype: 2 bp substitution

AA**AGGGTGGTTTTGGCTCTCGTGG**TGATGG**CGGTGGTGGCAGGGGCAAGGG**TAGTTGTGGT**GGACATCCATGGGCAGGGCGG**ACTGCTCA

Mutant, Phenotype: Loss barbel, Genotype: 2 bp insertion

AAGG**TGGTGGTTTTGGCTCTCGT****AGG**TGATGG**CGGTGGTGGCAGGGGCAAGGG**TAGTTGTGGT**GGACT****TATCCATGGGCAGGGCGG**ACTGCTCA

(B) CCL33.3

Wild type

TT**CCCGAACCCGTGTGCTTCAAT**TTCATTGACTTT**CCAATCCCAGCCAACAAAG**TAGTGAGTGCCGTGAGAACAGT**TTCACGCTGTGCTGTTAAAGGCATTGT**

Mutant, Phenotype: Curly barbel, Genotype: 5 bp deletion

TT**CCCGAACCCGTGT**-----**T**----**ATTCATTGACTTTCCAATCCCAGCCAACAAAG**TAGTGAGTGCCGTGAGAACAGT**TTCACGCTGTGCTGTTAAAGGCATTGT**

Mutant, Phenotype: Short barbel, Genotype: 7 bp deletion

TT**CCCGAACCCGTGT**-----**C--ATTT**----**TTGACTTTCCAATCCCAGCCAACAAAG**TAGTGAGTGCCGTGAGAACAGG**TTCACGCTGTGCTGTTAAAGGCATTGT**

Mutant, Phenotype: Loss barbel, Genotype: 3 bp deletion, 3 bp substitution

TT**CCCGAACCCGTGT**-----**TCAAT**TTCATTGACTTT**CCAATCCCAGCCAACAAA****AT**AGTGAGTGCCGTGA**AAACAGGTTCACGCTGTGATGTTAAAGGCATTGT**

Red, gRNA target sequence (plus strand); **Purple**, gRNA target sequence (minus strand); **Orange**, substitution; **Green**, insertion; --, deletion

Fig. S5. List of genotypes of *ccl33* mutants.

Table S1. GO terms that significantly enriched during barbel regeneration of channel catfish.

Time point	GO biological process	Fold Enrichment	<i>P</i> value
1d	GO:0045333 cellular respiration	3.59	0.003
	GO:0042060 wound healing	2.57	0.03
	GO:0009611 response to wounding	2.51	0.01
2d	GO:0032502 developmental process	1.49	0.0006
	GO:0007275 multicellular organism development	1.49	0.003
	GO:0044767 single-organism developmental process	1.48	0.001
3d	GO:0048856 anatomical structure development	2.04	0.005
	GO:0048568 embryonic organ development	1.79	0.001
7d	GO:0007155 cell adhesion	2.21	0.026
	GO:0022610 biological adhesion	2.2	0.028
	GO:0032502 developmental process	1.36	0.037
14d	GO:0007155 cell adhesion	2.64	0.00019
	GO:0022610 biological adhesion	2.63	0.0002
	GO:0044699 single-organism process	1.22	0.007

Table S2. Genes specific in channel catfish and were differentially expressed during barbel regeneration of channel catfish.

Gene ID	Name	Length
804886	ND6	171
108263381	pdss2	381
108266750	tex30	265
108263386	kmo	473
108270394	crls1	378
108266931	LOC108266931	197
100528215	emc4	187
108255681	LOC108255681	204
108263202	apopt1	198
108257472	edn1	195
108279850	LOC108279850	222
108275545	haus8	364
100528430	anapc13	74
108272348	cks1b	79
108276169	cenpw	75
100528304	ebag9	212
108266359	LOC108266359	332
108268882	tmsb15b	46
108257184	cap2	389
108268648	fanca	1141
108274164	bricd5	228
100528677	pdc5	126
108271425	tax1bp3	125
108262879	LOC108262879	329
108270530	LOC108270530	228
108280749	LOC108280749	510
804882	ND4	460
108258791	cytl1	192
108268228	ssx2ip	501
108270589	LOC108270589	463
100304887	cript	101
100528363	gins3	211
100528639	skp1	163
108279644	orc5	445
108264799	LOC108264799	383
108270120	LOC108270120	127
108274859	LOC108274859	226
108265590	epgn	153
108266789	LOC108266789	132
108262226	LOC108262226	186
108271379	slc39a3	315
108273999	ccser2	791
108271223	cep19	160
100528704	mzt1	75
108268687	LOC108268687	241
108272622	dctn3	185
108255763	LOC108255763	2337

108271519	snrpc	159
108261049	LOC108261049	163
108270478	cf12	166
108255701	LOC108255701	108
108271021	LOC108271021	119
108274856	LOC108274856	231
108259812	cchr1	692
108264363	tsnaxip1	548
100304584	LOC100304584	105
108280863	twsg1	223
108257874	atraid	230
108281088	LOC108281088	130
108260511	slc9b2	533
108268439	LOC108268439	78
108272615	pex11b	252
108256728	LOC108256728	157
108266662	dcaf17	529
108276694	prr11	299
108256526	polr1d	172
108268918	LOC108268918	115
108259227	cklf	143
108258502	p1d6	243
108256594	LOC108256594	83
108273111	ten1	126
108272082	LOC108272082	75
108260508	LOC108260508	170
108255648	LOC108255648	232
108265132	c1h8orf59	98
100304470	b2m	116
100305060	LOC100305060	78
100528356	brk1	75
108255519	LOC108255519	110
108260858	LOC108260858	231
108261098	LOC108261098	226
108269115	LOC108269115	86
100304587	LOC100304587	92
100304595	LOC100304595	119
100528727	arpc4	168
100529001	psmf1	270
108254817	LOC108254817	239
108255518	LOC108255518	112
108256245	LOC108256245	111
108264196	LOC108264196	231
108264531	LOC108264531	152
108266135	LOC108266135	268
100415908	LOC100415908	223
108255397	batf	124
108256237	azi2	353
108257382	LOC108257382	132
108258651	LOC108258651	186
108261625	LOC108261625	130

108262793	LOC108262793	259
108263248	LOC108263248	111
108265287	stap2	415
108267188	LOC108267188	101
108268115	LOC108268115	97
108268327	ltbr	353
108269344	LOC108269344	78
108271554	LOC108271554	223
108272639	LOC108272639	218
108273140	LOC108273140	158
108275426	LOC108275426	129
108277757	LOC108277757	281
108280668	mbp	133
108280775	LOC108280775	290
100304478	LOC100304478	60
100528404	rabif	133
100528408	tesc	123
100528986	znrd1	133
108256239	cmc1	107
108256026	carnmt1	418
108264825	LOC108264825	689
108275464	mdp1	160
108256275	hert	126
108265761	clps	136
108266496	LOC108266496	114
108269592	mtfr11	303
804876	ATP8	55
804879	ND5	607
804883	ND2	348
100528072	cf125	129
100528912	ndufb4	127
108254755	arv1	241
108259146	agrp	143
108263832	LOC108263832	112
108263890	LOC108263890	108
108267455	pex19	282
108268603	ndufc1	69
108272167	LOC108272167	76
108272602	atp5e	51
108274303	nt5m	230
108276884	LOC108276884	125
108280490	LOC108280490	55
108257443	LOC108257443	645
108274881	LOC108274881	128
108280026	LOC108280026	305
804878	ND3	116
108259575	pex11a	246
100528592	spcs1	101
108276972	LOC108276972	113
108278384	spcs2	219
100528255	cck	123

108270925	LOC108270925	193
108270874	tim13	95
108280891	LOC108280891	90
108255480	ascc2	763
108267567	LOC108267567	124
108271096	elp4	389
108271274	nr2c2ap	141
108269806	mrps5	400
108258002	LOC108258002	177
108273408	mrpl58	198
100528801	mrps14	133
108280907	mrpl34	115
108265414	tgoln2	307
804877	ATP6	227
804880	ND1	324
804885	COX3	261
108256436	pkiA	85
108264506	c4h15orf48	87
108272514	smdt1	97
108280886	LOC108280886	171
804875	COX2	230
804881	ND4L	98
100528515	vps28	223
108257495	tomm7	55
108264547	nutf2	127
108273994	tomm22	133
108276560	lyrm7	104
108277678	LOC108277678	916
108278988	slc31a2	138
108268928	LOC108268928	177
108259733	LOC108259733	219
108271080	LOC108271080	342
108272891	LOC108272891	300
108259533	LOC108259533	211
108260286	LOC108260286	1346
108264443	LOC108264443	449
108278302	LOC108278302	180
108263206	bore7	103
108263260	LOC108263260	100
108267632	LOC108267632	139
108268185	LOC108268185	215
108276417	LOC108276417	241
108278804	LOC108278804	365
100528339	dal1b	102
108257542	ociad2	160
108258663	LOC108258663	107
108259248	LOC108259248	227
108260035	LOC108260035	270
108260899	LOC108260899	264
108262451	LOC108262451	224
108266945	LOC108266945	224

108267969	LOC108267969	407
108268874	LOC108268874	222
108270531	tmem44	454
108271423	LOC108271423	222
108274023	c13h10orf128	91
108274089	LOC108274089	264
108274225	LOC108274225	394
108274412	smim22	81
108274670	LOC108274670	112
108278494	LOC108278494	142
108279884	ccdc167	100
108280991	LOC108280991	222
100305002	LOC100305002	184
100528681	cops9	57
100528768	lyrm5	85
108254720	LOC108254720	209
108257072	LOC108257072	267
108257214	LOC108257214	408
108258433	LOC108258433	378
108259252	LOC108259252	210
108259526	LOC108259526	249
108259529	fam207a	237
108259534	LOC108259534	211
108260421	LOC108260421	151
108262792	LOC108262792	416
108263973	LOC108263973	410
108264385	kiaa0513	421
108265078	LOC108265078	263
108265611	c5h9orf116	141
108265844	LOC108265844	209
108266094	LOC108266094	243
108266430	LOC108266430	258
108266545	LOC108266545	218
108266787	c6h21orf62	214
108267124	c7h22orf39	108
108267255	LOC108267255	124
108267286	LOC108267286	224
108267350	LOC108267350	203
108267429	LOC108267429	129
108267962	c7h4orf48	86
108268437	LOC108268437	595
108268660	LOC108268660	363
108269355	LOC108269355	204
108269361	c8h12orf73	64
108269490	tmem229b	185
108270539	c1h16orf91	155
108270745	LOC108270745	925
108270746	LOC108270746	921
108271006	LOC108271006	491
108271220	serf2	59
108272510	ccdc134	229

108272745	LOC108272745	363
108272856	hn1	139
108273013	LOC108273013	239
108273081	LOC108273081	173
108273612	LOC108273612	127
108274251	LOC108274251	425
108274403	c2h8orf82	213
108274852	LOC108274852	247
108275268	lrrc61	281
108275551	LOC108275551	326
108275692	LOC108275692	432
108276725	LOC108276725	114
108276957	LOC108276957	271
108277089	LOC108277089	87
108278455	LOC108278455	202
108278465	LOC108278465	209
108279232	c18h5orf15	290

Table S3. Chemokine *ccl33* in barbel-ed fish.

Species	Gene	Accession	Previous name	Reference
Zebrafish	<i>ccl33.2</i>	ENSDARG00000102519	CCL-chr25h	Ensembl GRCz10
Zebrafish	<i>ccl33.3</i>	ENSDARG00000099401	CCL-chr25a	Ensembl GRCz10
Channel catfish	<i>ccl33.1</i>	JT348749	SCYA121	(1, 2)
Channel catfish	<i>ccl33.2</i>	XP_017330655	SCYA117	(1, 2)
Channel catfish	<i>ccl33.3</i>	XP_017330182	SCYA111	(1, 2)
Channel catfish	<i>ccl33.4</i>	XP_017330183		(2)
Channel catfish	<i>ccl33.5</i>	XP_017329053		(2)
Channel catfish	<i>ccl33.6</i>	XP_017330184		(2)
Channel catfish	<i>ccl33.7</i>	XP_017328905		(2)
Channel catfish	<i>ccl33.8</i>	JT396455		(2)
Channel catfish	<i>ccl33.9</i>	ABA54969	SCYA122	(1, 2)
Common carp	<i>ccl33.1</i>	XP_018950216.1	CCL3	This study
Common carp	<i>ccl33.2</i>	XP_018950218.1	CCL4	This study
Common carp	<i>ccl33.3</i>	XP_018950217.1	CCL3	This study
Common carp	<i>ccl33.4</i>	XP_018980780.1	CCL4	This study
Golden line fish	<i>ccl33.1</i>	XP_016123015.1	CCL4	This study
Golden line fish	<i>ccl33.2</i>	XP_016123023.1	CCL4	This study
Golden line fish	<i>ccl33.3</i>	XP_016124759.1	CCL3	This study

Table S4. Sequences of the genomic target sites of zebrafish *ccl33* genes.

Target gene	Strand	Target sequence
<i>ccl33.2</i>	-	CCGCCCTGCCCATGGATGTCC
<i>ccl33.2</i>	-	CCCTTGCCCCTGCCACCACCG
<i>ccl33.2</i>	-	CCACGAGAGCCAAAACCACCA
<i>ccl33.3</i>	-	CCCGAACCTGTTGCTTCAAT
<i>ccl33.3</i>	-	CCAATCCCAGCCAACAAAGTA
<i>ccl33.3</i>	+	TTCACGCTGTGCTGTAAAGG

Table S5. Primers for antisense probe preparation for in situ hybridization analysis of *ccl33* genes in zebrafish embryos.

Primer name	Sequences
<i>ccl33.2F</i>	5'-CACCATGAGCAGTCCGCCCTGC-3'
<i>ccl33.2R</i>	5'-GGCCAGGAATGGA ACTTC-3'
<i>ccl33.3F</i>	5'-CTGCAATTCCCGAACCCCT-3'
<i>ccl33.3R</i>	5'-ACATGCCTTTAACAGCA-3'

Table S6. Sequences of zebrafish real-time PCR primers.

Primer name	Sequence (5' -> 3')
<i>ccl33.2_P1_F</i>	ACCAAATGAAGTATGGATAAAGGCT
<i>ccl33.2_P1_R</i>	TGTGCACAGTATTGTGTTGTGG
<i>ccl33.3_P1_F</i>	GTGTTTCTGCTGCTTGGACTG
<i>ccl33.3_P1_R</i>	GCAACAGGGTTCGGGAATTG
<i>18S_F</i>	TCGCTAGTTGGCATCGTTTATG
<i>18S_R</i>	CGGAGGTTCGAAGACGATCA
<i>b2m_F</i>	GCCTTCACCCCAGAGAAAGG
<i>b2m_R</i>	GCGGTTGGGATTTACATGTTG

Table S7. Sequences of the channel catfish real-time PCR primers.

Primer	Sequence (5'->3')
<i>ccl33.1F1</i>	GCAACACGTACGCACCATTC
<i>ccl33.1R1</i>	TCAGAATCACAGTGCAGCCT
<i>ccl33.2F1</i>	CTCAGACGCTCAGCCTTTTG
<i>ccl33.2R1</i>	GTCATGGCAGCCGGATTCT
<i>ccl33.3F2</i>	GCAGCTTTCACGCAACTGAA
<i>ccl33.3R2</i>	TGAAATGAAGCTTCGCACCG
<i>ccl33.4F2</i>	CGATAAAACCAGACCCCAACAAA
<i>ccl33.4R2</i>	GAGCGCCATGATCAGGACAA
<i>ccl33.5F2</i>	GCTCTACTCAGACGCTAAACC
<i>ccl33.5R2</i>	TGAAGCCTTGCTTTGGACAAC
<i>ccl33.6F1</i>	CGCTCTACTCAGACGCTATTCC
<i>ccl33.6R1</i>	GACAACGTGAGCCAGTCTTT
<i>ccl33.7F2</i>	CCAAACCCTGAGGACTGCTG
<i>ccl33.7R2</i>	TGAAGCCTTGCAGTGAACAG
<i>ccl33.8F1</i>	CGCTCCTTAGGTGTCGTAACA
<i>ccl33.8R1</i>	ATGATGCTGTCCTCAGATGCT
<i>ccl33.9F2</i>	TGGAATGTTTCCAGTGGGAGG
<i>ccl33.9R2</i>	AAATTTGCCTGAGCAGGGGT
<i>18S_f</i>	GAGAAACGGCTACCACATCC
<i>18S_r</i>	GATACGCTCATTCCGATTACAG
<i>gusb_f</i>	AAGATGGCCGATGTGTATCC
<i>gusb_r</i>	TATCCTGGGTTCTGGTGCAT

Table S8. Sequences of the PCR primers for amplifying the zebrafish target regions.

Target gene	Oligo Name	Sequence (5'→3')
<i>ccl33.2</i>	<i>ccl33.2</i> Primer3 F	AGCTCAATTTTCTCCGGGCT
<i>ccl33.2</i>	<i>ccl33.2</i> Primer3 R	TGACAGCAGAACTTACGGCCA
<i>ccl33.3</i>	<i>ccl33.3</i> Primer1 F	CCCGAACCCCTGTTGCTTCAA
<i>ccl33.3</i>	<i>ccl33.3</i> Primer1 R	ATGCACACATTCAAATGACATCAG

Additional dataset1 (separate file)

Genes present in channel catfish, and absent in bottlenose catfish.

Additional dataset2 (separate file)

Differentially expressed genes during barbel regeneration of channel catfish.

References

1. Bao B, *et al.* (2006) Characterization of 23 CC chemokine genes and analysis of their expression in channel catfish (*Ictalurus punctatus*). *Dev Comp Immunol* 30:783-796.
2. Fu Q, *et al.* (2017) The chemokine superfamily: II. The 64 CC chemokines in channel catfish and their involvement in disease and hypoxia responses. *Dev Comp Immunol* 73:97-108.