

Supplementary Methods

Processing of sequencing data

Nextera mate pair Illumina reads were screened for Nextera and Illumina adaptor sequences and trimmed accordingly by NextClip v1.3.2¹. The paired-end Illumina data had a tight insert size distribution around 300 bp and could be used without prior processing due to the high kmer assembly and correction steps during IDBA-UD assembly². Rare adaptor sequences left in the data did not hinder the assembly. They were too short to result in valid read overlaps with large kmer sizes. Pacific biosciences SMRT raw reads were corrected and trimmed by the CANU v1.0 assembler³.

Cost efficient reference assisted *de novo* assembly of *S. kneri*, *S. scherzeri* and *C. whiteheadi* based on short read sequencing

Our short reads genome assemblies relied on only two short read sequencing libraries, which can be obtained as highly complex libraries (low numbers of PCR duplicates) in most labs without expert knowledge on long distance library construction:

A) 2*150 bp paired end library (300bp insertsize); seq. cov. about 20×

B) 2*150 bp “gel-free” Nextera mate pair library (wide distribution of insertsizes, Peak ~3Kbp); seq. cov. about 20×

Short read data for *S. kneri*, *S. scherzeri* and *C. whiteheadi* was *de novo* assembled in a hybrid approach involving the kmer based *de Bruijn* graph assembler IDBA-UD and the overlap-layout-consensus (OLC) based NEWBLER v3.0 (Roche) assembler. We used a tweaked version of IDBA-UD allowing for kmers up to 252 nt and readlength of up to 300bp. We first assembled the Illumina paired end datasets (2*150bp, ~20×) using the following parameters:

```
--mink 54; --maxk 252; --seed_kmer 60; --similar 0.97; --no_coverage.
```

The resulting contigs were duplicated to produce a 2× coverage kmer backbone for the assembly of the mate pair data, which was then assembled in a second iteration of *idba_ud*:

```
-l “2xassembled_contigs.fa” --mink 206; --maxk 252; --step 20; --no_coverage; --similar 0.98; --seed_kmer 60
```

The resulting contigs and locally assembled contigs (local-contigs) of the different kmer iterations were splitted at unknown “n” bases and, if exceeding the NEWBLER V3.0 readlength limit, splitted into fragments of 29.000 bp with 4.000bp overlap. These splitted contigs were treated as “pseudo longreads” during the NEWBLER assembly.

To overcome the disadvantages of the broad distribution of insert sizes in Nextera gelfree mate pair library preparations, the mate pair data was size selected “*in-silico*”. This means the reads were mapped (BWA MEM) against the *sinChu7* reference genome and written to fastq files of distinct size classes. Mate pairs that could not be mapped consistently were written into a file with unknown size distribution. The mate pair data (reverse complemented orientation = FR is of importance for efficient scaffolding in this regard) was added to the NEWBLER assembler to produce a scaffolded OLC assembly.

To minimize misassemblies, scaffolds were checked for synteny by aligning them against the *sinChu7* reference assembly and scaffolds were splitted at detected putative inter-chromosomal fusions. Finally, we ordered the scaffolds by alignment to the *sinChu7* reference genome and applying RAGOUT, performed gap closure using *Platanus gap_close* of all data⁴ and joined neighbouring contigs, if overlaps were detectable. The derived assemblies were called *sinKne6*, *sinSch6* and *corWhi6*.

Repeat annotation

We used RepeatModeler v1.0.8 for *de novo* analysis of repeat sequences in the genome assembly sinChu7, sinKne6, sinSch6 and corWhi6. The resulting repeat sequence libraries were applied with RepeatMasker v4.0.6 to annotate/mask repetitive sequences in sinChu7, sinKne6 sinSch6 and corWhi6^{5,6}.

Gene model prediction in *S. chuatsi* by protein homology and evidence from RNAseq

We performed a homology based coding sequence (cds) prediction on the sinChu7 genome assembly first. We downloaded 1.765.230 Proteins assigned to Teleostei from the NCBI RefSeq database (date of download 03.05.2017). The proteins were aligned to the genome assembly by SPALN v2.06f to result in cds models for sinChu7 (parameters: -M4 -t 12 -O0 -Q7 -LS -pq)^{7,8}. As SPALN sometimes outputs cds models that harbor early stop codons, we re-calculated all ORFs using the TRANSDECODER (<https://transdecoder.github.io/>). The corrected genomic cds coordinates were converted to gtf format and combined into non-redundant cds models using TACO⁹. If a locus had several alternative models, the model with the longest cds sequence was chosen as representative gene model.

Subsequently, we used HISAT2¹⁰ to align RNAseq data from brain, muscle, liver and gut samples of *S. chuatsi* against the genome assembly. We supported HISAT2 mapping by adding a database of potential splice sites from the homology based cds prediction. STRINGTIE v1.2.3¹¹ was used to assemble the mapped RNAseq reads into transcript models. RNAseq transcript models and homology based CDS models were combined by TACO and TRANSDECODER was used to assign genomic cds- and UTR-exon coordinates to the resulting transcript models.

Annotation of gene function

For functional annotation we aligned protein sequences of the sinChu7 gene models against 4 fish species protein datasets that are relatively well annotated (RefSeq annotation: *Lates calcarifer*; ENSEMBL annotation: *Oreochromis niloticus*, *Gasterosteus aculeatus* and *Danio rerio*). We extracted the protein sequences of up to 100 best scoring matches per sinChu7 protein (some gene families have many similar scoring matches, it is hard to assign orthology), created multiple sequence alignments by MAFFT¹² and calculated a phylogenetic tree for each sinChu7 protein (FASTTREE2¹³). Gene descriptions and symbols were assigned from the protein match that had the smallest branch length distance to the sinChu7 protein (in most cases the gene description was assigned from the RefSeq annotation of the closely related *L. calcarifer*, while gene symbols were taken from the Ensembl annotations as they were not available for most *L. calcarifer* RefSeq proteins).

Transfer of gene annotation from sinChu7 to sinKne6, sinSch6 and corWhi6

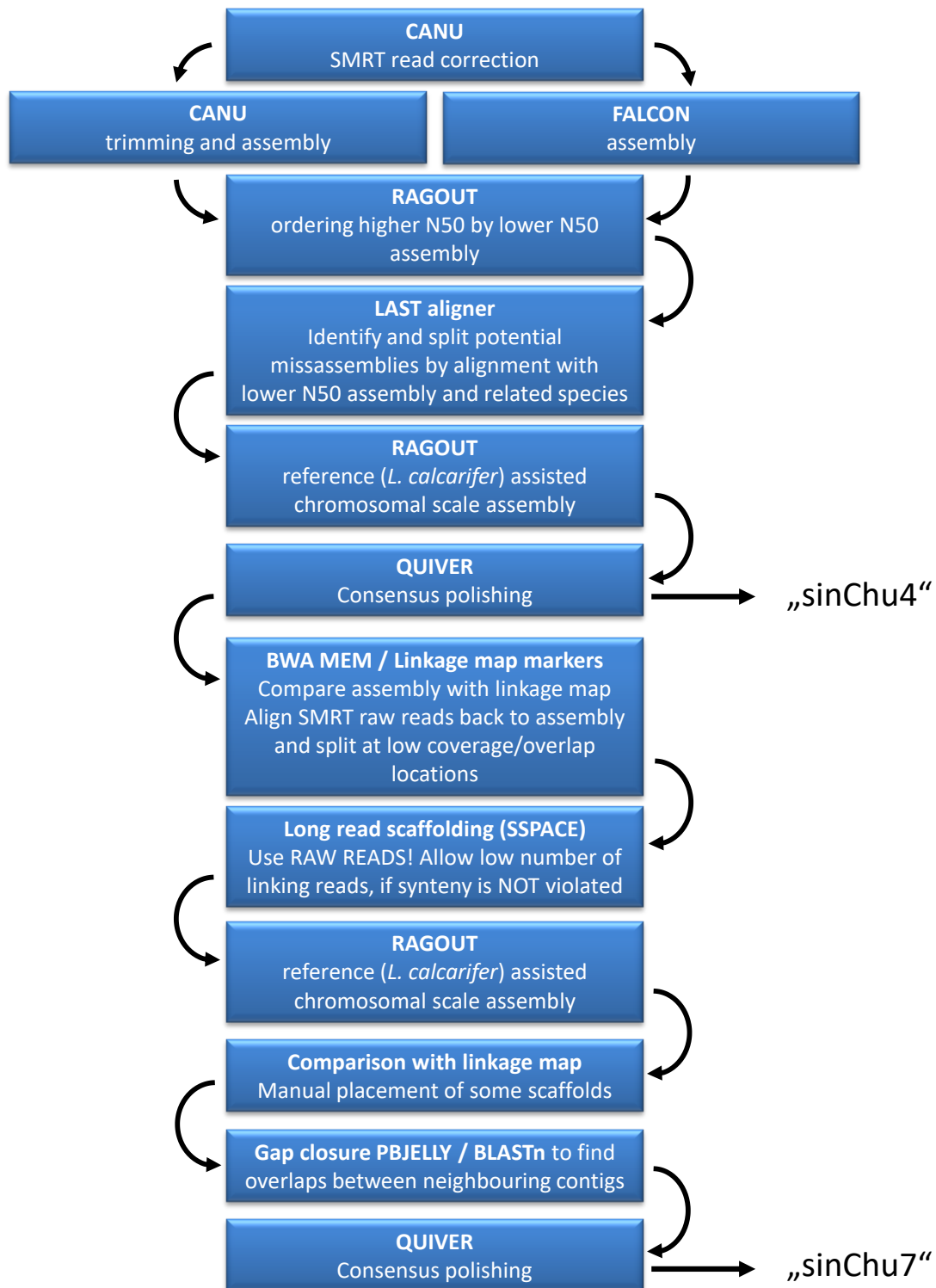
SPALN v2.06f^{7,8} was applied for spliced alignment of sinChu7 annotated mRNAs and corresponding proteins to the genome assemblies sinKne6, sinSch6 and corWhi6, respectively. The resulting mRNA and cds models were combined using TACO⁹. After calling the ORFs by TRANSDECODER (<https://transdecoder.github.io/>), the highest scoring transcript model per gene (score ~ cds length) was chosen as the reference gene model.

We assigned gene description and symbols from sinChu7 functional annotation and estimated orthology by micro synteny.

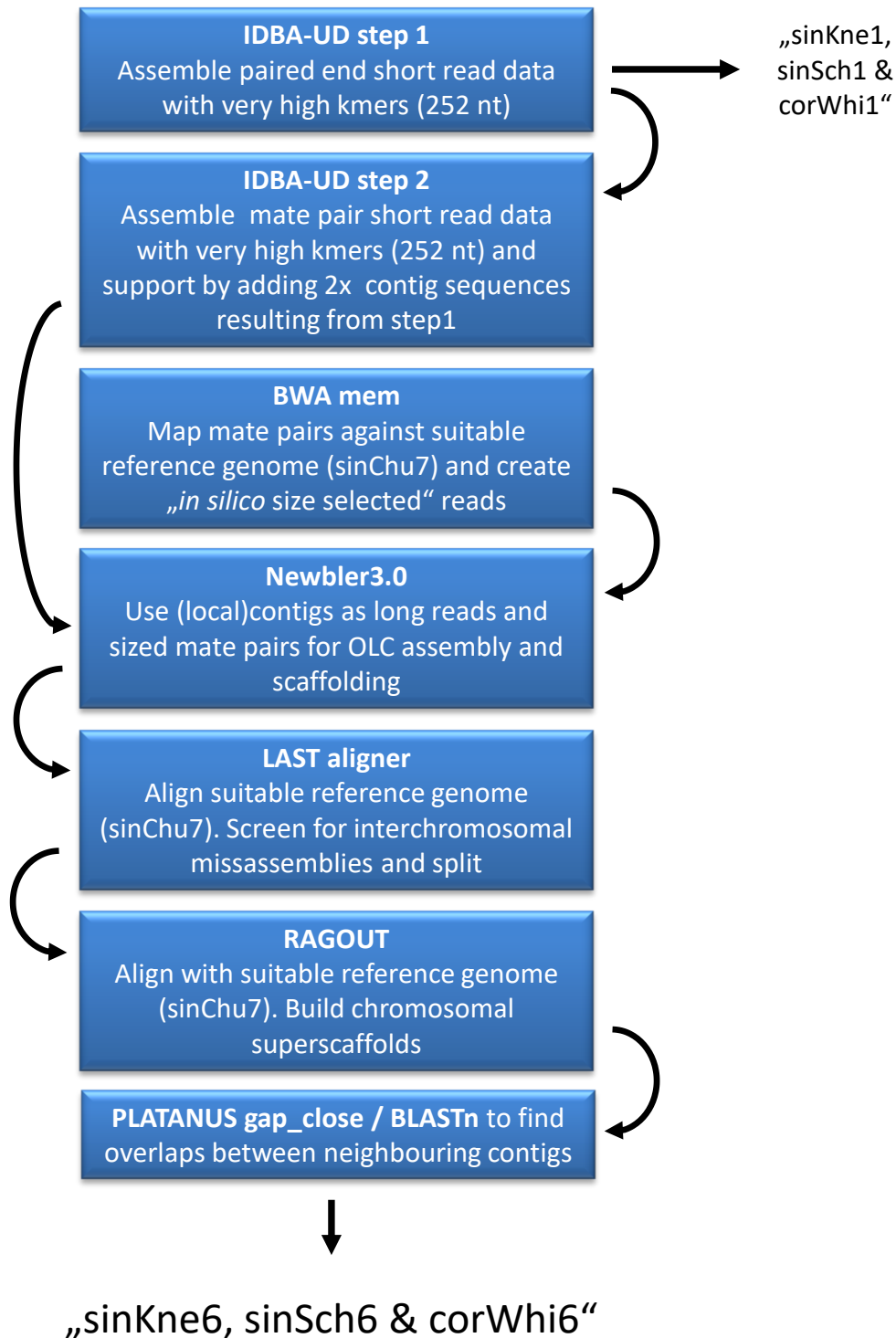
Whole genome alignment

Whole genome alignments were performed by LAST aligner and lastsplit¹⁴. MAF output files were converted to psl format using the maf-convert script. Blocks of shared collinearity between a pair of genomes were filtered by custom scripts (chaining local alignments, if they had consistent distances and orientation in both genomes in two iterations; iteration 1 maximum distance difference of blocks in both genomes 2,500 bp; iteration 2 removing blocks with length lower than 12,000 bp after iteration 1 and re-calculating blocks with maximum distance difference of 100,000 bp) using sinChu7 as the reference coordinates. If blocks of collinearity showed rearrangements, we calculated link coordinates ([Fig. 1](#)). The block and link coordinates were visualized by CIRCOS¹⁵.

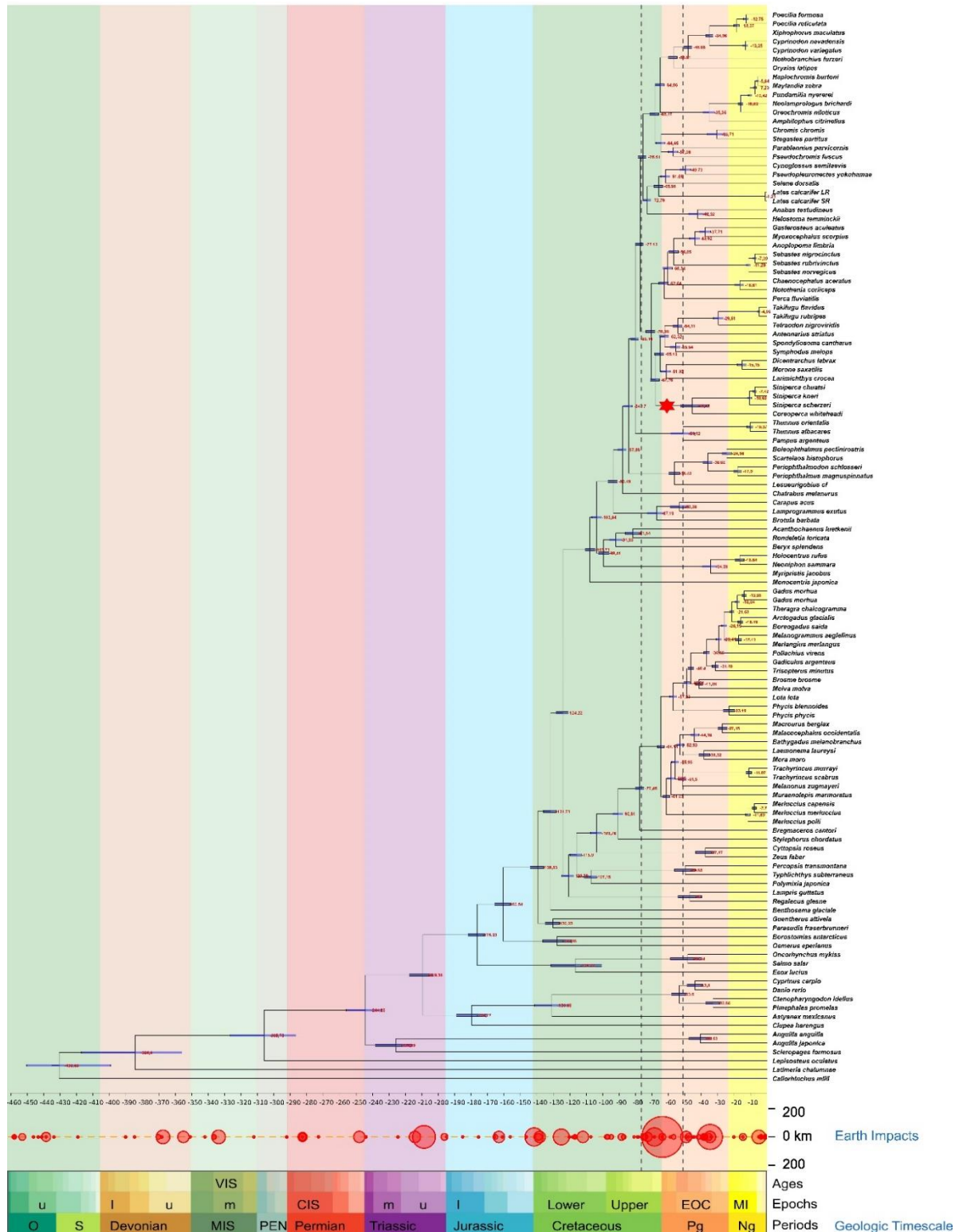
Supplementary Figures

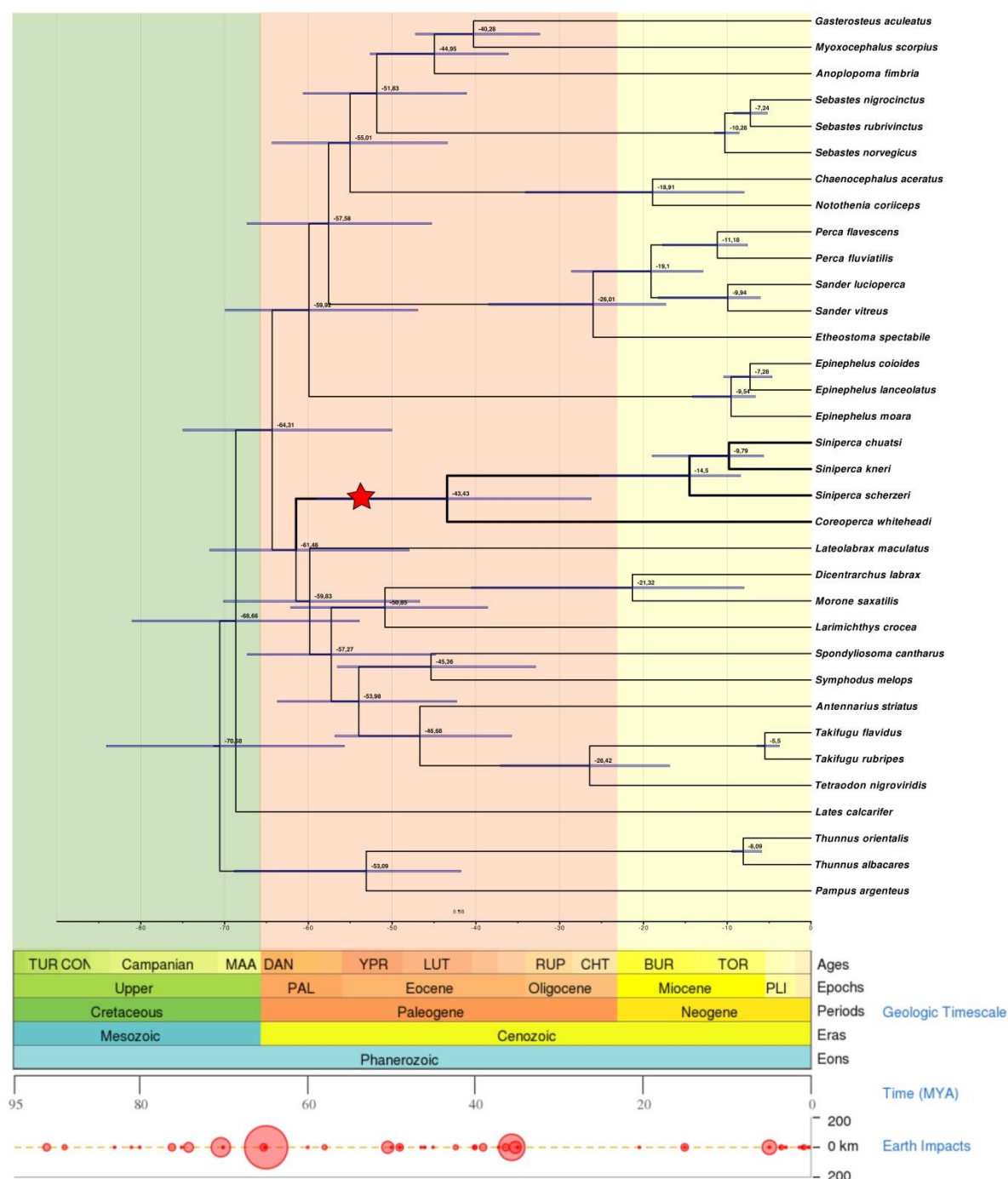


Supplementary Fig. 1 Overview of the bioinformatic pipeline for assembling the *S. chuatsi* genome. sinChu: *Siniperca chuatsi*.



Supplementary Fig. 2 Overview of the pipeline for assembling *S. kneri*, *S. scherzeri* and *C. whiteheadi* genomes. sinKne: *Siniperca kneri*, sinSch: *Siniperca scherzeri*, corWhi: *Coreoperca whiteheadi*.





Supplementary Fig. 4 Time-calibrated phylogenomic tree calculated from noncoding portions of whole-genome alignments. The SH-aLRT support was 100 for all branches. Divergence times (red or x-axis) were estimated by MCMCTree¹⁶ (clock=2 model) using a few calibration timepoints from www.timetree.org. The mandarin fish (Sinipercidae) clade is indicated with “★”.

D. labrax

LG1B



LG8



S. chuatsi

LG23



LG11

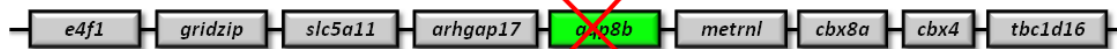


O. niloticus

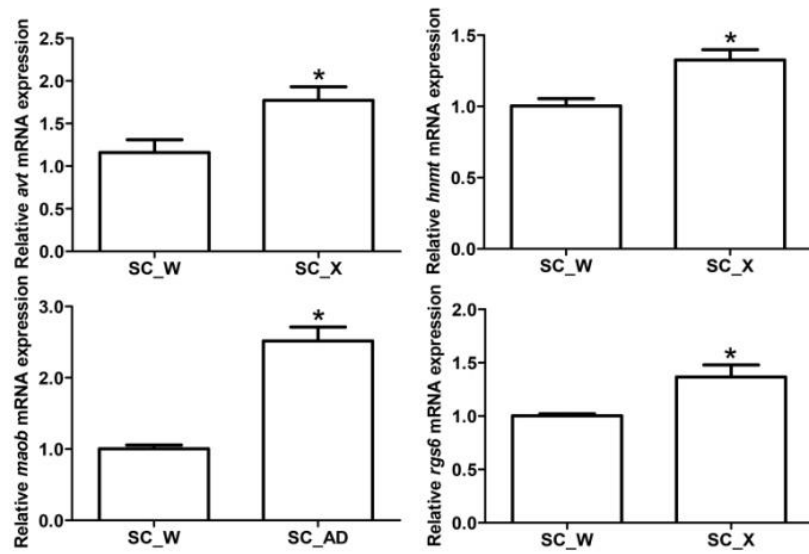
LG8



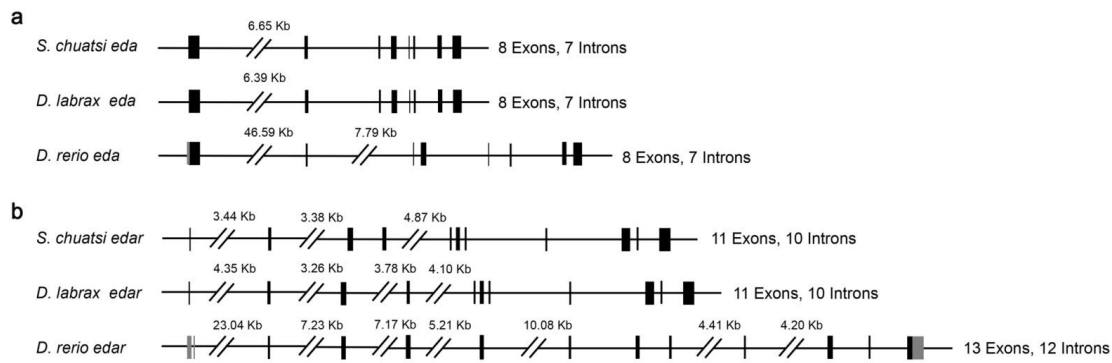
LG4



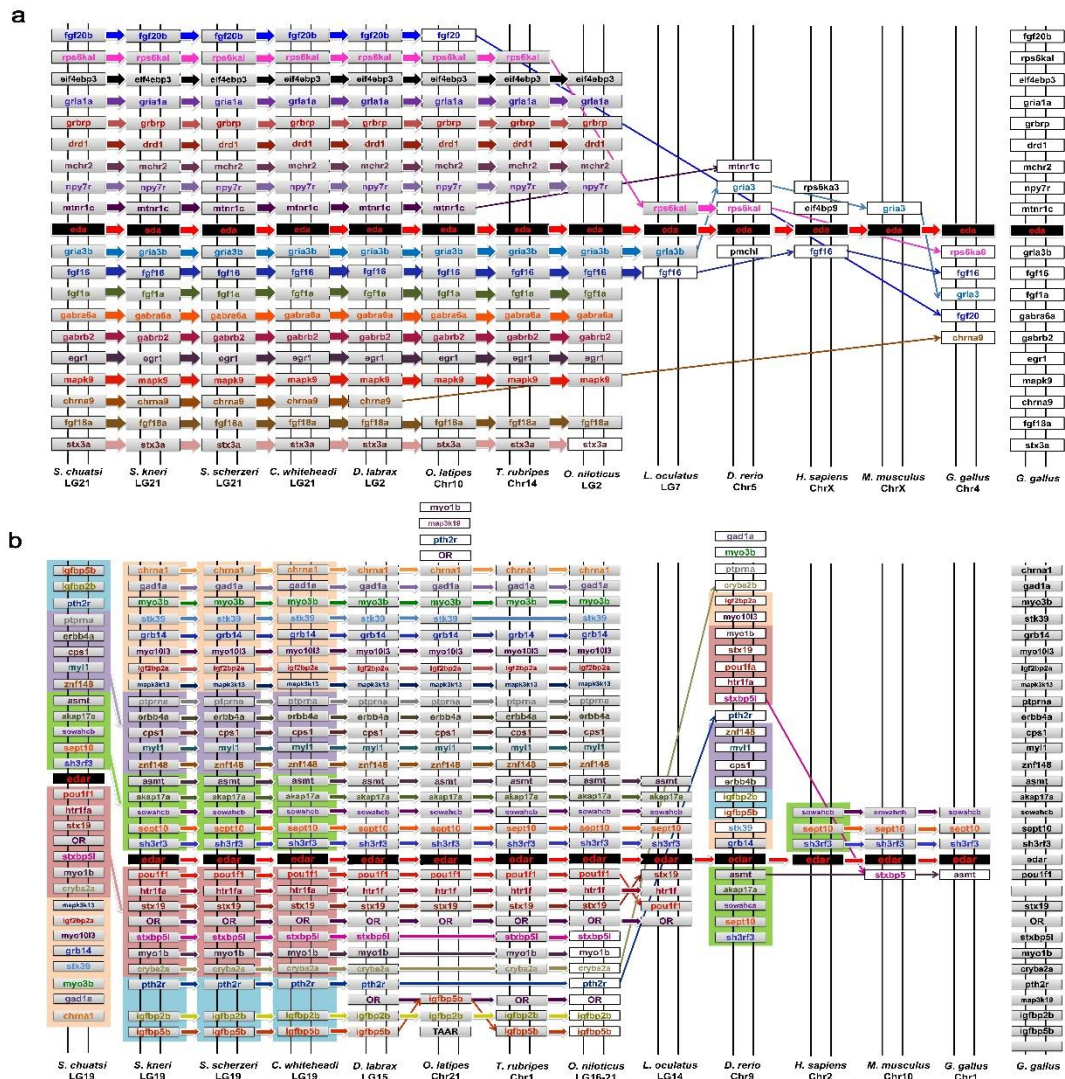
Supplementary Fig. 5 Synteny analysis of *aqp8* genes in *D. labrax*, *S. chuatsi* and *O. niloticus*. Synteny analysis was performed by searching flanking gene(s) of *aqp8s* using ensemble genome browser (<http://www.ensembl.org/index.html>), UCSC Genome Bioinformatics (<http://genome.ucsc.edu/index.html>) and Map Viewer (<http://www.ncbi.nlm.nih.gov/mapview/>).



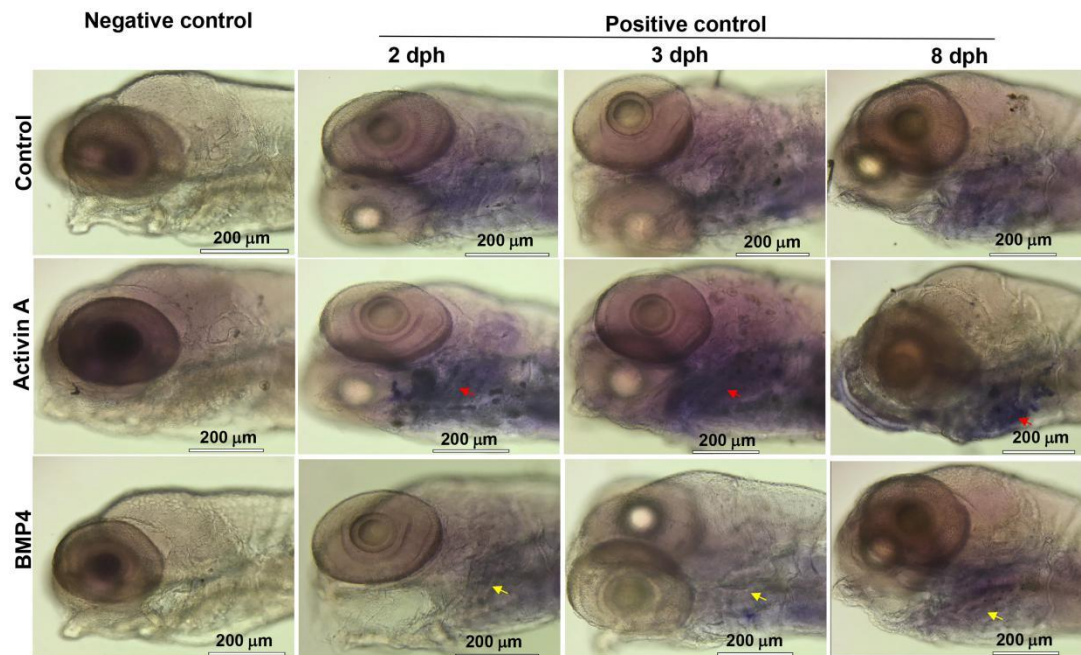
Supplementary Fig. 6 Validation of differentially expressed genes in nonfeeders (SC_W) and feeders (SC_X or SC_AD) with RT-QPCR. Brain tissues were used for analysis¹⁷. The amplification efficiencies and sequence information of primers were listed in Supplementary Table 14. Gene expression levels were quantified relative to the expression of *rpl13a* using the optimized comparative Ct ($2^{-\Delta\Delta C_t}$) value method¹⁸. Data were presented as means $\pm$ SEM (n = 6). *indicated significant difference ($P < 0.05$).



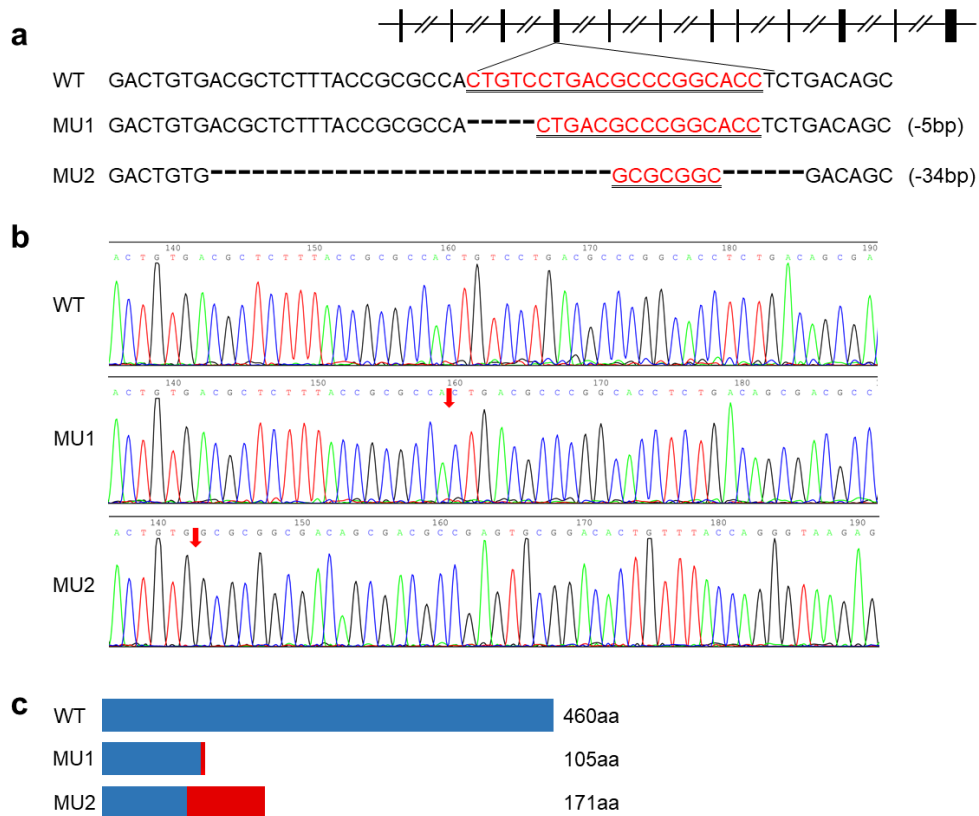
Supplementary Fig. 7 Genomic structures of *eda* and *edar* in *S. chuatsi*, *D. labrax* and *D. rerio*. The black and gray boxes indicate exons. The gray boxes represent 5'-UTR and 3'-UTR respectively.



Supplementary Fig. 8 Synteny analysis of *eda* (a) and *edar* (b). *Eda* and *edar* genes show conserved synteny across vertebrates.



Supplementary Fig. 9 Zebrafish *edar* expression assessed by whole mount *in situ* hybridization. Higher expression of *edar* (red arrowhead) were observed in the gill of fish treated with 1 ng/ml Activin A (*edar* activator), and lower expression (yellow arrowhead) were observed in fish treated with 50 ng/ml BMP4 (*edar* inhibitor).



Supplementary Fig. 10 Generation of *edar* knockout zebrafish. **a** The target site was underlined in red font. WT: wild type; MU1 and MU2: two mutants with 5bp and 34bp deletion, respectively. **b** Sequencing maps of WT, MU1 and MU2. The deletions in MU1 and MU2 were indicated by red arrows. **c**. Predicted amino acids of EDAR, blue rectangles were identical to WT EDAR, red rectangle indicated miscoding amino acids.

Supplementary Tables

Supplementary Table 1. Chromosomal/superscaffold only assembly statistics.

Assembly	sinChu7	sinKne6	sinSch6	corWhi6
Quality	"nearly finished"	HQ draft	HQ draft	HQ draft
DATA and approx. seq. coverage	SMRT ~50X	Illumina PE/MP ~30X	Illumina PE/MP ~30X	Illumina PE/MP ~40X
superscf. Count	24	34	28	34
superscf. length [bp]	730,055,992	714,256,286	722,311,289	691,332,895
Gap length [bp]	1,076,089	19,263,755	22,157,228	18,224,152
N50 superscf. length [bp]	30,577,383	29,890,789	30,166,107	28,748,119
N50 superscf. count	11	11	11	11
average superscf. length [bp]	30,419,000	21,007,538	25,796,832	20,333,320
largest superscf. [bp]	38,234,813	37,751,638	38,358,931	36,907,144
placed contig count	328	19,070	19,531	19,143
placed contig length [bp]	728,979,903	694,992,531	700,154,061	673,108,743
N50 placed contig length [bp]	12,304,016	78,643	85,698	78,820
N50 placed contig count	20	2,623	2,385	2,366
average placed contig length [bp]	2,222,500	36,444	35,848	35,162
largest placed contig [bp]	30,084,615	553,821	739,181	671,346
assembled nucleotides in chr./superscf.	96.68%	98.76%	98.18%	97.07%

sinChu: *Siniperca chuatsi*, sinKne: *Siniperca kneri*, sinSch: *Siniperca scherzeri*, corWhi: *Coreoperca whiteheadi*.

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Supplementary Table 2. Chromosomal/superscaffold + all unplaced scaffolds statistics

Assembly	sinChu7	sinKne6	sinSch6	corWhi6
Quality	"nearly finished"	HQ draft	HQ draft	HQ draft
DATA and approx. seq. coverage	SMRT ~50X	Illumina PE/MP ~30X	Illumina PE/MP ~30X	Illumina PE/MP ~35X
scf. count	1,156	1,854	2,826	2,615
scf. length [bp]	755,061,740	723,605,295	736,220,003	712,478,531
Gap length [bp]	1,078,130	19,895,218	23,119,548	
N50 scf. length [bp] incl. Map/Synten	30,508,166	29,777,865	30,166,107	28,603,870
N50 scf. Count incl. Map/Synten	12	12	11	12
N50 scf. length [bp] no Map/Synten	23,370,180	1,198,238	1,381,907	1,020,541
average scf. length [bp]	653,168	390,294	260,517	272,458
largest scf. [bp]	38,234,813	37,751,638	38,358,931	36,907,144
contig count	1,464	21,467	23,070	22,717
contig length [bp]	753,983,610	703,710,077	713,100,455	693,395,931
N50 contig length [bp]	12,191,788	77,505	83,589	76,435
N50 contig count	21	2,679	2,461	2,495
average contig length [bp]	515,016	32,781	30,910	30,523
largest contig [bp]	30,084,615	553,821	739,181	671,346

164 sinChu: *Siniperca chuatsi*, sinKne: *Siniperca kneri*, sinSch: *Siniperca scherzeri*, corWhi: *Coreoperca whiteheadi*.

Supplementary Table 3. Positively selected genes triggering enrichment of GO/MP terms related to observed biological traits

Traits	Branches involved in analysis	Total number of PSGs with assigned gene symbol	Identified GO/MP related to traits	Number of PSGs	p-value for enrichment	Genes triggering GO/MP term enrichment
Feeding habit	Branch 3 and <i>S. scherzeri</i>	544	learning or memory	20	7.30E-03	Abca7, B4gal2, Cacna1e, Cc, Dnah11, Ehmt2, Hif1a, Iga3a, Iga8b, Itp3c, Lamb1, Map1a, Nnan1, Nr1k1, Pabk6, Pclb1, Psen2, Ptn, Ptprz1, Sorcs3
			locomotion	102	2.00E-07	Abh3, Abhl1, Ace, Adgrb1, Adgrt3, Aire, Amot2, Ano6, Anxa1, Ash1l, Bves, Cacna1e, Ccbr8, Cd99, Cd99b2, Cdc42bpb, Cdh1, Cdh13, Cep131, Chm4, Cnkr1a, Ctk, Csf1r, Dce, Depdc1b, Dcl1, Dlg5, Dnah11, Dock2, Ednaa, F2hl, Pbln1, Fer, Fgfbp1, Flrt2, Flh1, Gd3, Glac5, Hif1a, Hoxa7, Ippp5b, Irt1, Irga2, Irga2b, Irga3, Irga4a, Irga6, Irg8b, Lama5, Lamb1, Lemt3, Lmo4, Lmo5, Magi2, Maf3, Mypc10, Myo9b, Mypn, Nlanc, Nplb, Nlcn, Notch1, Nr1k1, Pdr3, Pcsk5, Phlb2, PKC2b, PKC2c, PKCg, Pkn1, Pclb1, Pdl1, Plasn1, Ppp2ca, Prokr2, Psen2, Ptgdr2, Ptk2b, Ptk7, Ptn, Ptpn22, Ptprc, Ptprj, Ptprio, Ptprr2, Rerb1, Rspk9, Ryk, Scg2, Sema4a, Sema4d, Sema7a, Sep, 2Sod2, St14, Ssk10, Sun2, Tle1, Trim25, Trim35, Tnn, Vstm2l
			response to food	3	3.30E-02	Bcl10, Slc25a25, Pkicg
	Branch 3 (PSG p<0.005)	86	eating behaviour	2	3.10E-02	Lepr, Uchl3
	Growth	<i>S. chuatsi</i> and <i>S. kneri</i>	411	growth	42	2.30E-02
decreased circulating insulin-like growth factor I level				8	1.10E-03	Arid1b, Itpri2, Mbd5, Sirt1, Sk38a3, Spr, Suco, Tur4
decreased growth hormone level				5	9.90E-03	Lepr, Mbd5, Pou1f1, Sirt1, Zfhx3
<i>S. kneri</i>		206	proportional dwarf	3	7.80E-03	Nek1, Pou1f1, Wdr62
Pyloric caeca	Branch 5 and <i>C. whiteheadi</i>	1687	alimentary system mucosa	123	7.00E-07	Abca1, Abcf1, Acaa2, Acsf5, Adamts9, Aeep1, Ahnak, Ano8, Ano9, Apod, Aqp3, Arhgap29, Atptb1, Atna2, Bmp7, Bcc, Cast, Cdc6b0, Cdcpl, Cds4, Cep83, Chga, Coll18a1, Coll1a1, Colla2, Coll2a1, Col4a1, Col4a2, Col4a4, Col4a5, Col4a6, Cnabp1, Creb3l3, Cxcr4, Cyld, Cyp26a1, Dll4, Dnah12, Dxc2, Dsg2, Dsp, Dtl, Dnaa, Ecm1, Eln, Epa1, Epcam, Epr2, Esys3, Dsl, Eos1, F1fr, Fbn1, Fgf10, Fgf4, Fgfr1, Fnl1, Foxe1, Foxn1, Gqrl1, Gan, Has2, Hiplr, Hnf1a, Hnf4g, Hs6a13, Hspa8b, Hsp27, Igf2r, Igsb9, Irs1, Irs3, Irs5, Isll, Irga2, Irga3, Irga4, Irga6, Ilm2a, Jup, Krt15, Krt5, Lama3, Lama5, Lamb1, Lamb2, Lamb3, Mapkbp1, Matn4, Mmm2, Mpl22, Ncof6, Nedd8, Nid1, Notch2, Oma1, Oxc2, Pik3ip1, Pls1, Plasn1, Plasn2, Pparg1a, Prom2, Qsox1, Rab20, Rara, Rarb, Rfrf1, Robo2, Shroom2, Smad3, Smad4, Smad6, Snail, Sod3, Sox6, Spint2, Spo11, St14, Stard10, Syt1, Tacc1, Tbs22, Tfec, Tgfb2, Thbd, Tle3, Tmprss15, Tmx3, Trim24, Usp22, Vav3, Wnt4
			digestive/alimentary phenotype	164	0.198	Abca1, Acan, Ace2, Actn4, Acvrl1, Adam10, Adgrg7, Aeep1, Ahcyll1, Aicda, Angpt2, Aqp3, Aqp4, Arhgap29, Arf4, Bcam, Bmp7, Bcc2, Capn6, Capn8, Casp3, Cbx2, Cdc39, Cdc40, Cend1, Cn36, Cdc63, Cgn, Chst11, Chuk, Cldn18, Cntn1, Coll1a1, Coll1a1, Coll1a1, Col2a1, Col4a2, Col4a4, Col7a1, Cdh3, Ctndd1, Cxcr4, Cyld, Cyp26a1, Dce, Dher7, Dsp, Dst, Dylc1c1, Elf3, Enpp7, Epcam, Eya1, F2r, Fadd, Fads2, Fam20c, Fen1, Fga, Fgf10, Fgfr1, Fgf3, Foxc1, Foxe1, Foxt2, Galnt3, Gcgr, Gdnf4, Glna2, Gln2, Golgi1, Gsnrl, Gpc3, Hsd17b4, Hspg2, Hs98b, Il2g, Il6a1, Isll, Klf1lp, Krt15, Krt5, Krt5, Lama3, Lama5, Lamb3, Loh3, Lp4, Lp2, Lpdp4, Lnc1, Map3k4, Mdn, Mmp9, Mnr, Ncof6, Nod2, N2f2, Nkaf2, Nnm, Nsum2, Oxc2, P2zy4, Pcnt, Pcsk5, Pcsk6, Pdgrfa, Pex1, Pglym2, Pdl1, Plau, Pls1, Porcn, Pparg1a, Ptdm14, Pugs1, Phl1r, Qsox1, Rab34, Rb1cc1, Rbpj, Relb, Ripk3, Rmzb3, Runx2, Sc5d, Sdcagb8, Serpinf1, Shroom3, Slc10a2, Slc15a1, Slc18a2, Slc27a4, Slc2a12, Slc32a1, Slc5a2, Slc5a7, Slc6a19, Slc8a1, Smda3, Smda4, Socx1, Spic2, Sstr2, St14, Stard10, Supv3l1, Syt12, Tbc1d32, Tbs22, Tctn2, Tdp2, Tent5c, Tgfb2, Thbd, Tln1, Tlr2, Tm6a2, Tnfrsf11a, Tnfrsf1a, Tnki, Ttc7, Ulk4, Usp22, Wdr19, Wfs1, Xprnppep1
Salinity adaptation	Branch 7	918	ion transport	127	6.80E-05	Abca4, Abcb11, Ada, Ahnak, Ank3, Ano5, Ano6, Ano7, Apoa1, Apoa4, Apoc2, Apoe, Aqp8, Arg1, Asik1, Atpt1a3, Atpt1a2, Atpt4b, Atpt6va2, Atpt6vle1, Atpt8b5, Bn1, Cacna1a, Cacna1c, Cacna1d, Cacna1f, Cacna1g, Cacng6, Cacng7, Ccs, G236, Chchd10, Chma2, Chma7, Chma9, Chmd, Clec1, Cln3, Cnga1, Cpt1b, Cracr2b, Cyba, Cybb, Enpp1, Fgf12, Gabra6, Gabrg3, Gpr39, Hcn2, Hcn3, Homer2, Htr2a, Itpk62, Kcna1, Kcna2, Kcna7, Kcna8a3, Kcnh7, Kcnip2, Kcnj15, Kcnk18, Mif, Nlanc1, Nnt, Nsf, Opal1, Oprl1, Osxr1, P2ry2, Pcp4, Pdl1, Pln2, Plip, Pkica, Prkg2, Psap, Psen1, Pk2b, Rbp4, Rhag, Saraf, Scn1b, Scn4a, Scn8a, Sestd1, Shank3, Slc10a1, Slc12a6, Slc13a2, Slc15a1, Slc16a1, Slc20a1, Slc23a2, Slc24a2, Slc25a4, Slc27a2, Slc28a7, Slc27a2, Slc28a14, Slc28a15, Slc28a16, Slc28a17, Slc28a18, Slc28a19, Slc28a20, Slc28a21, Slc28a22, Slc28a23, Slc28a24, Slc28a25, Slc28a26, Slc28a27, Slc28a28, Slc28a29, Slc28a30, Slc28a31, Slc28a32, Slc28a33, Slc28a34, Slc28a35, Slc28a36, Slc28a37, Slc28a38, Slc28a39, Slc28a40, Slc28a41, Slc28a42, Slc28a43, Slc28a44, Slc28a45, Slc28a46, Slc28a47, Slc28a48, Slc28a49, Slc28a50, Slc28a51, Slc28a52, Slc28a53, Slc28a54, Slc28a55, Slc28a56, Slc28a57, Slc28a58, Slc28a59, Slc28a60, Slc28a61, Slc28a62, Slc28a63, Slc28a64, Slc28a65, Slc28a66, Slc28a67, Slc28a68, Slc28a69, Slc28a70, Slc28a71, Slc28a72, Slc28a73, Slc28a74, Slc28a75, Slc28a76, Slc28a77, Slc28a78, Slc28a79, Slc28a80, Slc28a81, Slc28a82, Slc28a83, Slc28a84, Slc28a85, Slc28a86, Slc28a87, Slc28a88, Slc28a89, Slc28a90, Slc28a91, Slc28a92, Slc28a93, Slc28a94, Slc28a95, Slc28a96, Slc28a97, Slc28a98, Slc28a99, Slc28a100, Slc28a101, Slc28a102, Slc28a103, Slc28a104, Slc28a105, Slc28a106, Slc28a107, Slc28a108, Slc28a109, Slc28a110, Slc28a111, Slc28a112, Slc28a113, Slc28a114, Slc28a115, Slc28a116, Slc28a117, Slc28a118, Slc28a119, Slc28a120, Slc28a121, Slc28a122, Slc28a123, Slc28a124, Slc28a125, Slc28a126, Slc28a127, Slc28a128, Slc28a129, Slc28a130, Slc28a131, Slc28a132, Slc28a133, Slc28a134, Slc28a135, Slc28a136, Slc28a137, Slc28a138, Slc28a139, Slc28a140, Slc28a141, Slc28a142, Slc28a143, Slc28a144, Slc28a145, Slc28a146, Slc28a147, Slc28a148, Slc28a149, Slc28a150, Slc28a151, Slc28a152, Slc28a153, Slc28a154, Slc28a155, Slc28a156, Slc28a157, Slc28a158, Slc28a159, Slc28a160, Slc28a161, Slc28a162, Slc28a163, Slc28a164, Slc28a165, Slc28a166, Slc28a167, Slc28a168, Slc28a169, Slc28a170, Slc28a171, Slc28a172, Slc28a173, Slc28a174, Slc28a175, Slc28a176, Slc28a177, Slc28a178, Slc28a179, Slc28a180, Slc28a181, Slc28a182, Slc28a183, Slc28a184, Slc28a185, Slc28a186, Slc28a187, Slc28a188, Slc28a189, Slc28a190, Slc28a191, Slc28a192, Slc28a193, Slc28a194, Slc28a195, Slc28a196, Slc28a197, Slc28a198, Slc28a199, Slc28a200, Slc28a201, Slc28a202, Slc28a203, Slc28a204, Slc28a205, Slc28a206, Slc28a207, Slc28a208, Slc28a209, Slc28a210, Slc28a211, Slc28a212, Slc28a213, Slc28a214, Slc28a215, Slc28a216, Slc28a217, Slc28a218, Slc28a219, Slc28a220, Slc28a221, Slc28a222, Slc28a223, Slc28a224, Slc28a225, Slc28a226, Slc28a227, Slc28a228, Slc28a229, Slc28a230, Slc28a231, Slc28a232, Slc28a233, Slc28a234, Slc28a235, Slc28a236, Slc28a237, Slc28a238, Slc28a239, Slc28a240, Slc28a241, Slc28a242, Slc28a243, Slc28a244, Slc28a245, Slc28a246, Slc28a247, Slc28a248, Slc28a249, Slc28a250, Slc28a251, Slc28a252, Slc28a253, Slc28a254, Slc28a255, Slc28a256, Slc28a257, Slc28a258, Slc28a259, Slc28a260, Slc28a261, Slc28a262, Slc28a263, Slc28a264, Slc28a265, Slc28a266, Slc28a267, Slc28a268, Slc28a269, Slc28a270, Slc28a271, Slc28a272, Slc28a273, Slc28a274, Slc28a275, Slc28a276, Slc28a277, Slc28a278, Slc28a279, Slc28a280, Slc28a281, Slc28a282, Slc28a283, Slc28a284, Slc28a285, Slc28a286, Slc28a287, Slc28a288, Slc28a289, Slc28a290, Slc28a291, Slc28a292, Slc28a293, Slc28a294, Slc28a295, Slc28a296, Slc28a297, Slc28a298, Slc28a299, Slc28a300, Slc28a301, Slc28a302, Slc28a303, Slc28a304, Slc28a305, Slc28a306, Slc28a307, Slc28a308, Slc28a309, Slc28a310, Slc28a311, Slc28a312, Slc28a313, Slc28a314, Slc28a315, Slc28a316, Slc28a317, Slc28a318, Slc28a319, Slc28a320, Slc28a321, Slc28a322, Slc28a323, Slc28a324, Slc28a325, Slc28a326, Slc28a327, Slc28a328, Slc28a329, Slc28a330, Slc28a331, Slc28a332, Slc28a333, Slc28a334, Slc28a335, Slc28a336, Slc28a337, Slc28a338, Slc28a339, Slc28a340, Slc28a341, Slc28a342, Slc28a343, Slc28a344, Slc28a345, Slc28a346, Slc28a347, Slc28a348, Slc28a349, Slc28a350, Slc28a351, Slc28a352, Slc28a353, Slc28a354, Slc28a355, Slc28a356, Slc28a357, Slc28a358, Slc28a359, Slc28a360, Slc28a361, Slc28a362, Slc28a363, Slc28a364, Slc28a365, Slc28a366, Slc28a367, Slc28a368, Slc28a369, Slc28a370, Slc28a371, Slc28a372, Slc28a373, Slc28a374, Slc28a375, Slc28a376, Slc28a377, Slc28a378, Slc28a379, Slc28a380, Slc28a381, Slc28a382, Slc28a383, Slc28a384, Slc28a385, Slc28a386, Slc28a387, Slc28a388, Slc28a389, Slc28a390, Slc28a391, Slc28a392, Slc28a393, Slc28a394, Slc28a395, Slc28a396, Slc28a397, Slc28a398, Slc28a399, Slc28a400, Slc28a401, Slc28a402, Slc28a403, Slc28a404, Slc28a405, Slc28a406, Slc28a407, Slc28a408, Slc28a409, Slc28a410, Slc28a411, Slc28a412, Slc28a413, Slc28a414, Slc28a415, Slc28a416, Slc28a417, Slc28a418, Slc28a419, Slc28a420, Slc28a421, Slc28a422, Slc28a423, Slc28a424, Slc28a425, Slc28a426, Slc28a427, Slc28a428, Slc28a429, Slc28a430, Slc28a431, Slc28a432, Slc28a433, Slc28a434, Slc28a435, Slc28a436, Slc28a437, Slc28a438, Slc28a439, Slc28a440, Slc28a441, Slc28a442, Slc28a443, Slc28a444, Slc28a445, Slc28a446, Slc28a447, Slc28a448, Slc28a449, Slc28a450, Slc28a451, Slc28a452, Slc28a453, Slc28a454, Slc28a455, Slc28a456, Slc28a457, Slc28a458, Slc28a459, Slc28a460, Slc28a461, Slc28a462, Slc28a463, Slc28a464, Slc28a465, Slc28a466, Slc28a467, Slc28a468, Slc28a469, Slc28a470, Slc28a471, Slc28a472, Slc28a473, Slc28a474, Slc28a475, Slc28a476, Slc28a477, Slc28a478, Slc28a479, Slc28a480, Slc28a481, Slc28a482, Slc28a483, Slc28a484, Slc28a485, Slc28a486, Slc28a487, Slc28a488, Slc28a489, Slc28a490, Slc28a491, Slc28a492, Slc28a493, Slc28a494, Slc28a495, Slc28a496, Slc28a497, Slc28a498, Slc28a499, Slc28a500, Slc28a501, Slc28a502, Slc28a503, Slc28a504, Slc28a505, Slc28a506, Slc28a507, Slc28a508, Slc28a509, Slc28a510, Slc28a511, Slc28a512, Slc28a513, Slc28a514, Slc28a515, Slc28a516, Slc28a517, Slc28a518, Slc28a519, Slc28a520, Slc28a521, Slc28a522, Slc28a523, Slc28a524, Slc28a525, Slc28a526, Slc28a527, Slc28a528, Slc28a529, Slc28a530, Slc28a531, Slc28a532, Slc28a533, Slc28a534, Slc28a535, Slc28a536, Slc28a537, Slc28a538, Slc28a539, Slc28a540, Slc28a541, Slc28a542, Slc28a543, Slc28a544, Slc28a545, Slc28a546, Slc28a547, Slc28a548, Slc28a549, Slc28a550, Slc28a551, Slc28a552, Slc28a553, Slc28a554, Slc28a555, Slc28a556, Slc28a557, Slc28a558, Slc28a559, Slc28a560, Slc28a561, Slc28a562, Slc28a563, Slc28a564, Slc28a565, Slc28a566, Slc28a567, Slc28a568, Slc28a569, Slc28a570, Slc28a571, Slc28a572, Slc28a573, Slc28a574, Slc28a575, Slc28a576, Slc28a577, Slc28a578, Slc28a579, Slc28a580, Slc28a581, Slc28a582, Slc28a583, Slc28a584, Slc28a585, Slc28a586, Slc28a587, Slc28a588, Slc28a589, Slc28a590, Slc28a591, Slc28a592, Slc28a593, Slc28a594, Slc28a595, Slc28a596, Slc28a597, Slc28a598, Slc28a599, Slc28a600, Slc28a601, Slc28a602, Slc28a603, Slc28a604, Slc28a605, Slc28a606, Slc28a607, Slc28a608, Slc28a609, Slc28a610, Slc28a611, Slc28a612, Slc28a613, Slc28a614, Slc28a615, Slc28a616, Slc28a617, Slc28a618, Slc28a619, Slc28a620, Slc28a621, Slc28a622, Slc28a623, Slc28a624, Slc28a625, Slc28a626, Slc28a627, Slc28a628, Slc28a629, Slc28a630, Slc28a631, Slc28a632, Slc28a633, Slc28a634, Slc28a635, Slc28a636, Slc28a637, Slc28a638, Slc28a639, Slc28a640, Slc28a641, Slc28a642, Slc28a643, Slc28a644, Slc28a645, Slc28a646, Slc28a647, Slc28a648, Slc28a649, Slc28a650, Slc28a651, Slc28a652, Slc28a653, Slc28a654, Slc28a655, Slc28a656, Slc28a657, Slc28a658, Slc28a659, Slc28a660, Slc28a661, Slc28a662, Slc28a663, Slc28a664, Slc28a665, Slc28a666, Slc28a667, Slc28a668, Slc28a669, Slc28a670, Slc28a671, Slc28a672, Slc28a673, Slc28a674, Slc28a675, Slc28a676, Slc28a677, Slc28a678, Slc28a679, Slc28a680, Slc28a681, Slc28a682, Slc28a683, Slc28a684, Slc28a685, Slc28a686, Slc28a687, Slc28a688, Slc28a689, Slc28a690, Slc28a691, Slc28a692, Slc28a693, Slc28a694, Slc28a695, Slc28a696, Slc28a697, Slc28a698, Slc28a699, Slc28a700, Slc28a701, Slc28a702, Slc28a703, Slc28a704, Slc28a705, Slc28a706, Slc28a707, Slc28a708, Slc28a709, Slc28a710, Slc28a711, Slc28a712, Slc28a713, Slc28a714, Slc28a715, Slc28a716, Slc28a717, Slc28a718, Slc28a719, Slc28a720, Slc28a721, Slc28a722, Slc28a723, Slc28a724, Slc28a725, Slc28a726, Slc28a727, Slc28a728, Slc28a729, Slc28a730, Slc28a731, Slc28a732, Slc28a733, Slc28a734, Slc28a735, Slc28a736, Slc28a737, Slc28a738, Slc28a739, Slc28a740, Slc28a741, Slc28a742, Slc28a743, Slc28a744, Slc28a745, Slc28a746, Slc28a747, Slc28a748, Slc28a749, Slc28a750, Slc28a751, Slc28a752, Slc28a753, Slc28a754, Slc28a755, Slc28a756, Slc28a757, Slc28a758, Slc28a759, Slc28a760, Slc28a761, Slc28a762, Slc28a763, Slc28a764, Slc28a765, Slc28a766, Slc28a767, Slc28a768, Slc28a769, Slc28a770, Slc28a771, Slc28a772, Slc28a773, Slc28a774, Slc28a775, Slc28a776, Slc28a777, Slc28a778, Slc28a779, Slc28a780, Slc28a781, Slc28a782, Slc28a783, Slc28a784, Slc28a785, Slc28a786, Slc28a787, Slc28a788, Slc28a789, Slc28a790, Slc28a791, Slc28a792, Slc28a793, Slc28a794, Slc28a795, Slc28a796, Slc28a797, Slc28a798, Slc28a799, Slc28a800, Slc28a801, Slc28a802, Slc28a803, Slc28a804, Slc28a805, Slc28a806, Slc28a807, Slc28a808, Slc28a809, Slc28a810, Slc28a811, Slc28a812, Slc28a813, Slc28a814, Slc28a815, Slc28a816, Slc28a817, Slc28a818, Slc28a819, Slc28a820, Slc28a821, Slc28a822, Slc28a823, Slc28a824, Slc28a825, Slc28a826, Slc28a827, Slc28a828, Slc28a829, Slc28a830, Slc28a831, Slc28a832, Slc28a833, Slc28a834, Slc28a835, Slc28a836, Slc28a837, Slc28a838, Slc28a839, Slc28a840, Slc28a841, Slc28a842, Slc28a843, Slc28a844, Slc28a845, Slc28a846, Slc28a847, Slc28a848, Slc28a849, Slc28a850, Slc28a851, Slc28a852, Slc28a853, Slc28a854, Slc28a855, Slc28a856, Slc28a857, Slc28a858, Slc28a859, Slc28a860, Slc28a861, Slc28a862, Slc28a863, Slc28a864, Slc28a865, Slc28a866, Slc28a867, Slc28a868, Slc28a869, Slc28a870, Slc28a871, Slc28a872, Slc28a873, Slc28a874, Slc28a875, Slc28a876, Slc28a877, Slc28a878, Slc28a879, Slc28a880, Slc28a881, Slc28a882, Slc28a883, Slc28a884, Slc28a885, Slc28a886, Slc28a887, Slc28a888, Slc28a889, Slc28a890, Slc28a891, Slc28a892, Slc28a893, Slc28a894, Slc28a895, Slc28a896, Slc28a897, Slc28a898, Slc28a899, Slc28a900, Slc28a901, Slc28a902, Slc28a903, Slc28a904, Slc28a905, Slc28a906, Slc28a907, Slc28a908, Slc28a909, Slc28a910, Slc28a911, Slc28a912, Slc28a913, Slc28a914, Slc28a915, Slc28a916, Slc28a917, Slc28a918, Slc28a919, Slc28a920, Slc28a921, Slc28a922, Slc28a923, Slc28a924, Slc28a925, Slc28a926, Slc28a927, Slc28a928, Slc28a929, Slc28a930, Slc28a931, Slc28a932, Slc28a933, Slc28a934, Slc28a935, Slc28a936, Slc28a937, Slc28a938, Slc28a939, Slc28a940, Slc28a941, Slc28a942, Slc28a943, Slc28a944, Slc28a945, Slc28a946, Slc28a947, Slc28a948, Slc28a949, Slc28a950, Slc28a951, Slc28a952, Slc28a953, Slc28a954, Slc28a955, Slc28a956, Slc28a957, Slc28a958, Slc28a959, Slc28a960, Slc28a961, Slc28a962, Slc28a963, Slc28a964, Slc28a965, Slc28a966, Slc28a967, Slc28a968, Slc28a969, Slc28a970, Slc28a971, Slc28a972, Slc28a973, Slc28a974, Slc28a975, Slc28a976, Slc28a977, Slc28a978, Slc28a979, Slc28a980, Slc28a981, Slc28a982, Slc28a983, Slc28a984, Slc28a985, Slc28a986, Slc28a987, Slc28a988, Slc28a989, Slc28a990, Slc28a991, Slc28a992, Slc28a993, Slc28a994, Slc28a995, Slc28a996, Slc28a997, Slc28a998, Slc28a999, Slc28a1000, Slc28a1001, Slc28a1002, Slc28a1003, Slc28a1004, Slc28a1005, Slc28a1006, Slc28a1007, Slc28a1008, Slc28a1009, Slc28a1010, Slc28a1011, Slc28a1012, Slc28a1013, Slc28a1014, Slc28a1015, Slc28a1016, Slc28a1017, Slc28a1018, Slc28a1019, Slc28a1020, Slc28a1021, Slc28a1022, Slc28a1023, Slc28a1024, Slc28a1025, Slc28a1026, Slc28a1027, Slc28a1028, Slc28a1029, Slc28a1030, Slc28a1031, Slc28a1032, Slc28a1033, Slc28a1

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Supplementary Table 4. Positively selected and differentially expressed genes between nonfeeders and feeders in Node 3

SC7 gene_ID	Gene symbol	Description	BMP4-initiated signaling molecules binding sites
SC7-LG05_06038	<i>nfatc3a</i>	nuclear factor of activated T-cells, cytoplasmic 3-like	
SC7-LG07_10081	<i>fam160b2</i>	protein FAM160B2-like isoform X3	
SC7-LG08_10806	<i>akap9</i>	A-kinase anchor protein 9	
SC7-LG09_12591	<i>hif1al</i>	hypoxia-inducible factor 1-alpha-like	
SC7-LG16_20555	<i>ly75</i>	lymphocyte antigen 75-like	
SC7-LG17_21189	<i>agmo</i>	alkylglycerol monooxygenase	Xvent-1
SC7-LG20_25777	<i>si:ch1073-396h14</i>	disintegrin and metalloproteinase domain-containing protein 10-like isoform X1	
SC7-LG20_25889	<i>vtg1</i>	vitellogenin-like isoform X3	
SC7-LG22_28752	-	uncharacterized protein LOC108891332 isoform X2	
SC7-LG23_29008	<i>abcc3</i>	canalicular multispecific organic anion transporter 2 isoform X1	
SC7-LG23_29346	<i>prodh2</i>	probable proline dehydrogenase 2	
SC7-LG24_30448	<i>slc22a13</i>	solute carrier family 22 member 13 isoform X3	

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Supplementary Table 5. Positively selected and differentially expressed genes between nonfeeders and feeders in *S. scherzeri*

SC7 gene_ID	Gene symbol	Description	BMP4-initiated signaling molecules binding sites
SC7-LG01_01389	<i>si:ch73-233k15</i>	uncharacterized protein LOC108888582	
SC7-LG02_01949	-	uncharacterized protein LOC108885782 isoform X5	
SC7-LG02_02163	<i>cnm1</i>	metal transporter CNNM1	
SC7-LG02_02680	<i>ncoa4</i>	nuclear receptor coactivator 4 isoform X1	
SC7-LG03_04139	<i>trim25</i>	E3 ubiquitin/ISG15 ligase TRIM25-like isoform X5	
SC7-LG03_04242	<i>mtmr7b</i>	myotubularin related protein 7b	
SC7-LG05_06904	<i>ighmbp2</i>	DNA-binding protein SMUBP-2	Smad3
SC7-LG06_08127	<i>dvl2</i>	segment polarity protein dishevelled homolog DVL-2	
SC7-LG08_11320	<i>asns</i>	asparagine synthetase	Xvent-1
SC7-LG09_11590	<i>ulk2</i>	serine/threonine-protein kinase ULK2	
SC7-LG09_11707	<i>rbp2a</i>	retinol-binding protein 2	
SC7-LG10_12878	<i>tfe3a</i>	transcription factor E3-like	
SC7-LG13_16974	<i>slc25a25b</i>	calcium-binding mitochondrial carrier protein SCaMC-2-like isoform X1	
SC7-LG13_17803	<i>zgc:171965</i>	proteinase-activated receptor 1-like	
SC7-LG15_19104	<i>bx548028</i>	-	
SC7-LG18_23562	<i>pard3</i>	partitioning defective 3 homolog isoform X10	
SC7-LG22_28439	<i>rnf14</i>	E3 ubiquitin-protein ligase RNF14 isoform X1	
SC7-LG23_29911	-	kinesin-like protein KIF20A isoform X3	Xvent-1
SC7-LG24_30940	-	zinc finger protein 708-like	
SC7-LG02_02157	-	uncharacterized protein C10orf12-like isoform X1	

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171 **Supplementary Table 6. Genes species-specific to *S. chuatsi* and differentially expressed between big-size and small-size group**

SC7 gene ID	Gene symbol	Description	BMP4-initiated signaling molecules binding sites
SC7-LG01_00687	<i>ighv1-2</i>	immunoglobulin heavy variable 1-2	
SC7-LG01_00731	<i>ighv5-3</i>	immunoglobulin heavy variable 5-3	Xvent-1
SC7-LG08_10356	<i>clec4e</i>	c-type lectin domain family 4 member e	Xvent-1
SC7-LG09_12253	<i>casr</i>	extracellular calcium-sensing receptor	
SC7-LG11_15157	<i>igic1s1</i>	immunoglobulin light iota constant 1	
SC7-LG12_16403	<i>ccl4l</i>	c-c motif chemokine 4 like	
SC7-LG16_19863	<i>muc5ac</i>	mucin 5 subtype ac	
SC7-LG16_20812	<i>b3galt2</i>	beta-1,3-galactosyltransferase 2	
SC7-LG18_23132	<i>muc2</i>	mucin 2e	
SC7-LG23_29695	<i>cd79b</i>	□□ cell antigen receptor complex-associated protein beta chain	

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173 **Supplementary Table 7. Positively selected and differentially expressed genes between big-size and small-size group in *S. chuatsi***

SC7 gene ID	Gene symbol	Description	BMP4-initiated signaling molecules binding sites
SC7-LG06_07835	<i>col4a6</i>	collagen-6 (IV) chain	
SC7-LG08_11253	<i>paqr7a</i>	membrane progesterin receptor-b	
SC7-LG06_08332	<i>dnah2</i>	dynein heavy chain 2, axonemal	
SC7-LG12_16478	<i>lgals3b</i>	galectin 3	Xvent-1
SC7-LG20_25596	<i>hmha1b</i>	histocompatibility (minor) ha-1b	
SC7-LG21_27349	<i>myot</i>	myopalladin	Xvent-1
SC7-LG22_28067	<i>ubash3ba</i>	ubiquitin-associated and sh3 domain-containing protein b	

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175 **Supplementary Table 8. Positively selected in *S. kneri* and differentially expressed genes between big-size and small-size group in**
176 ***S. chuatsi***

SC7 gene ID	Gene symbol	Description	BMP4-initiated signaling molecules binding sites
SC7-LG01_00815	<i>zp3</i>	zona pellucida sperm-binding protein 3	Xvent-1
SC7-LG02_01964	<i>ggps1</i>	geranylgeranyl pyrophosphate synthase	
SC7-LG02_02003	<i>capn8</i>	calpain-2 catalytic subunit	
SC7-LG04_05182	<i>ccnd3</i>	g1/s specific cyclin d3	
SC7-LG04_05407	<i>kif21b</i>	kinesin like protein kif21b	
SC7-LG05_06336	<i>mical2b</i>	molecule interacting with CasL 2	Xvent-1
SC7-LG07_08943	<i>aacs</i>	acetoacetyl-CoA synthetase	
SC7-LG10_13957	<i>krt5</i>	keratin 5	
SC7-LG12_16138	<i>efhc1</i>	ef-hand domain containing protein 1	
SC7-LG13_17161	<i>sh3bp2</i>	sh3 domain-binding protein 2	
SC7-LG13_17294	<i>slc46a2</i>	thymic stromal cotransporter homolog	
SC7-LG13_17920	<i>myo18b</i>	unconventional myosin-XVIIIb	
SC7-LG14_18276	<i>iqcb1</i>	iq calmodulin binding motif containing protein 1	
SC7-LG15_18918	<i>plekha5</i>	pleckstrin homology domain containing family a member 5	
SC7-LG17_21022	<i>muc1</i>	mucin 1	
SC7-LG18_22838	<i>magel2</i>	mage like protein 2	Xvent-1
SC7-UN_11_31167	<i>hla-dpa1</i>	hla class II histocompatibility antigen, dp alpha 1 chain	
SC7-UN_329_32169	<i>muc2</i>	mucin 2	

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Supplementary Table 9. Positively selected genes in *C. whiteheadi*

SC7 gene ID	Gene symbol	Description	BMP4-initiated signaling molecules binding sites
SC7-LG01_00371	<i>mcm5</i>	minichromosome maintenance complex component 5	Smad3, Smad4
SC7-LG01_00372	<i>gcat</i>	2-amino-3-ketobutyrate coenzyme A ligase, mitochondrial	Xvent-1, Smad4
SC7-LG01_00380	<i>mchr1b</i>	melanin-concentrating hormone receptor 1-like	Xvent-1, Smad4
SC7-LG01_00382	<i>bptf</i>	nucleosome-remodeling factor subunit BPTF-like	Xvent-2, Smad3, Smad4

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Supplementary Table 10. The number of *pepsin A*, *trypsin* genes and pyloric caeca in mandarin fish

Species	Number of intact <i>pepsin A</i>	Number of <i>pepsin A</i> pseudogenes	Number of pyloric caeca
<i>Siniperca chuatsi</i>	3	0	117~323
<i>Siniperca kneri</i>	3	0	62~100
<i>Siniperca scherzeri</i>	3	0	65~124
<i>Coreoperca whiteheadi</i>	2	1	3

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Supplementary Table 11. The number of Na⁺/K⁺-ATPase α -1 in selected fish species

Species	Salinity	<i>atp1a1</i>	<i>atp1a2</i>	<i>atp1a3</i>	<i>atp1b1</i>	<i>atp1b2</i>	<i>atp1b3</i>	<i>atp1b4</i>	total no.
<i>Cyprinodon variegatus</i>	SW	1	1	2	2	1	2	1	10
<i>Stegastes partitus</i>	SW	2	1	2	2	2	2	1	12
<i>Dicentrarchus labrax</i>	SW/BW	2	1	2	2	2	2	1	12
<i>Larimichthys crocea</i>	SW/BW	2	1	3	2	2	1	1	12
<i>Cynoglossus semilaevis</i>	FW/BW/SW	1	2	2	2	2	2	1	12
<i>Oreochromis niloticus</i>	FW/BW/SW	4	1	2	2	2	2	1	14
<i>Lates calcarifer</i>	FW/BW/SW	2	1	2	2	2	2	1	12
<i>Takifugu rubripes</i>	FW/BW/SW	2	1	2	2	2	2	1	12
<i>Oryzias latipes</i>	FW/BW	3	1	2	1	2	2	1	12
<i>Danio rerio</i>	FW	6	1	2	2	2	2	1	16
<i>Astyanax mexicanus</i>	FW	6	1	2	2	2	2	1	16
<i>Esox lucius</i>	FW	6	1	2	2	1	2	1	15
<i>Siniperca chuatsi</i>	FW	2	1	2	2	2	2	1	12
<i>Siniperca kneri</i>	FW	2	1	2	2	2	2	1	12
<i>Siniperca scherzeri</i>	FW	2	1	2	2	2	2	1	12
<i>Coreoperca whiteheadi</i>	FW	2	1	2	2	2	2	1	12

183 FW: freshwater, BW: brackish water, SW: sea water.

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Supplementary Table 12. The number of *aqp8* in selected fish species

Species	Salinity	<i>aqp8aa</i>	<i>aqp8ab</i>	<i>aqp8b</i>	total
<i>Larimichthys crocea</i>	SW/BW	1	1	1	3
<i>Lates calcarifer</i>	SW/BW/FW	1	1	1	3
<i>Dicentrarchus labrax</i>	SW/BW	1	1	1	3
<i>Siniperca chuatsi</i>	FW	--	1	1	2
<i>Siniperca kneri</i>	FW	--	1	1	2
<i>Siniperca scherzeri</i>	FW	--	1	1	2
<i>Coreoperca whiteheadi</i>	FW	--	1	1	2
<i>Maylandia zebra</i>	FW/BW	1	1	--	2
<i>Neolamprologus brichardi</i>	FW/BW	1	1	--	2
<i>Pundamilia nyererei</i>	FW/BW	1	1	--	2
<i>Haplochromis burtoni</i>	FW/BW	1	1	--	2
<i>Oreochromis niloticus</i>	FW/BW	1	1	--	2

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FW: freshwater, BW: brackish water, SW: sea water.

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Supplementary Table 13. Cruel genes and representative neurological pathways in hybrid *S. chuatsi* × *S. scherzeri*

Transcriptome accession no.	Gene symbol	Description	Pathway	log ₂ (SC_W RPKM/SC_X RPKM)	P-value	FDR	BMP4-initiated signaling molecules binding sites
Unigene99889_All	<i>adcy3</i>	Adenylate cyclase 3		1.8454	3.22E-05	9.58E-04	
Unigene9024_All	<i>avt</i>	Arginine vasotocin/Vasotocin-neurophysin VT 1		-2.331	2.42E-77	1.16E-74	
Unigene12884_All	<i>esr1</i>	Estrogen receptor alpha	HPG	-1.306	6.58E-12	6.50E-10	
Unigene50772_All	<i>esr1</i>	Estrogen receptor alpha	HPG	-2.015	3.33E-17	6.83E-15	
Unigene44248_All	<i>fshb</i>	Follicle-stimulating hormone beta		-1.717	9.05E-43	3.49E-40	
Unigene88134_All	<i>gad</i>	Glutamate decarboxylase		-12.41	1.34E-06	6.03E-05	
Unigene25448_All	<i>hnmt</i>	Histamine N-methyltransferase		-2.333	3.07E-13	3.99E-11	Xvent-1
Unigene95643_All	<i>htr1b</i>	5-hydroxytryptamine (serotonin) receptor 1B	5-HT	1.73	3.16E-05	9.46E-04	
Unigene5692_All	<i>kirrel3</i>	Kin of IRRE like protein 3		-2.855	9.33E-06	3.34E-04	Xvent-1
Unigene10121_All	<i>npas4</i>	Neuronal PAS domain-containing protein 4		1.0118	1.06E-08	6.97E-07	
Unigene51036_All	<i>oxt</i>	Isotocin-neurophysin IT 1		-1.072	4.76E-18	1.02E-15	Xvent-1
Unigene80547_All	<i>rgs6</i>	Regulator of G-protein signaling 6		-4.402	7.32E-06	2.69E-04	
Unigene64851_All	<i>rgs6</i>	Regulator of G-protein signaling 6		-2.798	1.73E-05	5.69E-04	
Unigene27397_All	<i>th</i>	Tyrosine hydroxylase/Tyrosine 3-monooxygenase	Dopamine	1.9508	2.92E-13	3.82E-11	Xvent-1
Unigene50578_All	<i>th</i>	Tyrosine hydroxylase/Tyrosine 4-monooxygenase	Dopamine	3.265	9.31E-07	4.36E-05	Xvent-1
Transcriptome accession no.	Gene symbol	Description	Pathway	log ₂ Fold Change (SC_AD/SC_W)	P value	FDR	BMP4-initiated signaling molecules binding sites
Unigene1344_All	<i>mao</i>	Amine oxidase [flavin-containing]		1.9346	0.037148		

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Supplementary Table 14. Primers used for RT-QPCR of cruel genes

Primer	Sequence (5'-3')	Product size (bp)	Annealing temperature (°C)	Amplification efficiency (%)
<i>sc-rpl13a</i> -F	TATCCCCCACCCTATGACA	100	60	100.57
<i>sc-rpl13a</i> -R	ACGCCCAAGGAGAGCGAACT			
<i>sc-hnmt</i> -F	CTACCATCAGCTTCTTCCAGAG	146	57	100.5
<i>sc-hnmt</i> -R	AGTGGTCACACACTGACTTATT			
<i>sc-avt</i> -F	TCAGAGCAGTAGGGTTAAGAGA	176	55	96
<i>sc-avt</i> -R	CCACCAGAGGACAGACTTAGTA			
<i>sc-maob</i> -F	GTTCTGAGTTGGTCCGATGTAA	152	57	100.2
<i>sc-maob</i> -R	GTTTGAGTGCAGCGAAGTTG			
<i>sc-rgs6</i> -F	ACAGTCTCAGAGTCCCATACA	160	55	94.8
<i>sc-rgs6</i> -R	GAACTGCTCAGTGTAGCTTATCA			

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Supplementary Table 15. Numbers of gill rakers in selected fish species

Species	Numbers of gill rakers
<i>Clupea harengus</i>	56-73
<i>Danio rerio</i>	13-15
<i>Cyprinus carpio</i>	20-25
<i>Ctenopharyngodon idellus</i>	18
<i>Sinocyclocheilus anshuiensis</i>	14
<i>Dicentrarchus labrax</i>	18-29
<i>Larimichthys crocea</i>	8-19
<i>Oreochromis niloticus</i>	30-36
<i>Siniperca chuatsi</i>	6-7
<i>Siniperca kneri</i>	4-7
<i>Siniperca scherzeri</i>	4-6
<i>Coreoperca whiteheadi</i>	7-9
<i>Psammoperca waigiensis</i>	2-7

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193 **Supplementary Table 16. Genomic fragments in reporter constructs**

Construct name	Sequence (5'- 3')	Size (bp)
pGL6-1	caccttatgcagagtcagttgcatgcgttttgtgtcacacatccatcatagtaaatacagggttcgcttaatatgtgttttgtccgttctgacagagcagccatcattctctcagcagaactcatcactcttctgttatcctctcctcatcctctctttatcctctgtcatctaatctcttctcctcacatcctcttttcagtcagggaagccccctggatcgacatcaatgagcctctacaagaaggacggagacagagagaagaagaaaaagagagagaggagaatggagaaaagagagaagggaacagagactgagagaggggacagagaaagagaagaataaagacagagtgaccaagaagagagaagaataatggggataaagagagaatgcacaaatggggacagaggggaagacagagacagagaggggagagtaagtaattacaggactcccagtgttttcaagccccctcttttatctcaccttctgtggctgagaaaaaggagaagcagcgggaaaagggaggaggaggagaggaagactaaagtccaagctttagtcacaaatcagacaacaggcaccagtcattccatcacacacacacacacagacacactcaactaatttatagcttctgtttcaagacattttatcatctgaacaagggtgtttgtgtgtgtgggtgtgggtctcatgtaaagtactttaatgggtgatagttgaagtattgtcagtaataatgcaacttttctgtcctttgaatattgtgggtattattatatatgttacatgtctgcctatacgttgtgctgctgacac	834
pGL6-2	aatcagacaacaggcaccagtcattccatcacacacacacacacacagacacactcaactaatttatagcttctgtttcaagacattttatcatctgaaacaagggtgtttgtgtgtgtgggtgtgggtctcatgtaaagtactttaatgggtgatagttgaagtattgtcagtaataatgcaacttttctgtcctttgaatattgtgggtattattatatatgttacatgtctgcctatacgttgtgctgctgacac	267

195 **Supplementary Table 17. Primers used for knockout and whole mount *in situ* hybridization of zebrafish *edar***

Purpose	Primer	Sequence (5'-3')
gRNA synthesis	zf-KO- <i>edar</i> -F1	TAATACGACTCACTATAGGTGCCGGGCGTCAGGACAG
	zf-KO- <i>edar</i> -F2	TAATACGACTCACTATAGGAGAATTTACCAGCCGGAC
	zf-KO-universal-R	GGCTGGAGGAGTACTTGATCTC
Mutation identification (nested PCR)	zf- <i>edar</i> -outside-F	TGTGTGTGTGTGTGTGTGTGT
	zf- <i>edar</i> -outside-R	CTTGCCATTCAGTTTCCTGTTG
	zf- <i>edar</i> -inside-F	CTCGTCTCAGTGAATGTGAGTT
	zf- <i>edar</i> -inside-R	GGCATCAATCTGCTCCTCTT
RNA probe synthesis	zf- <i>edar</i> -probe-F	TTGAATTTCGGCCATAAGAAAGATG
	zf- <i>edar</i> -probe-R	TCGGATCCTCTGGCTCACTC
		GCGUCCGUAUCUCAAGGAAGUACGAGAUCCGUCGACACAAAGACUGUGACGC
		UCUUUACCGCGCCACUGUCCUGACGCCCCGGCACCUCUGACAGCGACGCCGAGUGC
		GGACACUGUUUACCAGGGUACUACAUCCAGGAGAACCGGCCGCAGAAACAUUCUACG
		GUAUGGUGUGUCAUUCAUGCCAAAACGCUCCUCGAAACAUCAAAGAGUGCAUGA
		GGUCCACACCGCCAGCCUCAGGUCGAGCUCCCAGUGUUUCUCCAGCAGCACCAC
		UAUAUUCCCUCAGCCAGAGAAAGACCCAACAGGACAGGGUCACCUAGCAACGGCC
		CUCAUCAUCGCCAUGUCAACCAUCUUCAUCAUGGCCAUCGCUAUAGUGAUGAUCA
		UCAUGUUCUACAUCCUGAAGAGCAAACCGAGCGGACCAGUCUGCUGUUCUGGUCA
		GCUCAUCAAGCUGUAGAAGCUCAGACAAACAUGCAGGAGGAGAAGAAGGAGGC
		UCAGGAGAACGUGGUGAUCUUUCAGGAGAAGGACGAAUUGACAAACUCAAGCU
	UCCAUUCUCCAA	

196 Two gRNA targets located in the fourth coding exon were used in the present study. Targeting sites were indicated with underline.

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Supplementary Table 18. Primers used for absolute mRNA expression

Primer	Sequence (5'-3')	Product size (bp)	Annealing temperature (°C)	Amplification efficiency (%)
zf-RT- <i>eda</i> -F	GGTCCTACTTGACGGAACATA	101	55	102.2
zf-RT- <i>eda</i> -R	GAGTTTTGTCCACCATCACC			
zf-RT- <i>edar</i> -F	GCACCACCAACACCATCA	124	55	104.8
zf-RT- <i>edar</i> -R	CTCAGACCTTCCGCAACA			
zf-RT- <i>bmp4</i> -F	CGAGCCAACACCGTGAG	111	55	101
zf-RT- <i>bmp4</i> -R	TGGGATGCTGCTGAGATT			
sc-RT- <i>eda</i> -F	TGGGATTATTCCTGCTATCGC	286	58	99.5
sc-RT- <i>eda</i> -R	CTTTTCGCTCGCTGAGTTATG			
sc-RT- <i>edar</i> -F	GCGTCTGAGAAGCAAGGATT	214	58	99.8
sc-RT- <i>edar</i> -R	TGTGATTGGTGCTGGTGATG			
sc-RT- <i>bmp4</i> -F	CTGAGGAGAACGGAGCACAT	180	58	98.8
sc-RT- <i>bmp4</i> -R	GCTCGTCCTCTGGAATGCTTGT			

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