Expansion of sweet taste receptor genes in grass carp (Ctenopharyngodon idellus) coincided with vegetarian adaptation

Xiao-Chen Yuan
Huazhong Agricultural University
Xu-Fang Liang (xufang_liang@hotmail.com)
Huazhong Agriculture University
Wen-Jing Cai
Huazhong Agriculture University
Shan He
Huazhong Agricultural University
Wen-Jie Guo
Huazhong Agricultural University
Kang-Sen Mai
Ocean University of China

Research article

Keywords: Taste receptor, Gene expansion, Adaptive evolution, Food habit transition, Herbivory

Posted Date: August 30th, 2019

DOI: https://doi.org/10.21203/rs.2.13748/v1

Version of Record: A version of this preprint was published on February 11th, 2020. See the published version at https://doi.org/10.1186/s12862-020-1590-1.

Abstract

Background Taste is fundamental to diet selection in vertebrates. Genetic basis of sweet taste receptor in the shaping of food habits has been extensively studied in mammals and birds, but scarcely studied in fishes. Grass carp is an excellent model for studying vegetarian adaptation, as it exhibits food habit transition from carnivory to herbivory. Results We identified six sweet taste receptors (gcT1R2A-F) in grass carp. The four gcT1R2s (gcT1R2C-F) have been suggested to be evolved from and paralogous to the two original gcT1R2s (gcT1R2A and gcT1R2B). All gcT1R2s were expressed in taste organs and mediated glucose- or fructose-induced intracellular calcium signaling, revealing they were functional. Cells co-transfected with six gcT1R2s/gcT1R3 showed a greater response to glucose or fructose than those transfected alone, while a lower response to plant specific fructose than those co-transfected with the new four gcT1R2s/gcT1R3. Moreover, food habit transition from carnivory to herbivory in grass carp was accompanied by increased gene expression of certain gcT1R2s. Conclusions We suggested that the gene expansion of T1R2 in grass carp was an adaptive strategy to accommodate the change in food environment. Moreover, the selected gene expression of gcT1R2s might drive the food habit transition from carnivory to herbivory in grass carp. This study provided some evolutional and physiological clues for the formation of herbivory in grass carp.

Background

Taste perception, conveying important dietary information, is fundamental for the survival of animals ranging from insects to mammals [1, 2]. All tastes are combinations of five basic modalities: sweet, umami, bitter, salty, and sour [3, 4]. Sensory systems display remarkable flexibility across vertebrates, with some abandoning unnecessary sensory modalities [5–7] while others evolving new adaptive sensory modalities [8]. In vertebrates, sweet and umami tastes are identified by a class of G protein-coupled receptors (GPCRs) termed taste receptor type 1 (T1R) [2]. Most vertebrates have three T1Rs, with the T1R1-T1R3 heterodimer mediating umami taste and the T1R2-T1R3 heterodimer mediating sweet taste [2, 9].

Taste perception varies enormously across different lineages and species of vertebrates [1, 2]. Sweet taste “blindness” observed in some carnivorous mammals, such as domestic cat, California sea lion, southern fur seal, pacific harbor seal, Asian small-clawed otter, spotted hyena, fossa, banded linsang, bottlenose dolphin, and vampire bats, have been suggested to be the consequence of pseudogenization of T1R2 since they do not require the receptor for sweet food perception [6, 7, 10, 11]. However, almost all omnivorous and herbivorous mammals with the habit to consume sugars have a functional T1R2 structure [6, 7, 10, 12–15]. Unlike pseudogenization of T1R2 in some carnivorous mammals, the evolution of sweet taste in birds is in absence of T1R2 despite food habits [16–18]. Nevertheless, ancestral umami taste receptor has been repurposed to detect sweet in the hummingbird [17]. The absence or presence of intact T1R2 are concordant with food habits in mammals and birds, suggesting the adaption of T1R2 evolution to food habit formation and environmental change.

Teleost fishes represent about half of all living vertebrate species and provide important models for evolutionary study [19]. The food habits of fish, which are more sensitive to water-soluble chemicals than mammals, are demonstrated to be associated with chemosensory-mediated taste sense [20, 21]. Unlike pseudogenization or absence in mammals or birds, most fish have been shown to possess two or three T1R2s [16, 22, 23]. Whether the gene number of fish T1R2 genes related to the formation of food habits like mammals or birds is worthy of further exploration [24].

Constituting a member of the Cyprinidae family, grass carp (*Ctenopharyngodon idellus)* is typically herbivorous and an excellent model for studying the formation mechanism of herbivory as it shows the food habit transition from carnivory to herbivory [25, 26]. Grass carp is carnivorous when its total length is shorter than 3 cm, then fish of 3–5.5 cm undergo the food transition stage from zooplankton or benthos to aquatic macrophytes, whereas fish larger than 5.5 cm is completely herbivorous [25–27]. We hypothesized T1R2 gene might be involved in food habit transition and adaptation to a vegetarian diet observed in grass carp. For this purpose, six grass carp T1R2s (gcT1R2s) were identified from draft genome sequences and their evolutionary analysis was conducted among fish with different food habits. When transfected in Human embryonic kidney 293T (HEK293T) cells, gcT1R2/gcT1R3 responded to ubiquitous glucose and plant specific fructose in intracellular calcium signaling. Gene expressions of T1R2s in grass carp before and after food transition from carnivory to herbivory were also investigated. This study might provide new insights into the adaptive evolution of sweet taste receptors during food habit formation in fish.

Results

Characterization of gcT1R2 genes

Conducting a homology search, we sequenced six T1R2s gene, named gcT1R2A-F, from the grass carp genome sequence (Genbank no. in Table 1 and detail information in electronic supplementary dataset S1). The six T1R2s of grass carp showed higher than 78% identities with each other, higher than 73% identities with two T1R2s of zebrafish, whereas lower than 35% identities with human T1R2 (electronic supplementary material, table S4). The genomic structure of all gcT1R2s consisted of six coding exons and five introns like zebrafish T1R2s (figure 1).

Although several carnivorous and omnivorous fish species have two or three T1R2 genes, T1R2 genes have been highly duplicated in grass carp (table 1). Moreover, among Cypriniformes, highly gene duplication of T1R2s could only be observed in herbivorous grass carp and blunt snout bream.

Evolutionary analyses of gcT1R2s

Synteny analysis showed that the gcT1R2 genes are located on linkage group 21 (figure 2). The adjacent genes of gcT1R2A and gcT1R2B were identical with those of zebrafish T1R2.1 and T1R2.2. Interestingly, the order of adjacent genes of gcT1R2C-F were also identical with the order of the same genes in

zebrafish without any gene at the corresponding location. In addition, as the different color-marked gene groups shown in figure 2, gene translocation might be occurred among the four fished we selected.

Phylogenetic analysis showed that all fish T1R2s and mammalian T1R2 formed two independent clusters except coelacanth (figure 3). The coelacanth T1R2s are more closely related to mammalian T1R2. In fishes, spotted gar (*Lepisosteus oculatus)* which belongs to *Holostei* formed an independent cluster. For Teleostei, Ostariophysi, containing zebrafish, cavefish (*Astyanax mexicanus)* and grass carp, and Acanthopterygii, containing medaka, fugu, stickleback (*Gasterosteus aculeatus)* and tilapia (*Oreochromis niloticus)* formed two independent clusters. Notably, gcT1R2A and zebrafish T1R2.1 formed one cluster as well as the gcT1R2C-F), gcT1R2B and zebrafish T1R2.2 formed another cluster.

According to the TimeTree database, gcT1R2C-F were formed after the formation of gcT1R2A and gcT1R2B (figure 4). The estimated divergence time between the original formed two gcT1R2s and the new formed four gcT1R2s was around 34.7 million years ago.

Tissue distributions of gcT1R2s

By using geNorm software, the genes with the most stable expression across the experimental conditions was EF1 (electronic supplementary material, table S5). The mRNA tissue expression levels of gcT1R2swere analyzed by real-time PCR, using EF1 as an internal control (figure 5). The gene expression of gcT1R2A was the highest in gill filament and followed by tongue. The highest mRNA abundance of gcT1R2B was observed in gill filament, followed by tongue and pharynx, and the lower gene expressions were detected in brain and oral epithelium. GcT1R2C and gcT1R2D were prominently expressed in gill filament and tongue. The gene expression of gcT1R2E was the highest in tongue, and abundant in gill filament, gill raker, foregut, midgut, and hindgut. gcT1R2F was prominently expressed in the gill filament.

Response of gcT1R2s to glucose and fructose

Each gcT1R2/gcT1R3 could mediate glucose- and fructose-induced intracellular calcium signaling (figure 6). HEK293T cells transfected with gcT1R2E/gcT1R3 and gcT1R2F/gcT1R3 showed a greater response to glucose than those transfected alone. GcT1R2E/gcT1R3 also mediated more active fructose-induced intracellular calcium signaling. Upon stimulation with glucose and fructose, gcT1R2A-F/gcT1R3 mediated a more intensive and sustained calcium signal transduction than gcT1R2/gcT1R3 alone when transfected into HEK293T cells. Cells co-transfected with six gcT1R2s/gcT1R3 showed a lower response to plant specific fructose than those co-transfected with the new four gcT1R2s/gcT1R3.

Gene expression of T1R2s in grass carp of food habit transition from carnivory to herbivory

The gene expressions of gcT1R2s in the gut of grass carp fed with chironomid larvae (Groups A vs. Groups B) were significantly decreased during growth and development (figure 7). Fish fed with duckweed (Group C) had significantly higher gene expressions of gcT1R2C and gcT1R2E than those fed with chironomid larvae (Groups B) ($P < 0.05$). Moreover, compared with fish before food habit transition (Group A), the gene expression of gcT1R2E was significantly increased in the gut of fish after the food habit transition (Group C) from carnivory to herbivory.

Discussion

The genetic basis underlying the formation of food habits in fish is largely unknown [24]. The relationship between the evolution of sweet taste receptor and food habit in fish calls for further investigations. By screening the gene numbers of T1R2s in 15 teleost fishes with variant food habits from different orders, we found eight T1R2 genes and six T1R2 genes in morphologically and ecologically diverse threespine stickleback and typical herbivorous grass carp, in contrast to two or three T1R2 genes reported in some carnivorous and omnivorous fish species. Duplication of T1R2 genes of stickleback, resulting in enhanced perception for substances important for survival and reproduction, has been suggested as an adaptive strategy to varied environment [28]. In the present study, we observed that high T1R2 duplications in grass carp (six copies) as well as blunt snout bream are related to the vegetarian adaptation through the comparative analysis in cyprinid evolution [29]. Therefore, in addition to previously reported pseudogenization of T1R2 in mammals and gene loss in birds, adaptive gene expansion in fishes adds a new layer of complexity to the whole evolutionary story of sweet taste.

GcT1R2C-F were evolved from and paralogous to the two original gcT1R2s (gcT1R2A and gcT1R2B) according to evolutionary analyses. The estimated divergence time was around 34.7 million years ago. GcT1R2A and zebrafish T1R2.1, gcT1R2B and zebrafish T1R2.2 each formed an independent cluster, respectively. The original two gcT1R2s is paralogous to the new formed four gcT1R2s and orthologous genes to two zebrafish T1R2s according to the synteny analysis. Meanwhile, the transposition of genes nearby T1R2s among the four selected fishes might accelerate the duplication of T1R2s [30].

We found six gcT1R2s were all expressed in the taste organs such as tongue and gill. In mammals, T1R2 and T1R3 are expressed in glucose sensing cells of the gastrointestinal tract, where they play important roles in nutrient detection, perception and assimilation [31]. In the present study, gcT1R2s were expressed in not only taste organs but also intact gut, indicating their functions in glucose detection and perception. Intriguingly, low gene expression of gcT1R2B was detected in brain, suggesting that gcT1R2B might be involved in brain reward circuits that control food intake independently of palatability or functional taste transduction [32].

Signal transduction of gcT1R2s were determined through calcium imaging analysis in HEK293T cells for functional study. Each gcT1R2/gcT1R3 could mediate glucose- and fructose-induced intracellular calcium signaling. Previous studies also reported that sweet taste stimuli elicited an increase in Ca^{2+} concentration in mammalian taste cells [4, 33, 34]. Previous studies have reported that transient receptor

potential channel M5 and phospholipase C-beta 2 colocalized in fish taste receptor cells, indicating vertebrates share a common molecular component in taste signal transduction [22, 35–37]. Therefore, glucose and fructose activated the intracellular calcium signaling mediated by each gcT1R2/gcT1R3, indicating all gcT1R2s were functional genes.

Interestingly, upon stimulation with glucose and fructose, gcT1R2A-F/gcT1R3 mediated a more intensive and sustained calcium signal transduction than gcT1R2/gcT1R3 alone when transfected into HEK293T cells. Plant specific fructose stimulated a more active calcium signaling in cells transfected with gcT1R2C-F/gcT1R3 than gcT1R2A-F/gcT1R3, raising the possibility that change in food environment might be a major selective force shaping the adaptive evolution of the gcT1R2s. These results indicated that the gene expansion, especially the formation of new four gcT1R2s, was an adaptive strategy to dietary switch.

As grass carp goes through a transition from carnivory to herbivory during its life cycle, the gene expressions of gcT1R2s before and after the food habit transition should be detected to determine its contribution to diet selection. The gene expressions of gcT1R2s in the gut of grass carp were significantly decreased during growth and development, which was coincided with the less sensitivity of sweet taste in children compared with adults [38–41]. Despite the diminished sweet taste perception during development, food habit transition from carnivory to herbivory was accompanied by increased gene expression of certain gcT1R2s. In our previous study, we have declared the food habit transition from carnivory to herbivory in grass carp might be due to enhanced gut growth, increased appetite, resetting of circadian phase and enhanced digestion and metabolism, as well as extensive alternative splicing and novel transcript [25, 26]. Here, we found regulation of gcT1R2s expression also drove the food habit transition. In conclusion, both gene expansion and expression patterns of gcT1R2s contributed to food habit transition from carnivory to herbivory during the evolution of grass carp according to Haeckel's recapitulation law [42].

Conclusions

Six sweet taste receptors (gcT1R2A-F) were identified in grass carp, which is an excellent model for studying vegetarian adaptation as it exhibits food habit transition from carnivory to herbivory. The four gcT1R2s (gcT1R2C-F) have been suggested to be evolved from and paralogous to the two original gcT1R2s (gcT1R2A and gcT1R2B). All gcT1R2s were expressed in taste organs and mediated glucose- or fructose-induced intracellular calcium signaling, revealing they were functional. Cells co-transfected with six gcT1R2s/gcT1R3 showed a greater response to glucose or fructose than those transfected alone, while a lower response to plant specific fructose than those co-transfected with the new four gcT1R2s/gcT1R3, suggesting the gene expansion was an adaptive strategy to change in food environment. Moreover, despite diminished sweet taste perception during development, food habit transition from carnivory to herbivory was accompanied by increased gene expression of certain gcT1R2s, indicating gene expression also drove the food habit transition. Collectively, our studies provided some evolutional and physiological clues for the formation of herbivory in grass carp.

Methods

Data mining and DNA sequencing

A 0.9-Gb draft genome of a gynogenetic female grass carp adult and a 1.07-Gb genome of a wild male adult are available at the official National Center for Gene Research website (http://www.ncgr.ac.cn/grasscarp/). TBLASTN searches were conducted with *E*-value 10^{-10} against the genomic data using the available T1R2 coding sequence (CDS) of zebrafish *Danio rerio,* medaka *Oryzias latipes,* and fugu *Takifugu rubripes.* Each region of BLAST similarity was extended 5–10 kb in 5' and 3' directions to establish a detailed prediction of CDS. The screened sequences were estimated based on the profile hidden Markov model (HMM)-based gene prediction with the program WISE2 [43]. The exon-intron junctions were determined by comparing the genomic sequence with the cDNA sequence using SPIDEY. Then, the cDNA of grass carp tongue was used to verify the obtained sequences. The polymerase chain reaction (PCR) was conducted on Biometra Thermocyclers (Biometra, Germany) using Phanta® Super-Fidelity DNA Polymerase (Vazyme Biotech, Jiangsu, China) with the designed primers (electronic supplementary material, table S1). The sequences obtained from the genomic database were named as gcT1R2 genes.

The gene number of sweet taste receptors in teleost fishes was also investigated. By screening from GenBank and previous studies, we obtained the available sequences of T1R2 genes in 15 species of fishes with variant food habits from different orders.

Synteny analysis of T1R2 genes

To determine whether gcT1R2 genes are orthologous to other fish species, we performed a synteny analysis by searching T1R2 flanking genes of zebrafish, medaka and fugu using map viewer of NCBI.

Alignment and phylogenetic analysis

The T1R2s amino acid sequences of fishes and mammals used in this study are available in NCBI and Ensembl genome browser (electronic supplementary material, table S2). Amino acid sequence alignments were performed by ClustalW2.

The evolutionary history was inferred by using the Maximum Likelihood method based on the JTT matrix-based model [44]. Evolutionary analysis was conducted in MEGA7 [45].

Timetree analysis

The T1R2s nucleotide sequences of fishes selected are available in NCBI and Ensembl genome browser (electronic supplementary material, table S3). Nucleotide sequence alignments were performed by

ClustalW2.

A timetree inferred using the Reltime method [46] and the General Time Reversible model [47]. The coelacanth T1R2s were selected as the outgroup. Evolutionary analysis was conducted in MEGA7 [45].

T1R2s expressions in various tissues

Grass carp were obtained from the Fish Center of Xiantao, Hubei, China. The fish were fed to apparent satiation with a commercial diet (32.0% protein; 9.0% fat; 6.9% moisture; 7.6% ash) twice a day at 08:00 and 16:00 (Beijing time) under a standard laboratory condition. After the 2-week acclimation, six large grass carp (500.9 ± 57.6 g) used for tissue distributions of gcT1R2s were deeply anesthetized with MS222 (200 mg L^{-1}). The brain, lip, tongue, pharynx, oral epithelium, gill filament, gill raker, liver, foreogut, midgut, and hindgut samples were collected. RNA extraction and cDNA transcription were performed with Trizol reagent (Takara, Japan) and PrimeScript™ RT reagent Kit with gDNA Eraser (Takara) according to manufacturer's protocols.

The primer sets for T1R2s were designed (electronic supplementary material, table S1). A set of six housekeeping genes (β-actin, RPL13A, EF1, TUA, and GAPDH) were selected from the transcriptome assemblies [48] to test their transcription stability for tissue panel. GeNorm software was used to compute the expression stability values (M) for each gene where a lower M value corresponds to more stable gene expression.

Real-time PCR assays were carried out on a quantitative thermal cycler (MyiQ™ 2 Two-Color Real-Time PCR Detection System, BIO-RAD, USA) using AceQ® qPCR SYBR® Green Master Mix (Vazyme Biotech) with the designed primers (electronic supplementary material, table S1). The PCR parameters were 95 °C for 3 min followed by 40 cycles at 95 °C for 10 sec, annealing temperature for 30 sec, and a melt curve step. Primer PCR efficiencies of the genes ranged from 97.8 to 102.5%. Gene expression levels were quantified relative to the expression of housekeeping genes using the optimized comparative Ct ($2^{-\Delta\Delta Ct}$) value method [49].

Preparation of recombinant expressional vectors, cell culture and calcium imaging

The complete coding sequences of six gcT1R2s and gcT1R3 were subcloned into the pcDNA3.1 expression vector (Invitrogen, Carlsbad, CA) used ClonExpress™ II (Vazyme Biotech), respectively. HEK293T cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (Life Technologies, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (Sigma-Aldrich, Saint Louis, MO) at 37 °C in 5 % CO_2. The cells were plated at a density of 1×10^6 cells per 20-mm glass bottom cell culture dish the day before the experiment. After 14 h, the cells were transiently transfected 24 h before the experiment with the gcT1R2s/gcT1R3 recombinant expressional plasmids by using Lipofectamine 2000 reagent

(Invitrogen). For nutrient starvation experiments, HEK293T cells were placed in phenol-free/glucose-free DMEM (Life Technologies) for 3 h. The experiments were set up two parallel 10 groups: the first six groups were co-transfected with sole gcT1R2s and gcT1R3 (gcT1R2/gcT1R3); the 7th group was co-transfected with gcT1R2A, gcT1R2B and gcT1R3 (gcT1R2A-B/gcT1R3); the 8th group was co-transfected with gcT1R2C, gcT1R2D, gcT1R2E, gcT1R2F and gcT1R3 (gcT1R2C-F/gcT1R3); the 9th group was co-transfected with all six gcT1R2s and gcT1R3 (gcT1R2A-F/gcT1R3); the 10th group was transfected with pcDNA3.1 only.

After 3 h nutrient starvation experiments, cells were washed three times with Dulbecco's phosphate-buffered saline without calcium and magnesium (DPBS) (HyClone Lab, Logan, UT). Cells were loaded with 4 uM the calcium-bound Fluo–4 dye (Invitrogen) diluted in DPBS for 30 min at 37 °C in 5 % CO_2 and then washed three times with DPBS and incubated for an additional 30 min at 37 °C. Dishes were placed on the stage of an inverted confocal microscope (FluoView FV1000; Olympus, Tokyo, Japan). The dished were perfused with 200mM glucose or 200mM fructose at a rate of 2 mL/min after the first 3 pictures were taken. Baseline was established for at least 15 sec before stimulation. A series of 10 groups cell dishes were treated with 200mM glucose (Biosharp, Hefei, China) diluted in DPBS, and the other 9 groups were treated with 200mM fructose (Biosharp) diluted in DPBS. Images were recorded at 6.54 sec intervals up to 183.16 sec using 488 nm excitation filter and 516 nm emission filter and analyzed using FV10-ASW 3.1 Viewer software. The backgrounds of the emission intensities were subtracted. Data are expressed as the ratio of the fluorescence intensities of 20 single HEK293T cells per dish and initial intensity (F/F0).

T1R2s gene expression analysis of the food habit transition from carnivory to herbivory in grass carp

Fish and samples were prepared according to our previous experiments of He et al [25]. The fish embryos were obtained from Wuhan Academy of Agricultural Science and Technology (Wuhan, Hubei Province, China). Grass carp larvae were raised in tanks and fed with chironomid larvae (*Chironomus tentans)*.. At days 46 post-hatch (dph) (body weight 0.39 ± 0.05 g, body length 28.05 ± 0.99 mm), fish were randomly selected for sample collection as fish before food habit transition (Group A). The rest of the fish were randomly divided into two groups (n = 1000 for each group) fed with either chironomid larvae as fish without transition (Group B) or duckweed (*Lemna minor)* as fish after food habit transition to herbivory (Group C). An excess of food was offered 24 h a day and fed for 70 days. At 116 dph (body weight and body length for Group B were 2.97 ± 0.3 g and 53.96 ± 1.80 mm, respectively; those for Group C were 7.34 ± 1.43 g and 72.78 ± 6.15 mm, respectively), 6 fish were randomly selected from the groups for sample collection. The gut of grass carp was collected and then frozen in liquid nitrogen and stored at −80 ℃ for RNA. Total RNA was isolated, and cDNA synthesized as mentioned above.

To detect the gene expressions of T1R2s in grass carp of food habit transition from carnivory to herbivory, real-time PCR assays were carried out as mentioned above.

Abbreviations

β-actin: actin isoform β; CDS: coding sequence; DMEM: Dulbecco's phosphate-buffered saline without calcium and magnesium; EF1: elongation factor 1; GAPDH: glyceraldehyde–3-phosphate dehydrogenase;GPCRs: G protein-coupled receptors; HMM: hidden Markov model; PCR: polymerase chain reaction; RPL13A: ribosomal protein L13a; T1R: taste receptor type 1; T1R2: taste receptor type 1 member 2; TUA: tubulin alpha 1.

Declarations

Acknowledgements

We thank Dr. Lei Zhong for help with evolutionary analyses. We thank Ling Li, Jian Sun, Zheng-Yong Wen, Xiao-Yan Cheng, and Ai-Xuan Li for help with animal experiment.

Authors' contributions

XCY conceived the study, participated in the design of the study, carried out the laboratory work, participated in data analysis, and wrote the first draft of the manuscript. XFL designed the study and contributed to the final version. WJC carried out the laboratory work and assisted with data interpretation. SH collected the source animals and assisted with establishing the experimental treatments. WJG participated in data analysis. KSM participated in the design of the study. All authors have read and approved the manuscript.

Funding

This study was supported by the National Key R&D Program of China, Grant/Award Number: 2018YFD0900400 to XFL and the National Natural Science Foundation of China Grant/Award Number: 31502158 to WJG. The funding bodies for this study had no role in the design of the study, collection of data, data analysis and interpretation, or in writing the manuscript.

Availability of data and material

The datasets supporting this article have been uploaded as part of the supplementary material.

Ethics approval and consent to participate

All animals and experiments were conducted in accordance with the "Guidelines for Experimental Animals" of the Ministry of Science and Technology (Beijing, China). The study was approved by the

Institutional Animal Care and Use Ethics Committee of Huazhong Agricultural University. All efforts were made to minimize suffering.

Consent for publication

Not applicable

Competing interests

We have no competing interests.

References

1.Bachmanov AA, Beauchamp GK. Taste receptor genes. Annu Rev Nutr. 2007;27:389–414.

2.Yarmolinsky DA, Zuker CS, Ryba NJP. Common sense about taste: From mammals to insects. Cell. 2009;139(2):234–244.

3.Lindemann B. Taste reception. Physiol Rev. 1996;76(3):719–766.

4.Kinnamon SC, Margolskee RF. Mechanisms of taste transduction. Curr Opin Neurobiol. 1996;6(4):506–513.

5.Zhao HB, Yang JR, Xu HL, Zhang JZ. Pseudogenization of the umami taste receptor gene tas1r1 in the giant panda coincided with its dietary switch to bamboo. Mol Biol Evol. 2010;27(12):2669–2673.

6.Zhao HB, Zhou YY, Pinto CM, Charles-Dominique P, Galindo-Gonzalez J, Zhang SY et al. Evolution of the sweet taste receptor gene tas1r2 in bats. Mol Biol Evol. 2010;27(11):2642–2650.

7.Jiang P, Josue J, Li X, Glaser D, Li W, Brand JG et al. Major taste loss in carnivorous mammals. Proc Natl Acad Sci U S A. 2012;109(13):4956–4961.

8.Gracheva EO, Ingolia NT, Kelly YM, Cordero-Morales JF, Hollopeter G, Chesler AT et al. Molecular basis of infrared detection by snakes. Nature. 2010;464(7291):1006–1011.

9.Zhao GQ, Zhang YF, Hoon MA, Chandrashekar J, Erlenbach I, Ryba NJP et al. The receptors for mammalian sweet and umami taste. Cell. 2003;115(3):255–266.

10.Li X, Li W, Wang H, Cao J, Maehashi K, Huang L et al. Pseudogenization of a sweet-receptor gene accounts for cats' indifference toward sugar. PLoS Genet. 2005;1(1):27–35.

11.Bachmanov AA, Bosak NP, Lin C, Matsumoto I, Ohmoto M, Reed DR et al. Genetics of taste receptors. Curr Pharm Des. 2014;20(16):2669–2683.

12.Zimin AV, Delcher AL, Florea L, Kelley DR, Schatz MC, Puiu D et al. A whole-genome assembly of the domestic cow, *Bos taurus.* Genome Biol. 2009;10(4).

13.Daly K, Al-Rammahi M, Arora DK, Moran AW, Proudman CJ, Ninomiya Y et al. Expression of sweet receptor components in equine small intestine: relevance to intestinal glucose transport. Am J Physiol Regul Integr Comp Physiol. 2012;303(2):R199–208.

14.Liu G, Walter L, Tang S, Tan X, Shi F, Pan H et al. Differentiated adaptive evolution, episodic relaxation of selective constraints, and pseudogenization of umami and sweet taste genes TAS1Rs in catarrhine primates. Front Zool. 2014;11(1):79.

15.Zhu KL, Zhou XM, Xu SX, Sun D, Ren WH, Zhou KY et al. The loss of taste genes in cetaceans. Bmc Evol Biol. 2014;14.

16.Shi P, Zhang JZ. Contrasting modes of evolution between vertebrate sweet/umami receptor genes and bitter receptor genes. Mol Biol Evol. 2006;23(2):292–300.

17.Baldwin MW, Toda Y, Nakagita T, O'Connell MJ, Klasing KC, Misaka T et al. Sensory biology. Evolution of sweet taste perception in hummingbirds by transformation of the ancestral umami receptor. Science. 2014;345(6199):929–933.

18.Zhao HB, Li JW, Zhang JZ. Molecular evidence for the loss of three basic tastes in penguins. Curr Biol. 2015;25(4):R141-R142.

19.Volff JN. Genome evolution and biodiversity in teleost fish. Heredity. 2005;94(3):280–294.

20.Hara TJ. Olfaction and gustation in fish: an overview. Acta Physiol Scand. 1994;152(2):207–217.

21.Kasumyan AO, Doving KB. Taste preferences in fishes. Fish Fish. 2003;4(4):289–347.

22.Ishimaru Y, Okada S, Naito H, Nagai T, Yasuoka A, Matsumoto I et al. Two families of candidate taste receptors in fishes. Mech Dev. 2005;122(12):1310–1321.

23.Picone B, Hesse U, Panji S, Van Heusden P, Jonas M, Christoffels A. Taste and odorant receptors of the coelacanth-A gene repertoire in transition. J Exp Zool Part B. 2014;322(6):403–414.

24.Nei M, Niimura Y, Nozawa M. The evolution of animal chemosensory receptor gene repertoires: roles of chance and necessity. Nat Rev Genet. 2008;9(12):951–963.

25.He S, Liang XF, Li L, Sun J, Wen ZY, Cheng XY et al. Transcriptome analysis of food habit transition from carnivory to herbivory in a typical vertebrate herbivore, grass carp *Ctenopharyngodon idella.* BMC Genomics. 2015;16:15.

26.Wang YP, Lu Y, Zhang Y, Ning ZM, Li Y, Zhao Q et al. The draft genome of the grass carp (*Ctenopharyngodon idellus)* provides insights into its evolution and vegetarian adaptation. Nat Genet.

2015;47(6):625–631.

27.Watkins CE, Shireman JV, Rottmann RW, Colle DE. Food habits of fingerling grass carp. The Progressive Fish-Culturist. 1981;43(2):95–97.

28.Hashiguchi Y, Furuta Y, Kawahara R, Nishida M. Diversification and adaptive evolution of putative sweet taste receptors in threespine stickleback. Gene. 2007;396(1):170–179.

29.Liu H, Chen C, Gao Z, Min J, Gu Y, Jian J et al. The draft genome of blunt snout bream (*Megalobrama amblycephala)* reveals the development of intermuscular bone and adaptation to herbivorous diet. Gigascience. 2017;6(7):1–13.

30.Hao W, Golding GB. Does gene translocation accelerate the evolution of laterally transferred genes? Genetics. 2009;182(4):1365–1375.

31.Sternini C, Anselmi L, Rozengurt E. Enteroendocrine cells: a site of 'taste' in gastrointestinal chemosensing. Curr Opin Endocrinol. 2008;15(1):73–78.

32.de Araujo IE, Oliveira-Maia AJ, Sotnikova TD, Gainetdinov RR, Caron MG, Nicolelis MA et al. Food reward in the absence of taste receptor signaling. Neuron. 2008;57(6):930–941.

33.Huang YA, Roper SD. Intracellular Ca^{2+} and TRPM5-mediated membrane depolarization produce ATP secretion from taste receptor cells. J Physiol. 2010;588(Pt 13):2343–2350.

34.Kyriazis GA, Soundarapandian MM, Tyrberg B. Sweet taste receptor signaling in beta cells mediates fructose-induced potentiation of glucose-stimulated insulin secretion. Proc Natl Acad Sci U S A. 2012;109(8):E524–532.

35.Zhang YF, Hoon MA, Chandrashekar J, Mueller KL, Cook B, Wu DQ et al. Coding of sweet, bitter, and umami tastes: Different receptor cells sharing similar signaling pathways. Cell. 2003;112(3):293–301.

36.Yasuoka A, Aihara Y, Matsumoto I, Abe K. Phospholipase C-beta 2 as a mammalian taste signaling marker is expressed in the multiple gustatory tissues of medaka fish, *Oryzias latipes*. Mech Develop. 2004;121(7–8):985–989.

37.Yoshida Y, Saitoh K, Aihara Y, Okada S, Misaka T, Abe K. Transient receptor potential channel M5 and phospholipaseC-beta 2 colocalizing in zebrafish taste receptor cells. Neuroreport. 2007;18(15):1517–1520.

38.Mojet J, Christ-Hazelhof E, Heidema J. Taste perception with age: Generic or specific losses in threshold sensitivity to the five basic tastes? Chem Senses. 2001;26(7):845–860.

39.Mennella JA, Pepino MY, Lehmann-Castor SM, Yourshaw LM. Sweet preferences and analgesia during childhood: effects of family history of alcoholism and depression. Addiction. 2010;105(4):666–675.

40.Mennella JA, Lukasewycz LD, Griffith JW, Beauchamp GK. Evaluation of the monell forced-choice, paired-comparison tracking procedure for determining sweet taste preferences across the lifespan. Chem Senses. 2011;36(4):345–355.

41.Desor JA, Beauchamp GK. Longitudinal changes in sweet preferences in humans. Physiol Behav. 1987;39(5):639–641.

42.Richardson MK, Keuck G. Haeckel's ABC of evolution and development. Biol Rev Camb Philos Soc. 2002;77(4):495–528.

43.Birney E, Clamp M, Durbin R. GeneWise and Genomewise. Genome Res. 2004;14(5):988–995.

44.Jones DT, Taylor WR, Thornton JM. The rapid generation of mutation data matrices from protein sequences. Comput Appl Biosci. 1992;8(3):275–282.

45.Kumar S, Stecher G, Tamura K. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol. 2016;33(7):1870–1874.

46.Tamura K, Battistuzzi FU, Billing-Ross P, Murillo O, Filipski A, Kumar S. Estimating divergence times in large molecular phylogenies. P Natl Acad Sci USA. 2012;109(47):19333–19338.

47.Nei M, Kumar S: Molecular evolution and phylogenetics: Oxford University Press; 2000.

48.Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A et al. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol. 2002;3(7).

49.Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ Method. Methods. 2001;25(4):402–408.

Tables

Table 1. Numbers of sweet taste receptors and pseudogenes in fishes

Order	Species	Food habits	Numbers of T1R2	Accession no.
Coelacanthiformes	Coelacanth (*Latimeria chalumnae*)	Carnivore	2	XM_005986116 XM_005986117
Lepidosteiformes	Spotted gar (*Lepisosteus oculatus*)	Carnivore	2	XM_015337079 XM_015337086
Characiformes	Cavefish (*Astyanax mexicanus*)	Carnivore	2	ENSAMXG00000014366 ENSAMXG00000014380
Gasterosteiformes	Stickleback (*Gasterosteus aculeatus*)	Carnivore	8	ENSGACG00000006506 ENSGACG00000006510 ENSGACG00000006517 ENSGACG00000006525 ENSGACG00000006529 ENSGACG00000006534 ENSGACG00000006536 ENSGACG00000006762
Perciformes	European seabass (*Dicentrarchus labrax*)	Carnivore	2	DLAgn_00132730 DLAgn_00132740
Cyprinodontiformes	Guppy (*Poecilia reticulata*)	Omnivore	2	XM_008414738.2 XM_008413473.2
Cyprinodontiformes	Southern platyfish (*Xiphophorus maculatus*)	Omnivore	2	XM_005800149.1 XM_023341410.1
Beloniformes	Medaka (*Oryzias latipes*)	Omnivore	3	AB200906 AB200907 AB200908
Perciformes	Zebra mbuna (*Maylandia zebr*a)	Omnivore	2	XM_004554287.4 XM_004554097.2
Perciformes	Tilapia (*Oreochromis niloticus*)	Omnivore	3	XM_005478384 XM_003444871 XM_003444784
Tetraodontiformes	Fugu (*Takifugu rubripes*)	Omnivore	2	AB200911 AB200912
Cypriniformes	Common carp (*Cyprinus carpio*)	Omnivore	2	XM_019093519.1 XM_019091545.1
Cypriniformes	Zebrafish (*Danio rerio*)	Omnivore	2	NM_001039831 NM_001083856
Cypriniformes	Blunt snout bream (*Megalobrama amblycephala*)	Herbivore	3	SRP090157
Cypriniformes	Grass carp (*Ctenopharyngodon*	Herbivore	6	KU976430

idellus)	KU976431
	KU976432
	KU976433
	KU976434
	KU976435

Figures

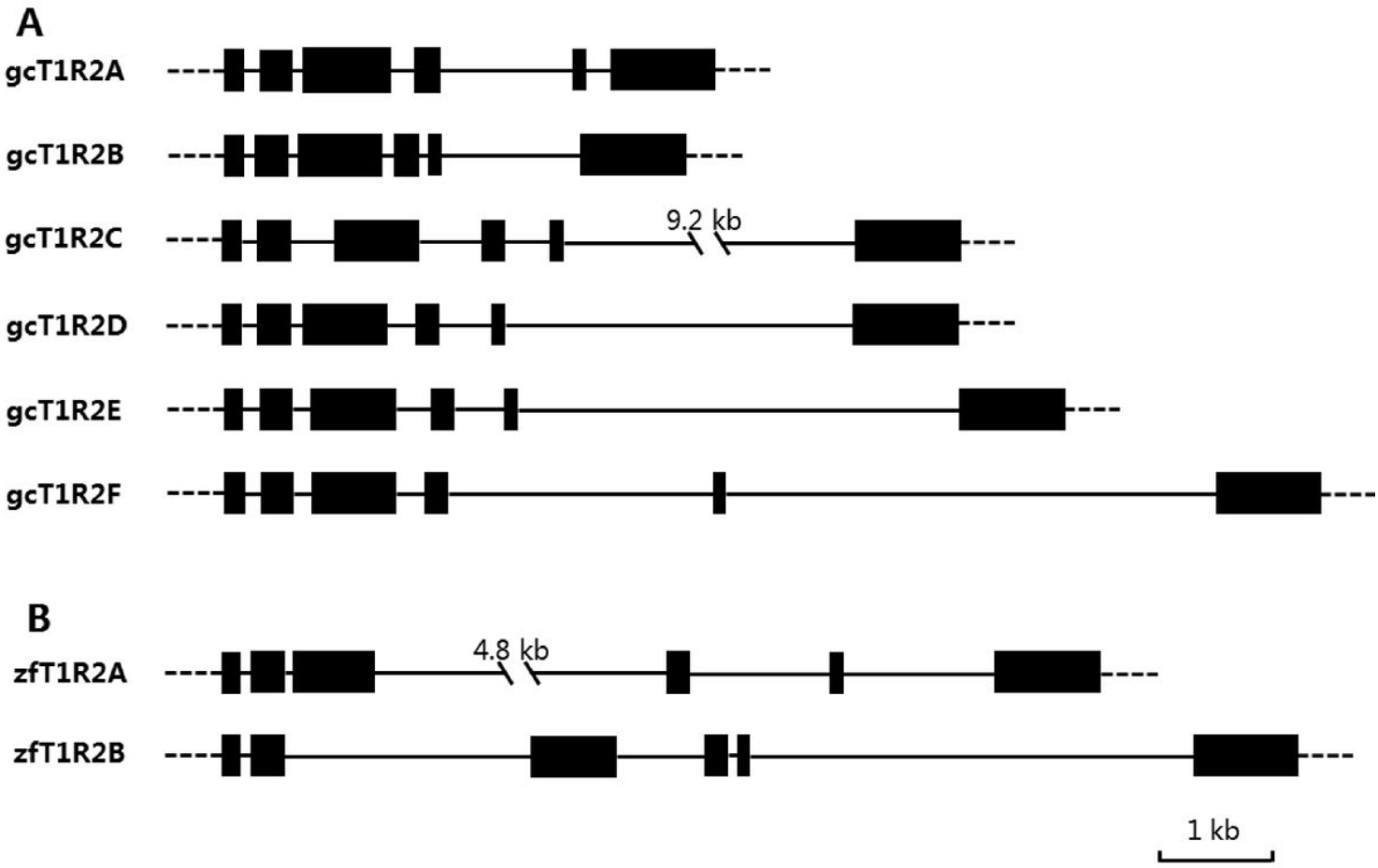

Figure 1

The gene structures of T1R2 genes in grass carp (A) and zebrafish (B). The black lines indicate introns, and the black boxes indicate exons. The six gcT1R2 genes we obtained contained 6 exons and 5 introns as well as the genomic structure of two zebrafish T1R2 genes.

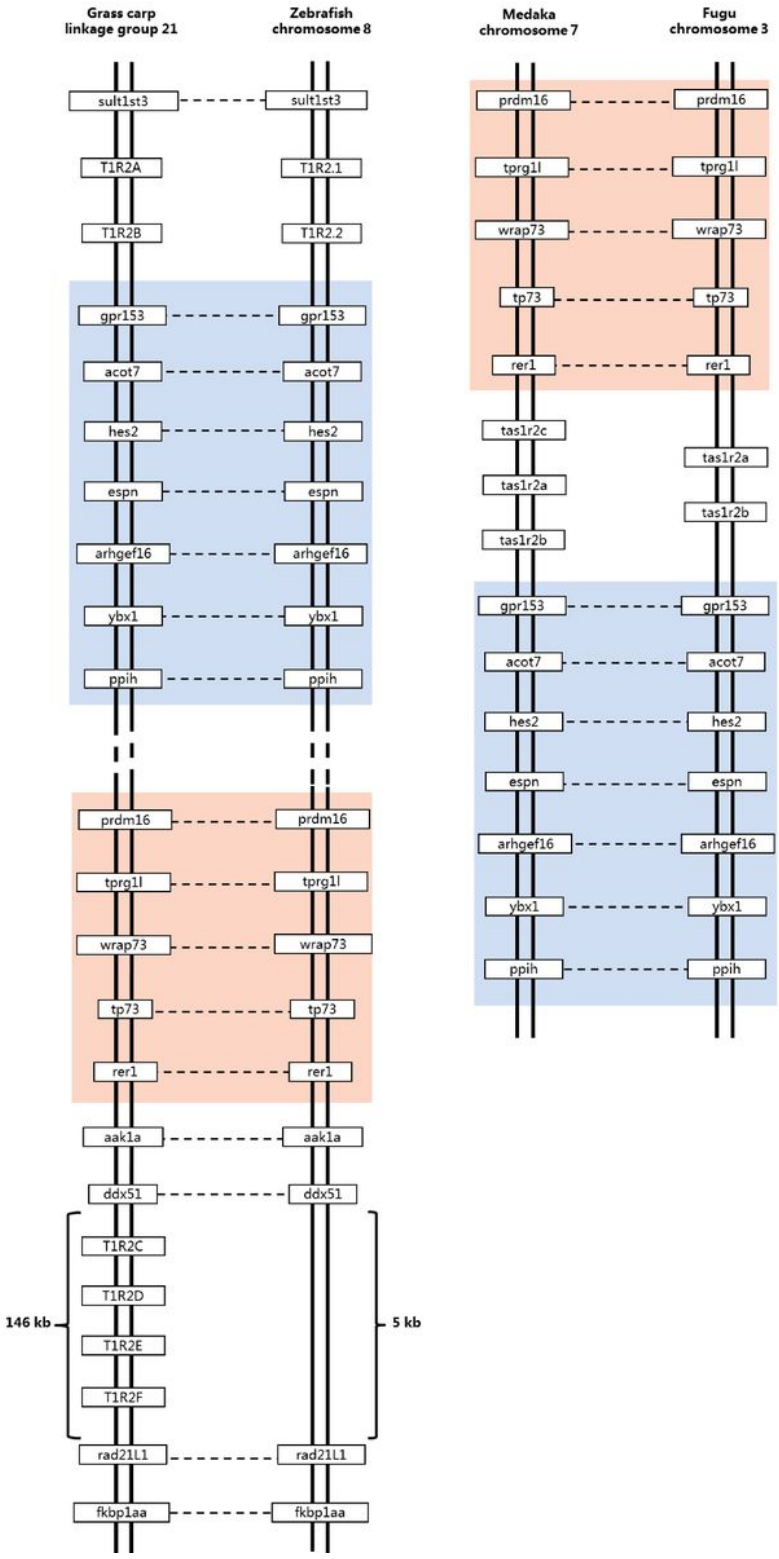

Figure 2

Synteny analysis of T1R2 genes. The synteny analysis performed by searching gene(s) flanking T1R2 in genomes of zebrafish, medaka and fugu using map viewer of NCBI. GenBank accession numbers of the adjacent genes of zebrafish T1R2.1 (NM_001039831.1) and T1R2.2 (NM_001083856.1) in the figure were as follows: sult1st3, NM_183348.2; gpr153, XM_009304261.1; acot7, NM001004617.1; hes2, NM_001045353.1; espn, NM_001123282.1; arhgef16, NM_001123283.1; ybx1, NM_001126457.1; ppih,

NM_001009902.2; prdm16; XM_005167301.2; tprg1l, XM_001922766.5; wrap73, NM_199893.1; tp73, NM_183340.1; rer1, XM_005167299.2; aak1a, XM_005167316.2; ddx51, NM_001003864.1; rad21L1, NM_001080050.1; fkbp1aa, NM199945.1. GenBank accession numbers of the adjacent genes of medaka tas1r2a (NM_001104858.1), tas1r2b (NM_001104723.1) and tas1r2c (NM_001104724.1) in the figure were as follows: prdm16, XM_011476903.1; tprg1l, XM_004070640.2; wrap73, XM_004070359.2; tp73, XM_004070358.2; rer1, XM_011476898.1; gpr153, XM_011476896.1; acot7, XM_011476894.1; hes2, XM_004070354.2; espn, XM_011476893.1; arhgef16, XM_011476892.1; ybx1, NM_001104673.1; ppih, XM_004070353.2. GenBank accession numbers of the adjacent genes of fugu tas1r2a (NM_001105217.1) and tas1r2b (NM_001105218.1) in the figure were as follows: prdm16, XM_003963257.1; tprg1l, XM_003963136.1; wrap73, XM_003963258.1; tp73, XM_003963138.1; rer1, XM_003963139.1; gpr153, XM_003963260.1; acot7, XM_003963140.1; hes2, XM_003963261.1; espn, XM_003963263.1; arhgef16, XM_003963264.1; ybx1, XM_003963141.1; ppih, XM_003963144.1.

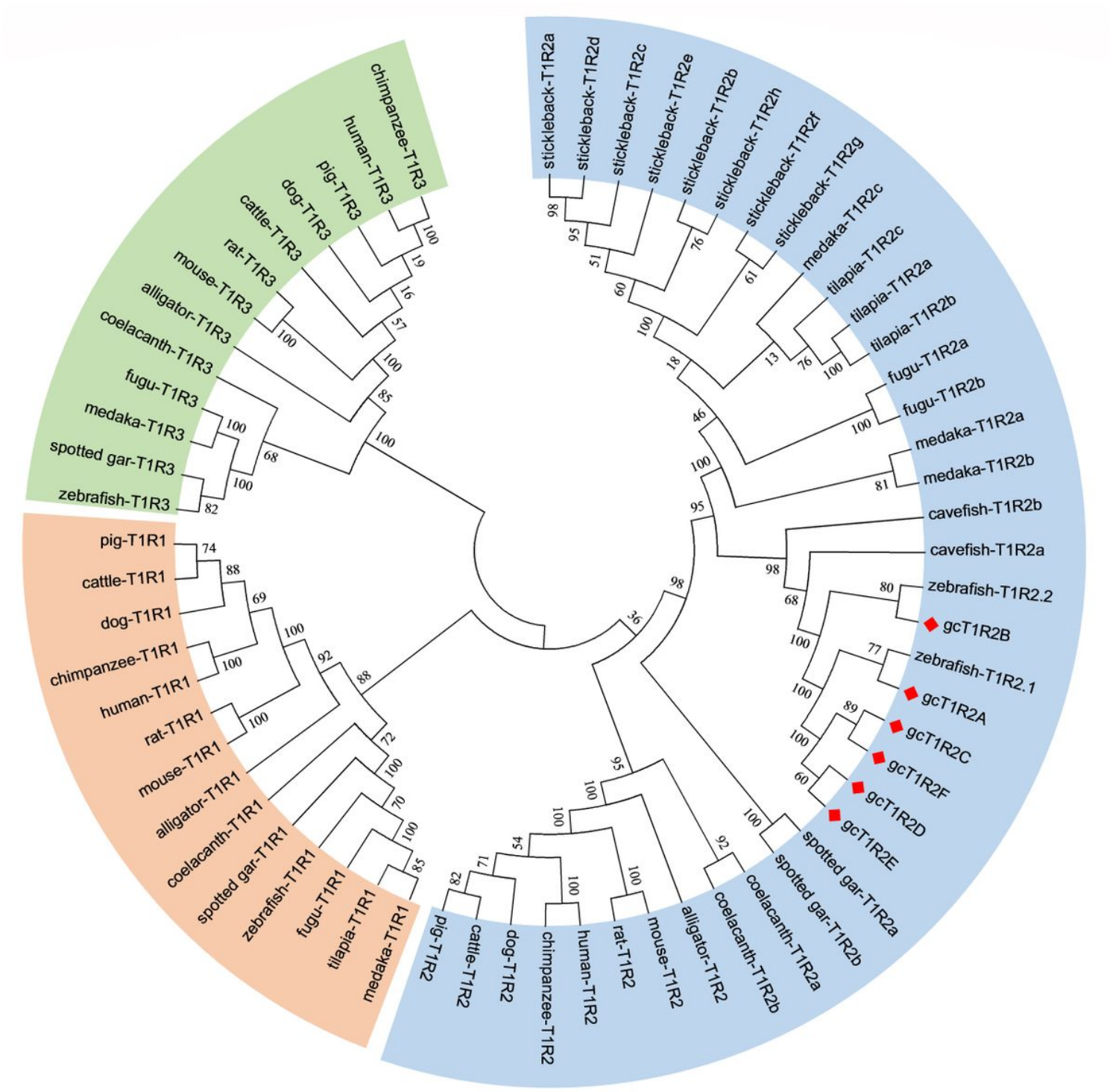

Figure 3

Molecular phylogenetic analysis by maximum likelihood method of T1R2 genes. The evolutionary history was inferred by using the Maximum Likelihood method based on the JTT matrix-based model. The tree with the highest log likelihood (-18992.7439) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using a JTT model, and then selecting the topology with superior log likelihood value. A

discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 1.8249)). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 0.8418% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 65 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 297 positions in the final dataset. Evolutionary analysis was conducted in MEGA7. The T1R2s amino acid sequences of fishes and mammals used are given in the electronic supplementary material, table S2.

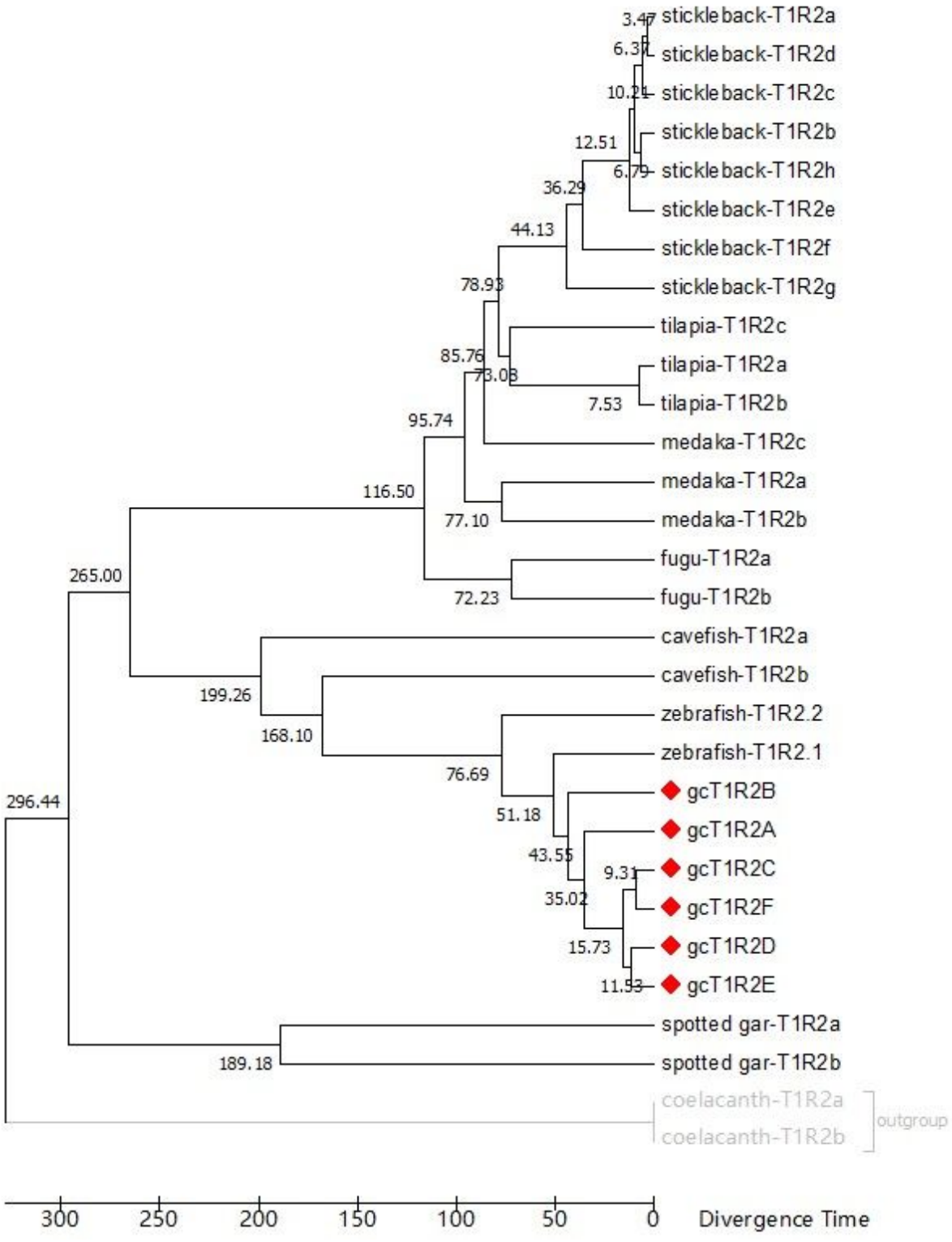

Figure 4

A timetree inferred using the reltime method and the general time reversible model of fish T1R2 genes. The timetree was computed using 4 calibration constraints. The estimated log likelihood value is -21587.6247. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 1.6461)). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 9.5713% sites). The analysis involved 30 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 1353 positions in the final dataset. Evolutionary analysis was conducted in MEGA7. The T1R2s nucleotide sequences of fishes used are given in the electronic supplementary material, table S3.

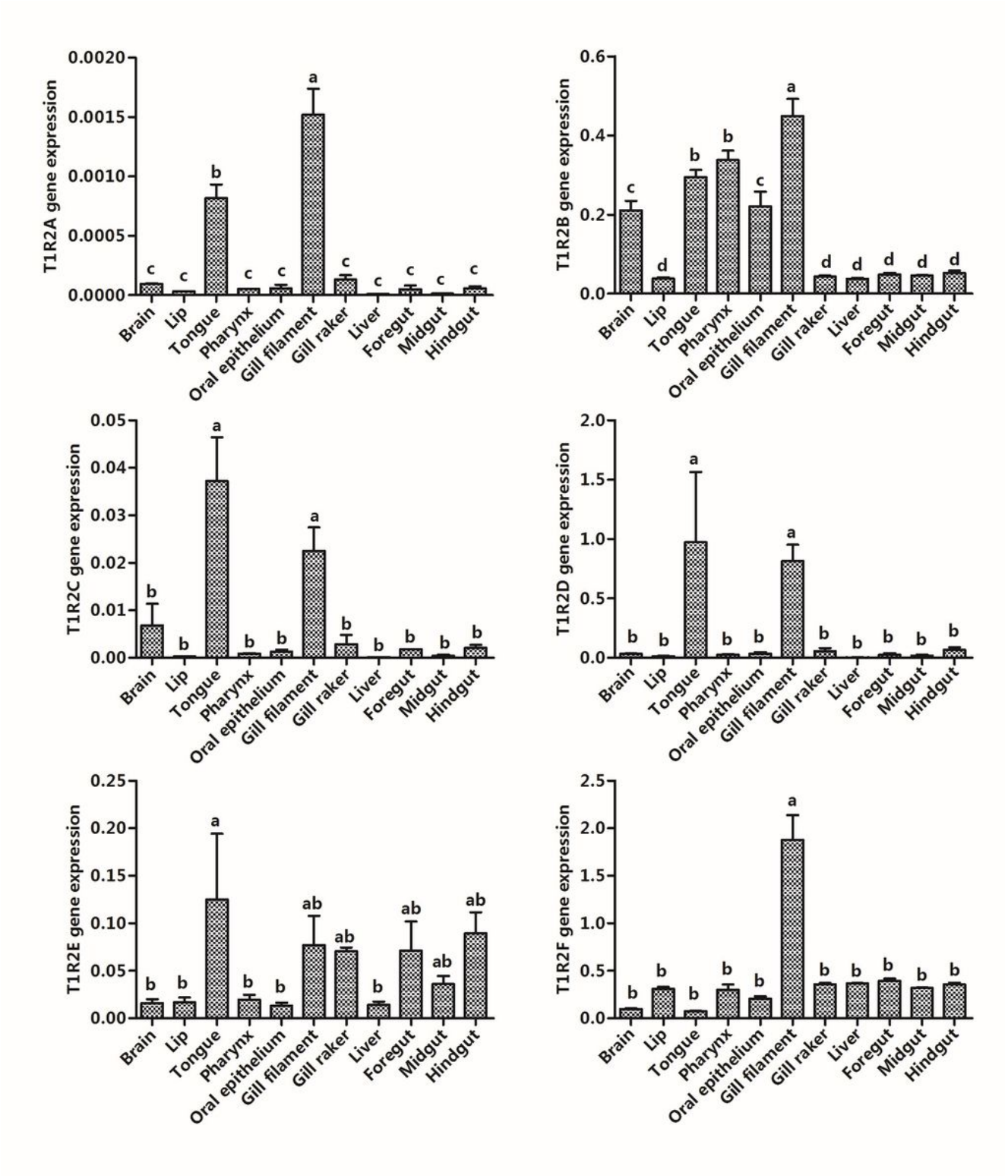

Figure 5

Tissue distributions of gcT1R2s. Relative mRNA expression was quantified using real-time PCR and normalized against EF1 as a housekeeping gene. All values represent the mean ± S.E.M. (n = 6).

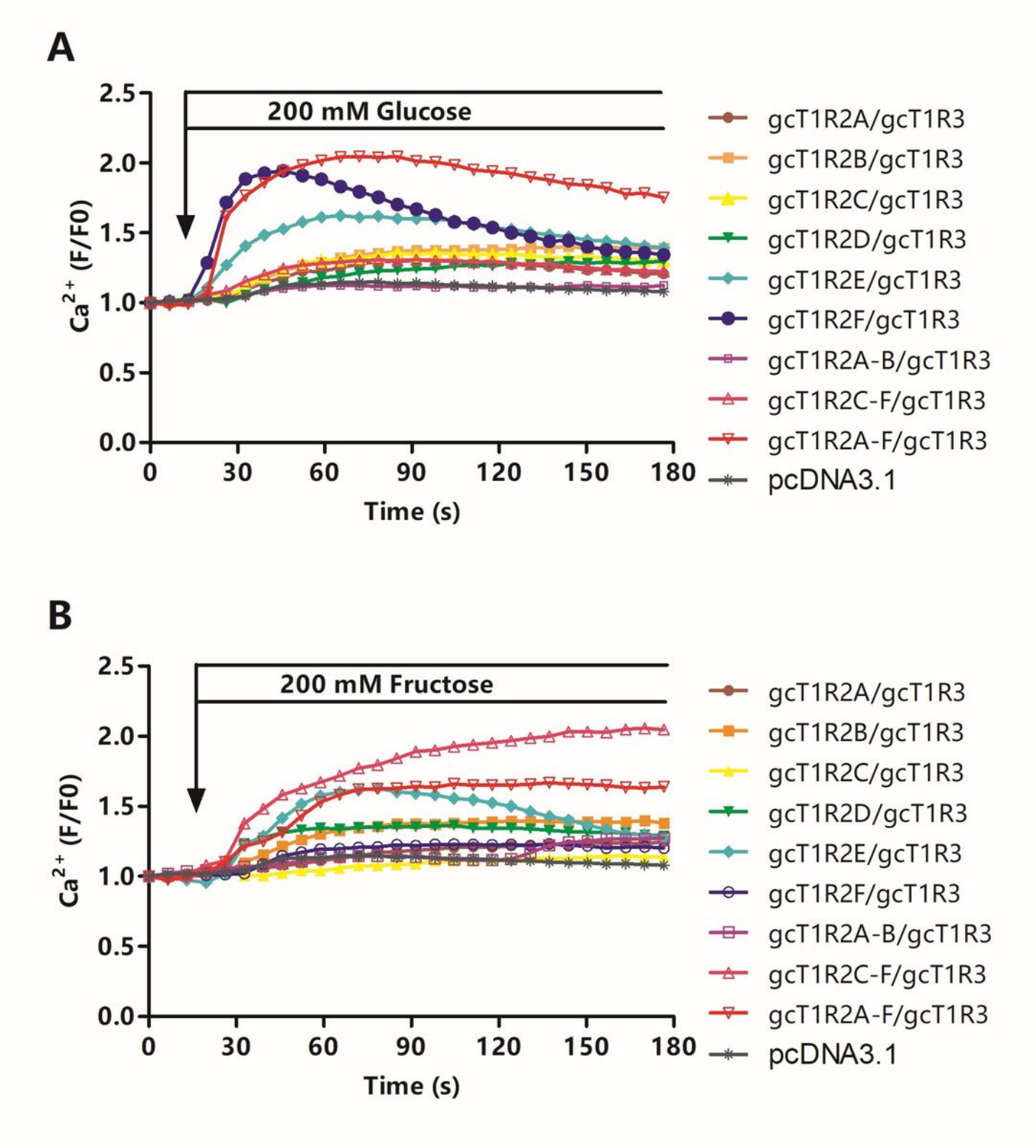

Figure 6

Ca2+ changes in fluorescence intensity of 20 single HEK293T cells upon glucose and fructose stimulations. HEK293T cells were stimulated with 200mM glucose and 200mM fructose. Images were recorded at 6.54 sec intervals up to 183.16 sec using 488 nm excitation filter and 516 nm emission filter and analyzed using FV10-ASW 3.1 Viewer software. The backgrounds of the emission intensities were

subtracted. Data are expressed as the ratio of the fluorescence intensities of 20 single HEK293T cells per dish and initial intensity (F/F0).

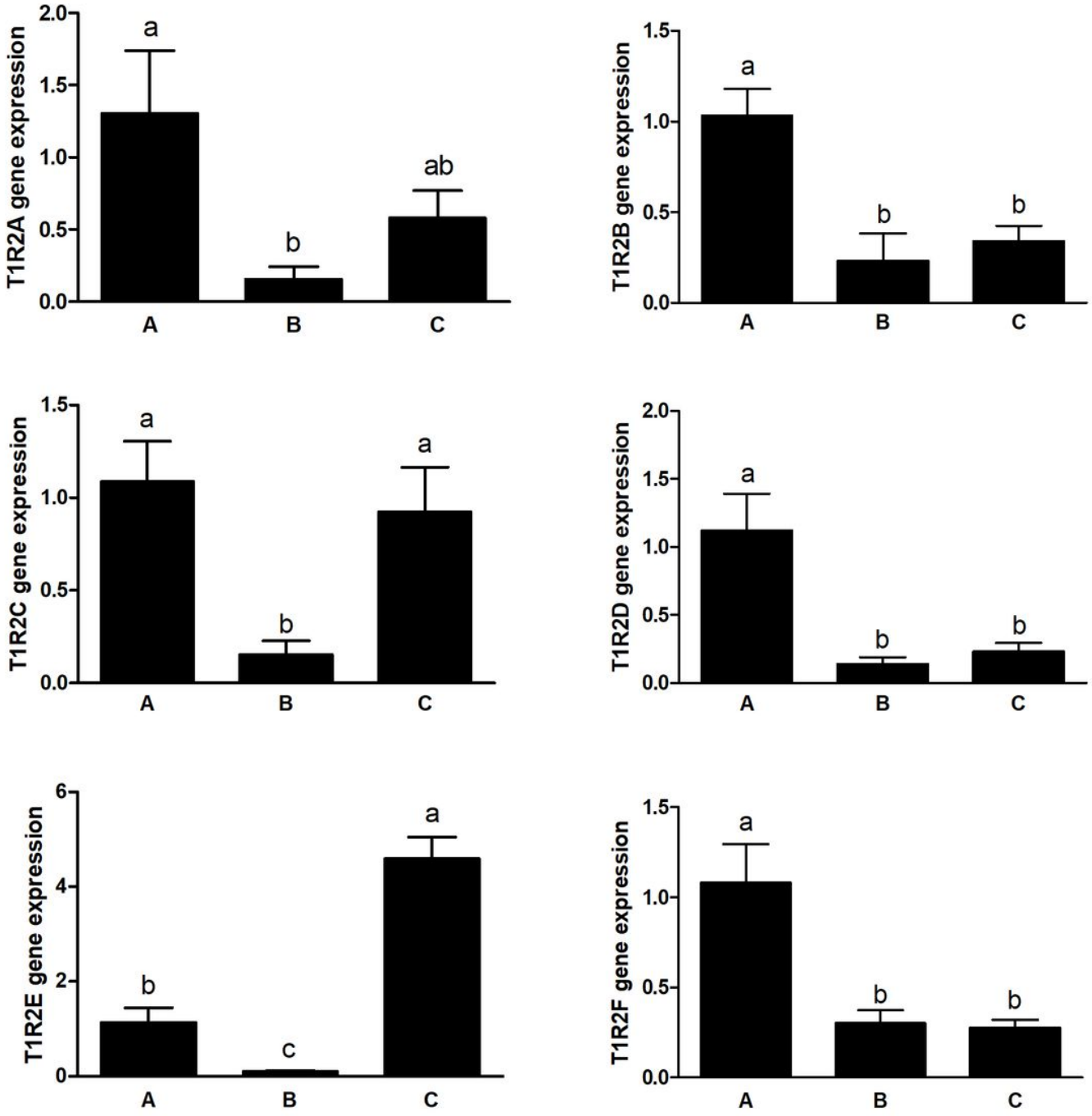

Figure 7

The gene expressions of gcT1R2s in the gut of grass carp transition from carnivory to herbivory. Relative mRNA expression was quantified using real-time PCR and normalized against EF1 as a housekeeping gene. All values represent the mean ± S.E.M. (n = 6).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplement1.rar