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Taste receptors and gustatory associated G proteins in channel catfish, *Ictalurus punctatus*

Sen Gao [a,1], Shikai Liu [a,1], Jun Yao [a], Tao Zhou [a], Ning Li [a], Qi Li [b], Rex Dunham [a], Zhanjiang Liu [a,*]

[a] The Fish Molecular Genetics and Biotechnology Laboratory, Aquatic Genomics Unit, School of Fisheries, Aquaculture and Aquatic Sciences, Auburn University, Auburn, AL 36849, USA
[b] Key Laboratory of Mariculture of the Ministry of Education, Ocean University of China, Qingdao, China

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ABSTRACT

Taste sensation plays a pivotal role in nutrient identification and acquisition. This is particularly true for channel catfish (*Ictalurus punctatus*) that live in turbid waters with limited visibility. This biological process is mainly mediated by taste receptors expressed in taste buds that are distributed in several organs and tissues, including the barbels and skin. In the present study, we identified a complete repertoire of taste receptor and gustatory associated G protein genes in the channel catfish genome. A total of eight taste receptor genes were identified, including five type I and three type II taste receptor genes. Their genomic locations, phylogenetic relations, orthologies and expression were determined. Phylogenetic and collinear analyses provided understanding of the evolution dynamics of this gene family. Furthermore, the motif and dN/dS analyses indicated that selection pressures of different degrees were imposed on these receptors. Additionally, four genes of gustatory associated G proteins were also identified. It was indicated that expression patterns of catfish taste receptors and gustatory associated G proteins across organs mirror the distribution of taste buds across organs. Finally, the expression comparison between catfish and zebrafish organs provided evidence of potential roles of catfish skin and gill involved in taste sensation.

1. Introduction

Taste sensations play pivotal roles for vertebrates' feeding decision after food searching and thereby for their survival (Bruch et al., 1988; Bachmanov and Beauchamp, 2007). They are mainly mediated by the taste receptors (TRs) in vertebrates. Vertebrate TRs are identified as seven trans-membrane G protein-coupled receptors and are expressed in the taste buds located within gustatory papillae. They could detect soluble stimuli and initiate signal transduction for the taste sensation (Mombaerts, 2004). There are five basic taste sensations in vertebrates, including sweet, bitter, umani, salty and sour (Chandrashekar et al., 2006). Three of these (sweet, bitter and umani) can be detected by TRs. The bitter sensation is considered to be the most important for survival in vertebrates since toxic and harmful substances usually taste bitter (Bachmanov and Beauchamp, 2007).

Two TR families are used for taste sensations, and they are expressed in different subsets of taste receptor cells (TRCs). The T1R family (taste receptor, type 1) was first discovered for the sweet sensation, and it has three subfamilies, including T1R1, T1R2 and T1R3 (Bachmanov and Beauchamp, 2007). Further analyses indicated that T1R3 was often co-expressed with T1R2 for the sweet sensation in responding to tastants like natural sugars, D-amino acids, sucrose, saccharin, dulcin and sweet proteins (Montmayeur et al., 2001; Nelson et al., 2001; Zhao et al., 2003), or was co-expressed with T1R1 for the umani sensation in responding to L-amino acids and monosodium L-glutamate (Mombaerts, 2004; Chandrashekar et al., 2006; Bachmanov and Beauchamp, 2007). The other TR family, T2R family (taste receptor, type 2), comprises the most members among the TR families (Adler et al., 2000; Chandrashekar et al., 2000). It was found that the main function of T2R family was related to bitter sensation even though the function of several members within T2R is still not clear (Lindemann, 2001; Mombaerts, 2004; Ishimaru et al., 2005; Bachmanov and Beauchamp, 2007). Another interesting finding was that several T2Rs were co-expressed with each other in the same TRCs. The number of vertebrate T2Rs is relatively small compared with that of bitter tasted chemical compounds found in nature (Jaggupilli et al., 2016), while another type of vertebrate chemosensory receptor, olfactory receptor, has a larger number in order to distinguish a variety of substances. All of these indicate that vertebrate T2Rs are more dedicated for sensing rather than distinguishing (Caprio, 1975; Adler et al., 2000; Lindemann, 2001; Bachmanov and Beauchamp, 2007).

Diverse tissues and organs in fish species, especially in catfish, were found to harbor TRs. For example, the entire external body surfaces of fish species, including the barbels and skin, were considered as the regions that express TRs (Caprio, 1975; Raji and Norozi, 2010). It was

* Corresponding author.
E-mail address: liuzhan@auburn.edu (Z. Liu).
[1] These authors contributed equally.

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indicated that solitary chemosensory cells, a type of cells distributed with high density in fish external body surface, expressed TRs as well (Hansen et al., 2002; Caprio and Derby, 2008; Hansen et al., 2014). In addition, TRs were also identified in fish gill, an organ important for breathing (Hansen et al., 2002; Caprio and Derby, 2008; Hansen et al., 2014). Although the diverse distribution of TRs in fish species has been widely studied in physiology, the expression profiling of fish TRs using RNA-Seq datasets is still lacking.

Except for TRs, some G proteins are also believed to be involved in gustatory activities. They were co-expressed with T1Rs for sweet sensing in the gut of mammals (Dyer et al., 2005; Margolskee et al., 2007). These proteins, especially the α subunit, were found to be involved in signal transduction pathways for both bitter and sweet in the taste buds of rat tongues (Shen et al., 2005). These studies indicated that G proteins might play significant roles involved in gustation than previously expected (Shigemura et al., 2008). However, expression analysis of gustatory associated G proteins remains largely unknown in fish species.

Thus, the TRs and gustatory associated G proteins in fish species become necessary to be further studied. Upon the completion of reference genome assembly (Liu et al., 2016) and availability of RNA-Seq datasets from various tissues in channel catfish (*Ictalurus punctatus*), the identification, annotation and expression profiling for the full set of TRs as well as gustatory associated G proteins become feasible, which have been performed in this study. Also, our project combined phylogenetic, orthogroup, collinearity, motif and dN/dS analyses to illustrate the evolutionary dynamics of vertebrate TRs across a broad range of chordate taxa.

2. Materials and methods

2.1. Retrieval of TR sequences of other vertebrates

Protein sequences of sixteen vertebrate species, including amazon molly (*Poecilia formosa*), cave fish (*Astyanax mexicanus*), cod (*Gadus morhua*), fugu (*Takifugu rubripes*), medaka (*Oryzias latipes*), platyfish (*Xiphophorus maculatus*), spotted gar (*Lepisosteus oculatus*), stickleback (*Gasterosteus aculeatus*), tetraodon (*Tetraodon nigroviridis*), tilapia (*Oreochromis niloticus*), zebrafish (*Danio rerio*), anole lizard (*Anolis carolinensis*), chicken (*Gallus gallus*), mouse (*Mus musculus*), cow (*Bos taurus*) and human (*Homo sapiens*), were downloaded from the ENSEMBL database. Besides, sequence descriptions were also downloaded using BioMart and were combined with protein sequences using a custom script. We then collected TRs through text searching. The query sequences were carefully selected using below standards: 1) only full-length TR protein sequences were used in our study; 2) only the longest sequences were selected when genes have multiple isoforms using custom script; 3) genes labeled with "pseudogene" were removed. The TR sequences used in the present project, including the sequences from channel catfish, were provided in Supplementary File 1.

2.2. Identification of TRs in channel catfish

We used an extensive computational method to identify potential TRs in the channel catfish genome. First, the draft genome sequences of channel catfish were masked using RepeatMasker (Smit et al., 2014), and the masked sequences were used to predict putative protein sequences using FGENESH embedded in MolQuest (Solovyev et al., 2006). All the predicted sequences aforementioned were annotated through BLAST against the NCBI non-redundant database. The catfish amino acid sequences with hits to well annotated TRs were then selected based on the annotation aforementioned, and used for further analysis in the project. At the same time, transcripts, corresponding to the annotated protein sequences, were generated from FGENESH and further used as reference sequences for the mapping of RNA-Seq reads from various organs.

2.3. Phylogenetic, orthogroup and collinear analyses

To provide a comprehensive understanding of the evolutionary dynamic of TRs in vertebrates, we conducted phylogenetic, orthogroup and collinear analyses in our project. First, full-length TR protein sequences were aligned using MUSCLE (Edgar, 2004) before the construction of phylogenetic tree using FastTree (Price et al., 2009, 2010) with default settings, based on the JTT model of amino acid evolution with maximum-likelihood rearrangements. A gamma-based Bayesian approach was used to assign each amino acid site. ProtTest was used to select the best model (Darriba et al., 2011). Gaps were ignored and the local support values were computed with 1000 replicates. The phylogenetic tree was visualized using MEGA6 (Tamura et al., 2013). Calcium-sensing receptors and frizzled receptors were selected as outgroups for T1Rs and T2Rs, respectively (Fredriksson et al., 2003). Second, all-versus-all comparisons were conducted among the genomes of the catfish and other 16 species using OrthoFinder (Emms and Kelly, 2015), and orthogroups that harbor TRs were extracted. Only orthogroup that consists of at least two orthologs or two paralogs were retained in our results. Third, collinear analysis was conducted between the catfish genome and zebrafish genome using MCScanX (Wang et al., 2012). Tandem duplicated genes in these two species were also identified.

2.4. Identification of conserved motifs

Motif analysis was conducted for fish TRs. We only selected amino acid sequences possessing the seven trans-membrane topology, and aligned them using MUSCLE (Edgar, 2004). The gaps were removed using trimAl (Capella-Gutierrez et al., 2009) for each TR subfamily. Finally, we conducted conserved motif identifications for T1Rs and T2Rs, respectively, using MEME (Bailey et al., 2009). The maximal length of each motif was set at 50. Only the top five best-conserved motifs were listed. The seven transmembrane domains were determined using Phobius based on multiple sequence alignments (Kall et al., 2007).

2.5. dN/dS Analysis

In order to measure the selection pressures imposed on each subfamily of fish TRs, we conducted the natural selection analysis based on the relative rates of synonymous and non-synonymous substitutions. The coding sequences, which corresponding to the amino acid sequences used for the motif identification, were collected to calculate the global and site-by-site dN/dS ratios using Datamonkey (Delport et al., 2010). Only subfamilies that have sequences originated from at least two species were selected.

2.6. Expression profiling of catfish TRs and G proteins

RNA-Seq datasets from five channel catfish organs under normal status, including barbel (SRA accession number SRR1984599), skin (SRA accession number SRR1984907), gill (SRA accession number SRR917958), liver (SRA accession number SRR917955) and intestine (SRA accession number SRR357322), were downloaded from the NCBI SRA database. Sequencing adaptors, low quality reads and reads with length lower than 36 bases were removed using Trimmomatic (Bolger et al., 2014). Clean reads were mapped to all the annotated transcripts generated from FGENESH using Bowtie 2, and expression values of the TRs and the gustatory associated G protein were extracted. FPKM (fragments per kilobase per million mapped reads) was calculated using RSEM (Li and Dewey, 2011). We selected ribosome protein S4 (RPS4) (Infante et al., 2008) as the housekeeping gene to eliminate expression variations among different tissues. Expression level of each catfish TR was determined based on the ratio of its FPKM to that of RPS4 in the same organ.

We also compared the expression levels of TRs between the catfish and zebrafish organs, including skin, gill, liver and intestine. RNA-Seq

Table 1
Genomic organization of TR genes in the catfish genome.

Gene name	Chromosome no.	Staring site	Ending site
T1R1	15	15,822,981	15,854,819
T1R2a	5	8,954,348	8,959,672
T1R2b	5	8,963,481	8,972,227
T1R2c	5	8,974,294	8,979,582
T1R3	21	10,218,388	10,242,169
T2R201a	6	5,333,686	5,335,131
T2R201b	6	5,343,501	5,344,343
T2R201c	6	5,369,269	5,370,202

dataset generated from the organs of zebrafish (SRA accession number SRP013931 and SRP048807) were downloaded from the NCBI SRA database. We used TPM (transcripts per million) instead of FPKM to conduct the comparisons. As reported before, TPM can effectively normalize the variations between samples of different origins with less bias compared with FPKM (Conesa et al., 2016).

3. Results and discussion

3.1. Identification and characterization of TR genes in channel catfish

Eight TR genes were identified in the channel catfish genome, of which one belonged to T1R1, three belonged to T1R2, one belonged to T1R3, and three belonged to T2R201 subfamilies. Each subfamily was located on a different chromosome, with T1R1 on chromosome 15, T1R2 on chromosome 5, T1R3 on chromosome 21, and T2R201 on chromosome 6. Their genomic locations were summarized in Table 1.

The copy number of each TR subfamily is variable between mammals and fish species. In the genomes of fish species, both T1R1 and T1R3 have a single copy as in mammals (Fig. 1; Supplementary Table 1). Several fish species, including catfish, zebrafish, and medaka, possessed several copies of T1R2, while mammals were found that only have a singe copy of T1R2. For T2Rs, mammals possess more members than that of fish species. For instance, there are about 50 T2Rs in the genome of mammals such as humans, mice and rats, while there are only about 10 T2Rs in the genome of fish species such as zebrafish, fugu and medaka (Adler et al., 2000; Ishimaru et al., 2005).

T2Rs in channel catfish were only identified in subfamily 201. There were no T2Rs belonging to the other two subfamilies, T2R200 and T2R202 (Fig. 2; Supplementary Table 1). Zebrafish is the only fish species that harbors all three T2R subfamilies, while it is a common phenomenon that other fish genomes lack one or two T2R subfamilies, for instance, T2R200 was not found in the cave fish genome, and both T2R200 and T2R202 were not found in the stickleback genome.

3.2. Phylogenetic, orthogroup and collinear analyses of TR genes

Two phylogenetic trees were constructed to delineate the evolution history of TRs in mammals and fish species (Fig. 1 and Fig. 2). In general, T1Rs and T2Rs were clustered into a different clade from calcium-sensing receptors and frizzled receptors, respectively, indicating that the phylogenetic analysis was robust. The fish T1Rs were clustered into a distinct cluster from their mammalian counterparts, and the same was true for T2Rs. This may be related to the different living environments of these two taxa, with mammals mainly live on land, while fish live in water. Environmental factors, high likely the pH (DeSimone et al., 2001; Lyall et al., 2001; Lin et al., 2004; Sakurai et al., 2009; Caprio et al., 2014), can affect not only the expression of TRs but also the divergence of TRs among fish species.

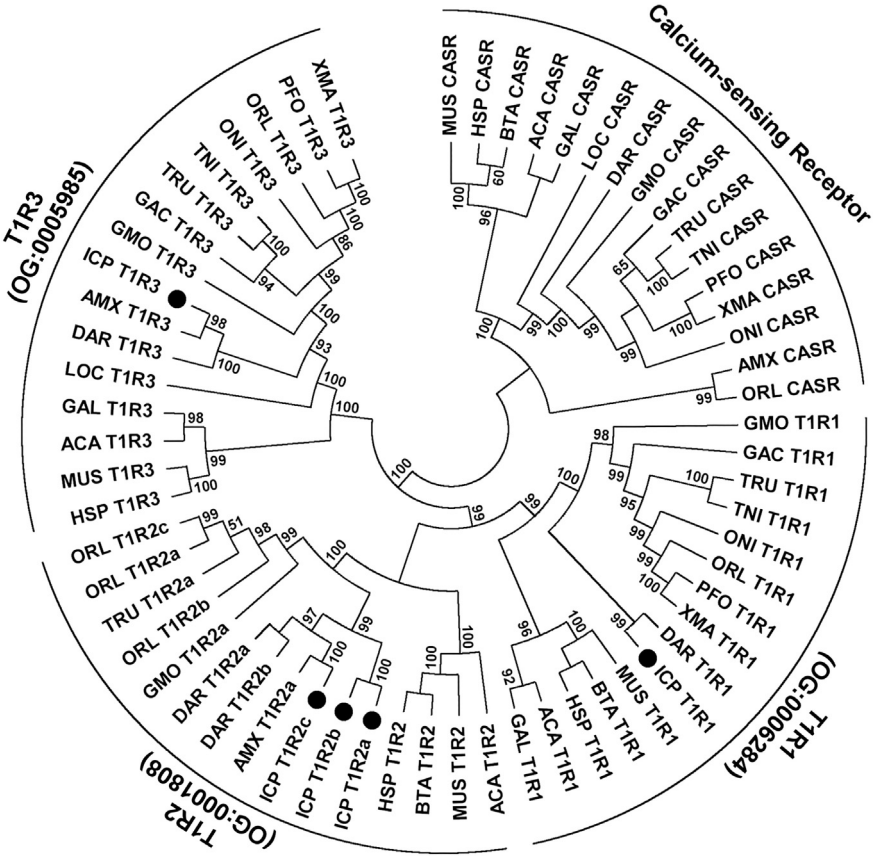

Fig. 1. A phylogenetic tree of the vertebrate T1Rs constructed using amino acid sequences. Members from the same subfamily are coverd with lines with subfamily names, while orthogroups are indicated in the parentheses. Abbreviations for species names: HSP, Human; MUS, Mouse; GAL, Chicken; BTA, Cow; ACA, Anole lizard; PFO, Amazon molly; AMX, Cave fish; GMO, Cod; TRU, Fugu; ORL, Medaka; XMA, Platyfish; LOC, Spotted gar; TNI, Tetraodon; GAC, Stickleback; ONI, Tilapia; DAR, Zebrafish; ICP, Channel catfish.

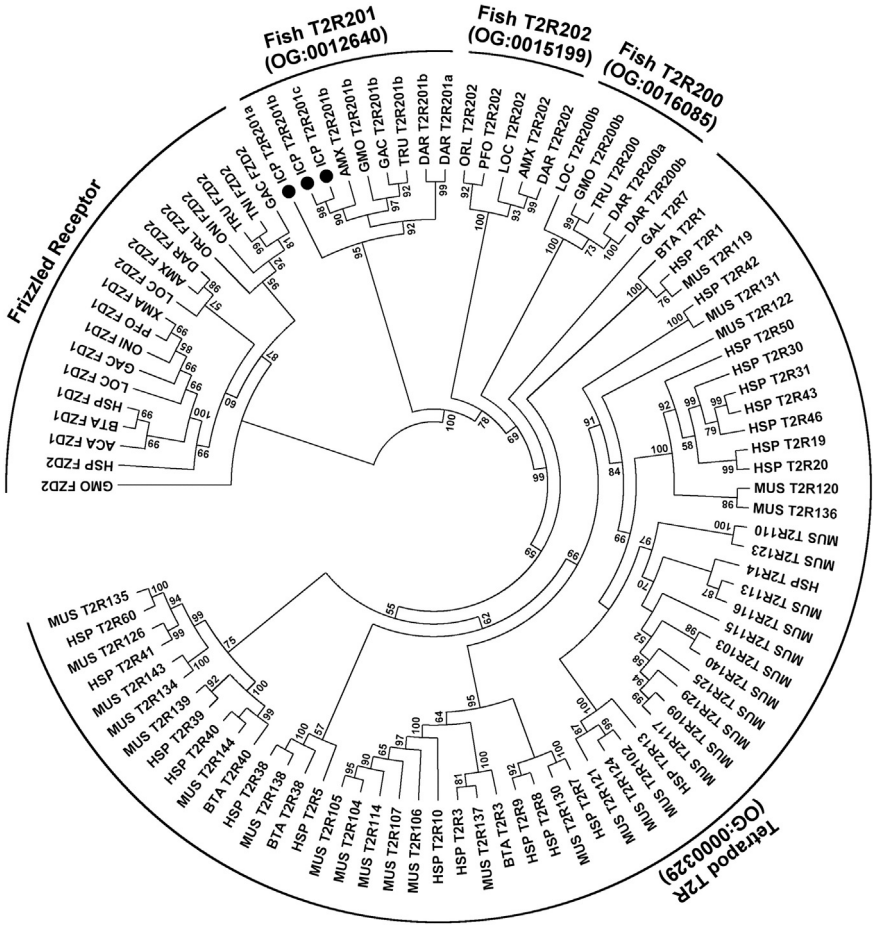

Fig. 2. A phylogenetic tree of the vertebrate T2Rs constructed using amino acid sequences. Members from the same subfamily are coverd with lines with subfamily names, while orthogroups are indicated in the parentheses. Abbreviations for species names: HSP, Human; MUS, Mouse; GAL, Chicken; BTA, Cow; PFO, Amazon molly; AMX, Cave fish; GMO, Cod; TRU, Fugu; ORL, Medaka; LOC, Spotted gar; GAC, Stickleback; DAR, Zebrafish; ICP, Channel catfish.

Orthogroup analysis was performed to validate the evolutionary relationships among various vertebrate TRs. Our results from orthogroup analysis was consistent with the phylogenetic analysis. For instance, each subfamily of fish T2Rs not only harbored a single clade in the phylogenetic tree, but also contained all members that from the same orthogroup.

Synteny analysis was further used to support orthology. In the present study, we used collinear relation, a special form of synteny to illustrate the orthologies for certain catfish TR subfamilies. Also, combined with the tandem duplication relationships identified in our analysis, the relative emerging time point for each member of these subfamilies could be inferred. Two pairs of conserved homologous regions were identified between the catfish genome and zebrafish genome, as each of them contained one pair of TRs as collinear genes (Fig. 3 and Supplementary Table 2). The catfish T2Ra and zebrafish T2Ra were listed as a pair of collinear genes, but their copies could not be listed as a pair of collinear genes. Between T1R2a and the next collinear gene, there were two other copies of T1R2 genes in catfish and one copy of T1R2 in zebrafish, however, they were not co-linear (not orthologous) since they were originated from tandem duplications. Thus, we inferred that T1R2a is the most ancient gene for catfish and zebrafish, and T1R2b and T1R2c in catfish, as well as the T1R2b in zebrafish are descendants of T1R2a after speciation (Fig. 3A). In other words, they may be derived from species-specific gene duplication in the form of tandem duplication. Similarly, T2R201a was the most ancient gene, and T2R201b was derived from species-specific tandem duplication (Fig. 3B).

3.3. Conserved motifs

Five best-conserved motifs were identified among T1R subfamilies in this study. The logo presentation and distribution of these five motifs were displayed in Fig. 4, where we used catfish T1R2a as an example. Two motifs (motif 1 and motif 2) were spanned on the extracellular N-terminal domain, which are the binding sites for ligands (Nie et al., 2006). As different fish T1Rs were used to detect different ligands, our results indicated that fish T1Rs possess the same binding sites or domains for detecting different ligands. The other conserved motifs were mainly resided on the trans-membrane domains (motif 3, motif 4 and motif 5). Previous studies reported that these domains could be interacted with sweetener to initiate enhancing effects for sweet sensation (Fujiwara et al., 2012). However, little is known about whether similar enhancing effects can be induced for umani sensation. Thus, we speculated that some tastants could induce similar promotion effects for umani sensation, just like sweetener to sweet sensation, but functional studies are still needed for validation in the future.

Similarly, the motif analysis for fish T2Rs was also conducted in this study (Fig. 5a). Unlike the T1Rs, the T2Rs have a much shorter extracellular N-terminal domain (Chaudhari and Roper, 2010). Thus, all the conserved motifs identified for T2Rs were resided close to or a part of the trans-membrane domains, intracellular and extracellular loops. Among fish T2Rs, five best motifs were found in subfamily 202 (Fig. 5b), while only two motifs (motif 1 and 3) were found in subfamilies 200 and 201 (Fig. 5c). As the results described above, these subfamilies were quite diverged from one another, as they were clustered into

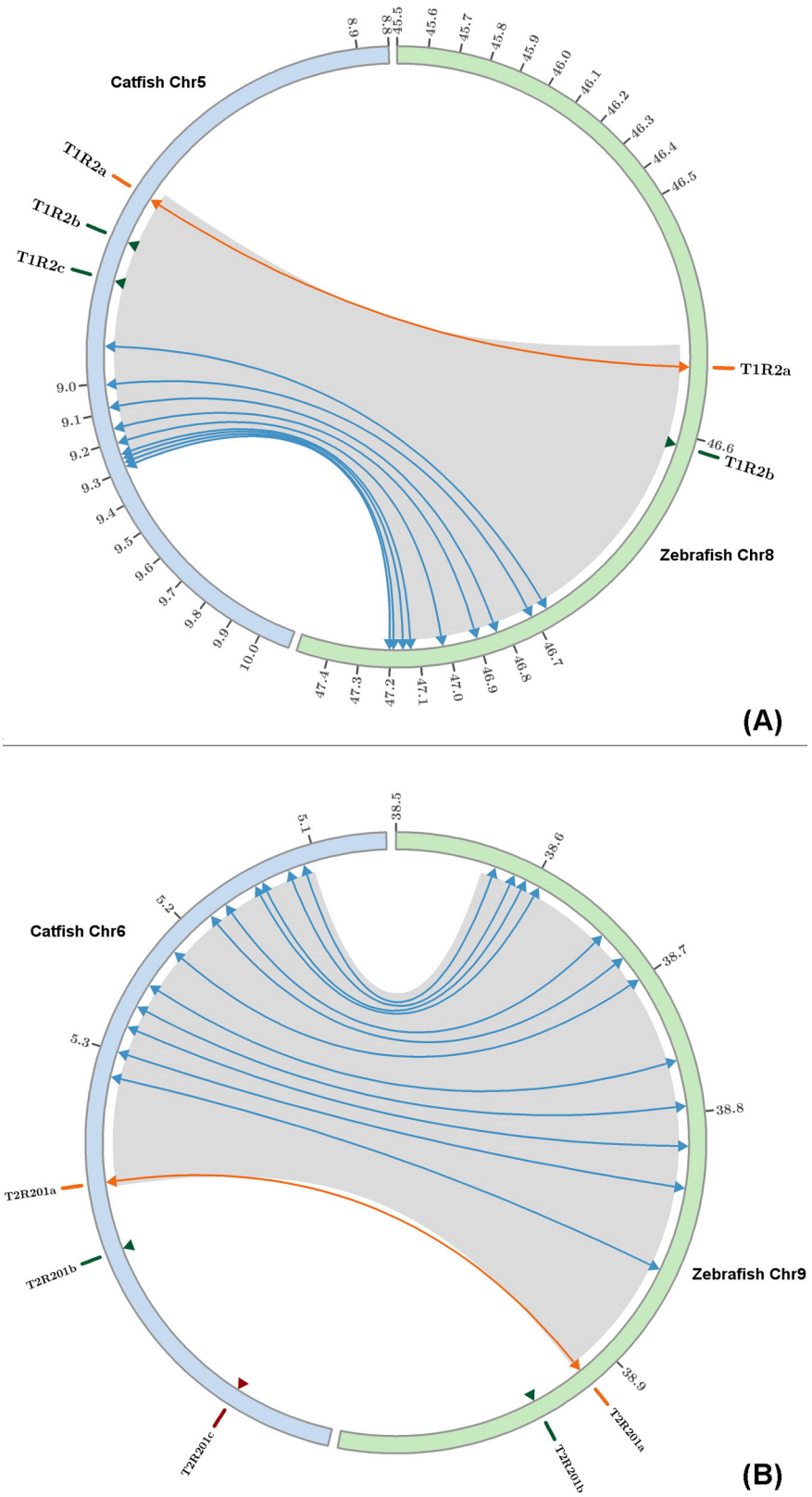

Fig. 3. Identification of two pairs of homologous chromosomal regions between channel catfish and zebrafish. The first pair of regions is between zebrafish chromosome 8 and catfish chromosome 5, and the second region is between zebrafish chromosome 9 and catfish chromosome 6. Anchor genes are linked with arrowed lines. TRs are linked with orange arrowed lines while other genes are linked with blue arrowed lines. Genomic positions for both fishes are indicated with numbers (in Mb) along its own chromosome. Tandem duplicated genes are indicated with green triangles. Catfish T2R201c is listed in dark red because it is not originated from tandem duplication. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

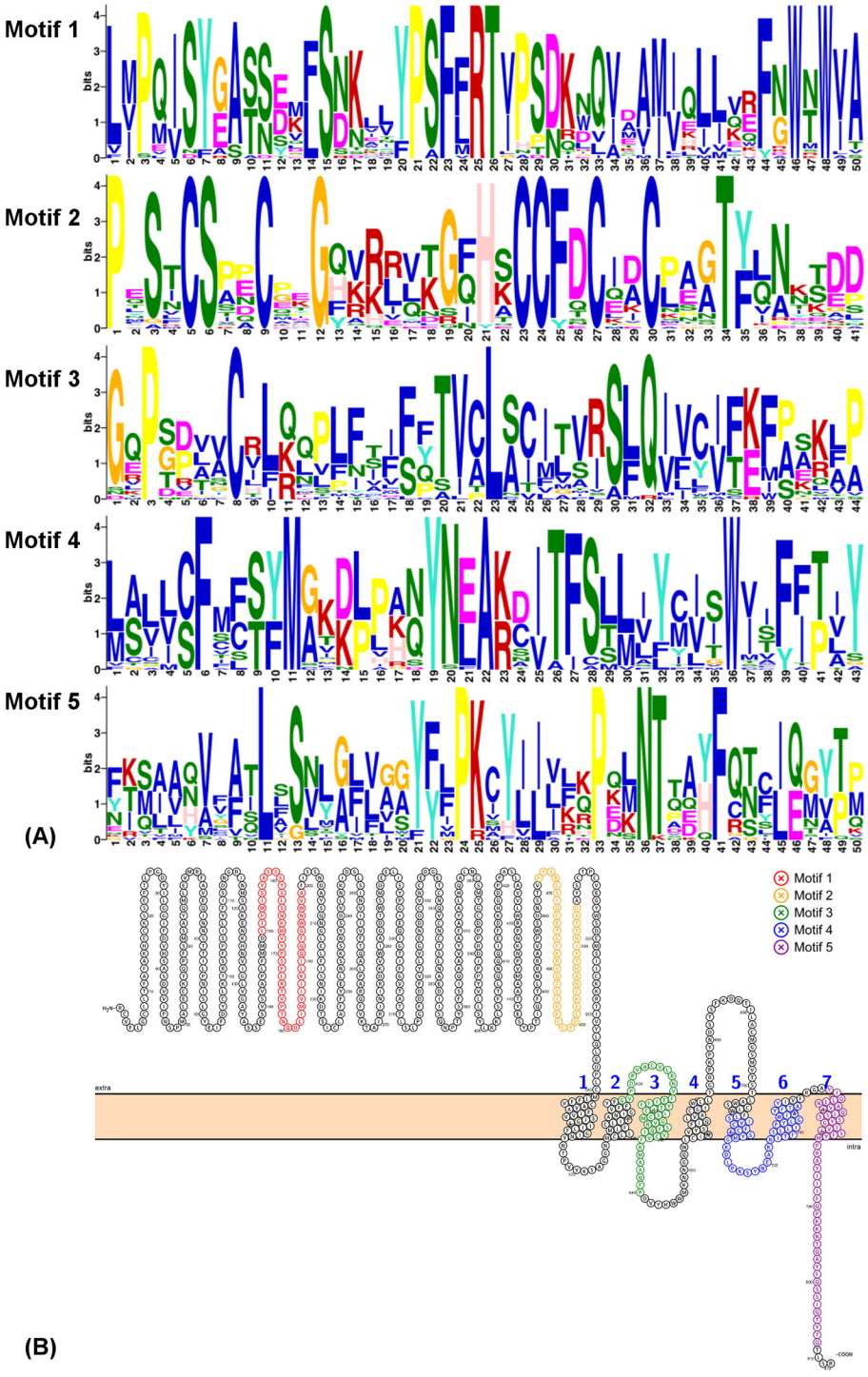

Fig. 4. Logo representation and distribution of the five best conserved motifs identified for teleost T1Rs. (A) Sequence logos of the conserved motifs, as the degree of conservation is indicated by the height of amino acid code. (B) The distribution of these motifs as displayed in the two-dimensional topology structure of T1Rs. The blue numbers represent the number of each trans-membrane domain. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

three distinct phylogenetic sub-clades and fell into three different orthogroups (Fig. 2). The phylogenetic and orthogroup analyses indicated the occurrence of divergences, while the motif analysis displayed the locations of divergences. The three motifs that were not found in subfamilies 200 and 201 were mainly resided on trans-membrane domains and extracellular loops, which were the regions that contain potential binding sites for T2Rs (Roper, 2007). Thus, we inferred that the fish T2R subfamilies were diverged from each other to increase their abilities for discriminating structurally distinct bitter tastants.

3.4. Ratios of dN/dS

Natural selection is the major force behind the frequent fluctuation of certain alleles within a group of taxa and can be measured by the relative ratios of synonymous (dS) and non-synonymous (dN) substitutions. Here, the global and site-by-site dN/dS analyses for all subfamilies of fish TRs were conducted to delineate the evolution dynamics. Generally, the average dN/dS ratio of T1Rs was smaller than that of T2Rs. This is mainly due to more negative selection sites were found in T1Rs than in

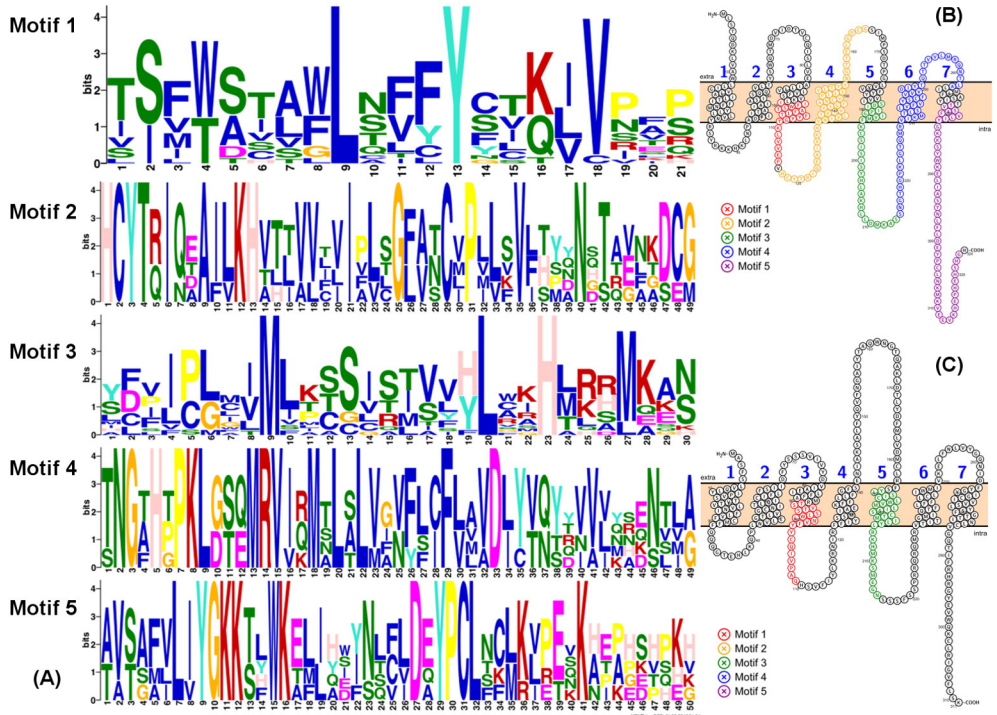

Fig. 5. Logo representation and distribution of the five best conserved motifs identified for teleost T2Rs. (A) Sequence logos of the conserved motifs, as the degree of conservation is indicated by the height of amino acid code. (B) The distribution of these motifs as displayed in the two-dimensional topology structure of T2Rs from subfamily 202. The blue numbers represent the number of each trans-membrane domain. (C) The distribution of these motifs as displayed in the two-dimensional topology structure of T2Rs from subfamilies 200 and 201. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

T2Rs (Table 2). A few positive selection sites were also found in T1Rs, however, the number is too small to lift the global dN/dS ratio of T1Rs. Interestingly, subfamilies 200 and 201 possessed the top two highest dN/dS ratios among all the fish subfamilies. As we mentioned above, these two subfamilies lost three conserved motifs, compared with subfamily 202. Considering the distribution of fish is so broad, each fish species was facing different environmental factors, especially substances taste bitter. Thus selective pressures of different degrees were imposed on various fish T2Rs. This makes fish species more adaptive to their living environments through avoidance of harmful substances. Furthermore, we observed copy number variation within certain TR subfamilies among fish species, and in large part, it was caused by tandem duplication. Combined with the dN/dS analysis, we concluded that selective pressure could be the main force of tandem duplication in various fish genomes, thus leading to the copy number variations.

3.5. Expression profiling of TRs across five different organs in channel catfish

The expression patterns of all TRs in channel catfish RNA-Seq datasets from various organs were characterized (Table 3; Supplementary Table 3). Generally, T1Rs, a group of receptors mainly expressed for sensation of umani and sweet, was expressed at higher levels than T2Rs, a group of receptors mainly expressed for sensation of bitter, in all organs analyzed. T2Rs were barely detected in the RNA-Seq datasets, suggesting extremely low expression. The expression levels of T1Rs were the highest in the barbel, followed by that in the gill, and then by that in the skin. Very low or no expression was detected in the intestine and liver. This pattern is in line with the distribution/density of taste buds in catfish, with barbels harboring the most taste buds, followed by gill and skin (Iwai, 1963; Northcutt, 2005; Raji and Norozi, 2010). Taken together, our study shows a unique biology character that the channel catfish mainly use TRs for the sensation of umani and sweet rather than for bitter, and barbel is the primary organ for taste sensation.

3.6. Identification and expression profiling of gustatory associated G protein genes in channel catfish

Previous studies have reported that TR associated proteins, like G proteins (including subunits α, β and γ), play pivotal roles in gustation

Table 2
A summary description of selection pressure for each subfamily of fish TR (dN/dS).

Gene	Global dN/dS	No. of positive sites	No. of negative sites
T1R1	0.253856	1	224
T1R2	0.315728	0	135
T1R3	0.314331	1	285
T2R200	0.405283	0	9
T2R201	0.480796	0	15
T2R202	0.258602	0	33

Table 3
Expression profiling of TRs across five different organs in catfish (Normalized FPKM $* 10^3$).

Gene	Barbel	Gill	Skin	Liver	Intestine
T1R1	2.77	1.71	0.84	0.24	0.08
T1R2a	1.74	0.28	0.08	0.00	0.02
T1R2b	2.34	0.05	0.02	0.00	0.00
T1R2c	0.11	0.05	0.02	0.00	0.00
T1R3	2.40	0.55	0.17	1.46	0.05
T2R201a	0.20	0.00	0.00	0.00	0.00
T2R201b	0.00	0.00	0.04	0.00	0.00
T2R201c	0.00	0.00	0.00	0.00	0.00

Table 4
Expression profiling of gustatory associated G protein genes across five different organs in catfish (Normalized FPKM $* 10^3$).

Gene name	Barbel	Skin	Gill	Liver	Intestine
gna14a	42.41	9.34	4.99	0.00	3.41
gnaia	3.64	17.55	25.66	0.57	1.36
gnb1	19.95	32.74	25.40	6.10	9.14
gng13	10.52	0.80	0.91	0.00	0.17

Table 5
Expression comparison of TR genes between catfish and zebrafish organs (TPM). The shadowed cells indicated at least five times higher expression in catfish than in zebrafish. Bold value indicated higher expression in zebrafish than in catfish.

Gene/Tissue	Skin		Gill		Liver		Intestine	
	Catfish	Zebrafish	Catfish	Zebrafish	Catfish	Zebrafish	Catfish	Zebrafish
T1R1	1.52	0.25	2.34	0.06	0.14	0.05	0.18	0.02
T1R2	0.22	0.00	0.50	0.01	0.00	0.00	0.04	0.00
T1R3	0.31	0.01	0.75	0.03	0.87	0.00	0.11	0.01
T2R201	0.08	0.00	0.00	**0.46**	0.00	0.00	0.00	0.00

based on the observations that they are co-expressed or coupled with TRs to initiate the gustatory signal cascade (Spielman, 1998). However, the canonical alpha subunit (gnat3) of G protein, which coordinates the gustatory process in mammals, is missing in fish species, presumably owing to the lineage-specific gene losses (Lagman et al., 2012). It has been proposed that gna14 and gnaia in fish may share the same gustatory associated roles with gnat3 in mammalian species (Oka and Korsching, 2011). We identified the catfish gustatory associated G proteins, and characterized their expression pattern using the channel catfish RNA-Seq datasets from various organs (Table 4; Supplementary Table 3). These genes, including gna14 and gnaia (alpha subunit), as well as gnb1 (beta subunit) and gng13 (gamma subunit), were identified in channel catfish.

The expression levels of all gustatory associated G protein genes were significantly higher than that of all TRs as revealed by FPKM. Among all the G proteins, only catfish gna14 exhibited the similar expression pattern with that of TRs across organs, following the descending order of barbel, gill and skin. This may suggest that fish gna14, at least for catfish, has functions involved in gustatory activities similar to the gnat3 in mammals.

3.7. Expression comparison between the channel catfish and zebrafish tissues for the TRs

Compared with other armored fish species, the scaleless characteristic makes catfish has more external body surfaces (mostly the skin) that are exposed to the environment. This may increase the ability of catfish for gustatory sensing. To explore this hypothesis, we compared the expression profiles of TRs between the channel catfish and zebrafish organs, including skin, gill, liver and intestine (Table 5). Our results indicated that all T1Rs were expressed at higher levels in the skin and gill of catfish than in that of zebrafish. This could mean that the catfish skin and gill are more involved in the gustation than that of zebrafish. However, T2R201 was expressed at similar levels between catfish and zebrafish tissues except that it was expressed higher in the zebrafish gill than in that of catfish.

4. Conclusions

Here, we identified and characterized the complete repertoire of channel catfish TRs and gustatory associated G protein genes. We conducted phylogenetic and orthogroup analyses to provide insights into the evolution dynamics of TRs across a broad range of chordate taxa. Our results indicated that vertebrate TRs were clustered into different clades. Second, two conserved homologous regions containing TRs as anchor genes were identified between catfish and zebrafish, providing hints for the relative emerging time of members of catfish subfamilies T1R2 and T2R201. Moreover, the motif analysis showed that three conserved motifs were missing in fish T2R subfamilies 200 and 201, which could reflect the divergence of fish T2Rs. The mechanism underlying this phenomenon was further uncovered by dN/dS analysis, which indicated that fish T2R subfamilies 200 and 201 were under greater selection pressure than any other TR subfamilies. Finally, expression pattern of catfish TRs across different organs mirrors the distribution of taste buds in catfish, with the highest expression in barbels, followed by gill and skin.

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