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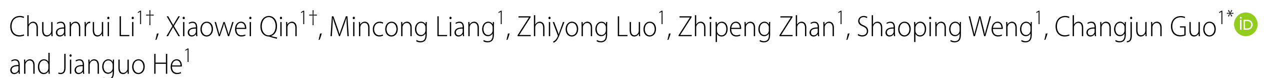
Genome-wide identification, characterization, and expression analysis of the transient receptor potential gene family in mandarin fish *Siniperca chuatsi*



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Abstract

Background Temperature is a crucial environmental determinant for the vitality and development of teleost fish, yet the underlying mechanisms by which they sense temperature fluctuations remain largely unexplored. Transient receptor potential (TRP) proteins, renowned for their involvement in temperature sensing, have not been characterized in teleost fish, especially regarding their temperature-sensing capabilities.

Results In this study, a genome-wide analysis was conducted, identifying a total of 28 TRP genes in the mandarin fish *Siniperca chuatsi*. These genes were categorized into the families of TRPA, TRPC, TRPP, TRPM, TRPML, and TRPV. Despite notable variations in conserved motifs across different subfamilies, TRP family members shared common structural features, including ankyrin repeats and the TRP domain. Tissue expression analysis showed that each of these TRP genes exhibited a unique expression pattern. Furthermore, examination of the tissue expression patterns of ten selected TRP genes following exposure to both high and low temperature stress indicated the expression of TRP genes were responsive to temperatures changes. Moreover, the expression profiles of TRP genes in response to mandarin fish virus infections showed significant upregulation for most genes after Siniperca chuatsi rhabdovirus, mandarin fish iridovirus and infectious spleen and kidney necrosis virus infection.

Conclusions This study characterized the TRP family genes in mandarin fish genome-wide, and explored their expression patterns in response to temperature stress and virus infections. Our work will enhance the overall understanding of fish TRP channels and their possible functions.

Keywords Transient receptor potential protein, *Siniperca chuatsi*, Gene family, Temperature

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Introduction

Temperature is a crucial environmental variable that profoundly shapes the physiological characteristics of both aquatic and terrestrial animals [1]. As poikilotherms, teleost fish heavily depend on ambient water temperature for their developmental processes, respiratory metabolism, and immune function [2, 3]. In the current era of global warming and increasingly frequent climate extremes, teleost fish are consistently subjected to temperature fluctuations during their breeding and cultivation cycles. Gaining insights into the mechanisms underlying how fish sense and respond to temperature variations could provide invaluable theoretical guidance for the scientific management of aquaculture. However, these mechanisms remain largely unexplored.

Transient receptor potential (TRP) proteins are identified as important temperature sensors in mammals and insects [4]. These proteins share a distinctive architectural blueprint, consisting of six transmembrane helical segments and diverse intracellular amino and carboxy terminal cytosolic domains. Central to their functionality, the pore region located between TM5 and TM6 grants most TRP proteins their non-selective cation properties and high Ca^{2+} permeability [5]. There are nine TRP subfamilies in animals. namely TRPA, TRPC, TRPN, TRPM, TRPS, TRPV, TRPVL, TRPP, and TRPML, among which six are found in mammals. TRP subfamilies in mammals can be broadly categorized into two major groups. Group 1 includes TRPA, TRPC, TRPM and TRPV subfamilies, while Group 2 comprises TRPP and TRPML subfamilies [5]. Among them, the TRPA, TRPV and TRPC subfamilies are characterized by their varying numbers of ankyrin repeats at the amino terminus, which are instrumental in ligand and protein interactions [6]. TRPC and TRPM proteins harbor a WKxxR motif, commonly referred to as the TRP domain or TRP box, in their carboxy terminals of the TM domain, which is instrumental for protein activation [7]. Moreover, other structural domains, including the coiled-coil domain, nudix hydrolase domain, and EF hand, also contribute significantly to modulating the function of TRP proteins [8]. The types of structural domains vary among different TRP proteins, reflecting their unique physiological roles and responses to environmental stress.

Mandarin fish, *Siniperca chuatsi*, is a highly commercially valuable teleost species that has undergone extensive artificial propagation and cultivation. Previous researches have indicated that numerous environmental factors, such as temperature, dissolved oxygen, and salinity, can influence the metabolism and immune response of mandarin fish [9–12]. Recently, the mandarin fish aquaculture industry has incurred significant losses due to viral diseases, notably infectious spleen and kidney necrosis virus (ISKNV), mandarin fish iridovirus (MRV), and Siniperca chuatsi rhabdovirus (SCRV), under high-density farming practices coupled with intense fluctuations in environmental temperature [13]. In this context, the identification of the TRP proteins and their roles in mandarin fish holds significant importance for enhancing aquaculture health, as well as for viral disease prevention and control.

In the present study, the identification, characterization, and expression of TRP family members in mandarin fish genome-wide were investigated, with the aim of understanding the roles of TRP protein families in teleost fish.

Materials and methods

Animals, cells and viruses

Mandarin fish (*S. chuatsi*) of approximately 50 ± 5 g were sourced from a farm in Foshan, Guangdong province, China. Prior to their use in the study, they were acclimatized for a two-week period at a temperature of 27 °C, as previously described [14]. Following this, six fish per group were randomly selected for pathogen detection, while five fish per group were selected for tissue expression analysis. Prior to handling, the fish were sedated using a solution of MS-222 (Sigma-Aldrich, USA) at a concentration of 40 to 50 mg/L in water. Mandarin fish fry (MFF-1) cells were cultured in Dulbecco's Modified Eagle's Medium (Gibco, USA), supplemented with 10% fetal bovine serum (HyClone, USA), and incubated at 27 °C in a humidified incubator with a 5% CO_2 atmosphere [15]. ISKNV strain NH-2005 (GenBank: OP896201.1), MRV strain NH-1609 (GenBank: MG941005.3), and SCRV strain NH-2103 (GenBank: PQ066876.1) were separated and stored in our laboratory [16]. For virus infection, cells were infected with the respective viruses at a MOI of 1. After 4 h of adsorption, the inoculum was removed, and the cells were washed twice with PBS. Fresh Dulbecco's Modified Eagle's Medium (10% fetal bovine serum) was then added. All the cells were harvested at different time points post infection for total RNA extraction [17] .

Gene identification and phylogenetic tree construction

To identify all TRP genes in mandarin fish, the genome and transcriptome database (GenBank: GCA_020085105.1, RefSeq: GCF_020085105.1) were utilized. The hidden Markov model profile of TRPs (Pfam: PF00917, PF02176) was downloaded from the Pfam database (http://pfam.xfam.org/) and used to search for TRP genes in the mandarin fish genome database. To construct the phylogenetic tree, TRP sequences were retrieved from human (*Homo sapiens*), mouse (*Mus musculus*), medaka (*Oryzias latipes*), fugu (*Takifugu rubripes*), spotted gar (*Lepisosteus oculatus*)

and stickleback (*Gasterosteus aculeatus*) from the NCBI (https://www.ncbi.nlm.nih.gov/) and Ensemble (http://www.ensembl.org) as query sequences (accession numbers of NCBI Reference Sequence or GenBank listed in Supplementary Material 1). Subsequently, TBLASTN was performed to the transcriptome database of *S. chuatsi* with an e-value of e^{-10} to collect target genes (accession numbers of NCBI Reference Sequence listed in Supplementary Material 2). Phylogenetic trees were constructed via the Molecular Evolutionary Genetics Analysis (MEGA) v10.0 software, utilizing 1000 bootstrap replicates, as previously described [18].

Gene characteristics, structure and conserved motif analyses

Physical parameters of putative proteins, including the lengths of amino acid sequences, mRNA sequences and molecular weights, were calculated using the ExPASy tool (http://www.expasy.org). To gain insights into the characteristics of TRP sequences in the *S. chuatsi* genome, a multiple sequence alignment of 28 TRP proteins was performed using Jalview software v2.10.5, followed by realignment with ClustalW2 v2.1 [19]. The exon-intron structures were visualized using the gene structure display server program (https://gsds.gao-lab.org/) by aligning of the CDSs of individual TRPs. The conserved motif domains within the *S. chuatsi* TRP proteins were identified by utilizing the MEME suite tool (https://meme.nbcr.net/). lastly, TBtools was employed to visualize patterns of gene structure, protein domain, and conserved motifs [20].

Table 1 Primers used for qRT – PCR

Genes	Primers	Sequences (5′–3′)	Primer efficiency
sctrpm3	Forward Reverse	CATCGCTTACATCTTTACCAACG TCCAGGACGGGTCTTCATTAG	0.96
sctrpm4a	Forward Reverse	CATACGACCCTCGCCTTGA ATGGTTTCCCACGTCATTAGTC	0.98
sctrpm6	Forward Reverse	AATTAACAGGATCAGAGCCA CAG GCCATCACTCGCCGCATA	0.98
sctrpm7	Forward Reverse	ACTCGGATGTGAAGGTGGGT CCTGCTTGATGCGTGGGT	0.99
sctrpv1	Forward Reverse	CGGCAGTGACATTCCTAACC CGGCTGTCCGTCCTTCTTA	0.96
sctrpml1a	Forward Reverse	GGCCATTCGCAGGAAGTTGA CGGCGTAGAAGATGTGGTCGT	0.97
sctrpml1b	Forward Reverse	AACAATGAAATCCCTGACTGC TACAC GCAGACAGACGAACGCCACC	0.98
scpkd1a	Forward Reverse	AGCCATACATCACAGCCTAC CTTC GACCCTTGTTGTCATGCCAAAT	0.96
scpkd2	Forward Reverse	TGGGACTACTGCCAATGTGAC CCTGAGGTTTTGCACTTCGC	0.97
sctrpc1	Forward Reverse	AGCCCTCCATTGCTAAACTGA CGTAGTTGTTCCTGTGGGCT	0.97
scβ-actin	Forward Reverse	CCCTCTGAACCCCAAAGCCA CAGCCTGGATGGCAACGTACA	0.98

Chromosomal localization of TRP genes

The chromosome locations of 28 TRP genes from the *S. chuatsi* transcriptome were derived. By aligning TRPs paralogs using the BLAST program, duplicate gene pairs were identified. Using the MCScanX toolkit [21], gene duplications and collinearity relationships among the TRP genes were investigated. Hypothetical gene duplication events were postulated when both the coverage and similarity of the aligned genes exceeded the 75% threshold [21]. Tandem duplications were recognized as genes duplicated on a solitary chromosome with less than one intervening gene; conversely, they were classified as segmental repeats in this investigation. Leveraging the TBtools software, a physical map of the TRP genes was generated, depicting their distribution across different mandarin fish chromosomes.

Temperature stress in mandarin fish and MFF-1 cells

The fish were randomly allocated to three treatment groups, each consisting of five biological replicates, and exposed to distinct temperature conditions (17±0.5 °C, 27±0.5 °C, 37±0.5 °C) [22]. On the seventh day post-stimulation, the tissues were collected from each group and promptly frozen in liquid nitrogen, then subsequently at −80 °C pending further analysis [22]. MFF-1 cells were counted to attain a concentration of 5×10^7 cells/mL and seeded in cell culture 12-well plates, with a final volume of 1 ml per plate. Following overnight culture, the plates were divided into three groups and incubated at different temperatures: 17 °C, 27 °C and 32 °C. Samples were collected at 0, 4, 8, 12, 24 and 48 h, followed by the isolation of total RNA.

RT – qPCR

RNAs isolations were carried out using a total RNA extraction kit (Promega, Beijing, China) following the manufacturer's protocol. Subsequently, the RNA samples were reverse transcribed into cDNA using a PrimeScript reverse transcription (RT) reagent kit (TaKaRa, China) [23]. The RT–qPCR reactions were performed with a SYBR premix ExTaq™ (Takara, Tokyo, Japan) on a LightCycler® 480 instrument (Roche Diagnostics, Switzerland) as previously described [24]. The gene-specific primers used for each gene are listed in Table 1.

Statistical analysis

Statistical analysis was conducted using GraphPad Prism software. The data were normalized and presented as means±standard deviations. All experiments were conducted in triplicate, unless otherwise specified. Group

comparisons were assessed using a two-tailed, unpaired Student's *t* test. *P* values less than 0.05 (denoted by a single asterisk (*) in the figures) were considered significant.

Results

Identification of TRP genes in mandarin fish

Using a homology-based prediction approach via BLASTP, we identified 28 TRP genes in mandarin fish. Upon further analysis of their structural specificity and conservation, we classified these genes into the following six categories: 1 TRPA (*trpa1b*), 8 TRPC (*trpc1*, *trpc2*, *trpc4a*, *trpc4b*, *trpc5a*, *trpc6a*, *trpc6b*, and *trpc7*), 8 TRPM (*trpm1*, *trpm2*, *trpm3*, *trpm4a*, *trpm4b*, *trpm5*, *trpm6*, and *trpm7*), 5 TRPML (*trpml1a*, *trpml1b*, *trpml2*, *trpml3a*, and *trpml3b*), 3 TRPP (*pkd1a*, *pkd2*, and *pkd2l1*), and 3 TRPV (*trpv1*, *trpv4*, and *trpv6*) (Supplementary Material 2). The number of identified TRP genes was comparable to that observed in other teleost fish species, such as *Danio rerio* (32 genes) and *Oryzias latipes* (36 genes).

Based on the TRP proteins from five vertebrate species (*Homo sapiens*, *Mus musculus*, *Gallus gallus*, *D. rerio*, and *O. latipes*), we constructed the phylogenetic tree of the TRP family (Fig. 1). Our analysis revealed six monophyletic subfamilies: TRPA, TRPC, TRPM, TRPML, TRPP and TRPV. Among these, the TRPM subfamily had the highest gene count, whereas the TRPA subfamily had the lowest. Within each subfamily branch, the TRP proteins of teleost fish formed monophyletic clusters, indicating sister-group relationships with the corresponding TRP proteins in other vertebrates. Specifically, within mandarin fish TRPA subfamily, TRPA1b formed a sister-group relationship with the TRPA1 of *O. latipes*. The TRPC subfamily included TRPC1, TRPC2, TRPC4a, TRPC4b, TRPC5a, TRPC6a, TRPC6b, and TRPC7. TRPC4a formed a clade with the TRPC of *D. rerio*, while the others formed sister-groups with *O. latipes*. The TRPM subfamily consisted of TRPM1 to TRPM7. Among them, TRPM1, TRPM4a, TRPM4b formed sister-groups with *D. rerio*, while the rest were sister to *O. latipes*. All members of the mandarin fish TRPML, TRPV, and TRPP subfamilies, excluding Pkd2, formed a sister-group relationship with those of *O. latipes*. With the exception for TRPM, most of the mandarin fish TRP subfamilies were more closely related to *O. latipes*, suggesting a relatively conserved gene structure within the TRP family between *S. chuatsi* and *O. latipes*.

Gene characteristics, structure and conserved motif analyses

To enhance our understanding of the TRP family in mandarin fish, a series of bioinformatics analyses were conducted. Through full-length alignments, we categorized the TRP family in mandarin fish into six subfamilies as

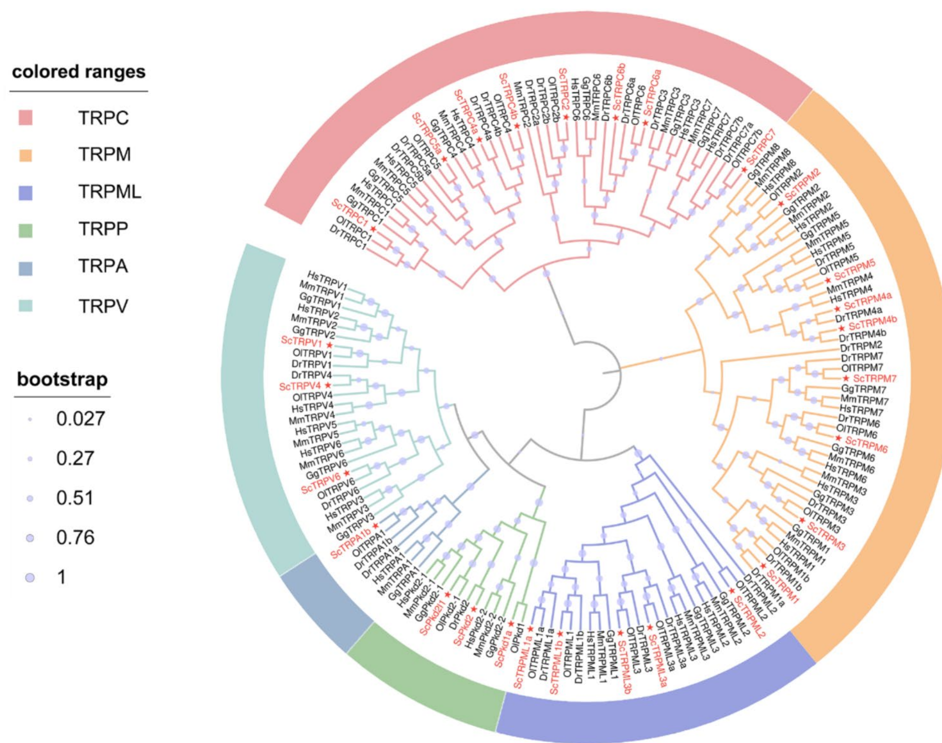

Fig. 1 Phylogenetic analysis of TRP proteins in *S. chuatsi* and other vertebrates. The tree was constructed using Mega v10.0 with 1000 bootstrap replicates based on the Maximum likelihood method. The different sizes of solid circles on branches denotes different levels of bootstrap support. Species abbreviations are Hs, *H. sapiens*, Mm, *M. musculus*, Gg, *G. gallus*, Dr, *D. rerio*, Ol, *O. latipes*, Sc, *S. chuatsi*

previously mentioned (Fig. 2A), thereby illustrating the evolutionary divergence and functional specialization within the broader TRP superfamily. To understand the potential function of these TRP subfamilies, we conducted a detailed motif analysis (Fig. 2B). Our results showed that closely related members shared common motifs, especially within the TRPC, TRPM, and TRPML subfamilies, although conserved motifs across different subfamilies exhibited notable variations. To gain a comprehensive understanding of the domain architecture of TRP proteins, we conducted a thorough analysis of their structures. Our analysis revealed the presence of critical domains, including ankyrin repeats and the TRP domains (Fig. 2C), which are essential for the functioning of TRP proteins [6, 7]. Furthermore, we observed variations in additional domains across the subfamilies, indicating their specialized functional roles. For instance, the LSDAT_euk domain, presumed to act as a ligand sensor and participate in a wide range of nucleotide-related activities [25], is conserved among all members of the TRPM subfamily. Each member of the TRPML subfamily uniquely possesses an ELD domain, which forms a tight tetramer crucial for full-length TRPML assembly and localization [26]. To delve into the transcriptional regulation and splicing diversity of TRP genes, we further investigated their exon-intron organization. Our results showed disparities in the exon-intron patterns among the subfamilies, particularly evident in *trpc* genes, which exhibit a more intricate structure compared to *trpml* genes (Fig. 2D). These observations contribute to an enhanced understanding of the genetic basis of TRP family genes.

Chromosome distribution of TRP Genes

By examining the physical positioning of genes within the genome of the mandarin fish (GCF_020085105.1), we were able to map the chromosomal locations of the TRP genes (Fig. 3). The results showed that the 28 TRP genes of mandarin fish were unevenly distributed across its fifteen chromosomes, with a significant portion of the TRPs localized in regions of high gene density. It is worth noting that Chr14 contained the highest number of TRP genes, with harboring four TRP genes. Moreover, Chr01, Chr03 and Chr05 were found to have three TRP gene members, while the rest chromosomes consisted of only 1–2 TRP genes. To gain deeper insights into the amplification and evolution of the TRP genes, we conducted an analysis of *S. chuatsi* gene duplication events using MCScanX. This analysis only revealed 2 duplication pairs, indicating that the presence of TRP genes in *S. chuatsi* is highly conserved in terms of gene duplication and tandem repetition.

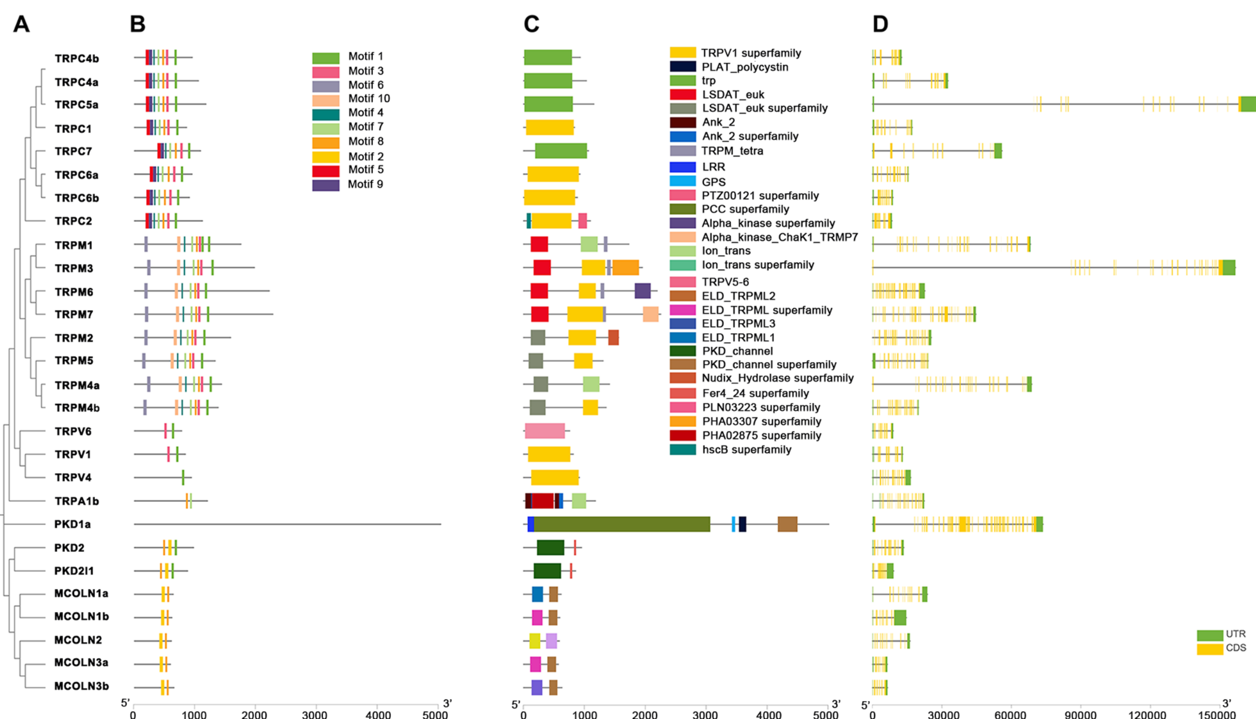

Fig. 2 Evolutionary relationships, motif patterns, conserved domains and gene structure of transient receptor potential family in mandarin fish. (**A**) Phylogenetic tree of 28 TRP proteins. (**B**) Motif pattern of TRP proteins. Ten presumptive motifs are indicated with boxes marked in different colors. (**C**) Distributions of conserved domains in TRP proteins. (**D**) The exon-intron architecture of TRP genes

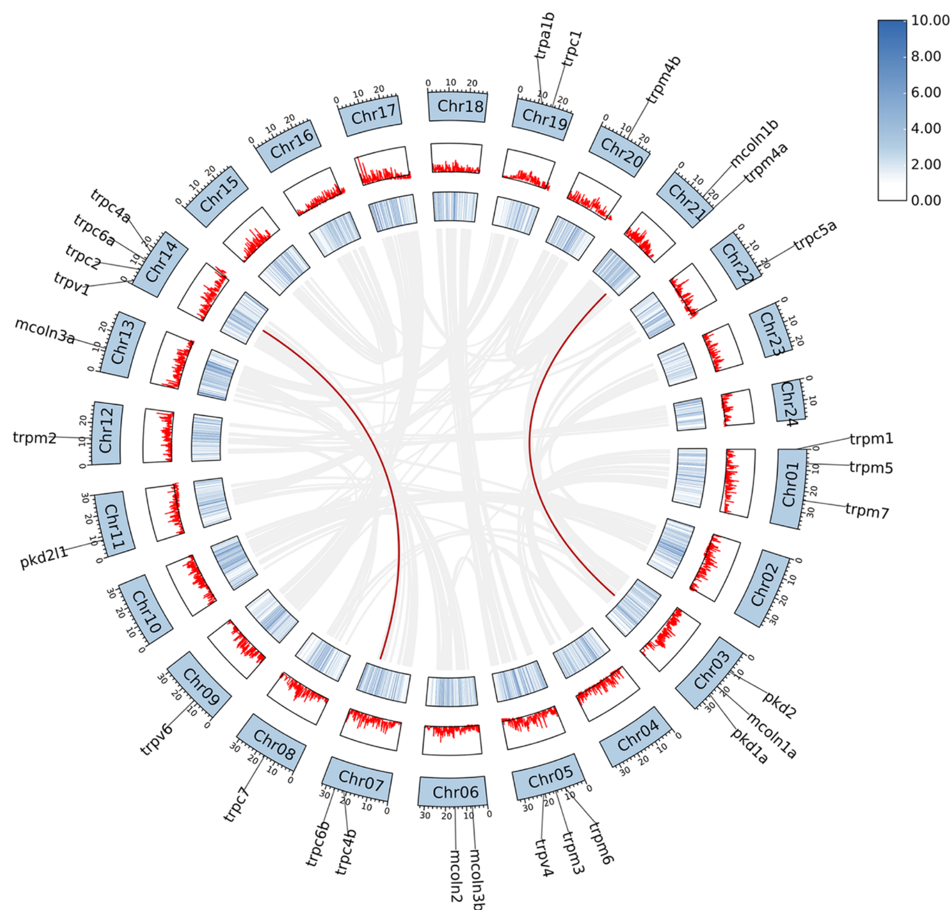

Fig. 3 Distribution and covariance of TRP genes on *S. chuatsi* genomic chromosomes. The red line indicates the collinearity between the TRP genes and that they are replication genes. The color gradient of chromosomes from white to blue represent gene density from low to high, the red line charts also represent the gene density of each respective chromosome

Relative expression of TRP family members in different tissue

To further investigate the potential functions of TRPs, we conducted an analysis of their expression profiles across 14 tissues, using RT−qPCR data obtained from mandarin fish. Our results revealed distinct expression patterns of TRPs (Fig. 4A). The majority of TRPs exhibited a constitutive expression pattern, with the exception of muscle; however, *trpml1b* was noted to be expressed at low levels ubiquitously, similar to its homology in zebrafish [27]. Furthermore, we found that *trpm3* and *pkd2* were highly expressed in a majority of the tissues examined. It is worth mentioning that tissue-specific expression patterns were also identified for certain TRPs, with *trpc1* being highly expressed exclusively in the brain, whereas *trpml1a* showed elevated expression in blood, spleen, and liver.

Further research has indicated that the transcription levels of these genes can vary in response to temperature alterations (Fig. 4B–K). For example, *trpml1a* is transcribed at significantly elevated levels at 17 °C compared to 27 °C in most tissues, achieving statistically significant at the $P<0.05$ level (Fig. 4G). Conversely, a majority of the genes, including *trpm6*, *trpml2*, *pkd1a*, *pkd2*, and *trpv1* (Fig. 4E and I−K), were significantly higher transcribed at 32 °C compared to 27 °C. Moreover, the expression patterns of certain genes, such as *trpm3*, *trpm4a* and *trpm7* (Fig. 4C−D, and F), are influenced by temperature variations, with their transcription levels being upregulated in response to both increasing and decreasing temperatures, suggestive of a complex regulatory mechanism. These transcription levels may regulate the function of these genes and their roles in temperature-mediated processes. These observations provide insights into the regulation of gene expression by temperature changes and their potential roles in biological processes.

Expression of TRP family members under temperature stress

To further obtain an understanding of the temporal dynamics of TRP gene expression influenced by temperature stress (17 °C, 27 °C, and 32 °C), in vitro experiments

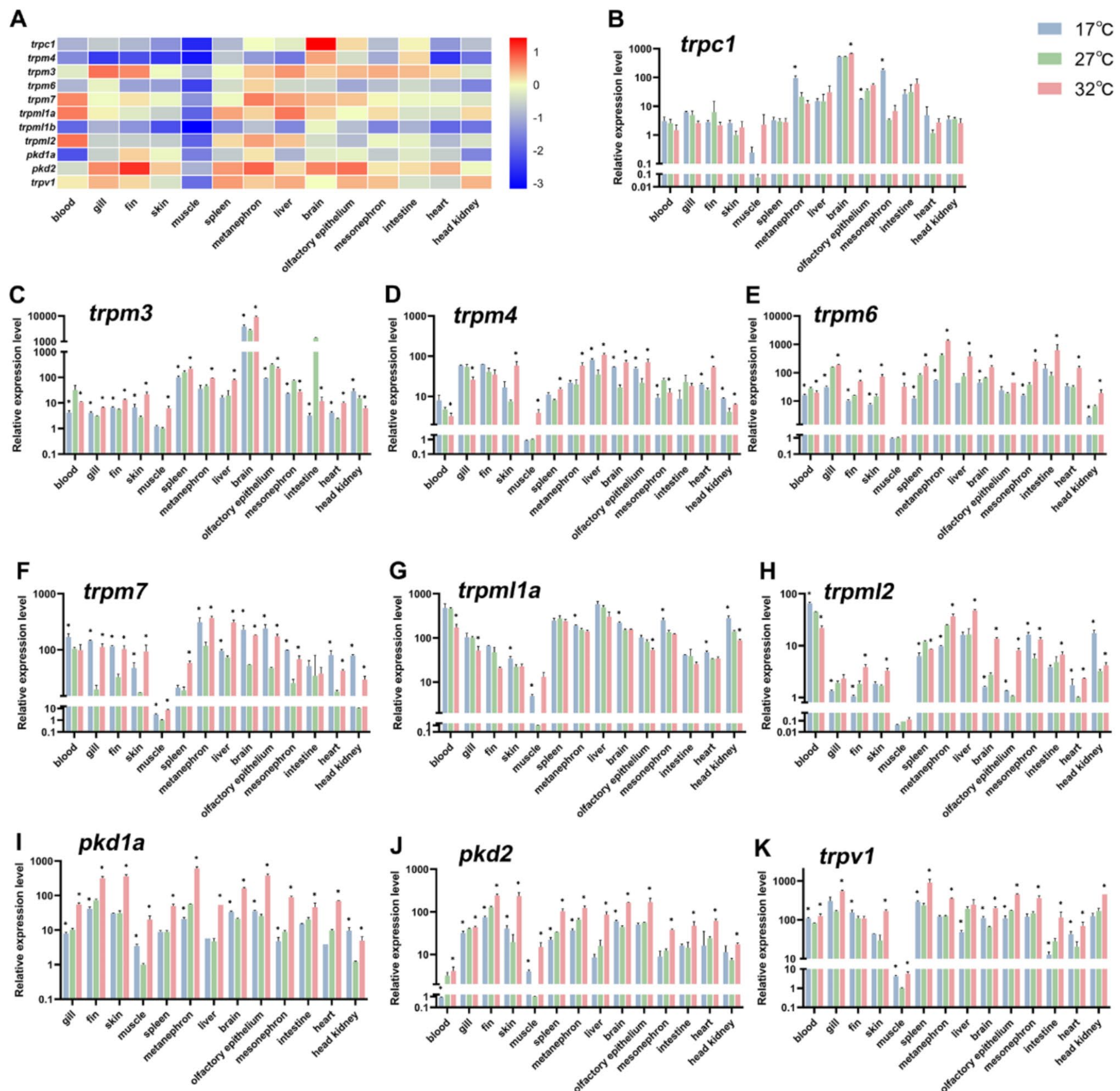

Fig. 4 Relative expression of TRP family members in *S. chuatsi*, including *trpc*, *trpm*, *trpp* and *trpv* genes. (**A**) Tissue distribution heatmap of the TRP genes mentioned above. The color gradient from blue to red signifies the gene relative expression level from low to high. (**B** – **K**) Relative expression levels of TRP family members in different tissues under temperature stress. The data is presented as the average ± standard error of the three independent biological replicates. A single asterisk (*) denotes a *P*-value below 0.05

were conducted using MFF-1 cells. When comparing gene expression levels across different temperature treatments, distinct patterns emerged for each gene. Specifically, the expression of the *trpml2*, *pkd1a*, *pkd2* was significantly downregulated at 17 °C and upregulated at 32 °C in most of the time within 4–24 h after temperature change (Fig. 5G – I), whereas the expression of the *trpm6* was oppositely regulated under the same conditions (Fig. 5C). Variations in expression levels were also observed for other TRP genes, including *trpc1*, *trpm4a*, *trpm7*, *trpml1a*, *trpml1b*, *trpv1* (Fig. 5A – B, D – F and J), under different temperature treatments, indicating their potential involvement in temperature-mediated responses. These results suggest that TRP genes play distinct roles in temperature-dependent regulation. Given the significant roles of the TRP proteins in pain perception, lysosome function, renal function, and other aspects [27–30], the observed alterations in gene expression

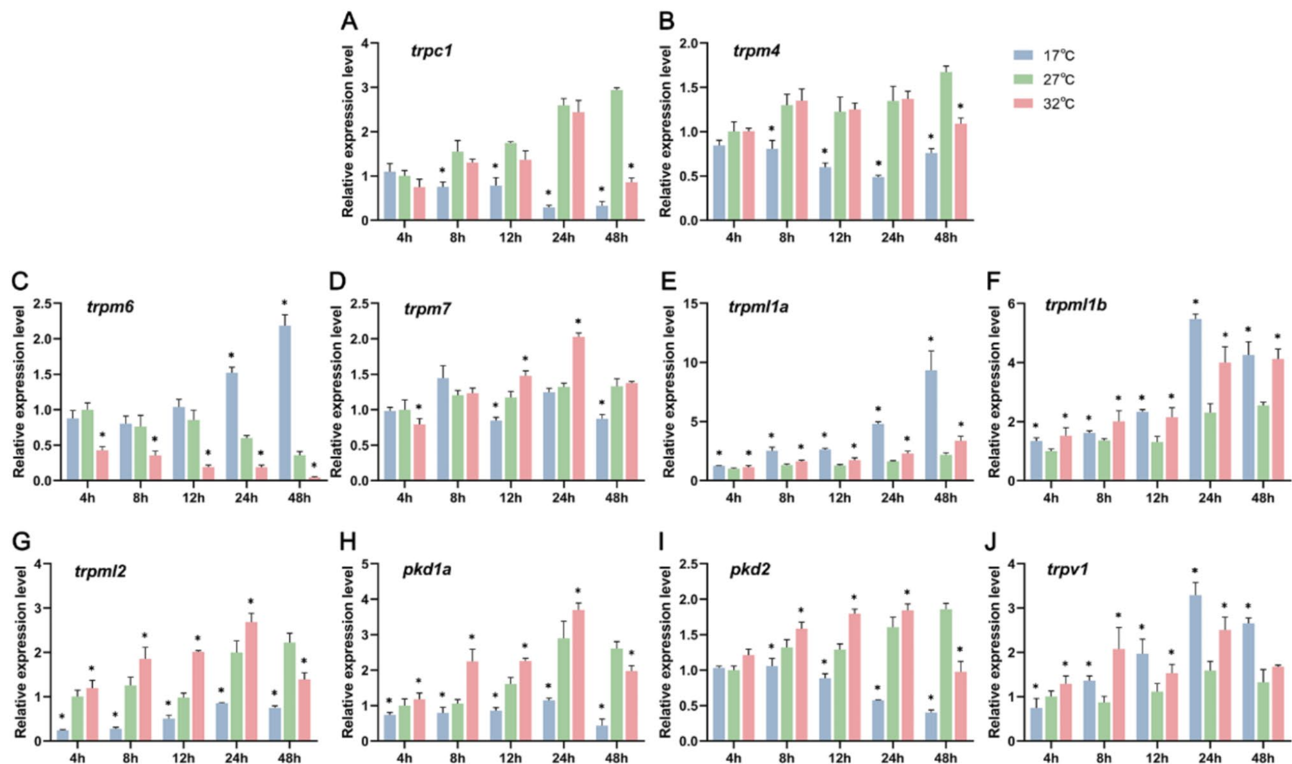

Fig. 5 Relative expression levels of TRP family members in cells under temperature stress. (**A**–**J**) Relative expression levels of TRP family members in MFF-1 cells after temperature stress. The data is presented as the average ± standard error of the three independent biological replicates. A single asterisk (*) denotes a *P*-value below 0.05

levels may have significant implications for elucidating the molecular mechanisms underlying temperature-mediated processes and diseases.

Expression patterns of TRP family members in response to fish virus

The TRP family members, as polymodal ion channels, possess a unique function in integrating diverse environmental stresses [31]. Upon activation, these TRP genes regulate the release of immunomodulatory neuropeptides, such as substance P and calcitonin gene-related peptide, through various pathways, thereby eliciting immunomodulatory effects on multiple levels [32]. Previous research indicates that the expression level of TRP proteins can also be influenced by multiple factors, including pathogenic or inflammatory stimuli, temperature and other environmental stresses [33–36]. Furthermore, variations in expression levels can have comparable effects to their activation or inhibition [37–39]. To investigate the role of TRP family proteins in the replication process of fish viruses, cells were infected with three distinct viruses (ISKNV, MRV, SCRV), and subsequent alterations in TRP gene expression levels were analyzed over time. Our results showed that TRP genes exhibited both upregulation and downregulation in response to these viral infections. MRV infection induced the most profound changes in gene expression (Fig. 6B), with a greater number of genes being significantly upregulated compared to SCRV (Fig. 6A) and ISKNV (Fig. 6C). SCRV infection had a relatively moderate impact on gene expression, with fewer genes being significantly altered compared to MRV (Fig. 6A). ISKNV infection exhibited the lowest impact on gene expression, with five of the eight genes showing significant changes (Fig. 6C). The experiments demonstrated that the expression levels of *trpm7*, *trpml1a*, *pkd1a and trpv1* were all changed in response to the infection of all three fish viruses. This observation indicates that these genes may possibly be involved in the complex interplay between the viruses and mandarin fish, potentially contributing to the host's defense mechanisms against viral infection or enhancing the replication ability of the viruses. These results suggest differential modulation of TRP genes in response to fish virus infections, implying their role in immune response, particularly *trpm7*, *trpml1a*, *pkd1a* and *trpv1*, which were consistently upregulated across all three viral infections.

Discussion

In the current era of global warming and the increasing frequency of extreme climate events, global aquaculture productivity faces mounting challenges posed by rising temperature, infectious diseases, salinity intrusion, and

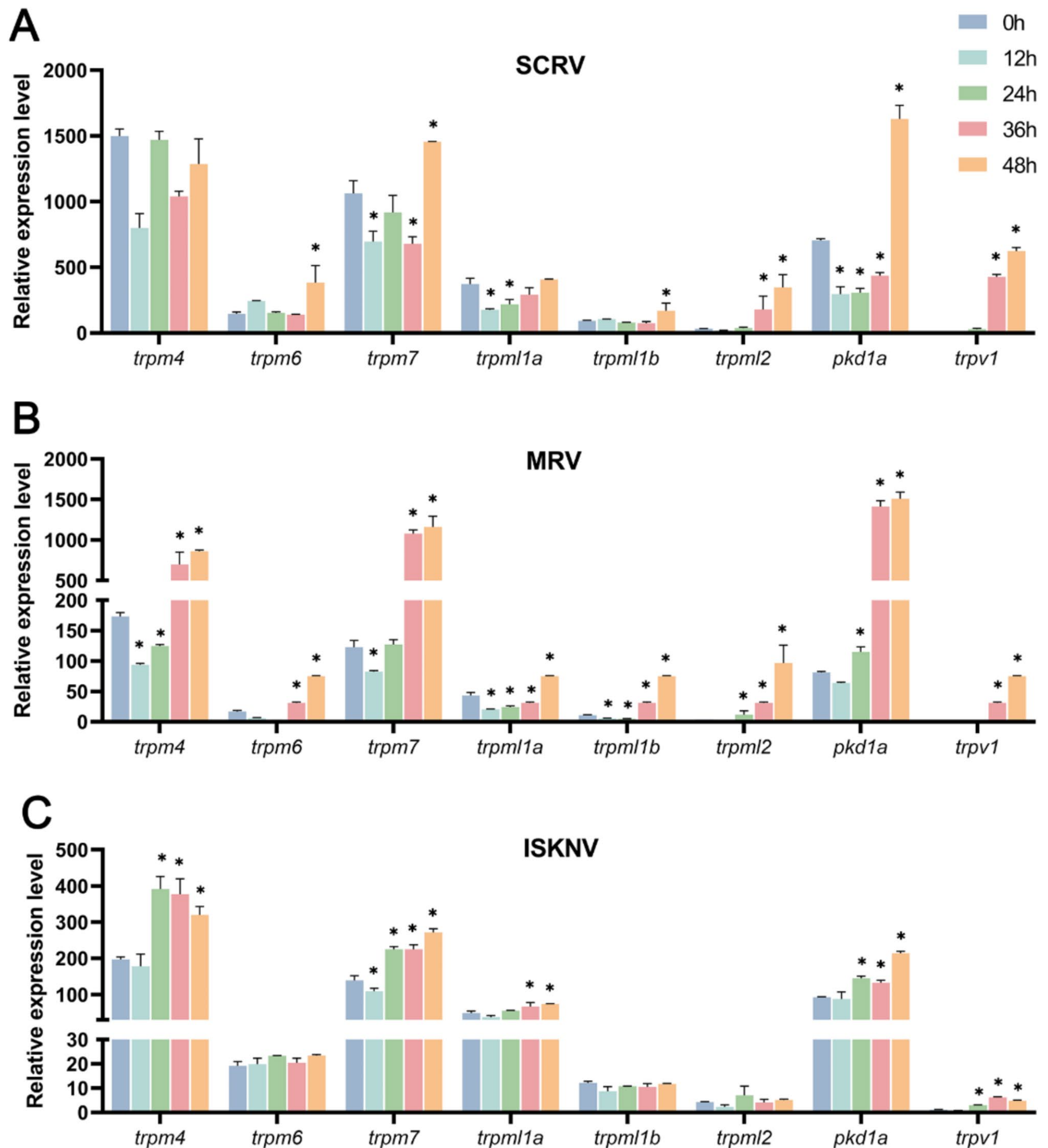

Fig. 6 Relative expression level of TRP genes in vitro at different time points after three specific mandarin fish viruses. (**A**) SCRV, (**B**) MRV, (**C**) ISKNV. Data are presented as the mean ± standard deviation of two independent biological replicates. A single asterisk (*) denotes a *P*-value below 0.05

other factors [40, 41]. The mandarin fish, an important and valuable native species in China's aquaculture industry, has experienced significant losses due to fish disease and thus confronts a grave threat from climate change [12, 42]. Given that the TRP protein can sense changes in environmental conditions and plays a regulatory role in various downstream pathways [31], identifying the TRP genes and its expression patterns in response to temperature variation and pathogen invasion is crucial for enhancing aquaculture health and infectious disease prevention and control.

In this study, we identified and characterized 28 TRP genes in mandarin fish, with nine belonging to the TRPC subfamily, eight to TRPM, five to TRPML, three to TRPP, three to TRPV and one to TRPA. The categories and numbers of TRP family protein members in mandarin fish are remarkably similar to those of its close relatives among bony and cartilaginous fish, particularly in the TRPC, TRPML, TRPM, and TRPV subfamilies. However, compared with mammals like humans and mice, the mandarin fish lacks *trpv2*, *trpv3* and *trpv5* in the TRPV subfamily and holds more paralogs in the TRPC (*trpc4a* and *trpc4b*, *trpc6a* and *trpc6b*) and TRPML (*trpml1a* and *trpml1b*, *trpml3a* and *trpml3b*) subfamilies, akin to other teleost fish [43]. As TRPML is known for its role in regulating lysosomal function and inflammation [44, 45], fish appear to mount a more robust immune response upon pathogen invasion. While *trpv2* and *trpv3* are crucial for temperature perception [46], the lack of certain TRPV genes in fish indicates a potentially slower perception and response to environmental shifts compared to mammals. We hypothesize that this may be the result of adaptive evolution in animals to maintain body temperature.

TRP proteins are unique among protein families due to their remarkable diversity in cation selectivities and specific activation mechanisms. They play critical roles in the responses to all major classes of external stimuli, such as light, sound, chemicals, temperature, and touch, highlighting their essential function in sensory perception [47]. In the mandarin fish, various TRP family members display significantly different patterns of tissue distribution and temperature response modes, reflecting the specific and diverse physiological roles within the organism. Notably, *trpc1* is highly expressed in the brain, yet its response to temperature is not significant. Given its homology in zebrafish is also highly expressed in the brain, retina, and inner ear [48], it seems that fish *trpc1* plays a role in various sensory organs and in the transmission and processing of sensory signals. However, the specific functions of *trpc1* remain unclear in current studies [31, 49].

Besides temperature responsiveness, we also investigated the expression patterns of TRP genes in the mandarin fish under pathogenic stimulation. Our results showed the temporal impact of three different viruses (SCRV, MRV, and ISKNV) on gene expression in the MFF-1 cells. We found that MRV infection elicits broad relative expression changes within the TRP family genes, whereas the changes induced by ISKNV infection within the TRP family are notably less pronounced, despite both MRV and ISKNV belonging to the family *Iridoviridae* and causing a fatality rate higher than 90% in the mandarin fish [50, 51]. SCRV infection also caused a broad response, with the upregulation of TRP family genes occurring approximately 12 h later compared to MRV infection. Contrary to the close genetic relationship between MRV and ISKNV, SCRV is an RNA virus that belongs to the *Rhabdoviridae* family. Given that SCRV also exhibits a high fatality rate and causes a rapid onset of illness [52], it is speculated that the differences in TRPs response patterns are possibly associated with the distinct pathogenic mechanisms between these two viruses. Furthermore, it was detected that the expression levels of *trpm7*, *trpml1a*, *pkd1a* and *trpv1* were all changed under the invasion of all three viruses. This suggested that these genes might be involved in the complex interplay between the viruses and mandarin fish [53, 54].

Conclusion

This study provides insightful perspectives on the functions of TRP proteins in the immune responses and adaptation mechanisms of mandarin fish to environmental stressors. The identification of TRP genes and the elucidation of their expression patterns in response to temperature variations and viral infections reveal the complexity and specificity of their physiological functions. These findings enhance our understanding of fish antiviral responses and viral pathogenesis, potentially laying the groundwork for developing effective strategies to combat fish virus diseases and enhancing aquaculture productivity. Further investigation into the functional attributes of TRP genes and their interplay with other cellular components is poised to yield a deeper comprehension of their involvement in fish immunology and disease resistance mechanisms.

Abbreviations

TRP	Transient receptor potential
ISKNV	Infectious spleen and kidney necrosis virus
MRV	Mandarin fish iridovirus
SCRV	Siniperca chuatsi rhabdovirus
MEGA	Molecular Evolutionary Genetics Analysis
RT – qPCR	Reverse transcription quantitative PCR

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

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Not applicable.

Author contributions

CRL, and XWQ conducted the experiments. SPW, and JGH provided the mandarin fish and virus samples. CRL, ZYL, MCL, and ZPZ performed the data and laboratory analyses. CJG, and CRL wrote the article. CJG conceived the study. CJG, and JGH provided Funding acquisition. All authors contributed to the discussion of the results and provided feedback on the manuscript.

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Data availability
The authors confirm that no new genes or proteins were generated in this study and all analyses were based on existing data in the databases. The data underlying the findings of this study are presented in the article and its supplementary materials. The accession numbers of NCBI Reference Sequence or GenBank for the vertebrate TRP proteins used in this study are provided in Supplementary Material 1, while those for the mandarin fish TRP proteins are listed in Supplementary Material 2.

Declarations

Ethics approval and consent to participate
All animal experiments were permitted by the Ethics Committee of Sun Yat-sen University (Approval No. 2023051701) and performed in accordance with the guide for the Care and Use of Laboratory Animals of Sun Yat-sen University. We have complied with all relevant ethical regulations for animal use.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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