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Identification and characterization of the *septin* gene family in rainbow trout (*Oncorhynchus mykiss*) and their expression profiles in response to *Vibrio anguillarum* and *Aeromonas salmonicida* infections

Mengqun Liu, Qiusheng Wang, Ziyi Zhao, Xin Qi, Haishen Wen *

Key Laboratory of Mariculture (Ocean University of China), Ministry of Education (KLMME), Ocean University of China, Qingdao, 266003, PR China

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ABSTRACT

Septins are cytoskeletal GTP-binding proteins that play critical roles in host defense against bacterial infections. Rainbow trout (*Oncorhynchus mykiss*), a commercially valuable and widely farmed salmonid species, has not been systematically investigated with regard to its *septin* gene family. In this study, we identified 34 *septin* genes in rainbow trout and categorized them into four subgroups based on phylogenetic relationships. Syntenic, structural, and motif analyses revealed their conserved features, with strong domain and sequence homology observed across species. Tissue- and gene-specific expression patterns were evaluated in healthy individuals. Moreover, expression profiling following bacterial challenge with *Vibrio anguillarum* and *Aeromonas salmonicida* revealed significant transcriptional changes in *septin* genes, suggesting their involvement in the regulation of innate immune responses. This study provides the first comprehensive characterization of the *septin* gene family in rainbow trout and highlights their complex and essential roles in host immunity, offering valuable insights into their immunoregulatory functions in teleosts.

1. Introduction

Septins are cytoskeletal GTP-binding proteins that can self-associate, polymerize, and bind to cell membranes [1–3]. Initially discovered in yeast (*Saccharomyces cerevisiae*) mutants with defects in cell cycle completion [4], they were later observed to localize at the septum during cell division, leading to the designation “septin” [5]. Despite the high conservation of septins from yeast to human (*Homo sapiens*) and their presence in nearly all eukaryotic species except higher plants [6], the quantity of *septin* genes exhibits remarkable variability, with only 2 in *Caenorhabditis elegans*, 7 in yeast, 14 in human, and 15 in channel catfish (*Ictalurus punctatus*) and zebrafish (*Danio rerio*) [7–9]. This variation is primarily attributed to species-specific differences in gene duplication, which have facilitated the functional diversification of septins in vertebrates [10]. In vertebrates, *septin* genes are categorized into four subgroups according to sequence homology: SEPT2, SEPT3, SEPT6, and SEPT7 [11].

Members of the *septin* gene family possess a highly conserved GTP-binding domain, also known as the G-domain or GTPase domain. This domain exhibits the utmost level of sequence conservation among septins and contains three prevalent GTP-binding motifs: G1 (GxxGxGKST), G3 (DxxG), and G4 (xKxD) [5,12]. The G1 motif includes a phosphate-binding loop (P-loop) that interacts with the phosphate groups of nucleotides [10,13,14]. The G3 motif, comprising several hydrophobic residues, binds Mg^{2+} and interacts with the β and γ phosphates of GTP. The G4 motif contributes to the selective binding of guanine nucleotides [5,10,15]. The septin unique element (SUE), a stretch of around 50 amino acids exclusive to septins, is located at the C-terminal end of the G-domain, differentiating them from other small GTP-binding proteins [16]. The SUE partially overlaps with the GTP-binding interface and has been implicated in septin polymerization [5,15,17]. Another distinguishing feature of Septins is their capacity to form oligomeric core complexes that eventually assemble into higher-order structures such as filaments, cages, rings, and gauzes, which are essential for their biological functions [18–21].

Septins participate in a wide array of biological processes, including cell division [18,22,23], cell migration [24], neurogenesis [11], cell polarity [25,26], apoptosis [27], exocytosis [28], autophagy [20,29], and phagocytosis [30]. Their involvement in bacterial infection was first proposed in 2002 based on studies of *Listeria monocytogenes* invasion [31]. Since then, septins have been implicated in host responses to various bacterial pathogens, including *Chlamydia trachomatis* [32],

* Corresponding author. Fisheries College, Ocean University of China, 5 Yushan Road, Qingdao, 266003, PR China.
E-mail address: wenhaishen@ouc.edu.cn (H. Wen).

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Clostridium difficile [33], *Salmonella* Typhimurium [34], enteropathogenic *Escherichia coli* [35], *Shigella flexneri* [36], *Staphylococcus aureus* [37], and *Pseudomonas aeruginosa* [38], where they play crucial roles in host cell infection processes [39–41]. However, these investigations have primarily concentrated on mammals, while the roles of *septin* genes in fish remain largely unexplored.

Rainbow trout (*Oncorhynchus mykiss*) is a commercially significant salmonid species in global aquaculture, with an annual production of approximately 1,000,000 tonnes in 2022 [42]. Outbreaks of infectious diseases such as vibriosis and furunculosis present serious challenges and result in significant economic losses in trout farming. *Vibrio anguillarum* and *Aeromonas salmonicida* are the pathogens responsible for these diseases, respectively, both associated with high mortality in infected populations [43,44]. To develop effective strategies for fish disease prevention and control, it is essential to comprehend the immune-related functional genes and their expression patterns during bacterial infections. However, the roles of *septin* genes in mediating immune responses to these infections in trout remain inadequately characterized and require further exploration.

Previous research have indicated that *V. anguillarum* infection triggers various pathological and immune phenotypes across different tissues in rainbow trout. For instance, infections lead to inflammation and metabolic disruption in the liver, while the intestine displays a robust immune response accompanied by accelerated lipolysis [45]. Moreover, *Vibrio*-rich bacterial communities were frequently detected in the spleens of trout with severe lesions, indicating the occurrence of serious bloodstream infections [46]. Based on these findings, we analyzed the expression profiles of *septin* genes in the liver, intestine, and spleen following *V. anguillarum* infection in rainbow trout. Furthermore, the gill serves as the first barrier against pathogen invasion and is a known colonization site for *A. salmonicida* in rainbow trout [47–49]. *A. salmonicida* infection activates toll-like receptor pathways, leading to neuroinflammation and neural dysfunction in the brain, perhaps causing anorexia and lethargy in infected trout. In the kidney, *A. salmonicida* infection triggers the activation of endocrine and immunomodulatory networks [50]. To further explore the involvement of *septin* genes in host defense, we also evaluated their expression profiles in the gill, brain, kidney, and spleen after *A. salmonicida* infection.

In this study, a complete set of 34 *septins* was identified and systematically characterized. To further investigate their immunological roles, the expression patterns of these genes were determined in healthy tissues and following bacterial challenge. This systematic research will enhance comprehension of the involvement of septins in the immune response of rainbow trout and other teleosts, serving as a basis for further functional investigations of trout *septin* genes.

2. Materials and methods

2.1. Ethics statement

All of the experiments in this study were performed in compliance with the regulations of the Animal Research and Ethics Committee of Ocean University of China (Permit Number: 2014201) and the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (NIH Publication No. 8023). This study did not include any endangered or protected species. Furthermore, all sampled rainbow trout were juveniles and not sexually mature; therefore, the influence of gender was excluded from the analysis.

2.2. Identification of rainbow trout septin genes

To identify *septin* genes in rainbow trout, the whole genome sequence database (GCA_013265735.2) of rainbow trout was queried via the TBLASTN program. The query sequences were sourced from NCBI databases (https://www.ncbi.nlm.nih.gov/) and corresponded to known Septin family members in humans and zebrafish. The E-value threshold of 1×10^{-5} was used to retrieve as many potential Septin sequences as possible. Redundant sequences were removed using TBLASTN and ClustalW alignment, resulting in an initial pool of candidate *septin* genes for further analysis. The identified candidate *septin* genes were converted into amino acid sequences utilizing Open Reading Frames (ORF) Finder (https://www.ncbi.nlm.nih.gov/orffinder/). Furthermore, the ProtParam tool (https://web.expasy.org/protparam/) was used to predict the molecular weight (MW) and theoretical isoelectric point (pI) of each putative Septin protein.

2.3. Phylogenetic analysis of septin genes

Phylogenetic analysis was conducted to validate the annotation of the identified *septin* genes in rainbow trout. Amino acid sequences of *septin* genes from representative vertebrate species, including human, chicken (*Gallus gallus*), mouse (*Mus musculus*), African clawed frog (*Xenopus laevis*), goldfish (*Carassius auratus*), zebrafish, medaka (*Oryzias latipes*), channel catfish, yellow catfish (*Tachysurus fulvidraco*), common carp (*Cyprinus carpio*), and Atlantic salmon (*Salmo salar*), were retrieved from the NCBI or Ensembl databases (http://asia.ensembl.org/index.html) for phylogenetic tree construction (Fig. 1). Multiple sequence alignments were performed utilizing ClustalW with default parameters. Phylogenetic relationships were deduced employing the neighbor-joining (NJ) method and the Jones-Taylor-Thornton (JTT) model as implemented in MEGA 7.0. The reliability of the tree was assessed with 1000 bootstrap replicates, and gaps were eliminated via pairwise deletion. The Interactive Tree of Life (ITOL) tool (https://itol.embl.de/login.cgi) was employed to refine the visualization of the phylogenetic tree. Additionally, rainbow trout *septin* genes were named according to existing vertebrate counterparts whenever possible.

2.4. Analysis of conserved motifs, gene structure and sequences in septin genes

Conserved motifs were identified utilizing the MEME tool (https://meme-suite.org/meme/tools/meme). Exon and intron information of each *septin* gene were retrieved from the rainbow trout reference genome, and gene structures were visualized using the Gene Structure Display Server (Gene Structure Display Server 2.0). Furthermore, multiple sequence alignments of the GTP-binding domains of Septins were conducted with ClustalW and graphically illustrated using ESPript tool (ESPript 3.x/ENDscript 2.x). Residues of structural or functional significance, G-motifs prevalent among most small GTP-binding proteins, septin-specific motifs, and characteristic Septin features, were annotated based on References [5,51].

2.5. Syntenic, collinear and chromosomal locations analysis

To explore gene identities and orthologies, syntenic analysis was conducted. Syntenic blocks were constructed to visualize the genomic positions of genes flanking the *septin* genes. Conserved syntenic regions of *septin* genes in zebrafish, human, medaka, fugu, and rainbow trout were identified using the Genomicus databases (v92.01) [52] and NCBI genome annotation databases. Intraspecific collinearity analyses were performed and visualized using TBtools [53,54]. The chromosomal positions of the *septin* genes were determined based on the coordinates provided in the rainbow trout genome assembly. These genes were subsequently mapped to specific chromosomes, and their genomic distribution was visualized using TBtools software [54].

2.6. Transcriptomic analysis following V. anguillarum infection

To examine *septin* gene expression across various tissues and in response to *V. anguillarum* infection, publicly available transcriptomic datasets were retrieved from the NCBI database (accession numbers: PRJNA667799, PRJNA867038, PRJNA753277, PRJNA866872,

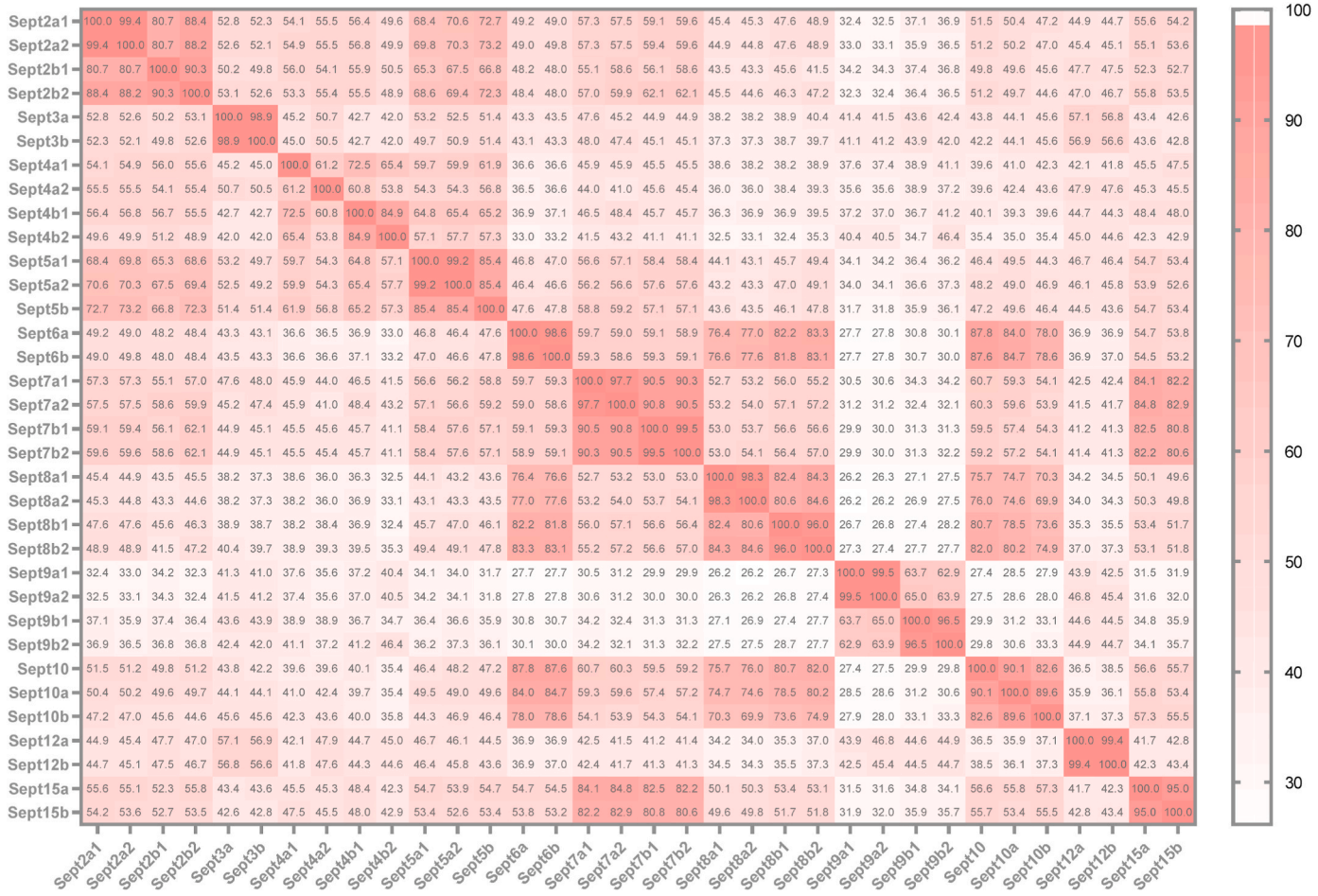


Fig. 1. Consistency of amino acid sequences of Septins in rainbow trout. Amino acid sequences of Septins in rainbow trout were obtained from NCBI databases. The data in cells indicates the percentages of consistency of amino acid sequences of Septins. "Sept" represents "Septin".

PRJNA866205, and PRJNA865462), as described in our previous studies [45,50,55]. Briefly, brain, kidney, liver, spleen, gill, and intestine of rainbow trout were sampled from three experimental groups: control (CG), symptomatic (SG), and asymptomatic (AG), following *V. anguillarum* infection for RNA-Seq analysis. Clean reads were aligned to the rainbow trout reference genome (GCA_013265735.2) utilizing Hisat2 (v2.2.1) [56]. Gene expression levels were quantified using FeatureCounts [57] and normalized as fragments per kilobase of transcript per million mapped reads (FPKM). Differential expression analysis was conducted with the DESeq2 R package [58]. *Septin* genes with a *p*-value <0.05 and an absolute $\log_2$ fold change ($|\log_2 FC|$) greater than 1 were deemed significantly differentially expressed.

2.7. Expression analysis of septin genes after A. salmonicida challenge

Juvenile rainbow trout (~10 g) were obtained from a trout farm in Linqu (Weifang, China) and randomly assigned to either the infected or control group. Based on our previous study [59], fish in the infected group (two replicates, n = 20 per replicate) were intraperitoneally injected with 0.2 mL of *A. salmonicida* suspension (1.0×10^8 CFU/mL), while fish in the control group were given an equal volume of sterile phosphate-buffered saline (PBS). At 0, 12, 24, 48, and 72 h post-injection (hpi), fish from both groups were euthanized using MS-222. Brain, kidney, spleen, and gill were collected, snap-frozen in liquid nitrogen, and stored at −80 °C for subsequent analysis.

Total RNA was isolated from brain, gill, spleen, and kidney tissues utilizing TRIzol (Invitrogen, USA) in accordance with the manufacturer's protocol. First-strand cDNA was synthesized from total RNA utilizing HiScript III RT SuperMix reagent kit (Vazyme, China) for qPCR analysis. Primers for rainbow trout *septin* genes were designed using Primer 5 (Table S1). *β-actin* served as the internal reference gene [59]. qPCR was performed on an StepOnePlus System (Applied Biosystems, USA) in a 10 μL reaction mixture comprising 1 μL cDNA, 5 μL SYBR ®FAST qPCR Master Mix, 0.2 μL of each primer, and 3.6 μL RNase-free water. Reactions were conducted in technical triplicates. Thermal cycling parameters were as follows: initial denaturation 95 °C for 30s, followed by 40 cycles of 95 °C for 10s, annealing at the primer-specific temperature (Tm) for 30s, and extension at 72 °C for 30s. Relative gene expression levels were calculated utilizing the $2^{-\Delta\Delta CT}$ method [60].

2.8. Statistical analysis

Data are presented as mean ± standard error of the mean (SEM). Statistical analyses were performed using SPSS 21.0 software (SPSS Inc., USA). One-way analysis of variance (ANOVA) was conducted to evaluate differences in relative mRNA expression levels obtained via qPCR. Fisher's LSD multiple comparisons were applied under the assumption of ANOVA, while Dunnett's T3 method was used when the assumption of ANOVA was not met. Statistical significance was defined as $p < 0.05$.

3. Results

3.1. Identification and localization of septin genes

To identify *septin* genes in rainbow trout, protein sequences from human and zebrafish were utilized as queries for BLAST searches. As a

result, a total of 34 *septin* genes were found in the genome of rainbow trout. Detailed characteristics of these *septins* are summarized in Table 1. Based on sequence homology, the 34 *septins* were classified into four subgroups, including SEPT2, SEPT3, SEPT6, and SEPT7. The mRNA lengths of the identified *septins* range from 1409 to 4657 bp. The predicted molecular weights (MW) of the encoded proteins ranged from 40.04 to 70.94 kDa, and their predicted isoelectric points (pI) values range from 5.40 to 9.37.

High amino acid sequence similarity was observed among several duplicated *septin* gene pairs in rainbow trout. Specifically, Septin2a1/Septin2a2 (99.4%), Septin5a1/Septin5a2 (99.2%), Septin7b1/Septin7b2 (99.5%), Septin9a1/Septin9a2 (99.5%), and Septin12a/Septin12b (99.4%) shared nearly identical amino acid sequences. Additionally, other pairs also exhibited strong similarity, including Septin3a/Septin3b (98.9%), Septin6a/Septin6b (98.6%), Septin7a1/Septin7a2 (97.7%), Septin8a1/Septin8a2 (98.3%), Septin8b1/Septin8b2 (96.0%), Septin9b1/Septin9b2 (96.5%), and Septin15a/Septin15b (95.0%) (Fig. 1).

3.2. Phylogenetic analysis of septin genes

To validate the annotation and examine the evolutionary relationships of *septin* genes, the predicted amino acid sequences of rainbow trout, along with those from selected representative vertebrates, were utilized to construct a phylogenetic tree. As shown in Fig. 2, the rainbow trout *septin* genes clustered with their orthologs in other species, forming four major clades: the SEPT2 (*septin1/2/4/5*), SEPT3 (*septin3/9/12*), SEPT6 (*septin6/8/10/11/14*) and SEPT7 (*septin7/15*) subgroups. This phylogenetic structure provides strong support for the accurate annotation and classification of rainbow trout *septin* genes. Furthermore, gene duplication events were evident in several rainbow trout *septins*. Specifically, *septin2*, *septin4, septin7, septin8,* and *septin9* each had four duplicate members; *septin3, septin6*, and *septin10* had three; and *septin5, septin12*, and *septin15* each had two. In contrast, *septin1* and *septin14*, which are exclusive to mammals, were not detected in rainbow trout. Likewise, *septin11* was absent not only in rainbow trout but also in other teleosts. *Septin13*, found only in humans, has been reclassified as a SEPT7-related pseudogene [7]. Notably, duplicated *septin* genes appear to be largely restricted to teleost fish.

3.3. Syntenic analysis of septin genes

To further support orthologies, syntenic analysis was conducted by comparing the genomic neighborhoods of rainbow trout *septin* genes with those in zebrafish, channel catfish, medaka, fugu, Atlantic salmon, and human. Syntenic blocks surrounding *septin2, septin4, septin5, septin6, septin7, septin8, septin9*, and *septin12* provided additional evidence for accurate annotation in rainbow trout (Fig. 3, S1). For instance, rainbow trout *septin6a* and *septin6b* shared neighboring genes with human (*rpl39, upf3b, ube2a, nkrf*), zebrafish (*rpl39, upf3b*), and medaka (*upf3b, nkrf, ube2a, elf1, pcdh19, nkap*), indicating that the genomic regions surrounding the *septin6* genes are highly conserved across species and strongly support the orthology of rainbow trout *septin6* genes to their vertebrate counterparts. Collectively, integration of phylogenetic and syntenic analyses revealed that trout *septin* genes exhibit strong orthology with those in other vertebrates.

3.4. Exon-intron structure and motif analysis of septin genes

To gain a deeper understanding of the structural diversity within rainbow trout *septin* gene family, exon-intron structures were analyzed in the context of their phylogenetic relationships. Structure analyses of the 34 *septin* genes indicated that the majority of *septin* genes contained 9 to 15 exons (excluding untranslated regions) separated by 8–14 introns

Table 1
Summary of *septin* genes identified in the rainbow trout genome and the characteristics and GenBank accession numbers of their transcripts. Chr* stands for chromosome. pI* stands for theoretic isoelectric points of proteins. MW* represents molecular weight.

Subgroup	Gene	Gene ID	Location	Chr*	Protein length (aa)	MW* (kDa)	pI*	mRNA (bp)
SEPT2	*septin2a1*	110525804	33,227,813–33,278,199	6	348	40.10	6.11	3732
	septin2a2	110536196	62,558,296–62,597,845	11	348	40.04	6.00	2706
	septin2b1	110508962	31,524,273–31,540,827	28	403	45.90	5.54	2854
	septin2b2	110530084	52,733,010–52,756,602	8	371	42.35	5.95	2808
	septin4a1	110490731	43,723,357–43,737,731	15	504	57.89	5.65	2342
	septin4a2	110490109	43,759,762–43,788,637	15	405	46.96	6.51	1443
	septin4b1	110503875	16,833,518–16,913,657	24	502	57.86	5.62	2506
	septin4b2	110507706	19,571,623–19,641,866	27	571	65.02	5.60	3611
	septin5a1	110536199	62,600,731–62,635,778	11	377	43.88	6.17	3304
	septin5a2	110525803	33,213,385–33,227,655	6	377	43.88	6.00	2526
	septin5b	110523411	21,578,990–21,597,442	5	371	43.22	6.10	2362
SEPT3	*septin3a*	110485707	13,822,748–13,850,213	13	369	41.41	6.77	2909
	septin3b	110493458	21,505,853–21,529,488	17	369	41.36	6.77	2960
	septin9a1	110516610	86,607,875–8,677,033	12	635	70.94	9.37	3109
	septin9a2	110516540	57,303,445–57,459,461	13	633	70.74	9.37	4657
	septin9b1	110493563	26,844,294–26,928,278	17	591	66.03	8.80	2035
	septin9b2	110485820	18,995,717–19,079,223	13	590	65.69	8.90	2091
	septin12a	118937800	96,221,848–96,301,339	12	463	52.59	5.74	1775
	septin12b	118938378	71,981,149–72,163,427	13	466	52.87	5.74	1554
SEPT6	*septin6a*	110488975	20,332,797–20,374,856	14	434	49.86	6.40	1547
	septin6b	110505183	41,245,345–41,274,161	31	434	49.82	6.53	2151
	septin8a1	110533358	12,581,314–12,628,444	10	473	54.72	6.38	3761
	septin8a2	110537756	52,629,937–52,677,747	12	474	54.89	6.07	2921
	septin8b1	110504542	9,560,101–9,586,391	31	454	52.30	5.79	2596
	septin8b2	110487785	34,307,144–34,339,240	14	441	50.97	5.40	3192
	septin10a	110527515	22,460,310–22,470,522	7	445	51.42	7.23	2818
	septin10b	110495902	25,263,995–25,272,511	18	468	54.56	8.56	1409
	septin10	110496360	45,342,003–45,347,556	18	425	48.96	6.69	2824
SEPT7	*septin7a1*	110488148	24,273,279–24,317,171	32	442	51.43	8.07	2954
	septin7a2	110496429	49,876,477–49,922,726	18	442	51.59	8.08	3003
	septin7b1	110488078	34,384,506–34,396,588	2	426	49.56	8.49	2333
	septin7b2	110515724	25,811,027–25,823,121	3	426	49.62	8.32	2527
	septin15a	110494104	66,266,850–66,298,314	2	465	54.23	7.97	3226
	septin15b	110514277	24,507,626–24,550,216	1	477	55.52	8.22	1652

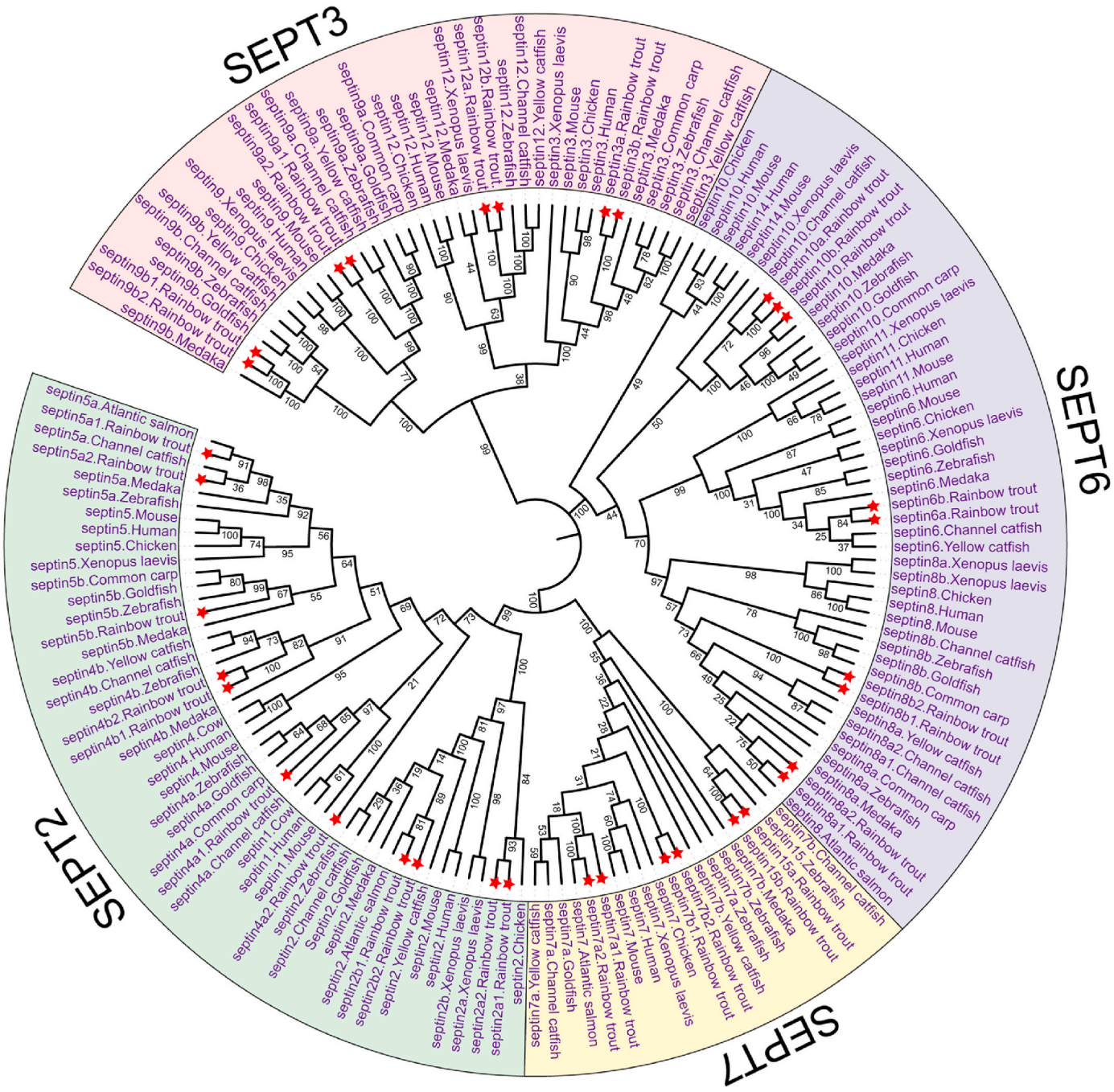


Fig. 2. Phylogenetic relationships of *septin* genes in rainbow trout and selected vertebrate species. The outer ring marks the name of septin subgroups, and the different subgroups were indicated in different colors. Bootstrap (1000 replications) support values appear on the branches, and the rainbow trout *septin* genes were marked with a red pentagram. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

(Fig. 4A). The exon-intron structures were relatively conserved within each Septin subgroup, indicating a high degree of structural conservation among phylogenetically related members.

To further characterize structural features of Septin proteins, motif analysis was performed using MEME, and sequence logos of motifs 1–16 were generated to determine the conserved amino acid residues (Fig. 4B). Nine motifs (1–7, 9, and 11) were shared across all Septin proteins. In the SEPT2 subgroup (Septin2/4/5), ten conserved motifs (1–9, 11, and 12) were identified, while the SEPT6 subgroup (Septin6/8/10) and SEPT7 subgroup (Septin7/15) exhibited eleven conserved motifs (1–9, 11, and 15). In contrast, unique motifs were identified in specific members of the SEPT3 subgroup; for instance, motif 13 was exclusively present in Septin9, whereas motif 12 was uniquely detected in the SEPT2 subgroup. These distinct motif patterns may contribute to the functional divergence of *septin* genes in rainbow trout.

3.5. Chromosomal location and interchromosomal relationship of septin genes

A collinearity map of *septin* genes in rainbow trout was constructed, excluding chromosomes without *septin* genes for clarity (Fig. 5A). The analysis identified fifteen occurrences of *septin* gene segmental duplication in rainbow trout. Members of each Septin subgroup exhibited pairing relationships and were distributed across different

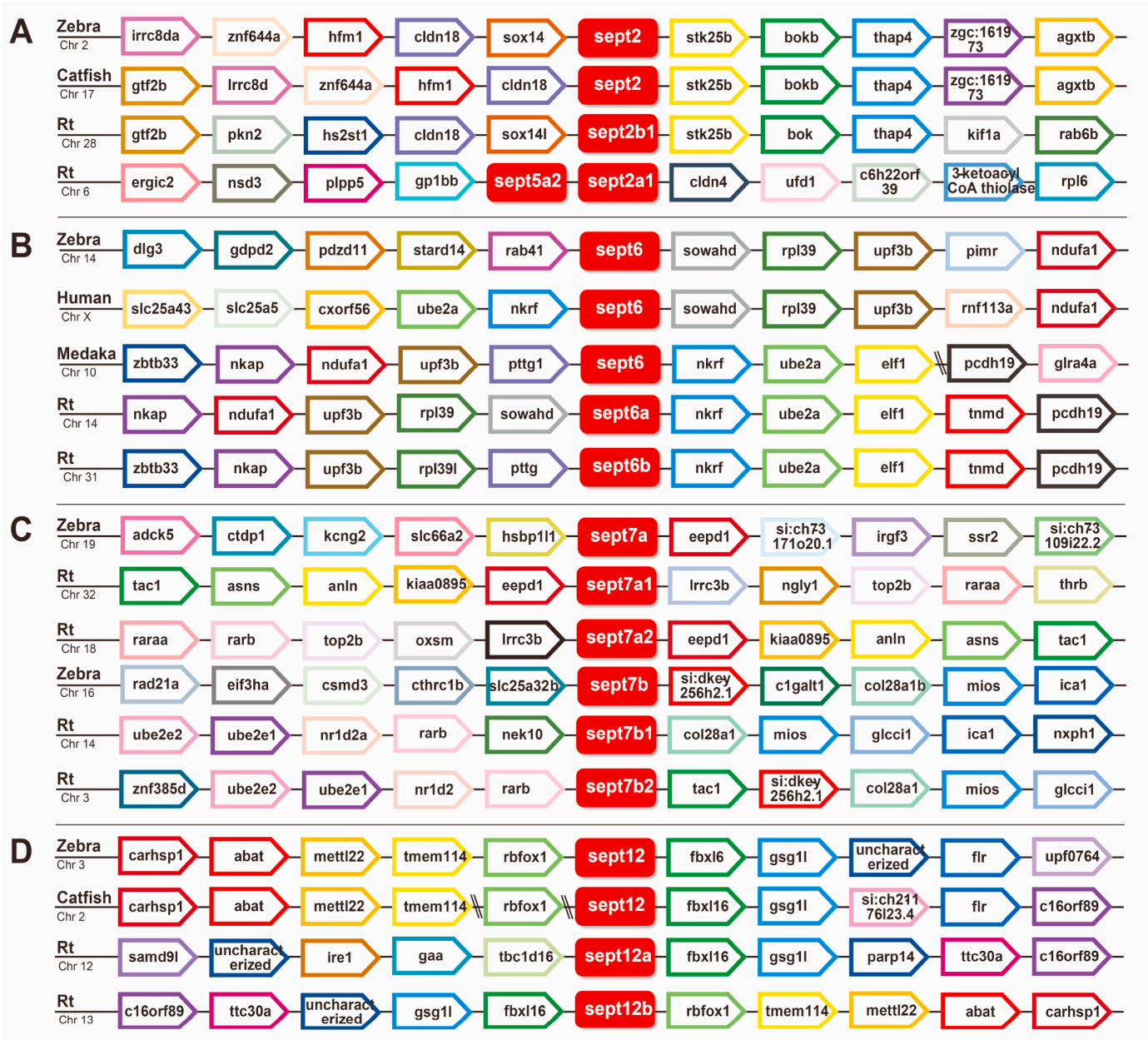


Fig. 3. Conserved synteny analysis of *septin* genes from rainbow trout using the genomes of four selected vertebrate species (human, zebrafish, channel catfish and medaka). Genes were represented by pentagons, and the direction in which the pentagon points does not indicate the direction of the reading frames. The syntenies were generated with the information obtained from NCBI and the Genomicus databases (v 92.01). The *septin* genes were marked with red-filled rectangles, and double slashes represented a few omitted genes. (A) *septin2,* (B) *septin6,* (C) *septin7,* (D) *septin12*. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

chromosomes. This pattern is consistent with previous findings suggesting that the separation of subgroup members across chromosomes may contribute to maintaining functional integrity [61]. Chromosomal mapping further indicated that the 34 *septin* genes were distributed across 20 chromosomes in rainbow trout (Table 1, Fig. 5B). Notably, *septin2a1* and *septin5a2*, as well as *septin2a2* and *septin5a1*, were found in adjacent positions on the same chromosome. Two closely related *septin* genes, *septin4a1* and *septin4a2*, were found on the same chromosome in adjacent locations (Fig. 5, S1). Furthermore, the number of *septin* genes varied considerably among chromosomes: seven chromosomes contained two genes, two chromosomes harbored three genes, and chromosome 13 possessed the highest number (n = 4). The remaining chromosomes each carried a single *septin* gene. These dispersed distributions suggest that interchromosomal duplication events have contributed to the expansion of the *septin* gene family.

3.6. Multiple sequence alignment analysis of septin domain

Multiple sequence alignment was performed to analyze the sequence features of the functional Septin domain (mainly including the GTP-binding domain) among the 34 *septin* genes in rainbow trout (Fig. 6 and S2). The alignment revealed a high degree of conservation across Septin domains, with an average sequence identity of 73.4%. Conserved sequence motifs were clearly observed, including G-motifs (G1, G3, and G4), which are characteristic of most small GTP-binding proteins [5]. Additionally, septin-specific motifs (Sep1, Sep2, Sep3, and Sep4), together with the SUE motif, were also detected. These features are considered signature elements that distinguish Septins from other small

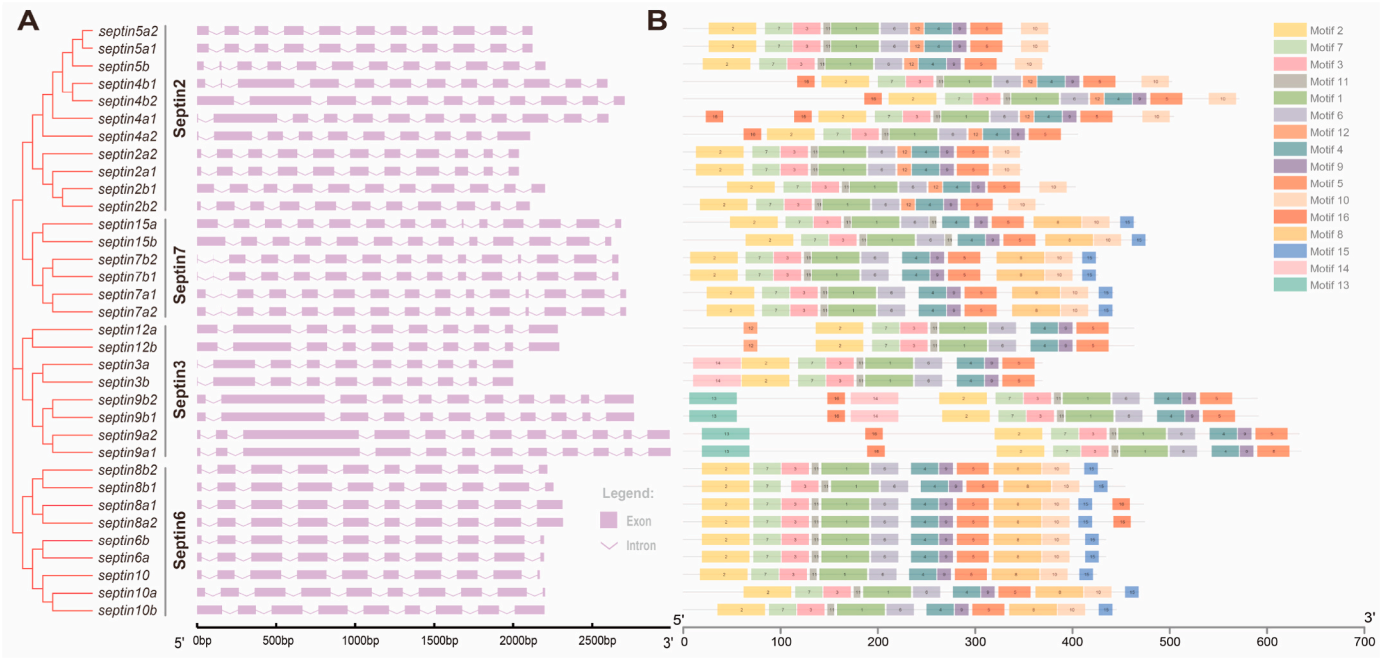


Fig. 4. Exon-intron structure and conserved motif analysis of *septin* genes in rainbow trout. (A) Exon-intron structure. The phylogenetic tree was constructed using the neighbor-joining method with JTT model and 1,000 bootstrap replicates in MEGA 7.0. Red rectangles represent exons, and purple broken lines represent introns. All introns were displayed at uniform length for visualization. (B) Conserved motif architecture. Distinct motifs are indicated by different colors. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

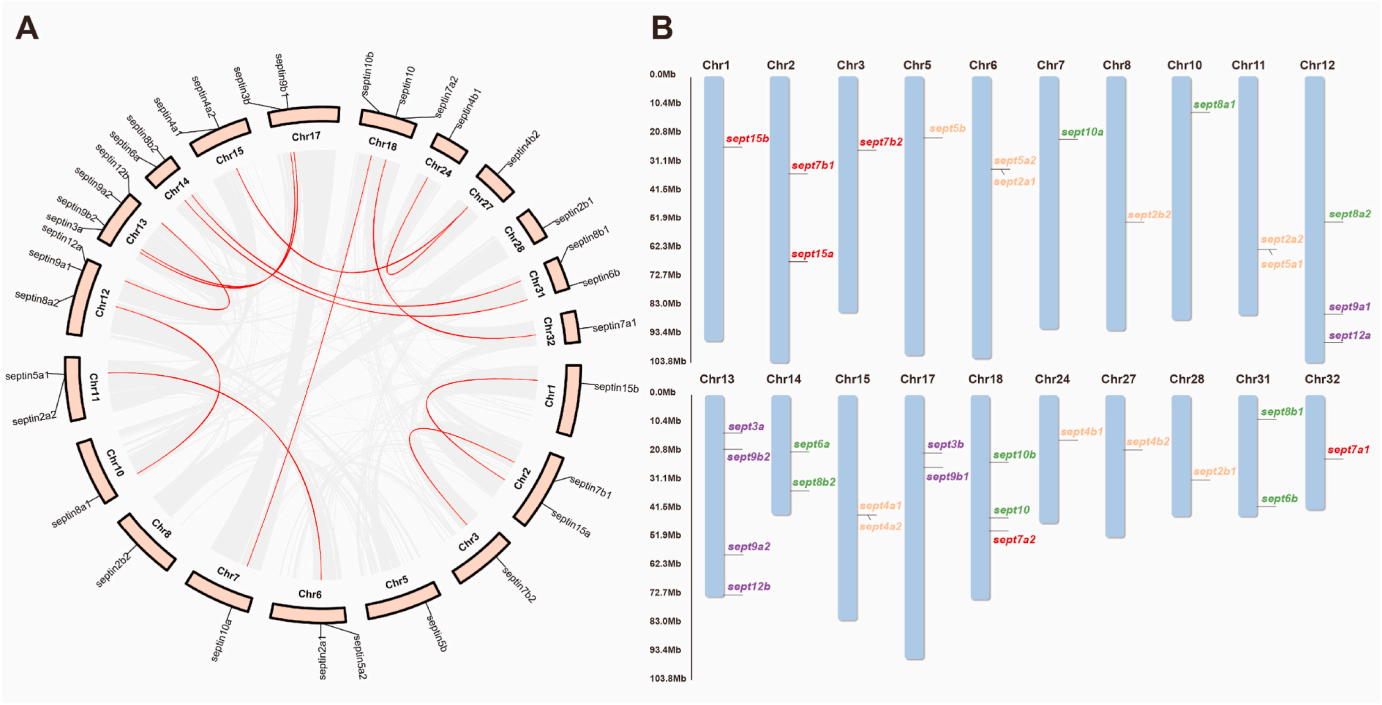
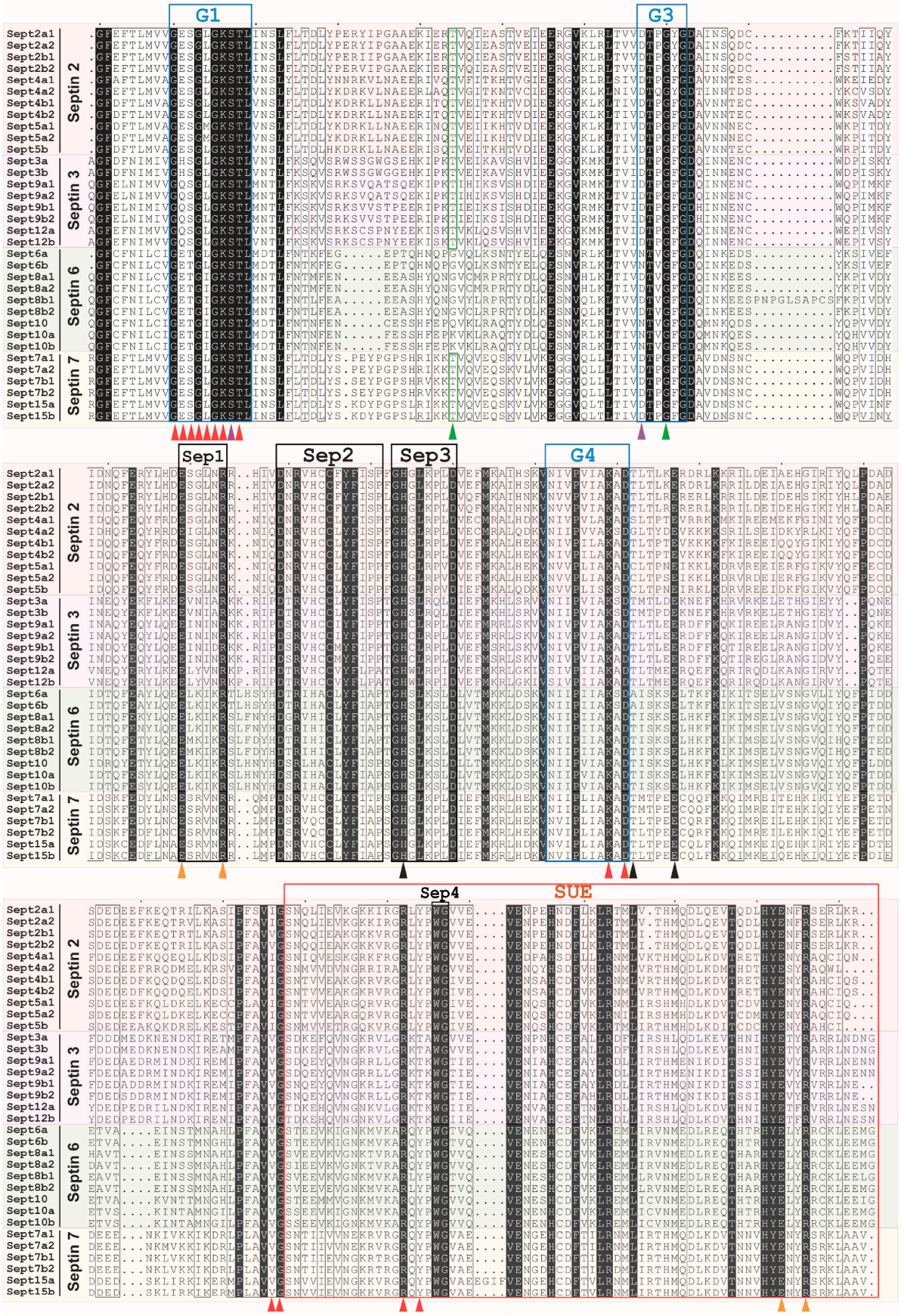


Fig. 5. (A) Interchromosomal relationships of *septin* genes in rainbow trout. Grey lines represent genome-wide collinear blocks extracted using MCScanx in TBtools. Orange square indicates chromosomes, and segmental duplication *septin* genes are connected with solid red lines. (B) Chromosomal distribution of *septin* genes in rainbow trout. A total of 34 *septin* genes with confirmed chromosomal locations were mapped onto 20 chromosomes. Each gene is color-coded according to its corresponding subfamily. The scale bar represents physical position in megabases (Mb). Gene names are shown in bold on the right side of each chromosome, and black lines indicate their precise chromosomal positions. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

GTPases. Furthermore, several conserved amino acid residues associated with key structural or functional roles were annotated, including: (i) residues directly interacting with ligands, (ii) residues forming bridging contacts with ligands from adjacent subunits across the G interface, (iii) Mg^{2+}-binding residues, (iv) universal switch residues, such as the catalytic threonine, and (v) residues involved in salt bridge formation at the



(caption on next page)

Fig. 6. Sequence analysis of the Septin domain in rainbow trout septin proteins. Residues highlighted in black are fully conserved among all analyzed septin members. G-motifs, common to most small GTP-binding proteins, are indicated with blue boxes, while septin-specific motifs [5,51] are marked with black boxes. These septin-specific motifs, along with the SUE motif (red box), are distinguishing features of *septins* that set them apart from other small GTPases. Residues with structural or functional importance are annotated with colored triangles, as described below: Red triangles represent direct interactions with ligands; Black triangles represent bridging contacts to ligands from adjacent subunits across the G interface; Purple triangles represent Mg^{2+}-binding residues; Green triangles represent universal switch residues, including the catalytic threonine (green boxes); Orange triangles represent residues involved in salt bridge formation at the NC interface. The annotations are based on Reference [5,71]. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

NC interface. Notably, the conserved threonine residue considered essential for catalytic function is present in all rainbow trout septins except those of the SEPT6 subgroup. This observation is consistent with previous reports that SEPT6 members typically lack the catalytic threonine [5], and it reinforces the subgroup-specific evolutionary and structural divergence of septins in rainbow trout.

3.7. Expression patterns of septin genes in different tissues

The analysis of tissue expression profiles is an effective way to further understand the physiological functions of genes. The expression profiles of *septin* genes were evaluated using previously published transcriptomic data from six tissues of rainbow trout, including the brain, spleen, gill, liver, kidney, and intestine under normal physiological conditions (Fig. 7). All identified *septin* genes were ubiquitously expressed across the examined tissues, although their expression levels varied substantially. Notably, *septin2b1, septin2b2, septin7b1, and septin7b2* exhibited relatively high expression in most tissues, whereas *septin4a1, septin4a2*, and *septin9a1* showed consistently low expression levels. In general, most *septin* genes tended to be more highly expressed in the spleen and brain, while lower expression levels were observed in the kidney. Furthermore, several *septin* genes exhibited distinct tissue-specific expression patterns. For example, *septin15a* exhibited the highest expression in the brain; *septin2b2* was most abundant in the liver and spleen; *septin2b1* was predominantly expressed in the kidney and intestine; and *septin7b2* showed the highest expression in the gill.

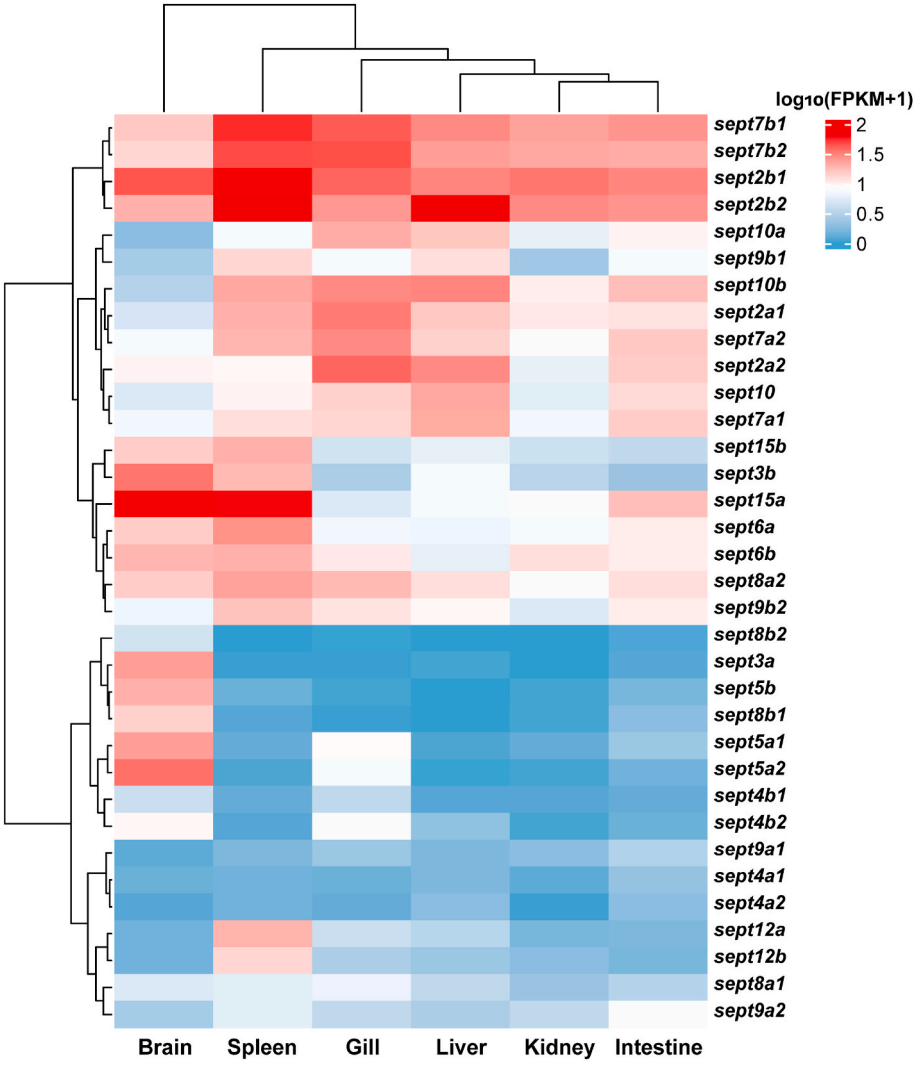


Fig. 7. Heatmap of rainbow trout *septin* gene expression in different tissues. The examined tissues include the brain, spleen, gill, liver, kidney, and intestine. The color depth indicates the level of expression, with red representing relatively high expression and blue representing relatively low expression. Each cell with different color has a concrete value of $\log_{10}$(FPKM+1) to represent the expression level. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Additionally, certain *septin* genes displayed distinct tissue specificity: *septin5b* was not expressed in the liver, *septin8b2* was absent from the kidney, *septin12b* was highly expressed in the spleen, and both *septin5b* and *septin8b1* were predominantly expressed in the brain while exhibiting low expression in other tissues.

3.8. Expression of septin genes after V. anguillarum infection

To investigate the potential roles of *septin* genes in immunological responses, the expression profiles of all 34 *septin* genes were examined following *V. anguillarum* infection using RNA-Seq datasets. In the spleen, *septin2b2* and *septin15b* were significantly downregulated in AG compared to CG, and *septin15b* was also significantly downregulated in SG relative to CG (Fig. 8A and B). In the kidney, *septin15b* showed significant downregulation in both SG and AG (Fig. 8C). In the intestine, *septin3b* was downregulated in SG, whereas *septin5a1* was significantly upregulated in SG compared to CG (Fig. 8D and E). In the liver, *septin3b* and *septin9a2* were significantly upregulated in SG relative to AG, whereas *septin9b1* and *septin15b* were significantly downregulated. Additionally, *septin4b2*, *septin9b1*, *septin15a,* and *septin15b* were significantly downregulated in SG compared to CG (Fig. 8F). Most other *septin* genes did not exhibit significant differential expression in response to infection (Fig. 8).

3.9. Expression profiles of septin genes after A. salmonicida infection

To further explore the immune-related functions of *septin* genes, we examined the temporal expression patterns of *septin2b1, septin3, septin4a2, septin5a1, septin6, septin7b1, septin8b, septin9b, septin10, septin12,* and *septin15* in the brain, kidney, spleen, and gill following *A. salmonicida* infection by qPCR (Fig. 9). In general, *septin* gene expression levels varied among tissues, and their responses to infection were both gene- and tissue-specific, indicating that *septin* genes may play a complex regulatory role in the host immune defense of rainbow trout.

In the brain, all *septin* genes exhibited differential expression following *A. salmonicida* infection. The expression levels of *septin9b*, *septin10,* and *septin15* exhibited an upregulated trend at 24, 48, and 72 hpi, respectively. Conversely, other *septin* genes were initially downregulated at 12 or 24 h but their expression either returned to baseline or exceeded control levels by 72 h. In the kidney, all *septin* genes were differentially expressed following *A. salmonicida* infection, except for *septin5a1* and *septin15*. *Septin8b* and *septin12* exhibited an upregulated trend at 24 and 48 h, respectively, but returned to baseline levels by 72 h. Other genes were initially downregulated at 12, 24 or 48 h. Additionally, *septin3*, *septin4a2*, and *septin9b* remained significantly lower than control levels at 72 h, whereas *septin2b1* and *septin10* were significantly upregulated at that time point. In the spleen, most *septin* genes were differentially expressed after infection, with the exception of *septin7b*, *septin9b*, *septin10,* and *septin12*. *Septin2b1*, *septin3*, *septin4a2*, and *septin15* were initially downregulated at 12 or 24 h, while *septin5a1*, *septin6*, and *septin8b* were significantly induced. In the gill, a similar expression pattern was observed. Most *septin* genes were differentially expressed, except for *septin3*, *septin4a2*, *septin7b*, and *septin9b*. *Septin2b1*, *septin10,* and *septin15* were downregulated at 12 or 24 h, whereas *septin5a1*, *septin6*, *septin8b*, and *septin12* were significantly induced. Interestingly, *septin8b* consistently showed upregulation across most time

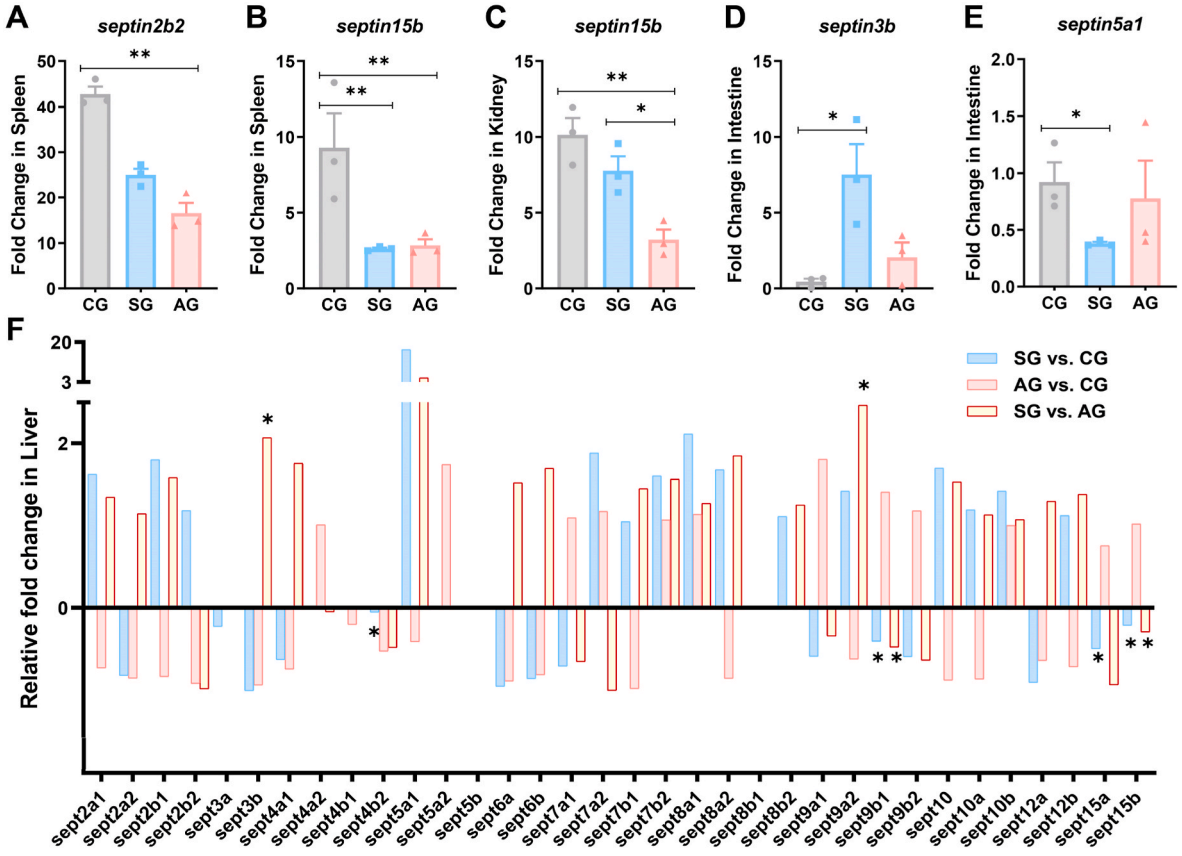


Fig. 8. Differential expression of *septin* genes in rainbow trout following *V. anguillarum* infection. (A, B) Expression of *septin2b2* and *septin15b* in the spleen; (C) *septin15b* in the kidney; (D, E) *septin3b* and *septin5a1* in the intestine; (F) *septin* gene expression in the liver across different groups. Gene expression levels are presented as fold changes relative to the control group (CG). The symptomatic group (SG) comprised the first three moribund fish exhibiting erratic swimming behavior, while the asymptomatic group (AG) included three surviving individuals without clinical symptoms at 120 h post-infection (hpi). Asterisks indicate statistically significant differentially expressed genes ($p < 0.05$, and $|\log_2 FC| > 1$).

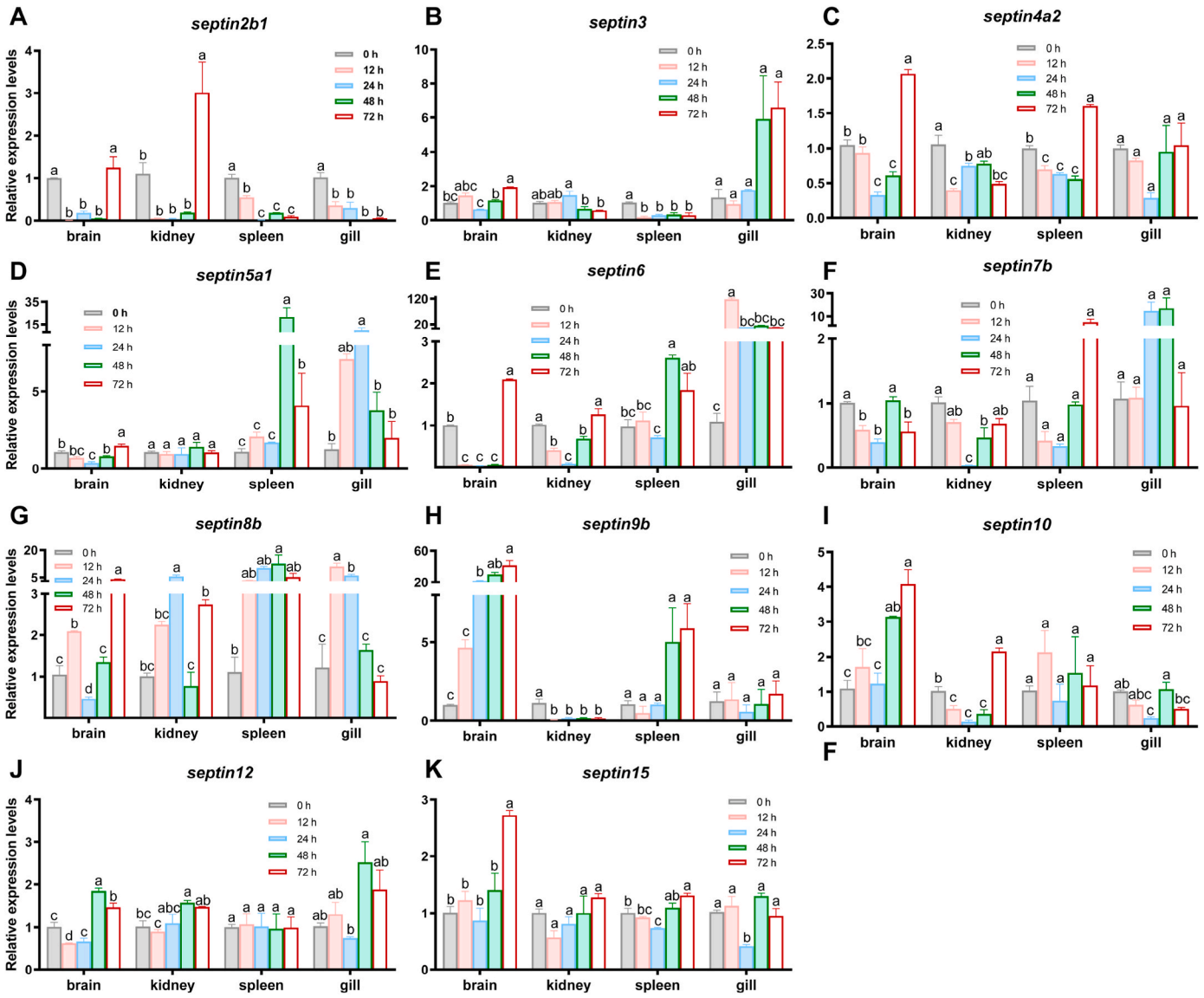


Fig. 9. Relative expression levels of selected *septin* genes in the brain, kidney, spleen, and gill of rainbow trout at 0 h, 12 h, 24 h, 48 h, and 72 h post *A. salmonicida* infection. (A) *septin2b1*, (B) *septin3*, (C) *septin4a2*, (D) *septin5a1*, (E) *septin6*, (F) *septin7b*, (G) *septin8b*, (H) *septin9b*, (I) *septin10*, (J) *septin12*, (K) *septin15*. Gene expression levels are shown relative to the control group (0 h) and presented as the mean ± standard error of the mean (SEM) from three biological replicates. Different letters indicate statistically significant differences between time points ($p < 0.05$).

points, whereas *septin2b1* and *septin4a2* displayed a generally down-regulated trend across all four examined tissues.

To further explore the possibility of functional Septin complex formation, co-expression clustering analyses were performed (see Discussion and Supplementary Figs. S3 and S4).

4. Discussion

Gene duplication has been recognized as a crucial source of evolutionary novelty [62], as it generates new gene copies that may acquire subfunctional or neofunctional roles to adapt to changing environments [63,64]. In vertebrates, the expansion of the *septin* gene family has primarily occurred through the duplication of pre-existing genes rather than the emergence of new *septin* subgroups [65]. A similar pattern is evident in rainbow trout genome, where most *septin* paralogs appear to have arisen from whole-genome duplication (WGD) events [66,67]. Variations in *septin* gene copy number between teleosts and tetrapods provide important insights into the evolutionary history of the *septin* family. Except for a couple genes in *Xenopus laevis*, gene duplication has been predominantly observed in teleost fish, with the duplicated *septin* genes likely originating from the teleost-specific WGD events [9,68]. In comparison to other teleost species, most of which have fewer than 15 *septin* genes [9], rainbow trout exhibit a markedly expanded *septin* repertoire (34 genes), likely resulting from salmonid-specific WGD events [69,70]. Furthermore, collinearity analysis revealed evidence of interchromosomal segmental duplications (Fig. 5A), and syntenic analysis confirmed these findings while also identifying additional tandem duplication events involving *septin4a1* and *septin4a2* in rainbow trout (Fig. 3, S1). Collectively, these findings suggest that the lineage-specific expansions of *septin* genes in rainbow trout are primarily driven by WGD, segmental duplication, and tandem duplication. Additionally, *septin1, septin11*, and *septin14*, which are present in mammals [71], are absent in teleosts such as rainbow trout, zebrafish, channel catfish [9], and starry flounder (*Platichthys stellatus*) [72]. The absence of *septin11, septin13, and septin14* in certain teleost species is likely the result of gene loss events during evolution [63,73].

Based on gene structure and functional domain analyses of the *septin* genes in rainbow trout, we found that closely related members within

the same subgroup exhibited similar exon-intron architectures (Fig. 3A), motif compositions (Fig. 3B), and domain structures (Fig. S2). These conserved features may provide important clues regarding the potential functional roles of *septin* genes. The number of introns in rainbow trout *septin* genes ranges from eight (e.g., *septin10b*) to fourteen (e.g., *septin7a1*, *septin7a2*, *septin7b1,* and *septin7b2*), a pattern of intron reduction also observed in *Channa argus* [74]. Intron loss, as a form of genomic variation, may influence gene regulation and expression, thereby contributing to the evolutionary diversification of eukaryotic genes [74,75]. In rainbow trout, such intron loss may reflect an adaptive response during evolutionary processes. Furthermore, domain and structural analyses revealed that rainbow trout Septin proteins display a conserved classical architecture similar to their mammalian counterparts [5,71]. Specifically, trout Septins harbor conserved G-motifs (G1, G3, and G4), septin-specific motifs (Sep1–Sep4), the SUE motif, and several highly conserved amino acid residues critical for maintaining protein structure and function. Additionally, several duplicated *septin* gene pairs (Septin2a1/2a2, Septin5a1/5a2, Septin7b1/7b2, Septin9a1/9a2, Septin12a/12b, Septin3a/3b, Septin6a/6b, Septin7a1/7a2, Septin8a1/8a2, Septin8b1/8b2, Septin9b1/9b2, and Septin15a/15b) exhibited more than 95% amino acid sequence identity (Fig. 1), consistent with earlier observations of other gene family in rainbow trout [55]. Together, these findings suggest that the *septin* gene family in rainbow trout has been highly conserved and extensively retained throughout evolutionary history, supporting its evolutionary conservation across vertebrates.

The transcript level expression of *septin* genes has been extensively studied in humans [71,76–78], but remains largely unexplored in fish, where limited information is available. In this study, most *septin* genes in rainbow trout exhibit distinct tissue- and gene-specific expression patterns (Fig. 7), suggesting their potential involvement in diverse physiological processes. Specifically, *septin2b* was highly expressed in all examined tissues, particularly in the spleen, while *septin2a* showed peak expression in the gill, liver, and spleen. Similar tissue-specific expression patterns of *septin2* were observed in other teleosts, including *Labeo rohita* (liver, skin, and kidney) [79], starry flounder (trunk kidney, heart, and peripheral blood leukocytes) [72], and channel catfish (spleen, kidney, and gill) [9]. Other *septin* genes also exhibited species- and tissue-specific patterns. *Septin7b* showed consistently high expression in both channel catfish and rainbow trout. Conversely, *septin5* showed low expression in rainbow trout but high expression in channel catfish and the kidney of starry flounder. *Septin3*, strongly expressed in the liver of starry flounder, showed relatively low expression in both rainbow trout and channel catfish. Similarly, *septin8* and *septin9*, which are highly expressed in the gills of starry flounder, were weakly expressed in rainbow trout and channel catfish [9,72]. Collectively, these findings highlight the tissue- and species-specific expression patterns of *septin* genes across teleost species.

Bacterial infections are known to induce inflammatory and apoptotic responses in rainbow trout [45,55,59]. However, the involvement of *septin* genes in immune responses remains largely uncharacterized in this species and other teleosts. In the present study, six *septin* genes—*septin2b2, septin4b2, septin5a1, septin9b1, septin15a*, and *septin15b*—were significantly downregulated in SG or AG compared to CG following *V. anguillarum* infection (Fig. 8). Similarly, all tested *septin* genes, except *septin8b*, were significantly downregulated in at least one tissue following *A. salmonicida* infection (Fig. 9). Comparable downregulation patterns of immune-related genes, particularly within cytoskeletal and lysosomal/phagosomal pathways involving septins, have been reported in channel catfish infected with enteric septicemia. As cytoskeletal components, septins facilitate endocytosis and macrophage uptake. Their downregulation may reflect pathogen-induced cytoskeletal disruption or immune suppression [9,80,81]. Consistent findings were reported in starry flounder, where *septin2* was significantly downregulated in the spleen after exposure to viral hemorrhagic septicemia virus (VHSV) [72], and in rainbow trout following *A. salmonicida* infection (Fig. 9A). Conversely, *septin2* was upregulated in multiple tissues following viral, ectoparasitic, or bacterial infections in starry flounder [72], *L. rohita* [79], and channel catfish [9], suggesting that *septin2* may play divergent immunomodulatory roles across teleost species and infection models. Additionally, *septin4* was downregulated in mouse liver 12 weeks after *Schistosoma japonicum* infection, coinciding with parasite-induced hepatic fibrosis progression. As *septin4* promotes apoptosis, its reduction may contribute to fibrosis development, indicating a potential protective role via apoptosis-mediated regulation [9,82]. Comparable trends have been reported in other fish species. In channel catfish, *septin4b* and *septin5* were significantly downregulated in the gill at 72 hpi after *Edwardsiella ictaluri* infection. Similarly, in blue catfish (*Ictalurus furcatus*), *septin5* was significantly downregulated in the skin at 2, 12, and 24 hpi with *Aeromonas hydrophila* [9,83], suggesting a partially conserved role for septins in antibacterial immune responses across teleosts. Notably, in zebrafish, *septin15* is essential for host defense against *Shigella flexneri* infection [84]. Its depletion during *S. flexneri* infection leads to severe neutropenia and high mortality of zebrafish [85]. In our study, *septin15b* was significantly downregulated in response to *V. anguillarum* but upregulated in the brain after *A. salmonicida* challenge, highlighting a complex role of *septin15* in antimicrobial immunity in teleosts.

In contrast to the observed downregulation, several *septin* genes were significantly upregulated at 12, 24, or 48 hpi following *A. salmonicida* challenge. Specifically, *septin9b*, *septin10*, and *septin15* were upregulated in the brain; *septin2b1*, *septin3*, *septin8b*, and *septin12* in the kidney; *septin5a1, septin6,* and *septin8b* in the spleen; and *septin5a1, septin6, septin8b,* and *septin12* in the gill (Fig. 9). Additionally, in response to *V. anguillarum* infection, only *septin3b* was significantly upregulated in the intestine of symptomatic fish compared to controls (Fig. 8). These tissue-specific expression patterns suggest that certain septin members may be involved in immune activation during bacterial infection. Previous studies have revealed that *A. salmonicida* can invade the fish hosts through the skin, gut, or gills, subsequently spreading to and colonizing the head kidney, liver, spleen, and brain [86]. Septins are known to play important roles in autophagy and phagocytosis [30,72,87,88]. They can assemble into cage-like structures that entrap intracellular bacteria, targeting them for autophagic degradation and restricting their dissemination within the host [87]. In zebrafish, such septin cages have been shown to function as a significant defense mechanism for *in vivo* clearance of intracellular pathogens [29]. Moreover, septins form collar-like structures at the base of the phagocytic cup in macrophages and neutrophils to enable phagocytosis [89]. Septin2 is particularly essential for efficient phagocytosis activity, a role that has also been validated in aquatic species such as *Apostichopus japonicus* [30] and starry flounder [72]. Consistent with our findings, *septin* gene upregulation has been observed in other teleost species during pathogen challenge. Specifically, *septin5, septin6, septin8a2, septin9, septin10,* and *septin12* were markedly upregulated in the gills of channel catfish in response to *Flavobacterium columnare* challenge [9]; *septin3, septin5,* and *septin8* were significantly upregulated in the spleen of starry flounder after VHSV and *Streptococcus parauberis* infection [72]; and *septin6* was induced in the skin of blue catfish after *A. hydrophila* infection [83]. Collectively, these observations support a conserved role for *septin* gene upregulation in mediating host defense and suggest that the *septin* gene family contributes to bacterial clearance and immune defense in rainbow trout.

Given the complex roles of septins in cellular architecture and host defense, understanding their higher-order assembly is essential. Septins are known to form multimeric complexes, and their size and composition vary among species. These oligomeric assemblies, typically composed of paralogous septins, may consist of four, six, or eight subunits [90]. In vertebrates, both hexameric and octameric forms have been well characterized, with the SEPT2–SEPT6–SEPT7 hexamer being the best-characterized structure [21,91]. Functional interchangeability among subgroup members has also been documented. For instance, SEPT2 can be replaced by SEPT1, SEPT4, or SEPT5, while SEPT6 may be

substituted by SEPT11, SEPT8, or SEPT10 [3,90,92]. This molecular flexibility allows for compositional diversity in septin complexes, potentially enabling species- or tissue-specific adaptations. To further explore the functional implications of *septin* gene expression in fish, we analyzed the co-expression profiles of *septin* genes across infection-relevant tissues and time points. Following *V. anguillarum* infection, coordinated expression of SEPT2, SEPT6, and SEPT7 members was observed in gill, liver, and intestinal tissues (Fig. S3). Representative combinations included *septin2b2* (SEPT2), *septin7a1* (SEPT7), and *septin6b* (SEPT6); *septin4b2* (SEPT2), *septin7b1* and *septin7b2* (SEPT7), and *septin10b* (SEPT6); as well as *septin5a1* (SEPT2), *septin7a2* (SEPT7), and *septin10a* (SEPT6). Similarly, in response to *A. salmonicida* infection, co-expression of these subgroups was also detected across several tissues. In the brain (Fig. S4A), *septin2b*, *septin7b1*, and *septin6b* were co-expressed; in the spleen (Fig. S4C), *septin4a2*, *septin7b1*, and *septin6b* were simultaneously upregulated. In the gills (Fig. S4D), *septin5a1*, *septin7b1*, and *septin8a* (SEPT6) showed similar expression profiles. These consistent patterns suggest that genes from these three subgroups may be transcriptionally co-regulated in response to bacterial infection, thereby supporting the possible formation of hexameric septin complexes in teleost fish. Furthermore, the co-expression of SEPT3 subgroup members, such as *septin3a, septin9a2,* and *septin9b2*, together with SEPT2, SEPT6, and SEPT7 genes following *V. anguillarum* infection, may reflect the potential formation of octameric complexes under specific immune conditions.

In summary, 34 *septin* genes were identified for the first time in rainbow trout and classified into four subgroups. Comprehensive analyses, including phylogenetic reconstruction, synteny comparison, gene structure and motif characterization, collinearity analysis, and conserved domain identification, provided strong evidence supporting their annotation. Molecular evolutionary analysis indicated that *septin* genes in rainbow trout are highly conserved and exhibit strong homology with those in other teleosts. Expression profiling indicated that the majority of *septin* genes were ubiquitously expressed across six tissues and were significantly regulated in response to bacterial infections, suggesting their roles in host immune defense. Collectively, these findings offer important insights into the evolutionary conservation and immunological functions of the *septin* gene family in rainbow trout.

CRediT authorship contribution statement

Mengqun Liu: Writing – original draft, Data curation, Methodology, Software, Visualization. **Qiusheng Wang:** Visualization, Investigation. **Ziyi Zhao:** Visualization, Investigation. **Xin Qi:** Formal analysis, Writing – review & editing, Validation, Supervision. **Haishen Wen:** Conceptualization, Validation, Writing – review & editing, Funding acquisition, Project administration.

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Declaration of competing interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fsi.2025.110546.

Data availability

Data will be made available on request.

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