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Identification and Expression Profiling of Scorpion Ionotropic Receptors and Gustatory Receptors Based on the Transcriptomic Analysis

Xinrong Wang[1] | Sijia Lu[1] | Yitong Li[1] | Haozhen Zhang[1] | Hengwu Jiao[2] | Yingliang Wu[1]

[1]College of Life Sciences, Wuhan University, Wuhan, China | [2]Key Laboratory of Pesticide & Chemical Biology of Ministry of Education, Hubei Key Laboratory of Genetic Regulation and Integrative Biology, Institute of Evolution and Ecology, School of Life Sciences, Central China Normal University, Wuhan, China

Correspondence: Hengwu Jiao (hwjiao@ccnu.edu.cn) | Yingliang Wu (ylwu@whu.edu.cn)

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ABSTRACT

Chemical signal perception plays a crucial role in arthropod reproduction and survival. The scorpion is one of the oldest terrestrial arthropods; however, its knowledge of the chemosensory genes remains unclear. Based on the transcriptomic analysis of widely distributed *Mesobuthus martensii* in China, 46 candidate gustatory receptors (GRs) and 80 candidate ionotropic receptors (IRs)/ionotropic Glutamate receptors (iGluRs) overall showed similar expression trends in different tissues between the third-instar and adult scorpions, which suggested that young scorpions possessed a relatively complete chemical perception capability. Most GRs showed higher transcript accumulation in the pectines, rather than other tissues, aligning with their known chemosensory function and highlighting the remarkable significance of pectines as crucial sensory organs. Conversely, IRs/iGluRs overall exhibited a more widespread distribution throughout the different tissues of the scorpion body. The phylogenetic tree further elucidated the evolutionary relationships among these chemosensory genes in arthropods. These findings contributed to a better understanding of the functionality and evolution of the chemosensory systems in scorpions, which would accelerate the functional investigations of scorpion chemosensory genes in the future.

1 | Introduction

The scorpion is one of the oldest terrestrial arthropods with a well-known morphological similarity between the Paleozoic and extant scorpions. The interest shown by most people usually stems from the scorpion venom system. In the Silurian, the aquatic and/or amphibious scorpions developed an extant scorpion-like venomous telson with a distinct aculeus (Waddington et al. 2015; Figure 1A). In Paleozoic scorpions, the origin of the venom system was likely related to the ion channel modulation by scorpion hemolymph and its defensin ingredients (Meng et al. 2020). Recently, the similar neurotoxin expression profiles between the second, third, fourth instar, and adult scorpions further highlighted the importance of the venom system (Guo et al. 2024). These progresses indicate that the venom system has played a critical role in capturing prey and defending against predators during the long-term evolutionary process.

In addition to the venom gland, the pectines are also unique organs that show a comb-like structure feature (Figure 1B). During movement on the ground, scorpion pectines are easily found to sweep across the substrate, especially during the mating process, possibly for spermatophore deposition on the ground and following the insemination process. Therefore, the pectines

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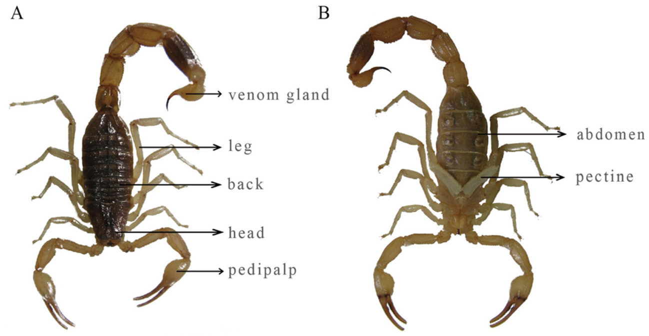

FIGURE 1 | Different positions of *Mesobuthus martensii* sampled for Illumina sequencing and assembly. (A) Dorsal side of *M. martensii*; (B) ventral side of *M. martensii*.

likely have a chemosensory function (Polis 1990). Some earlier behavior experiments suggested that the pectines were responsible for humidity detection since scorpions had a preference for moist areas when they were exposed to a humidity gradient (Sreenivasa-Reddy 1959). When using the extracts of the cuticle of prey insects, pectine-specific searching responses increased more remarkably after touching the prey marks, which indicated the chemosensitivity of the pectines (Krapf 1986). The subsequent electrophysiological experiments further indicated near-range stimulation of pectines by volatile alcohols, aldehydes, ketones, esters, and carboxylic acids produced dose-dependent patterns of neural response (Gaffin and Brownell 1997). However, the chemoreceptors responsible for detecting chemical simulation remain elusive in scorpion pectines nowadays.

Besides the scorpion pectines, some other parts, including the mouthparts, pedipalps, and legs, could possibly detect chemical stimulation. In 1957, scorpions were reported to reject food and began cleaning the chelicerae once tinctura quassiae from bitter ash was dropped onto food that the scorpions were feeding upon (Alexander and Ewer 1957). Later, scorpions were observed to also reject food treated with the concentrated sodium chloride solution (Abushama 1964). Meanwhile, when scorpion pedipalps were painted over, they were found to lose sensitivity to some airborne chemicals, which indicated their chemosensory capability. If the leg tarsi were covered with nail polish, scorpions would not prefer humid regions and would choose a dry environment, which indicated the humid detecting capability was impaired (Abushama 1964). All these behavior observations showed that chemoreception likely played critical roles in the scorpion's life history. However, the knowledge of chemosensory genes in different parts of scorpions remains highly limited.

In this work, the widely distributed *Mesobuthus martensii* in China were used to investigate the expression profiles of candidate chemosensory genes based on the transcriptomic analysis since the current sequencing techniques has greatly accelerated the extensive characterization of the chemosensory genes of many insects and non-insect organisms, which have been found to harbor members of the gustatory receptor (GR) and ionotropic receptor (IR) families in their genomes (Montagné et al. 2015; Gulia-Nuss et al. 2016; Zhao et al. 2021; Stern et al. 2010; Colbourne et al. 2011; Chipman et al. 2014). Transcriptomic studies of lobsters and hermit crabs further support the presence of IR genes, highlighting the crucial role of this gene family in the olfaction of crustaceans (Kelly et al. 2023; Kozma et al. 2018; Groh-Lunow et al. 2015) In 2018, comparative genomics revealed thousands of chemosensory genes and massive changes in chemoreceptor repertoires across chelicerates. 973 and 496 chemosensory sequences were, respectively, found in *C. exilicauda* and *M. martensii* scorpions through an exhaustive search strategy, which would accelerate the understanding of scorpion chemosensory receptors (Vizueta et al. 2018). In this work, the transcriptomic analysis of different instars and body parts of *M. martensii* was used, and the differential expression profiles of 46 candidate GRs and 80 candidate IRs/ionotropic glutamate receptors (iGluRs) were characterized among the different tissues and between the different instar stages. Real-time quantitative PCR was further employed to detect the transcriptional expression of key chemosensory protein genes in various tissues of third-instar and adult scorpions. These findings would prompt further investigations into the molecular mechanisms of the scorpion chemosensory system.

2 | Materials and Methods

2.1 | Animals and Tissue Collection

M. martensii were obtained from the scorpion breeding base of Shiyan city in Hubei Province, China, and maintained in the laboratory in plastic boxes with a continuous supply of water and yellow mealworms. Each summer, the newborn *M. martensii* were collected and gradually became second instars, third instars, and fourth instar *M. martensii* after continuous molting (Guo et al. 2024). Five different tissues of the head, pedipalps, legs, pectines, and venom glands from the adult scorpions were sampled with three biological replicates for each tissue. For the third-instar scorpions, seven different tissues of the head, pedipalps, legs, pectines, back, abdomen, and venom glands were sampled with three biological replicates per tissue. All these tissue samples were rapidly frozen in liquid nitrogen and stored at −80°C.

2.2 | Illumina Sequencing and Assembly

De novo transcriptome assembly of *M. martensii* was performed using Trinity software based on the clean short reads (Grabherr et al. 2011), followed by optimization of the initial assembly with filters from TransRate and CD-HIT programs (Li and Godzik 2006; Smith-Unna et al. 2016). The assembly was further evaluated using the BUSCO software (Simão et al. 2015). The longest open reading frame (ORF) for each gene of *M. martensii* was determined using the NCBI ORF Finder tool. The final dataset of *M. martensii* comprised the transcripts and unigenes, which were selected from the longest sequence of each transcript. Transcript annotation of *M. martensii* was performed by searching the Nr (non-redundant), STRING, Swiss-Prot, KEGG, and other databases using BLASTX. A cut-off e-value of < 1e−5 was applied. The BLAST annotation results were further processed using the Blast2GO program to obtain functional classifications and descriptions based on the unigene annotations of *M. martensii* (Ashburner et al. 2000; Conesa et al. 2005; Gotz et al. 2008). The expression level of each unigene was quantified using its FPKM value (fragments per kilobase of exon model per million mapped

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reads). Higher FPKM values indicated higher gene expression levels, and these values were calculated using the RSEM program (Trapnell et al. 2010).

2.3 | Annotation of Chemoreceptors

We used two methods to identify the GRs and IRs/iGluRs in *M. martensii*. First, all the *Drosophila melanogaster* receptors from the National Center for Biotechnology Information as a database were downloaded for the tBLASTn searches against the assembled *M. martensii* scorpion transcriptome. The ORF Finder online software was used to predict the ORFs of *M. martensii* scorpion genes. Second, the InterProScan (v5.28) (Jones et al. 2014) searches were used in the protein database, in which the PF08395 and PF06151 represent the GRs, while PF02949 represents ORs. For IRs, the PF00060 predicts the S2 and ICD domains, and PF10613 predicts the S1 of the LBD (Finn et al. 2016). Transmembrane domain predictions were processed using TMHMM v2.0 (Krogh et al. 2001) and deeptmhmm programs. For GRs, sequences shorter than 300aa were removed, and sequences with both predicted TM values less than 5 or greater than 8 were also discarded in this work. However, computational predictions of transmembrane domains may exhibit systematic biases, such as limited accuracy in identifying atypical regions and misclassification of unusually long or short hydrophobic segments (Benton et al. 2020).

2.4 | Analysis of Differentially Expressed Genes

Differential expression genes (DEGs) of scorpion chemosensory genes were analyzed using edgeR software, with filtering criteria $|\text{Log2FC}| > 1$, adjusted p value < 0.05, and FDR < 0.05. The DEG heatmap for different parts of third-instar and adult scorpions was plotted using the R heatmap package.

2.5 | Phylogenetic Analyses

Prior to phylogenetic tree reconstruction, we filtered the GR sequences from all target species by removing those shorter than 300 amino acids to ensure sequence quality for the subsequent evolutionary analysis. For the phylogenetic analysis, amino acid sequences of scorpion chemosensory genes were aligned using the MUSCLE program. The final alignments of scorpion chemosensory genes were trimmed with TRIMAL v1.4 (Capella-Gutiérrez et al. 2009), employing automatic trimming. The best substitution model was determined using ModelFinder (Kalyaanamoorthy et al. 2017), and maximum likelihood phylogenetic trees were constructed with IQ-Tree (Nguyen et al. 2015; Trifinopoulos et al. 2016) using 1000 bootstraps. Tree figures of scorpion chemosensory genes were generated using FIGTREE v1.4.

2.6 | qPCR

Primers for PCR were designed using Primer3 and purchased from TsingkeBiotechnology. qPCR amplification was carried out using thermal cyclers Mastercycler SXI (Eppendorf AG, Hamburg, Germany) and C1000 Touch (Bio-Rad Laboratories, Hercules, California). The qRT-PCR reaction program comprised an initial denaturation at 95°C for 30 s, followed by 40 cycles of 95°C for 5 s and 60°C for 34 s. Subsequently, a melting curve analysis (55–95°C) was performed to detect single-gene-specific peaks and to examine the presence of primer dimers. All experiments were performed with three independent sample replicates. Gene expression levels were analyzed using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001).

3 | Results

3.1 | Transcriptome Sequence and Assembly

Since the IRs and GRs were found to distribute in the different tissues of various insects (Chen and Dahanukar 2020; Montell 2021), five different tissues, including the head, pedipalps, legs, pectines, and venom glands (Figure 1), were sampled for the transcriptome sequence in the adult *M. martensii*. To investigate the effect of scorpion growth and development on the expression of IRs and GRs genes, the above five corresponding tissues, together with two additional back and abdomen tissues, were collected for the transcriptome sequence in the third-instar scorpions (Figure 1). By using fastp, low-quality reads less than 20 nt in length were trimmed. The quality control statistics were summarized, with the distribution of Q20 (the percentage of bases with an accuracy rate of over 99%) range from 95.27% to 96.63%, while Q30 (the percentage of bases with an accuracy rate of over 99.9%) range from 88.79% to 94.45% (Table S1). Principal component analysis (PCA) showed that the three biological replicates of the different tissues indicated strong reproducibility, which well supported the identification and analysis of candidate scorpion IRs and GRs based on the transcriptomic data (Figure S1).

3.2 | Candidate Chemoreceptor Gene Families

To find candidate scorpion chemoreceptor genes, two strategies were used in this work: one is the tBLASTn search against the assembled *M. martensii* scorpion transcriptome using all the known *D. melanogaster* receptors as the reference sequences, and the other is an InterProScan search of target sequences in the protein database. Among 46 qualified potential GR protein sequences, 4 GRs had PF06151, and 42 GRs had PF08395. The identity values between the GRs of *M. Martensii* and *D. melanogaster* were around 30%, but their TM7 regions appeared highly conserved. For IRs/iGluRs, a total of 80 IRs/iGluRs protein sequences were identified, with 17 protein sequences containing three domains: PF01094 amino-terminal domain (ATD-domain), PF10613 ligand-binding (LBD-domain), and PF00060 ligand-gated channel (LCD-domain). Among them, 51 proteins had both PF10613 and PF00060 domains, which were classified as IRs. Most identified IR sequences showed identities ranging from 20% to 30%, with only one protein having an identity of 55.30% with IR25a in *D. melanogaster*, which was considered a conservative homolog of the IR25a subfamily. Interestingly, no candidate odorant receptors (ORs) were found in the scorpion transcriptomic data. All these candidate GRs and IRs/iGluRs FASTA sequences are provided in File S1.

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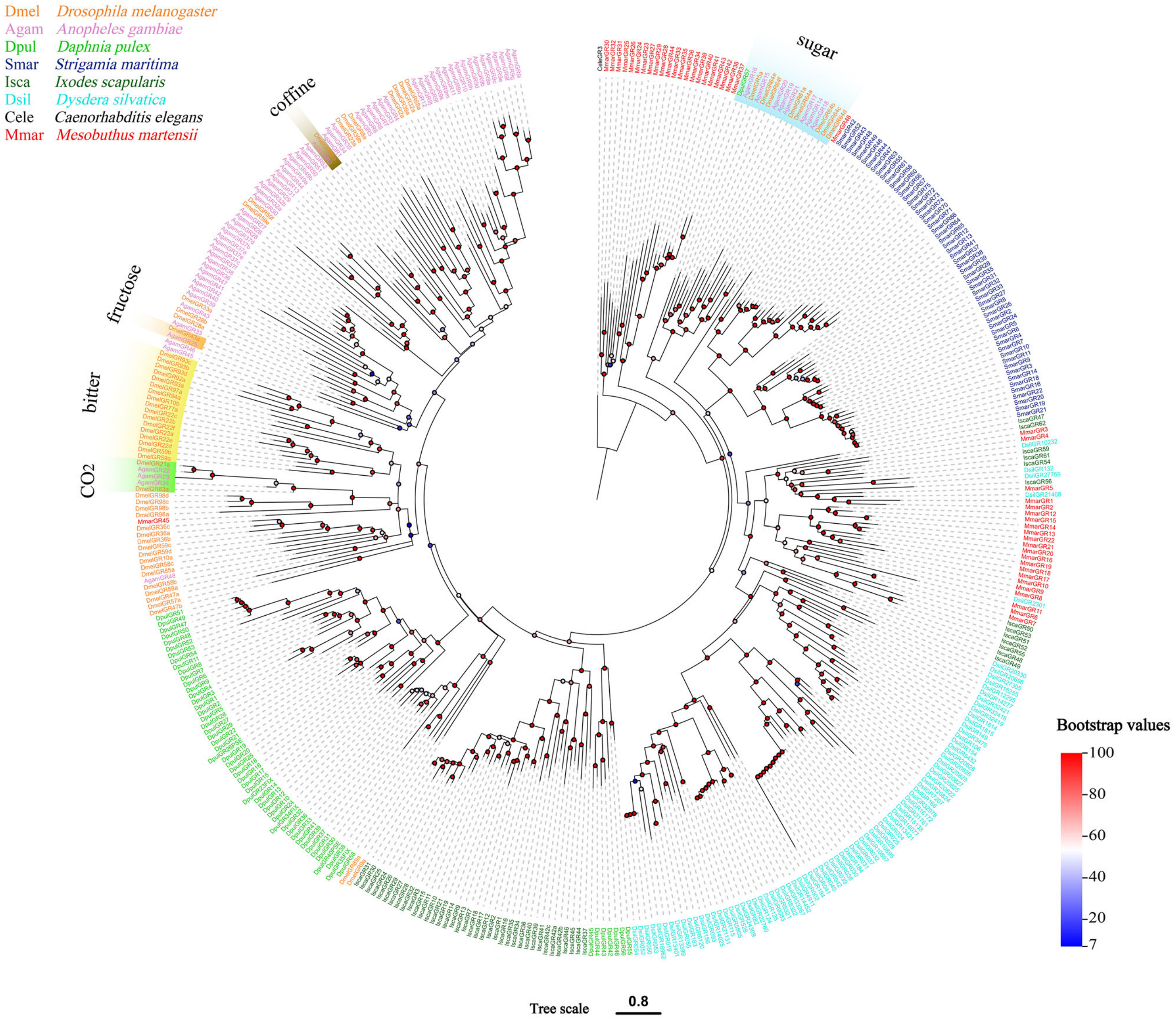

FIGURE 2 | The maximum likelihood phylogenetic tree of GRs. The functionally identified clades were highlighted in colors. The phylogenetic tree was constructed with *Drosophila melanogaster* (Dmel, orange), *Anopheles gambiae* (Agam, purple), *Daphnia pulex* (Dpul, light green), *Ixodes scapularis* (Isca, dark green), *Strigamia maritima* (Smar, dark blue), *Dysdera silvatica* (Dsil, light blue), *Caenorhabditis elegans* (Cele, black), and *Mesobuthus martensii* (Mmar, red), with the JTT+F+R7 model and 1000 bootstrap replications. The bootstrap values of each branch are indicated by the color of the circles, with the colors transitioning from blue to red as the values increase.

3.3 | Phylogenetic Analysis of Scorpion Chemoreceptor Genes

Based on the 46 GRs in the *M. martensii*, a phylogenetic analysis was conducted by incorporating the GRs of *M. martensii* and other representative arthropods (Figure 2 and File S2). Among insect GR lineages, the highly conserved CO_2, sugar, and fructose receptor clades (Robertson 2019) were highlighted in our phylogenetic tree (Figure 2). In *D. melanogaster*, the CO_2 receptor clade comprises Gr21a and Gr63a (Jones et al. 2007; Kwon et al. 2007), while in *Anopheles gambiae*, it consists of Gr22–Gr24 (Hill et al. 2002; Lu et al. 2007). The sugar receptor clade includes eight genes in *D. melanogaster*—Gr5a, Gr61a, and Gr64a–Gr64f (Dahanukar et al. 2001; Slone et al. 2007). The fructose receptor is represented by DmGr43a (Robertson 2019). Notably, none of these three conserved GR clades show clear one-to-one orthology with any GRs identified in the *M. martensii* scorpion. This lack of obvious orthologs is not unexpected, given that these clades are scarcely identifiable even in basal insect lineages such as *Zootermopsis nevadensis* (Terrapon et al. 2014) and *Calopteryx splendens* (Ioannidis et al. 2017). However, GR1–GR22 and GR46 in the *M. martensii* scorpion might be related to sugar detection, as they clustered relatively close to the conserved sugar receptor branch of *Drosophila* (Figure 2). In other non-insect arthropods—including *Daphnia pulex, Ixodes scapularis, Strigamia maritima*, and *Dysdera silvatica*—most GRs form their own distinct branches, which might indicate rapid sequence evolution within the phylum Arthropoda and lineage-specific expansions of GR subfamilies.

For the phylogenetic analysis of IRs/iGluRs, their sequences were obtained from the mollusk *Aplysia californica*, the nematode *Caenorhabditis elegans*, insects within the subclass Pterygota, including *D. melanogaster*, the centipede *S. maritima*, the crus-

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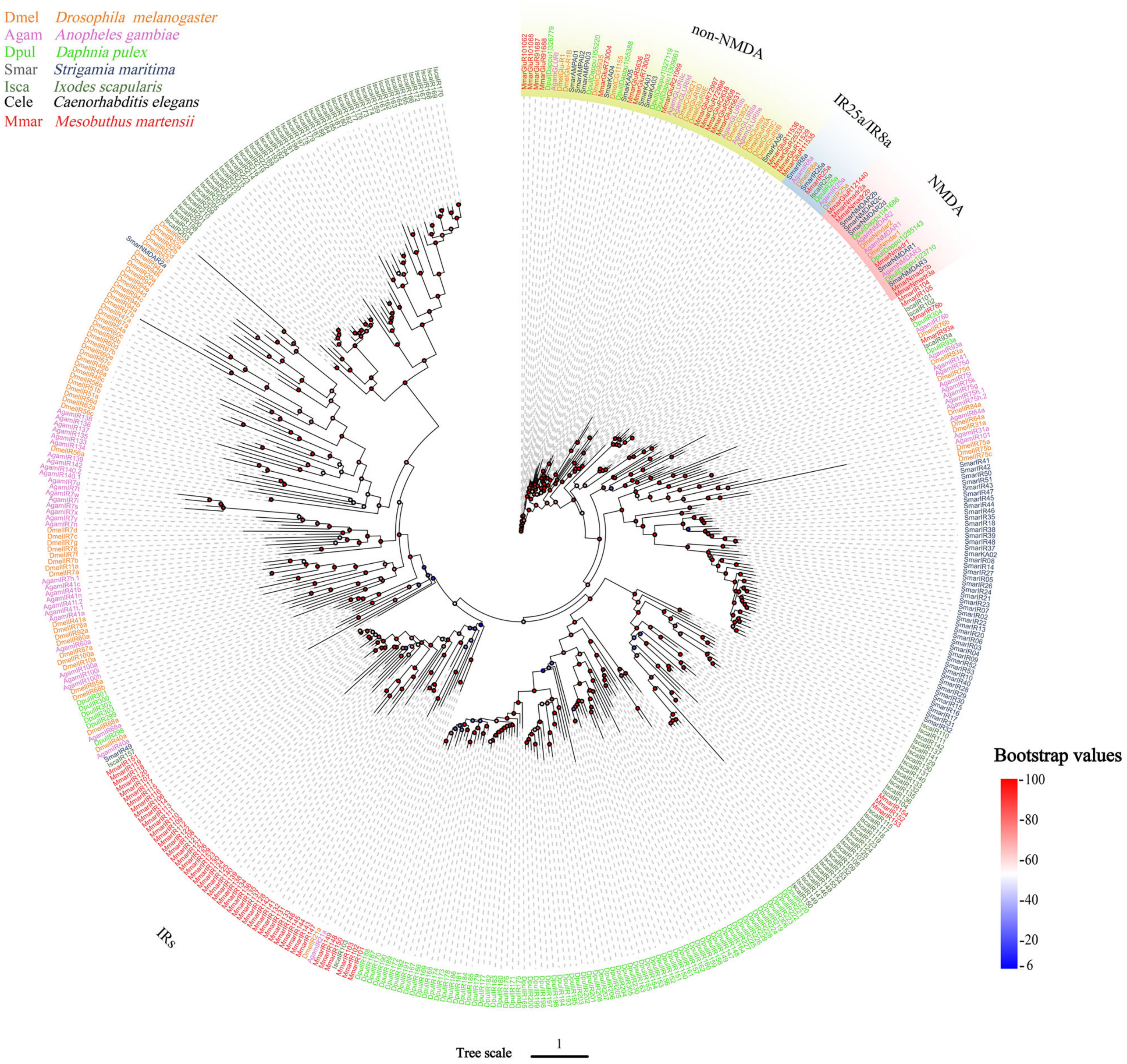

FIGURE 3 | Maximum likelihood phylogenetic tree of the IRs/iGluRs. The deorphaned receptor clades are highlighted in colors. The phylogenetic tree was constructed with *Drosophila melanogaster* (Dmel, orange), *Anopheles gambiae* (Agam, purple), *Daphnia pulex* (Dpul, light green), *Strigamia maritima* (Smar, dark blue), *Ixodes scapularis* (Isca, dark green), *Caenorhabditis elegans* (Cele, black), and *Mesobuthus martensii* (Mmar, red), with the LG+F+R8 model and 1000 bootstrap replications. The bootstrap values of each branch are indicated by the color of the circles, with the colors transitioning from blue to red as the values increase.

tacean *Da. pulex*, and the arachnid *I. scapularis* (Figure 3) and File S3.

IRs represent a highly diversified subfamily of iGluRs, with Drosophila possessing 63 IRs, including four co-receptors (IR8a, IR25a, IR76b, and IR93a) and 59 modulatory receptors (Benton et al. 2009). iGluRs include sequences for both NMDA and non-NMDA receptors (Stern et al. 2010). The *M. martensii* were found to possess major representative IRs/iGluRs subfamilies, including IR25a, IR93a, IR76b, NMDA, and non-NMDA receptors. The IR25a is a receptor found in all investigated arthropods to date, widely expressed as a protein crucial for the functional role of other IRs involved in olfactory and gustatory perception (Eyun et al. 2017). The homolog of IR76b in most arthropods serves as an auxiliary receptor for other IRs and is associated with the taste function of detecting low salt concentrations (Zhang et al. 2013). The presence of IR25a and IR76b in the *M. martensii* further highlighted their irreplaceable functional roles across the arthropods (Figure 3). In *D. melanogaster*, IRs are identified up to IR100a. To avoid confusion with *Drosophila* IRs, we designated newly identified scorpion IR genes starting from IR101 in this work. This naming convention aligned with the approach used by Terrapon et al. (2014) and Hoy et al. (2016). The IRs of these species were overall from the distinct branches, indicating powerful evidence of the high adaptive potential of molecular

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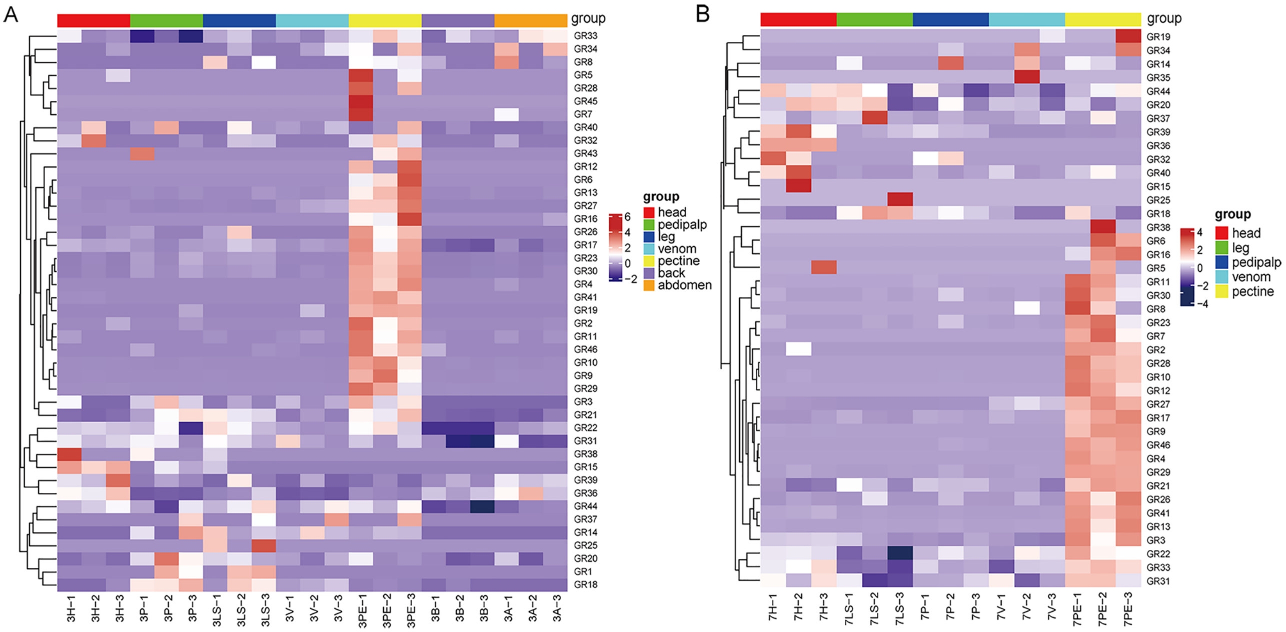

FIGURE 4 | Tissue-specific expression profiles of *Mesobuthus martensii* GR genes. (A) Seven tissues in the third-instar scorpions: heads (H), pedipalps (P), legs (LS), venom (V), pectines (PE), back (B), and abdomen (A). (B) Five tissues in adult scorpions: heads (H), pedipalps (P), legs (LS), venom (V), and pectines (PE). Data are shown as the mean value ($n = 3$).

components in arthropod taste systems (Figure 3; Torres-Oliva et al. 2016).

3.4 | Tissue-Specific Expression Profiles of Scorpion Chemoreceptors

Next, the RNAseq analysis was employed to study the expression patterns of GR genes in different tissues and developmental stages of *M. martensii*. As depicted in the heatmap, a majority of GRs exhibit relatively high expression levels in the pectines from both the third-instar and adult stages (Figure 4). Specifically, out of 46 GR genes, 28 and 27 genes showed high expression in the pectines of young and adult scorpions, respectively. In the pedipalps and legs, the expression of GR genes was slightly higher in third-instar than adult scorpions, suggesting that scorpions likely placed greater importance on perceiving chemical signals through their pedipalps and legs during their juvenile stages than adulthood.

IRs are widely expressed in arthropod olfactory organs, involved in chemical perception and serving as co-receptors for sensing humidity and temperature preferences (Stern et al. 2010). Both young and adult scorpions exhibited the predominant IR expression in the pedipalps, legs, and pectines, whereas most iGluRs showed relatively high expression levels in the head, with some also highly expressed in the pedipalps, legs, and venom glands (Figure 5). Among the identified receptors, IR25a, IR93a, and IR76b were notably expressed at high levels in the pectines (Figure 5), which highlighted their critical importance in the pectines as the chemosensory function (Polis 1990; Sreenivasa-Reddy 1959; Krapf 1986; Gaffin and Brownell 1997). Additionally, some individual IRs were highly expressed in the abdomen of young scorpions, but no IRs showed relatively high expression in the back (Figure 5), which implied that scorpions would also use the abdomen rather than the back to probe the chemical signals.

Except in the pectines, the expression levels and FPKM values of most GRs were notably low (Figure S2). Of the 46 GRs, the FPKM values of only 6 and 8 GRs were more than 10 in the young and adult scorpions, respectively. Most IRs also presented low expression levels, while iGluRs generally showed higher expression levels compared to IRs in the young and adult scorpions (Figure S2).

The expression level and tissue distribution of the chemosensory proteins were found to be generally consistent between the young and adult scorpions. The differential expression analysis based on the transcriptomic data of GRs/IRs/iGluRs in the head, pedipalps, legs, venom glands, and pectines between young and adult scorpions and instars was conducted using the edgeR program. The genes showing $|\log_2 FC| > 1$, $p < 0.05$, and $FDR < 0.05$ are listed in Table S2. It was easily found that many receptor genes themselves exhibited low expression levels. For instance, the average FPKM value of GR36 was 3 and 0 in the venom glands of young and adult scorpions, respectively. The genes with the FPKM differences greater than 30 were bolded in Table 1. Overall, the similar expression trends of chemical sensor genes between the young and adult scorpions suggested that different instar scorpions already possessed a relatively complete chemical perception capability.

3.5 | Quantitative-PCR Analysis for Relative Gene Expression

The expression levels of some representative chemoreceptor genes were further validated by quantitative PCR (qPCR) analysis in this work. The GR36 gene, showing the most significant

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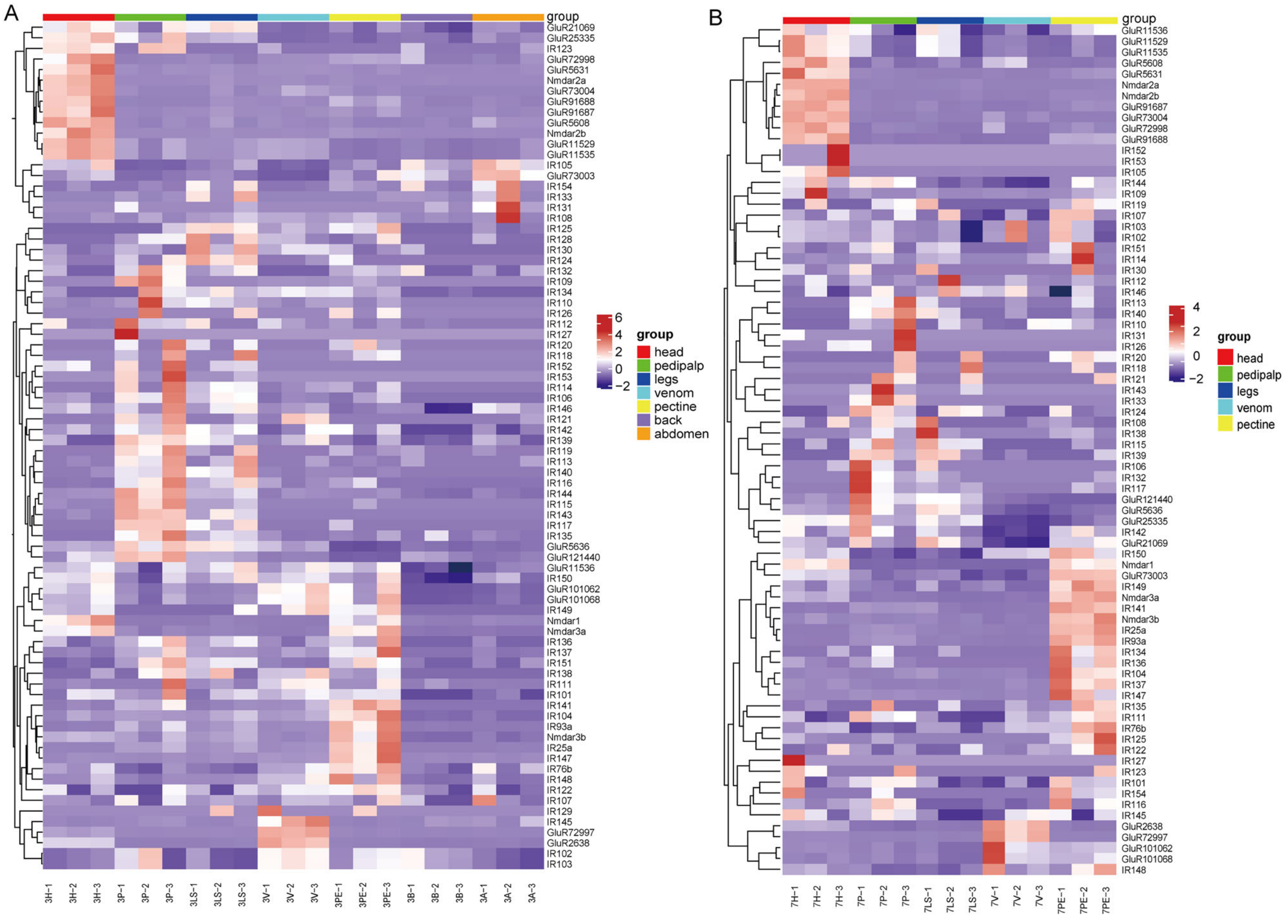

FIGURE 5 | Tissue-specific expression profiling of *Mesobuthus martensii* IRs/iGluRs. (A) Seven tissues in the third-instar scorpions: heads (H), pedipalps (P), legs (LS), venom glands (V), pectines (PE), back (B), and abdomen (A). (B) Five tissues in adult scorpions: heads (H), pedipalps (P), legs (LS), venom glands (V), and pectines (PE). Data are shown as the mean value ($n = 3$).

TABLE 1 | Differentially expressed chemosensory genes based on the FPKM values in the five positions between the third-instar and adult scorpions. The genes with the FPKM differences greater than 30 are in bold.

Tissue	Highly expressed genes in the third-instar scorpions	Highly expressed genes in adult scorpions
Head	iGluR21069, Nmdar2b	iGluR11536
Pedipalps	GR44	GR44, iGluR11536, iGluR11529, iGluR11535
Legs	**GR36, IR105**	GR44, iGluR11529, iGluR11535, iGluR11536, **iGluR121440**, iGluR25335
Venom glands	GR36, iGluR5636, iGluR21069	iGluR11536, iGluR11535, iGluR11529, iGluR72997, iGluR101062
Pectines	**GR36, GR9,** GR10, GR29, GR41, GR30, GR23	GR44, iGluR11535, iGluR11529

difference, and GR9, GR10, and IR25a genes, showing specific and high expression in the pectines, were selected for the qPCR validation. As shown in Figure 6A, the GR36 gene showed high expression in the head, legs, venom gland, and pectines of the third-instar scorpions, whereas it exhibited high expression only in the head of adult scorpions among the five examined tissues. The qPCR results of GR9, GR10, and IR25a genes indicated the high expression in the pectines, which were well consistent with the transcriptomic data (Figure 6B–D), confirming the critical role of pectines in chemical sensing.

4 | Discussion

The chemosensory system is essential for arthropod reproduction and survival, which is well illustrated by the extensive characterization of the chemosensory genes of many insects and non-insect organisms (Montagné et al. 2015; Gulia-Nuss et al. 2016; Zhao et al. 2021; Stern et al. 2010; Colbourne et al. 2011; Chipman et al. 2014). These chemosensory genes usually include GRs and IRs/iGluRs, which are sometimes distributed in the different arthropod tissues (Foelix and Müller-Vorholt 1983; Stocker 1994;

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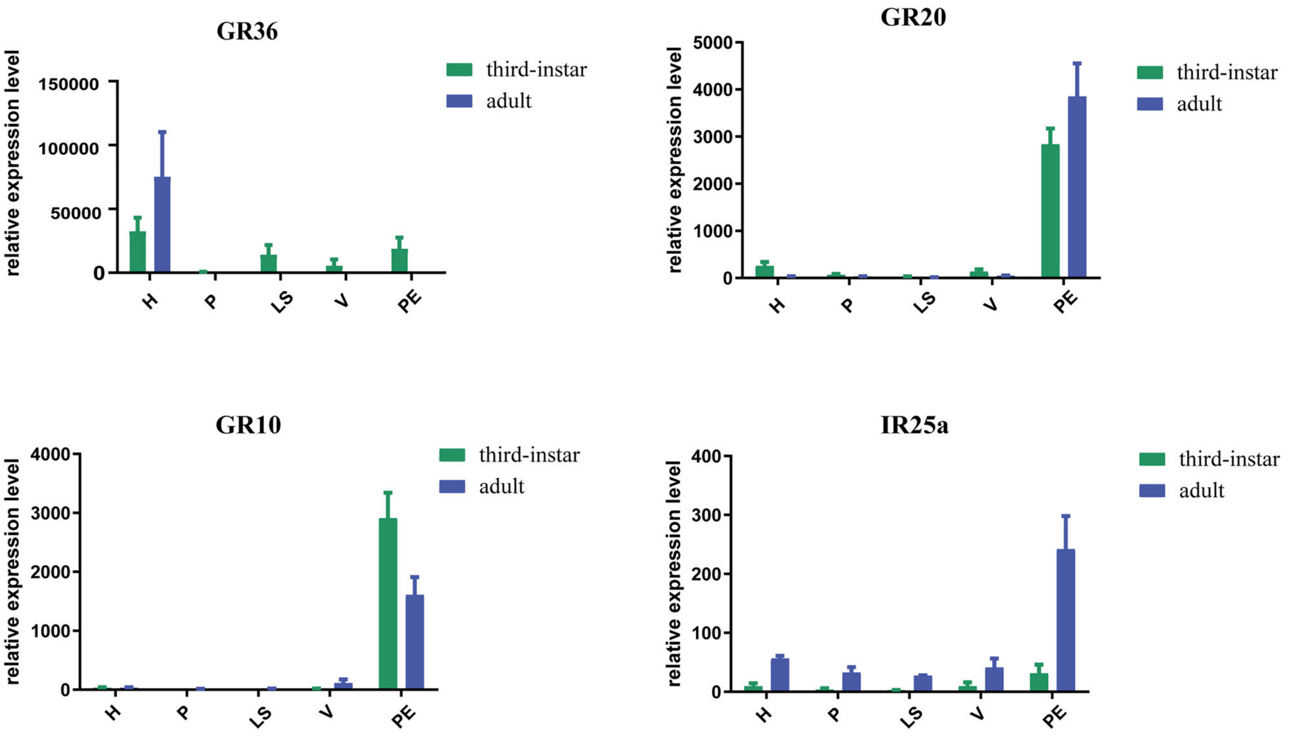

FIGURE 6 | The qPCR validation expression profiles of four genes in different tissues of the third-instar and adult scorpions. The *X*-axis represents five different tissues: heads (H), pedipalps (P), legs (LS), venom glands (V), and pectines (PE). The *Y*-axis shows the mRNA relative expression levels of the target genes. Data are presented as mean ± SEM ($n = 3$).

Edgecomb and Murdock 1992; Newland 1998; de Brito Sanchez et al. 2014; Pelosi 1996; Shanbhag et al. 2001). The scorpion is one of the oldest terrestrial arthropods with the well-known morphological similarity between the Paleozoic and extant scorpions (Waddington et al. 2015). Based on the different scorpion behavior experiments stimulated by the various chemical materials (Gaffin and Brownell 1997; Knowlton and Gaffin 2010), the expression profiles of candidate chemosensory genes of scorpions were first investigated based on the transcriptomic analysis in this work.

Using widely distributed *M. martensii* in China as the experimental materials, 46 candidate GR and 80 candidate IRs/iGluRs proteins were identified based on the transcriptomic data (Table S1), which showed similar to the number of chemosensory genes in *D. melanogaster* (66 GRs and 80 IRs/iGluRs) (Chen and Dahanukar 2020), *S. maritima* (77 GRs and 69 IRs/iGluRs) (Chipman et al. 2014), and *Da. pulex* (58 GRs and 93 IRs/iGluRs) (Kelly et al. 2023). Using an exhaustive search strategy, thousands of chemosensory genes and massive changes in chemoreceptor repertories across chelicerates were revealed based on the comparative genomics, and hundreds of chemosensory sequences were found in *C. exilicauda* and *M. martensii* scorpions (Vizueta et al. 2018), which suggested the potential effect of different methods on the exploring chemosensory sequences.

The GRs of scorpions and the conserved carbon dioxide- and sugar-sensing subfamilies in insects showed no detectable homologous sequences, with extremely low sequence similarity (e.g., only 8% amino acid identity with *D. melanogaster* GR proteins; Figure 2). This pattern of "functional conservation coupled with sequence divergence" suggested that adaptive diversification within the phylum Arthropoda has driven rapid sequence evolution. Furthermore, it implies that the GR repertoire in chelicerates has evolved independently, forming a monophyletic clade distinct from other arthropods (Figure 2). Besides the GRs, *M. martensii* possessed IR25a, IR93a, IR76b, NMDA, and non-NMDA receptors, which were widely distributed in other arthropods, such as *A. gambiae*, *Ap. californica*, and *D. melanogaster* (Figure 3; Stern et al. 2010). Notably, IR25a, the sole antennal IR of protostome origin, was conserved across all arthropods (including scorpions) and played a central role in olfactory and gustatory signal transduction (Joseph and Carlson 2015). Similarly, IR93a and IR76b in *M. martensii* were widely retained in arthropods for possible salt and humidity sensing (Zhang et al. 2013). The iGluRs, evolutionarily conserved across arthropods, originally functioned as neurotransmitter receptors but have acquired chemosensory roles in some lineages. In insects, the glutamate-gated chloride channel (GluCl) gene is known to be a primary molecular target for avermectin (Wang et al. 2023; Meyers et al. 2015). In scorpions, iGluRs might participate in peripheral chemical sensing.

Based on these *M. martensii* scorpion chemosensory genes, high expression levels of GRs and IRs/IGluRs were found in the pectines in both the third-instar and adult scorpions (Figures 4 and 5), which well confirmed the critical importance in the pectines as the chemosensory function from the behavior experiments (Polis 1990; Sreenivasa-Reddy 1959; Krapf 1986; Gaffin and Brownell 1997). Similar expression trends of chemical sensor genes in the different tissues between the young and adult scorpions further indicated that different instar scorpions already possessed a relatively complete chemical perception capability-specific gene expression profiles for their survival (Figures 4–6; Table 1).

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Most arthropods possess taste receptors on their legs and mouthparts (Foelix and Müller-Vorholt 1983; Stocker 1994; Edgecomb and Murdock 1992; Newland 1998; de Brito Sanchez et al. 2014), while olfactory receptors are typically distributed to varying degrees on appendages (Wolf 2017). In *D. melanogaster*, olfactory receptors are concentrated on the antennae and maxillary palp, whereas taste receptors are distributed across various body parts such as the proboscis, legs, and the leading edge of wings (Pelosi 1996; Shanbhag et al. 2001). In *M. martensii*, GRs are predominantly present in the pectines, followed by the head, and much fewer receptors are distributed in the pedipalps and legs (Figure 4). This feature suggested a potentially diminished gustatory function in the mouthparts of scorpions and might retain minimal GR-mediated taste perception. Regarding IRs/iGluRs, their distribution in the back, abdomen, and venom glands of scorpions at the third-instar was nearly negligible, whereas they were more abundant in the head, pedipalps, legs, and pectines, similar to IRs distribution in *D. melanogaster* (Chen and Dahanukar 2020). Such a distribution feature indicated that they likely served various functions, including perception of acids, salts, aldehydes, amines, temperature, humidity, and tastes.

Due to the slow research progress on the chemosensory genes of the chelicerates, the functions of many GRs and IRs/iGluRs remain unclear in *M. martensii* (Figures 2 and 3). These findings indicated the highly dynamic evolution of these receptors, which likely suggested the adaptive potential of molecular components in arthropod chemosensory systems (Torres-Oliva et al. 2016).

Finally, the pectines emerged as an organ with the highest expression of chemosensory-related genes (GRs, IRs, iGluRs), which was well consistent with previous behavior investigations. The pectines in scorpions are specialized appendages located on the abdomen with the many "teeth," and each tooth contained approximately 10 chemosensory neurons (Figure 1; Gaffin 2021), which were found to be able to exhibit the distinct electrophysiological responses to water, acids, EtOH, and KCl soluble substances, respectively (Knowlton and Gaffin 2010). They also responded to close-range olfactory stimuli, eliciting compound discharges in three types of sensory neurons (A, B, and C cells) in response to different categories of chemicals (aldehydes, alcohols, ketones, and esters; Knowlton and Gaffin 2010). Our findings suggested that pectines likely play a predominant role in detecting chemical molecules as well as environmental variables such as humidity and temperature. Further behavioral studies and experiments at the molecular level are needed to validate these findings.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All data used in this research are publicly available (sources provided in the Methods). The fish catch data are available from the Iranian Fisheries Organization.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

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