Identification of Candidate Olfactory Genes in *Scolytus schevyrewi* Based on Transcriptomic Analysis

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The bark beetle, *Scolytus schevyrewi* (*S. schevyrewi*), is an economically important pest in China that causes serious damage to the fruit industry, particularly, in Xinjiang Province. Chemical signals play an important role in the behavior of most insects, accordingly, ecofriendly traps can be used to monitor and control the target pests in agriculture. In order to lay a foundation for future research on chemical communication mechanisms at the molecular level, we generate antennal transcriptome databases for male and female *S. schevyrewi* using RNA sequencing (RNA-seq) analysis. By assembling and analyzing the adult male and female antennal transcriptomes, we identified 47 odorant receptors (ORs), 22 ionotropic receptors (IRs), 22 odorant-binding proteins (OBPs), and 11 chemosensory proteins (CSPs). Furthermore, expression levels of all the candidate OBPs and CSPs were validated in different tissues of male and female adults by semiquantitative reverse transcription PCR (RT-PCR). ScosOBP2 and ScosOBP18 were highly expressed in female antennae. ScosCSP2, ScosCSP3, and ScosCSP5 were specifically expressed in the antennae of both males and females. These results provide new potential molecular targets to inform and improve future management strategies of *S. schevyrewi*.

Keywords: *Scolytus schevyrewi*, transcriptome, olfactory genes, expression analysis, antennae

INTRODUCTION

Olfaction serves to detect environmental chemical information necessary for insect behavior such as finding food sources, mates, and oviposition sites (Hanson, 1999; Clyne et al., 2000). Insects have a sophisticated olfactory system that begins with the reception of odorants at the peripheral chemosensory system. Insect olfaction is dependent on olfactory receptor neurons (ORNs) in sensilla (Leal, 2013) distributed mainly in antennae and also in maxillary palps or labial palps (Stoker et al., 1990). The research of molecular mechanisms of olfactory reception in insects has predominantly been in the model organism *Drosophila melanogaster*. These studies have shown diverse olfactory genes encoding proteins, such as odorant receptors (ORs), ionotropic receptors (IRs), odorant-binding proteins (OBPs), and chemosensory proteins (CSPs), involved in different chemical signal transduction processes (Benton et al., 2009; Wilson, 2013; Xiao et al., 2019).

Odorant receptors play a critical role in recognizing thousands of odor molecules in the insect olfactory system. Insect ORs were first identified in *Drosophila* which has the characteristic feature...
of a seven-transmembrane domain (TMD) structure that is unrelated to the ORs in vertebrates (Clyne et al., 1999; Benton et al., 2006). Every ORN can express a single or two OR genes (Vosshall and Hanson, 2011). Specificity of OR relies on the ligand-banding ORs (Dobrissa et al., 2003; Elmore et al., 2003; Hallem et al., 2004), while Orco functions as an obligatory chaperon for the Orco-OR complex (Larsson et al., 2004; Benton et al., 2006; Stengl, 2017).

Evolved from the ionotropic glutamate receptor superfamily, IRs have been shown to be involved in odor reception. They are expressed in the sensory neurons that respond to many distinct odors, such as acids, amines, and other chemicals that cannot be recognized by ORs (Benton et al., 2006). Aside from olfaction, IRs serve various functions, such as cool sensation (Ni et al., 2015), hygrosensation (Knecht et al., 2016), circadian clock (Chen et al., 2015), and detection of carbon dioxide (CO₂) (Breugel et al., 2018).

In addition to ORs and IRs, other multigene families encode proteins that also play critical roles in olfaction. OBPs are small soluble proteins secreted in the sensillar lymph. They are characterized by an N-terminal signal peptide sequence and a set of six conserved cysteine residues that form three disulfide bridges (Pelosi et al., 2005, 2006). Studies of defective mutants and wild-type counterparts of OBP76a (also known as LUSH) in Drosophila have shown that this protein has a key role in the perception of alcohol and 11-cis vaccenyl acetate (Kim et al., 1998; Xu et al., 2005; Gomez-Diaz et al., 2013). OBPs have also been reported as a pheromone-binding protein in Lepidoptera (Jing et al., 2019). Some OBPs operate similar to LUSH in response to pheromones. In vivo studies have shown that OBPs significantly affect pheromone perception in moths. Knocking out these OBPs significantly reduced electrophysiological responses to pheromones in several species, such as Helicoverpa armigera (Ye et al., 2017), Spodoptera litura (Liu et al., 2012; Zhu et al., 2016a), and Chilo suppressalis (Dong et al., 2017).

Chemosensory proteins are also small soluble proteins but are shorter in amino acid sequence length than that of OBPs, and CSPs share the same structure of having four conserved cysteines forming two disulfide bridges (Pelosi et al., 2005, 2006; Honson et al., 2015). As semiochemical carriers, some CSPs are involved in chemodetection (Pelosi et al., 2018; Li et al., 2021) because CSPs are abundant in the lymph of chemosensory hairs (Angeli et al., 1999; Jacquin-Joly et al., 2001; Monteforti et al., 2002; Sun et al., 2014). Some of them already have been reported to function such as OBPs, e.g., CSP3 of the honeybee, which specially binds some components of brood pheromone (Briand et al., 2002).

Bark beetles (Coleoptera; Curculionidae; Scolytinae) feed on woods and several of them pose serious threats to forestry, e.g., Ips typographus (Wermelinger, 2004), Dendroctonus ponderosae (Andersson et al., 2013). Since their host-finding relies on chemical communication, e.g., aggregation behavior based on male-produced pheromone (Schlyter et al., 1987), pheromone-based technique could be used for the detection and control of this pest. In order to develop this technique efficiently, one way is to exploit olfactory genes that are critical for successful mate and host finding. Transcriptomic and genomics studies have been performed for searching olfactory genes in bark beetles (Andersson et al., 2013, 2019; Mitchell et al., 2019), and functional studies were limited to only seven ORs (Hou et al., 2021; Yuvaraj et al., 2021). Scolytus schevyrewi (S. schevyrewi) (Cleoptera: Scolyliidae) is one of the most destructive insect pests of fruit trees in China. It has a wide host range and has been reported to attack several families of trees in Xinjiang province (Li et al., 1995). Several studies have focused on the identification and field bioassay of chemical attractants in the bark beetle (Fan et al., 2014). In order to provide a molecular basis for gene targets for putative chemical lures of this pest, we performed Illumina Hiseq 2000 sequencing of the transcriptome of adult male and female antennae samples.

MATERIALS AND METHODS

Insect Rearing and Tissue Collection

Scolytus schevyrewi larvae were reared on the branches of their host plants (Armeniaca vulgaris) collected from Baren County, Xinjiang province, China (39.0°N, 75.8°E) and maintained in the lab under the following conditions of 26.5 °C, a cycle of 14-h light:10-h dark, and 65% relative humidity. Pupae were placed on a branch and the emerged adults were collected every day. Two-day-old adults were used to collect male and female antennae, heads (without antennae), thorax, abdomen, legs, and wings using the fine-tip forceps, immediately frozen in liquid nitrogen and stored at −80 °C until RNA isolation.

RNA Extraction

Total RNA from different tissues of S. schevyrewi was obtained using TRIZol reagent (Invitrogen, Carlsbad, California, USA) following the instruction of manufacturer. The total RNA from each pair of antennae, legs, and wings were separately obtained from each adult, totaling 300 males and 300 females. Heads (without antennae), thoraxes, and abdomens were separately collected from 20 to 30 adult males and 20 to 30 females. Total RNA was dissolved in RNase-free water, and RNA integrity was verified by gel electrophoresis. RNA concentration and purity were determined on the Nanodrop ND-2000 spectrophotometer (NanoDrop products, Wilmington, DE, USA).

cDNA Library Construction and Sequencing

A total of 1 μg of total RNA of each sample of male and female antennae were used to construct two separate cDNA libraries, one for each sex. Paired-end reads of 100 bp were sequenced using the Illumina HiSeq 2000 platform to obtain library-sequencing information at Beijing Genome Institute (Shenzhen, China). The detailed protocols for cDNA library construction and sequencing applied have been described in the previous studies (Cao et al., 2014; Zhang et al., 2015). The raw data were uploaded to the NCBI SRA database (Accession: PRJNA732801, https://www.ncbi.nlm.nih.gov/sra).

Assembly

Low-quality reads were filtered out, low-quality nucleotides at each end were trimmed, and 3′ adapters and poly-A/T tails were trimmed.
Identification of Olfactory Genes

Unigenes were annotated using blastx against NCBI nonredundant (nr) sequences with $e < 1e^{-5}$. The blast results were then imported into the Blast2Go (version 3.1) with default parameters (Conesa et al., 2005). OR, IR, OBP, and CSP genes of the candidates were selected according to the nr sequence annotation results in the remote server from the lab. All candidate olfactory genes were manually checked using the blastx program against the nr sequence database. The open-reading frames (ORFs) of the putative olfactory genes were predicted using the ExPASy (Expert Protein Analysis System) translate tool (https://web.expasy.org/translate/). The TMDs of ORs and IRs were predicted using TMHMM server version 2.0 (http://www.cbs.dtu.dk/services/TMHMM). Putative N-terminal signal peptides of OBPs and CSPs were predicted using the SignalP 4.0 server (http://www.cbs.dtu.dk/services/SignalP-4.0/) with default parameters.

Phylogenetic Analysis

Olfactory genes from S. schevyrewi, Ips typographus, Dendroctonus ponderosae (Andersson et al., 2013), and Holotrichia parallela (Yi et al., 2018) were selected for the phylogenetic analysis. Sequence information was listed in Supplementary Table 2. Amino acid sequences were aligned by MAFFT (https://www.ebi.ac.uk/Tools/msa/mafft/). Phylogenetic trees of olfactory genes were constructed using RAxML version 8 with the Jones-Taylor-Thornton amino acid substitution model. Node support was assessed using a bootstrap method based on
### TABLE 1 | Unigenes of candidate odorant receptors.

| Unigene reference | Gene name | Length (bp) | ORF(aa) | Blastx best hit (Reference/Name/Species) | E-value | Full length | TMD |
|--------------------|-----------|-------------|---------|------------------------------------------|---------|-------------|-----|
| CL839.Contig1_All  | ScosOR1   | 735         | 244     | XP_019768579.1 PREDICTED: odorant receptor 94a-like [Dendroctonus ponderosae] | 1.00E-129 | No 3       |
| CL839.Contig2_All  | ScosOR2   | 1,143       | 380     | XP_019768579.1 PREDICTED: odorant receptor 94a-like [Dendroctonus ponderosae] | 2.00E-144 | Yes 6      |
| CL1001.Contig1_All | ScosOR3   | 1,251       | 416     | XP_019754377.1 PREDICTED: putative odorant receptor 92a [Dendroctonus ponderosae] | 5.00E-92 | No 6       |
| CL1001.Contig2_All | ScosOR4   | 1,365       | 454     | XP_019762540.1 PREDICTED: odorant receptor 49b-like [Dendroctonus ponderosae] | 4.00E-92 | No 7       |
| CL1025.Contig1_All | ScosOR5   | 1,173       | 390     | XP_019765587.1 PREDICTED: odorant receptor Or2-like [Dendroctonus ponderosae] | 5.00E-57 | No 5       |
| CL1025.Contig2_All | ScosOR6   | 996         | 331     | XP_019765587.1 PREDICTED: odorant receptor Or2-like [Dendroctonus ponderosae] | 3.00E-45 | No 4       |
| CL1127.Contig1_All | ScosOR7   | 1,131       | 376     | XP_019762033.1 PREDICTED: odorant receptor 4-like isoform X2 [Dendroctonus ponderosae] | 4.00E-133 | Yes 5      |
| CL1127.Contig2_All | ScosOR8   | 1,155       | 384     | XP_019762033.1 PREDICTED: odorant receptor 4-like isoform X2 [Dendroctonus ponderosae] | 2.00E-144 | Yes 6      |
| CL1127.Contig4_All | ScosOR9   | 1,149       | 382     | XP_019762033.1 PREDICTED: odorant receptor 4-like isoform X2 [Dendroctonus ponderosae] | 5.00E-144 | Yes 6      |
| CL1283.Contig1_All | ScosOR10  | 1,188       | 395     | XP_019755291.1 PREDICTED: odorant receptor 47b-like [Dendroctonus ponderosae] | 4.00E-63 | No 4       |
| CL1283.Contig2_All | ScosOR11  | 774         | 257     | AKK25156.1 odorant receptor 15 [Dendroctonus ponderosae] | 3.00E-64 | No 4       |
| CL1562.Contig1_All | ScosOR12  | 891         | 296     | XP_018564120.1 PREDICTED: odorant receptor 85b-like [Anoplophora glabripennis] | 6.00E-19 | No 6       |
| CL1562.Contig2_All | ScosOR13  | 1,156       | 364     | XP_018564120.1 PREDICTED: odorant receptor 85b-like [Anoplophora glabripennis] | 2.00E-33 | No 7       |
| CL2075.Contig3_All | ScosOR14  | 1,455       | 484     | ALR72569.1 odorant receptor OR26 [Colaphellus bowringi] | 2.00E-57 | No 7       |
| CL2243.Contig2_All | ScosOR15  | 1,191       | 396     | XP_019754447.1 PREDICTED: odorant receptor 49b-like [Dendroctonus ponderosae] | 9.00E-59 | No 7       |
| CL2311.Contig1_All | ScosOR16  | 1,137       | 378     | XP_019770928.1 PREDICTED: odorant receptor 4a-like isoform X2 [Dendroctonus ponderosae] | 6.00E-127 | Yes 7      |
| CL2593.Contig1_All | ScosOR17  | 1,173       | 390     | AKK25156.1 odorant receptor 15 [Dendroctonus ponderosae] | 1.00E-20 | No 5       |
| CL2643.Contig2_All | ScosOR18  | 1,176       | 391     | XP_019874891.1 PREDICTED: odorant receptor 67c-like [Aethina tumida] | 3.00E-27 | No 6       |
| CL2759.Contig1_All | ScosOR19  | 377         | 125     | XP_019768086.1 PREDICTED: odorant receptor Or2-like [Dendroctonus ponderosae] | 1.00E-29 | No 2       |
| CL2885.Contig1_All | ScosOR20  | 1,206       | 401     | XP_019771895.1 PREDICTED: odorant receptor 67c-like, partial [Dendroctonus ponderosae] | 3.00E-106 | Yes 7      |
| CL3312.Contig1_All | ScosOR21  | 1,176       | 391     | XP_018567969.1 PREDICTED: odorant receptor Or2-like [Anoplophora glabripennis] | 4.00E-54 | Yes 6      |
| Unigene7_All       | ScosOR22  | 1,179       | 392     | XP_019772797.1 PREDICTED: odorant receptor Or2-like [Dendroctonus ponderosae] | 3.00E-18 | No 7       |
| Unigene529_All     | ScosOR23  | 367         | 122     | AKK25157.1 odorant receptor 17, partial [Dendroctonus ponderosae] | 6.00E-15 | No 2       |
| Unigene2373_All    | ScosOR24  | 1,167       | 388     | XP_019761187.1 PREDICTED: odorant receptor Or2-like [Dendroctonus ponderosae] | 8.00E-75 | Yes 4      |
| Unigene2403_All    | ScosOR25  | 1,179       | 424     | XP_019753281.1 PREDICTED: odorant receptor 47b-like [Dendroctonus ponderosae] | 5.00E-92 | Yes 6      |
| Unigene3424_All    | ScosOR26  | 1,170       | 389     | XP_019761187.1 PREDICTED: odorant receptor Or2-like [Dendroctonus ponderosae] | 4.00E-199 | Yes 6      |
| Unigene3466_All    | ScosOR27  | 1,134       | 377     | XP_019770928.1 PREDICTED: odorant receptor 4a-like isoform X2 [Dendroctonus ponderosae] | 1.00E-138 | No 7       |
| Unigene3624_All    | ScosOR28  | 1,179       | 392     | AKK25156.1 odorant receptor 15 [Dendroctonus ponderosae] | 9.00E-72 | No 5       |

(Continued)
TABLE 1 | Continued

| Unigene reference | Gene name | Length (bp) | ORF(aa) | Blastx best hit (Reference/Name/Species) | E-value | Full length | TMD |
|-------------------|-----------|-------------|---------|----------------------------------------|---------|-------------|-----|
| Unigene3644_All   | ScosOR29  | 1,176       | 391     | XP_019756949.1 PREDICTED: odorant receptor 67c-like isofrm X2 [Dendroctonus ponderosae] | 3.00E-119 | Yes         | 7   |
| Unigene4009_All   | ScosOR30  | 1,185       | 394     | XP_018571501.1 PREDICTED: odorant receptor 67c-like [Anoplophora glabripennis] | 1.00E-49  | No          | 6   |
| Unigene6079_All   | ScosOR31  | 1,122       | 373     | XP_019768655.1 PREDICTED: odorant receptor 49b-like [Dendroctonus ponderosae] | 1.00E-23  | No          | 3   |
| Unigene7306_All   | ScosOR32  | 1,188       | 395     | XP_019768655.1 PREDICTED: odorant receptor 49b-like [Dendroctonus ponderosae] | 1.00E-26  | No          | 6   |
| Unigene8744_All   | ScosOR33  | 372         | 123     | XP_019771464.1 PREDICTED: odorant receptor 49b-like isofrm X3 [Dendroctonus ponderosae] | 3.00E-31  | No          | 0   |
| Unigene9796_All   | ScosOR34  | 1,160       | 385     | XP_01975672.1 PREDICTED: odorant receptor 4 [Dendroctonus ponderosae] | 7.00E-133 | Yes         | 6   |
| Unigene10776_All  | ScosOR35  | 1,158       | 385     | XP_019759347.1 PREDICTED: odorant receptor 30a-like [Dendroctonus ponderosae] | 8.00E-24  | No          | 6   |
| Unigene12156_All  | ScosOrco  | 1,467       | 488     | ACO03528.3 olfactory co-receptor [Rhynchophorus ferrugineus] | 0.00E+00  | Yes         | 7   |
| Unigene12163_All  | ScosOR36  | 1,184       | 393     | XP_015836240.1 PREDICTED: odorant receptor 49b [Tribolium castaneum] | 1.00E-114 | No          | 6   |
| Unigene12204_All  | ScosOR37  | 1,134       | 378     | XP_019874691.1 PREDICTED: odorant receptor 67c-like [Aethina tumida] | 2.00E-24  | No          | 5   |
| CL90.Contig6_All  | ScosOR38  | 1,236       | 411     | XP_019768012.1 PREDICTED: odorant receptor 83a-like isofrm X2 [Dendroctonus ponderosae] | 2.00E-170 | No          | 6   |
| CL90.Contig22_All | ScosOR39  | 927         | 308     | XP_019768012.1 PREDICTED: odorant receptor 83a-like isofrm X2 [Dendroctonus ponderosae] | 7.00E-82  | No          | 2   |
| Unigene17267_All  | ScosOR40  | 1,041       | 346     | AKK25154.1 odorant receptor 7, partial [Dendroctonus ponderosae] | 9.00E-08  | No          | 2   |
| Unigene18514_All  | ScosOR41  | 370         | 123     | XP_019768655.1 PREDICTED: odorant receptor 49b-like [Dendroctonus ponderosae] | 1.00E-37  | No          | 1   |
| CL110.Contig4_All | ScosOR42  | 900         | 299     | EFA01416.1 odorant receptor 283 [Tribolium castaneum] | 1.00E-06  | No          | 4   |
| CL110.Contig8_All | ScosOR43  | 825         | 273     | EFA01423.1 odorant receptor 293 [Tribolium castaneum] | 4.60E+00  | No          | 4   |
| CL548.Contig1_All | ScosOR44  | 1,161       | 386     | XP_015837918.1 PREDICTED: putative odorant receptor 85d [Tribolium castaneum] | 7.00E-26  | No          | 7   |
| CL584.Contig4_All | ScosOR45  | 1,179       | 392     | XP_019772797.1 PREDICTED: odorant receptor Or2-like [Dendroctonus ponderosae] | 2.00E-18  | No          | 8   |
| CL733.Contig1_All | ScosOR46  | 1,176       | 391     | XP_019768655.1 PREDICTED: odorant receptor 49b-like [Dendroctonus ponderosae] | 6.00E-25  | No          | 4   |

1,000 replicates. The trees were visualized, and color-coded in FigTree 1.4.3. For ORs, the tree was rooted in the Orco lineage.

Expression Analysis of the Candidate OBPs and CSPs by Semiquantitative Reverse Transcription PCR (RT-PCR)

Reverse transcription-PCR was performed to verify the expression patterns of OBPs and CSPs of S. schevyrewi. Total RNA from male and female antennae, heads (without antennae), thoraxes, legs, abdomens, and wings were used to synthesize cDNA by RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, Waltham, MA, USA). Gene-specific primers were designed using Primer 5 and synthesized by Sangon Biotech Co., Ltd. (Shanghai, China) (Supplementary Table 1). PCR was performed with the Veriti Thermal Cycler (Applied Biosystems, Carlsbad, CA, USA) under the following conditions: 95°C for 3 min, 25 cycles at 95°C for 30 s, 55°C for 30 s, 72°C for 30 s, and 72°C for 10 min. PCR amplification products were run on a 2% agarose gel. Because it is difficult to acquire massive amounts of RNA from antennae samples of S. schevyrewi, only two technical repeats were performed for each gene. Uncropped gel images were uploaded as supplements (Supplementary Figure 2).

RESULTS

Transcriptome Assembly

The transcriptomes of male and female S. schevyrewi antennae were separately sequenced by the Illumina HiSeq 2000 platform.
Then after filtering, 26,804,894 and 29,176,485 clean reads with 98.60 and 98.55% Q20 scores were generated for male and female samples, respectively. The clean reads were assembled subsequently and generated 40,666 and 36,216 unigenes, respectively. After merging and clustering, a final transcript dataset was revealed with 34,098 unigenes consisting of 14,071 clusters and 20,027 distinct singletons. The dataset was 46.7~57.4 Mb in size and with unigenes having a mean length of 1,684 bp and N50 of 3,179 bp.

**Gene Identification and Functional Annotation**

The functional annotations of the unigenes were performed mainly based on the blastx results against the nr sequence database. We matched 22,815 (66.9%) unigenes to known proteins by blastx. Among those annotated genes, 16,725 (73.3%) unigenes showed strong homology (e-values lower than 1e−45), while 6,090 (26.7%) unigenes showed poor matches with e-values between 1e−15 and 1e−5. The similarity analysis showed that 11,514 (50.5%) unigenes had more than 60% similarity with known proteins. Most of the annotated unigenes were matched to *Tribolium castaneum* (67.3%), followed by *D. ponderosae* (13.7%) and others species (19.0%).

Gene ontology (GO) annotations of the entire set of unigenes were performed using the Blast2GO pipeline based on the blastx searches against nr sequences. A total of 12,720 unigenes were assigned various GO terms. In the molecular function category, genes involved in the binding activity and catalytic activity were most abundant. In the cellular component category, genes involved in cell, cell part, macromolecular, membrane, organelle, and organelle part were enriched. In the biological process category, genes involved in the cellular process, metabolic process and single-organism process were the most represented.

**Identification of Candidate Odorant Receptors**

The candidate ORs were identified by keyword search of the blastx annotations. We identified 47 putative OR genes. Thirteen

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**FIGURE 2** | Phylogenetic tree of candidate ScosIRs with ionotropic receptor sequences from other insects. Tcas, *T. castaneum*; Dpon, *D. ponderosae*; Hpar, *H. parallela*; Ityp, *I. typographus*; Dmel, *D. melanogaster*; Scos, *S. schevyrewi*.
| Unigene reference | Gene name | Length (bp) | ORF(aa) | Blastx best hit (Reference/Name/Species) | E-value | Full length | TMD |
|-------------------|-----------|-------------|---------|------------------------------------------|---------|-------------|-----|
| Unigene10667_All  | ScosIR1   | 1,497       | 498     | XP_019865961.1 PREDICTED: LOW QUALITY PROTEIN: glutamate receptor 2-like [Aethina tumida] | 4.00E-102 | No          | 2   |
| CL3060.Contig1_All| ScosIR2   | 927         | 308     | ABD36125.1 glutamate receptor Gr2 [Bombyx mori] | 2.00E-101 | No          | 7   |
| CL146.Contig15_All| ScosIR3   | 1,836       | 611     | AML72541.1 ionotropic receptor IR2 [Colaphellus bowringi] | 5.00E-172 | No          | 5   |
| Unigene2622_All   | ScosIR4   | 1,803       | 600     | XP_019865961.1 PREDICTED: LOW QUALITY PROTEIN: glutamate receptor 2-like [Aethina tumida] | 5.00E-108 | No          | 4   |
| Unigene14518_All  | ScosIR5   | 319         | 106     | XP_018563257.1 PREDICTED: glutamate receptor ionotropic, NMDA 2D-like [Anoplophora glabripennis] | 5.00E-62  | No          | 1   |
| CL296.Contig4_All | ScosIR8a  | 2,649       | 882     | XP_019770830.1 PREDICTED: ionotropic receptor 25a [Dendroctonus ponderosae] | 0.00E+00  | No          | 3   |
| Unigene4531_All   | ScosIR21a | 2,370       | 789     | XP_019753472.1 PREDICTED: ionotropic receptor 21a [Dendroctonus ponderosae] | 0.00E+00  | No          | 3   |
| CL3341.Contig1_All| ScosIR25a | 2,796       | 931     | XP_019763174.1 PREDICTED: ionotropic receptor 25a [Dendroctonus ponderosae] | 0        | No          | 3   |
| Unigene6808_All   | ScosIR40a | 539         | 179     | XP_019764671.1 PREDICTED: glutamate receptor ionotropic, delta-2-like [Dendroctonus ponderosae] | 2.00E-97  | No          | 0   |
| CL16.Contig22_All | ScosIR64a | 1,860       | 619     | XP_019770931.1 PREDICTED: glutamate receptor-like [Dendroctonus ponderosae] | 5.00E-164 | No          | 3   |
| Unigene8833_All   | ScosIR64b | 1,863       | 620     | XP_019770931.1 PREDICTED: glutamate receptor-like [Dendroctonus ponderosae] | 7.00E-173 | No          | 0   |
| Unigene11157_All  | ScosIR68a | 357         | 118     | XP_015839172.1 PREDICTED: glutamate receptor ionotropic, kainate 5 [Tribolium castaneum] | 1.00E-27  | No          | 1   |
| Unigene5334_All   | ScosIR75a | 540         | 180     | XP_021192228.1 glutamate receptor ionotropic, delta-1-like isoform X2 [Helicoverpa armigera] | 1.00E-22  | No          | 3   |
| Unigene11324_All  | ScosIR75b | 1,533       | 510     | XP_015836653.1 PREDICTED: glutamate receptor 2-like [Tribolium castaneum] | 2.00E-72  | No          | 4   |
| CL266.Contig1_All | ScosIR75c | 1,854       | 617     | AKC58589.1 chemosensory ionotropic receptor 75q, partial [Anomala corpulenta] | 3.00E-85  | No          | 3   |
| Unigene2679_All   | ScosIR75d | 390         | 129     | APC94258.1 ionotropic receptor 2, partial [Pyrrhalta maculicollis] | 6.00E-06  | No          | 1   |
| Unigene6978_All   | ScosIR75e | 1,893       | 630     | XP_018572783.1 PREDICTED: glutamate receptor 1-like [Anoplophora glabripennis] | 4.00E-77  | No          | 3   |
| CL1275.Contig2_All| ScosIR75f | 1,626       | 541     | XP_018572783.1 PREDICTED: glutamate receptor 1-like [Anoplophora glabripennis] | 4.00E-92  | No          | 3   |
| Unigene11968_All  | ScosIR76b | 1,668       | 555     | XP_019762016.1 PREDICTED: LOW QUALITY PROTEIN: glutamate receptor ionotropic, kainate 1-like [Dendroctonus ponderosae] | 0.00E+00  | Yes         | 5   |
| Unigene2912_All   | ScosIR87a | 352         | 117     | XP_017770100.1 PREDICTED: glutamate receptor ionotropic, NMDA 2B-like isoform [Nicrophorus vespilloides] | 1.00E-101 | No          | 0   |
| Unigene1422_All   | ScosIR87b | 1,848       | 615     | OW45511.1 putative chemosensory ionotropic receptor IR68a [Danaus plexippus] | 2.00E-054 | No          | 5   |
| Unigene874_All    | ScosIR93a | 2,598       | 865     | XP_018576793.1 PREDICTED: glutamate receptor ionotropic, delta-1 isoform X2 | 0.00E+00  | No          | 1   |
**FIGURE 3** | Sequence alignment of candidate ScosOBPs. The six conserved cysteine residues are marked as C1-C6.
of them were full-length putative OR genes ranging from 1,100 to 1,400 bp with complete ORFs and 5 to 7 TMDs, which are characteristics of typical insect ORs. This includes the full-length ScosOrco gene encoding 488 amino acids. Seven of the predicted incomplete ORs were shorter in length and contained a deduced protein longer than 300 amino acids. Four of the predicted incomplete ORs were even shorter than 200 amino acids.

The blastx results indicated that the identities of the most predicted ORs shared with known insect ORs were very low, ranging from 24 to 49%. Nine predicted ORs (ScosOR1, ScosOR27, ScosOR7, ScosOR38, ScosOR39, ScosOR2, ScosOR8, ScosOR9, and ScosOR34) had greater identity (52–62%) with the OR from *D. ponderosae*. ScosOrco had 88% identity with the Orco from *Rhynchophorus ferrugineus*. Phylogenetic analysis was performed with ORs from *D. ponderosae*, *I. typographus*, *H. parallela*, and *S. schevyrewi* (Figure 1). A branch for Orco was identified in the phylogenetic tree. Two expanded branches in this species relative to others in the comparison were also identified. One branch consisted of ScosOR5, ScosOR6, ScosOR10, ScosOR11, ScosOR25, and ScosOR28 and the other consisted of ScosOR17, ScosOR18, Scos22, Scos31, Scos32, Scos37, Scos40, and Scos45. Most of the branches in the tree were supported by high-local support values and few branches were not reliable.

Information on unigene reference, length, and best blastx hit of all 47 ORs are listed in Table 1.

**Identification of Candidate Ionotropic Receptors**

Bioinformatics analysis identified 22 putative IRs in the *S. schevyrewi* transcriptome. Only ScosIR76b was a full-length sequence with 555 amino acids and five TMDs; the other IRs were incomplete due to the lack of the 5′ or 3′ terminus.

The blastx results showed that more than half of the predicted IRs (ScosIR1, ScosIR75a, ScosIR75b, ScosIR75c,
TABLE 3 | Unigene of candidate odorant binding proteins.

| Unigene reference | Genename | Length (bp) | ORF(aa) | Blastx best hit | E-value | Full length | Signal peptide |
|-------------------|-----------|-------------|---------|-----------------|---------|-------------|----------------|
| CL1164.Contig1_All| ScosOBP1  | 441         | 146     | ALR72497.1 odorant binding protein 9 [Colaphellus bowringi] | 6.00E-24 | No          | Yes            |
| CL2073.Contig1_All| ScosOBP2  | 489         | 162     | AAA96921.1 odorant-binding protein RpalOBP4*, partial [Rhynchophorus palmarum] | 6.00E-48 | Yes         | Yes            |
| CL2693.Contig1_All| ScosOBP3  | 402         | 133     | AKK25145.1 odorant binding protein 21 [Dendroctonus ponderosae] | 4.00E-39 | No          | Yes            |
| CL3244.Contig1_All| ScosOBP4  | 426         | 141     | ALM64971.1 odorant binding protein 13 [Dendroctonus armandi] | 2.00E-32 | Yes         | Yes            |
| CL3634.Contig1_All| ScosOBP5  | 402         | 133     | ALM64972.1 odorant binding protein 14 [Dendroctonus armandi] | 9.00E-44 | No          | Yes            |
| CL3848.Contig1_All| ScosOBP6  | 489         | 162     | APG73064.1 pheromone binding protein 3 [Cyrtotrachelus buqueti], partial | 1.00E-27 | No          | Yes            |
| Unigene1743_All  | ScosOBP7  | 358         | 119     | ARL83754.1 odorant binding protein 3 [Anoplophora glabripennis] | 9.00E-09 | Yes         | Yes            |
| Unigene1760_All  | ScosOBP8  | 411         | 136     | AGO5185.1 odorant-binding protein 9 [Dendroctonus ponderosae] | 2.00E-42 | Yes         | Yes            |
| Unigene1792_All  | ScosOBP9  | 393         | 130     | AQY118983.1 odorant-binding protein [Galeruca daurica] | 8.00E-18 | Yes         | Yes            |
| Unigene4680_All  | ScosOBP10 | 429         | 142     | AKK25140.1 odorant binding protein 16 [Dendroctonus ponderosae] | 3.00E-64 | Yes         | Yes            |
| Unigene5401_All  | ScosOBP11 | 411         | 136     | AKK25135.1 odorant binding protein 9, partial [Dendroctonus ponderosae] | 8.00E-44 | Yes         | Yes            |
| Unigene6017_All  | ScosOBP12 | 537         | 178     | ARIH65471.1 odorant binding protein 16 [Anoplophora glabripennis] | 9.00E-69 | No          | Yes            |
| Unigene7865_All  | ScosOBP13 | 531         | 176     | AMK468566.1 odorant-binding protein, partial [Rhynchophorus ferrugineus] | 7.00E-24 | No          | Yes            |
| Unigene9055_All  | ScosOBP14 | 429         | 142     | AHE13793.1 odorant binding protein [Lissorhoptrus oryzophilus] | 2.00E-75 | Yes         | Yes            |
| Unigene9643_All  | ScosOBP15 | 405         | 134     | AHE13799.1 odorant binding protein [Lissorhoptrus oryzophilus] | 9.00E-40 | Yes         | Yes            |
| Unigene9999_All  | ScosOBP16 | 429         | 142     | AHE13800.1 odorant binding protein, partial [Lissorhoptrus oryzophilus] | 6.00E-50 | Yes         | Yes            |
| Unigene10379_All | ScosOBP17 | 381         | 126     | ALM64966.1 odorant binding protein 6 [Dendroctonus armandi] | 7.00E-22 | No          | Yes            |
| Unigene11422_All | ScosOBP18 | 423         | 140     | ALM64970.1 odorant binding protein 8 [Dendroctonus armandi] | 3.00E-09 | No          | Yes            |
| Unigene11547_All | ScosOBP19 | 489         | 162     | AG05181.1 odorant-binding protein 11 [Dendroctonus ponderosae] | 1.00E-18 | Yes         | Yes            |
| Unigene12124_All | ScosOBP20 | 726         | 241     | AG05158.1 odorant-binding protein 21 [Dendroctonus ponderosae] | 1.00E-43 | Yes         | Yes            |
| Unigene17771_All | ScosOBP21 | 242         | 80      | ANE37553.1 odorant binding protein 9 [Rhynchophorus ferrugineus] | 1.00E-24 | No          | Yes            |
| CL142.Contig2_All| ScosOBP22 | 414         | 137     | AFI45061.1 odorant-binding protein [Dendroctonus ponderosae] | 8.00E-60 | Yes         | Yes            |

ScosIR75d, ScosIR75e, ScosIR75f, ScosIR3, ScosIR4, ScosIR64a, ScosIR64b, and ScosIR87b) shared low identity (24–49%) with known insect IRs. A total of 10 predicted IRs (ScosIR68a, ScosIR93a, ScosIR76b, ScosIR2, ScosIR21a, ScosIR8a, ScosIR40a, ScosIR87a, ScosIR25a, and ScosIR5) had greater identity (54–95%) with known insect IRs, most of which were from D. ponderosae. Candidate genes with high identity (87%) to DponIR25a were deemed IR 25a homologs. A phylogenetic tree was constructed based on the IR sequences from D. ponderosae, I. typographus, H. parallela, and S. schevyrewi (Figure 2). ScosIR8a and ScosIR25a were identified as putative IR8a and IR25a homologs due to the IR8a/IR25a branch.

Information on unigene reference, length, and best blastx hit of all 22 IRs are listed in Table 2.

Identification of Odorant-Binding Proteins

In the S. schevyrewi antennal transcriptome, 22 different sequences encoding putative OBPs were identified. More than half of them (ScosOBP2, ScosOBP4, ScosOBP8, ScosOBP9,
ScosOBP10, ScosOBP11, ScosOBP14, ScosOBP15, ScosOBP16, ScosOBP19, ScosOBP20, and ScosOBP22) were identified as full-length sequences. The lengths of all full-length ScosOBPs ranged from 130 to 241 amino acids.

Nearly, half of the predicted OBPs (ScosOBP7, ScosOBP18, ScosOBP9, ScosOBP1, ScosOBP20, ScosOBP19, ScosOBP17, ScosOBP4, ScosOBP4, ScosOBP6, and ScosOBP8) shared relatively low identity with known insect OBPs (31–49%). A total of 12 predicted OBPs (ScosOBP13, ScosOBP15, ScosOBP11, ScosOBP16, ScosOBP21, ScosOBP5, ScosOBP2, ScosOBP3, ScosOBP22, ScosOBP12, ScosOBP10, and ScosOBP14) had greater identity (51–83%) with known OBPs, a majority of which were D. ponderosae OBPs. Sequence alignment showed that 10 OBPs (ScosOBP1, 2, 3, 10, 12, 14, 16, 17, 18, and 22) matched in amino acid sequence to the sequence structure of classic OBPs, and eight OBPs (ScosOBP4, 5, 8, 9, 11, 13, 15, and 19) matched the sequence structure of Minus-C OBPs (Figure 3). Other OBPs were not analyzed by sequence alignment due to their limited sequence lengths. In the phylogenetic analysis of OBPs of different coleopterans, ScosOBPs were found across various branches and generally formed small subgroups together with OBPs from other coleopterans (Figure 4). No species-specific branch was discovered.

Information on unigene reference, length, and best blastx hit of all 22 OBPs are listed in Table 3.

**Identification of Putative Chemosensory-Binding Proteins**

A total of 11 putative CSPs were identified from the S. schevyrewi antennal transcriptome. Seven of them had full-length ORFs and nine of them had the predicted signal peptide. All of them shared the typical structure of a CSP except ScosCSP3 and ScosCSP10 because these two lacked the signal peptide.
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FIGURE 6 | Sequence alignment of candidate ScosCSPs. The four conserved cysteine residues are marked as C1–C4. Additional residues that conserved in this species were also marked.

All of the predicted CSPs shared relatively high identity (57–100%) with known insect CSPs. The phylogenetic analysis of the CSPs in different beetles showed that most of the ScosCSPs were clustered with orthologs of D. ponderosae, I. typographus, and H. parallela in a separate clade (Figure 5). Only ScosCSP2 and ScosCSP3 formed a small subgroup.

Information on sequence alignment, unigene reference, length, and best blastx hit of all 11 CSPs are shown in Figure 6 and Table 4.

Tissue- and Sex-Specific Expression of Candidate ScosOBP and ScosCSP Genes

The expression patterns of ScosOBPs and ScosCSPs were analyzed by RT-PCR and are shown in Figures 7, 8. ScosOBP1, 2, 3, 7, 9, 10, 16, 17, 18, 20, and 22 were highly expressed or specifically expressed in the antennae and head tissues. Among them, ScosOBP2 and OBP18 expressed at higher levels in female antennae than in male antennae. ScosOBP4, 5, 6, 11, 12, 13, 15, and 19 were generally expressed in multiple tissues. Among them, ScosOBP12 and ScosOBP19 expressions were stronger in the female than in the male antennae. ScosOBP8 and ScosOBP21 were not detected by RT-PCR possibly because their expression levels were too low to detect.

ScosCSP2, ScosCSP3, and ScosCSP5 were specifically expressed in the male and female antennae. Other ScosCSPs were expressed in multiple tissues. Among them, ScosCSP1 was not detected in male antennae and ScosCSP10 was not detected in the antennae of both the sexes. Potentially due to undetectable expression levels, ScosCSP6, ScosCSP8, and ScosCSP9 were not detected by RT-PCR.

DISCUSSION

The genes reported in our study provide additional knowledge on the pool of identified olfactory genes in coleopterans. Compared with a large number of studies on Lepidopteran species, the current understanding of olfactory genes in Coleoptera is mainly sourced from a few reported studies on T. castaneum (Engsontia et al., 2008), Megacyllene cyrcae (Mitchell et al., 2012), I. typographus, and D. ponderosae (Andersson et al., 2013), Leptinotarsa decemlineata (Liu et al., 2015), H. parallela (Yi et al., 2018), Rhynchophorus ferrugineus (Antony et al., 2016; Gonzalez et al., 2021), etc. S. schevyrewi belongs to the genus of bark beetles and shares similar biology with the related species that are destructive forest pests, such as I. typographus and D. ponderosae. Aggregation behaviors are critical for bark beetle survival and rely on chemical communication (Byers, 1989). The genes we
TABLE 4 | Unigene of candidate chemosensory binding proteins.

| Unigene reference | Gene name | Length (bp) | ORF(aa) | Blastx best hit | E-value | Full length | Signal peptide |
|-------------------|-----------|-------------|---------|----------------|---------|-------------|----------------|
| CL2121.Contig1_All | ScosCSP1 | 948         | 315     | XP_008193776.1 PREDICTED: chemosensory protein 6 isoform X1 [Tribolium castaneum] | 3.00E-51 | No          | Yes            |
| CL3677.Contig1_All | ScosCSP2 | 408         | 135     | AFI45003.1 chemosensory protein [Dendroctonus ponderosae] | 6.00E-65 | Yes         | Yes            |
| CL3677.Contig2_All | ScosCSP3 | 207         | 68      | ALR25256.1 chemosensory protein 12 [Calophellus bowringi] | 3.00E-35 | No           | No             |
| Unigene2372_All   | ScosCSP4 | 381         | 126     | AHE13802.1 chemosensory protein 6 [Lasiorhoptrus oryzophilus] | 1.00E-45 | Yes          | Yes            |
| Unigene3205_All   | ScosCSP5 | 315         | 104     | AG05163.1 chemosensory protein 11 [Dendroctonus ponderosae] | 4.00E-46 | No           | No             |
| Unigene3968_All   | ScosCSP6 | 381         | 126     | AG204932.1 chemosensory protein 4 [Laodelphax striatella] | 3.00E-86 | Yes          | Yes            |
| Unigene4248_All   | ScosCSP7 | 390         | 180     | AG05162.1 chemosensory protein 6 [Dendroctonus ponderosae] | 1.00E-58 | Yes          | Yes            |
| Unigene5446_All   | ScosCSP8 | 372         | 123     | AG204930.1 chemosensory protein 2 [Laodelphax striatella] | 5.00E-56 | Yes          | Yes            |
| Unigene5467_All   | ScosCSP9 | 393         | 130     | AG204940.1 chemosensory protein 12 [Laodelphax striatella] | 7.00E-78 | Yes          | Yes            |
| Unigene5845_All   | ScosCSP10 | 333      | 110     | AG05172.1 chemosensory protein 2 [Dendroctonus ponderosae] | 1.00E-52 | No           | No             |
| Unigene12055_All  | ScosCSP11 | 381       | 126     | AHE13803.1 chemosensory protein 8 [Lasiorhoptrus oryzophilus] | 9.00E-67 | Yes          | Yes            |

identified might contribute to aggregation behavior and provide molecular targets for novel pest management techniques.

We identified a total of 47 OR genes in the S. schevyrewi antennae transcriptome. In another coleopteran, 265 candidate OR genes were annotated in the T. castaneum genome (Richards, 2008), which is much more than the known number of OR genes reported by other beetles. The numbers of ORs in M. caryae (Mitchell et al., 2012), I. typographus (Andersson et al., 2013), D. ponderosae (Andersson et al., 2013), and H. parallela (Yi et al., 2018) range from 43 to 57. The number of ScosORs identified in this study is consistent with that identified in these reports. Most of the predicted ORs in S. schevyrewi share greater identity with ORs of D. ponderosae, another bark beetle, indicating that these two species may be able to share the same ecological environments and detect similar semiochemicals. Functional studies in OR from bark beetles were relatively limited to only seven ORs (Hou et al., 2021; Yuvaraj et al., 2021). ItypOR46 and ItypOR49 were responsive to single enantiomers of the common bark beetle pheromone compound ipsenol and ipsdienol, respectively (Yuvaraj et al., 2021). The other five ItypORs were responsive to monoterpenoids of different ecological origins (Hou et al., 2021). Future studies should be focused on deorphinized ScosORs with similar functions to provide potential molecular targets for detection and control methods.

We identified, in total, 22 IR genes in the S. schevyrewi antennae transcriptome. ScosIR8a and ScosIR25a were identified as coreceptors. The numbers of IR genes in I. typographus, D. ponderosae, and H. parallela (Yi et al., 2018) are 7, 15, and 27, respectively (Andersson et al., 2013; Yi et al., 2018). The number of ScosIRs identified in this study is considerable compared with the numbers reported in the previous studies. More than half of the predicted IRs shared relatively low identity with other coleopteran IRs. These IRs with low identity were probably not conserved in Coleoptera, and they might serve diverse functions in S. schevyrewi.

Within the S. schevyrewi antennae transcriptome, a total of 22 OBPs were predicted. Genome annotation indicated that 46 OBPs were identified in T. castaneum (Richards, 2008). Fewer OBPs were found in other coleopteran antennae transcriptomes. In D. ponderosae, I. typographus, and H. parallela, respectively, 31, 15, and 25 OBPs were annotated (Andersson et al., 2013; Ju et al., 2014). Our number of ScosOBPs is consistent with the numbers stated in these reports.

A total of 11 CSPs were identified in the S. schevyrewi antennae transcriptome. In the T. castaneum genome, a total of 40 CSPs were identified (Richards, 2008). Other coleopterans have fewer CSPs in their antennae transcriptomes; in D. ponderosae, I. typographus, and H. parallela, respectively, 11, 6, and 16 were annotated, respectively (Andersson et al., 2013; Ju et al., 2014). Our recorded number of ScosCSPs is comparable with these reports. The high level of sequence conservation (57–100%) indicates the function of CSPs is likely conserved among coleopterans.

All ScosOBPs and ScosCSPs contain a typical OBP and CSP motif, respectively. ScosOBP1, 2, 3, 10, 12, 14, 16, 17, 18, and 22 generally possessed the “classic” OBP motif C1-X22−44-C2-X3-C3-X21−42-C4-X4−12-C5-X4−6-C6 of coleopteran insects (Xu et al., 2009). ScosOBP4, 5, 8, 9, 11, 13, 15, and 19 generally contained the “minus-C” OBP motif C1-X30−32-C2-X38-C3-X16−18-C4 (Ju et al., 2014). The cysteine-spacing pattern of ScosOBP20 followed
FIGURE 7 | Sex- and tissue-specific expression of candidate ScosOBPs. A, antennae; H, head; T, thorax; Ab, abdomen; L, leg; W, wing; ck, control [ultra-pure water (germ-free)].

FIGURE 8 | Sex- and tissue-specific expression of candidate ScosCSPs. A, antennae; H, head; T, thorax; Ab, abdomen; L, leg; W, wing; ck, control [ultra-pure water (germ-free)].

the “plus-C” OBP motif C_{1}-X_{24–28}-C_{2}-X_{3}-C_{3}-X_{43–44}-X_{13–15}-C_{64–65}-C_{5}-X_{9}-C_{9}-C_{10} from D. melanogaster and Anopheles gambiae (Zhou et al., 2008). All the CSPs were conserved in having the motif C_{1}-X_{65}-C_{2}-X_{158}-C_{3}-X_{2}-C_{4} (Xu et al., 2009).

Scolytinae beetles respond to volatiles that emanate from both the host and non-host plants (Zhang and Schlyter, 2004; Erbilgin et al., 2007; Andersson et al., 2010). However, most individuals locate target trees by relying on an important signal called an aggregation pheromone released by beetles that have already attacked a tree (Andersson et al., 2013). Thus, olfactory signals and proteins serve critical roles in insect behavior. In this study, ScosOBP1, 2, 3, 7, 9, 10, 16, 17, 18, 20, and 22 might be important in odor perception because they were only expressed in the antennae and head, especially, ScosOBP2 and ScosOBP18. These two may be the key proteins in female olfactory behavior based on the specificity of protein expression we observed. ScosCSP2, ScosCSP3, and ScosCSP5 might also be important in olfaction due to their antennae-specific expression. Other ScosOBPs and ScosCSPs might not be involved in odor reception. Studies have shown a multitude of other roles that insect OBPs and CSPs have in Pelosi et al. (2018) releasing semiochemicals in pheromone glands (Benton, 2007), regeneration and development (Cheng et al., 2015), anti-inflammatory action (Isawa et al., 2002), nutrition (Zhou et al., 2016), carrying visual pigments (Wang et al., 2007), and insecticide resistance (Bautista et al., 2015).

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA732801.

AUTHOR CONTRIBUTIONS

XZ and WL designed the research, analyzed the data, and wrote the paper. YL gave a lot of advices and help to revise the paper. AK, BS, and HC provided biological samples. XZ, BX, and ZQ performed the experiment. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2021.717698/full#supplementary-material
**Supplementary Figure 1** | GO classification analysis of unigenes in all-unige. GO functions are shown in the X-axis. The right Y-axis shows the number of genes that have the GO function, and the left Y-axis shows the percentage.

**Supplementary Figure 2** | Uncropped gel images of candidate ScosOBPs and candidate ScosOSPs. A, antennae; H, head; T, thorax; Ab, abdomen; L, leg; W, wing; ck, control [ultra-pure water (germ free)].

**Supplementary Figure 3** | Uncropped gel images for candidate ScosOBPs and ScosOSPs.

**Supplementary Table 1** | Forward and reverse primer sequence used in RT-PCR.

**Supplementary Table 2** | Sequence information.

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