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ORIGINAL ARTICLE

Antennal transcriptome analysis of *Psyttalia incisi* (silvestri) (Hymenoptera: Braconidae): identification and tissue expression profiling of candidate odorant-binding protein genes

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Abstract

Background Olfaction plays an important role in host-seeking by parasitoids, as they can sense chemical signals using sensitive chemosensory systems. *Psyttalia incisi* (Silvestri) (Hymenoptera: Braconidae) is the dominant parasitoid of *Bactrocera dorsalis* (Hendel) in fruit-producing regions of southern China. The olfactory behavior of *P. incisi* has been extensively studied; however, the chemosensory mechanisms of this species are not fully understood.

Results Bioinformatics analysis of 64,515 unigenes from the antennal transcriptome of both male and female adults *P. incisi* identified 87 candidate chemosensory genes. These included 13 odorant-binding proteins (OBPs), seven gustatory receptors (GRs), 55 odorant receptors (ORs), 10 ionotropic receptors (IRs), and two sensory neuron membrane proteins (SNMPs). Phylogenetic trees were constructed to predict evolutionary relationships between these chemosensory genes in hymenopterans. Moreover, the tissue expression profiles of 13 OBPs were analyzed by quantitative real-time PCR, revealing high expression of seven OBPs (1, 3, 6, 7, 8, 12, and 13) in the antennae.

Conclusion This study represents the first identification of chemosensory genes and the determination of their expression patterns in different tissues of *P. incisi*. These results contribute to a better understanding of the function of the chemosensory system of this parasitoid species.

Keywords *Psyttalia Incisi* · Antennal transcriptome · Olfactory genes · Expression profile · Phylogenetic analysis

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Abbreviations

OBPs	Odorant-binding proteins
GRs	Gustatory receptors
ORs	Odorant receptors
IRs	Ionotropic receptors
SNMPs	Sensory neuron membrane proteins
CSPs	Chemosensory proteins
PBPs	Pheromone-binding proteins
GOBPs	General odorant-binding proteins
NCBI	NR-NCBI non-redundant
NCBI	NT-NCBI nucleotide
GO	Gene Ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes
Pfam	Protein Family
KOG/COG	EuKaryotic Ortholog Groups/Clusters of Orthologous Groups
ORFs	Open reading frames
TMDs	Transmembrane domains
pI	Theoretical isoelectric point
Mw	Molecular weight
TMDs	Transmembrane domains

Introduction

Insects depend on olfaction to recognize pheromones and volatile compounds, find mates, oviposition sites, and food sources, and avoid natural enemies and toxins [1]. Olfactory receptors are distributed in different parts of an insect's body, such as the antennae, head, abdomen, wings, and legs [2]. The proteins involved in chemosensory recognition process within the antennal olfactory system include odorant-binding proteins (OBPs), chemosensory proteins (CSPs), odorant receptors (ORs), gustatory receptors (GRs), ionotropic receptors (IRs), and sensory neuron membrane proteins (SNMPs) [3–7]. OBPs, in particular, play a significant role in the detection and recognition of odor molecules [8]. Once bound by OBPs, hydrophobic odor molecules traverse the the insect's lymphatic system, ultimately reaching the dendritic cell membrane. Subsequently, the olfactory receptors, namely ORs, convert chemical signals into electrical impulses that are transmitted to the nerve center, triggering behavioral responses [4, 9]. Following signal transmission, the ORs dissociate from the odor molecules, which are then degraded by odorant-degrading enzymes, regulating the dynamics of olfactory processing [10, 11].

OBPs are water-soluble acidic proteins that are present in the lymph of chemosensillar [12]. They can be divided into two primary subfamilies: pheromone-binding proteins (PBPs) and general odorant-binding proteins (GOBPs) [13]. Furthermore, OBPs are classified into five groups based on the number of conserved cysteine residues that form an α-helix: typical, plus-C, minus-C, dimer, and atypical [14–16]. Typical OBPs, which are the most common, consist of six conserved cysteine residues that form three disulfide bonds, six α-helices, and a hydrophobic pocket responsible for binding small odor molecules [14, 15, 17]. The sequence and structure of OBPs can vary significantly among different insects species. For instance, *Trichogramma japonicum* (Hymenoptera: Trichogrammatidae) possesses typical OBPs [18], whereas *Aenasius bambawalei* (Hymenoptera: Encyrtidae) has both typical and minus-C OBPs [19]. OBPs are predominantly expressed in the antennae, head, thorax, abdomen, and legs of insects [20]. Further, the expression of OBPs varies according to the insect's sex and developmental stage.

OBPs play a crucial role in the olfactory responses of insects by facilitating the transport of hydrophobic odor molecules through an aqueous lymph to odorant receptors [21]. In 1981, Vogt and Riddi-Ford employed an isotopic labeling method to identify an OBP in the antennae of male *Antheraea polyphemus* (Lepidoptera: Saturniidae) [6]. Subsequently, antennal binding proteins were discovered in *Drosophila melanogaster* (Diptera: Drosophilidae) and *Bombyx mori* (Lepidoptera: Bombycidae) [22, 23]. Numerous OBP genes have been identified in *D. melanogaster* (51) [14], the tobacco cutworm *Spodoptera litura* (38) (Lepidoptera: Noctuidae) [24], and over 300 in more than 20 parasitoids of Hymenoptera [25]. OBPs are responsible for binding to and transporting odor molecules, thereby regulating olfactory responses [12, 23]. In addition, these proteins have the ability to recognize phytochemical substances and volatile organic compounds emitted by plants. For instance, in *A. bambawalei*, OBP28 can bind to 15 compounds at pH 7.4, including 1-octen-3-one and diethyl sebacate [26].

Bactrocera dorsalis (Hendel) (Diptera: Tephritidae) poses a significant economic threat to the fruit and vegetable industry worldwide due to its broad range of hosts and high reproductive capacity. It infests over 300 types of produce, including citrus, pomegranate, apple, banana, and cucumber [27]. The primary method for controlling this pest is through chemical insecticide spraying, which contributes to issues such as insecticide resistance, environmental contamination, and food contamination [28]. To mitigate the negative impact of chemical pesticides on human health and the environment, biological control methods utilizing parasitoids have been widely adopted. These approaches are sustainable and do not result in environmental contamination [29]. For instance, following an outbreak of *B. dorsalis* in 2005 in Fujian Province, China, parasitoids such as *Fopius arisanus* (Hymenoptera: Braconidae) native to Hawaii, along with other parasitoids including *Fopius vandenboschi*, *Psyttalia incisi*, and *Diachasmimorpha longicaudata* (Hymenoptera:

Braconidae), were introduced to reduce the field population of *B. dorsalis* [30].

Psyttalis incisi is a solitary opiine parasitoid that displays a preference for early larval instars of *B. dorsalis* [31]. In southern China, *P. incisi* is the predominant parasitoid species, accounting for 77.6% of the population that targets *B. dorsalis* in orchards located in Zhangzhou City, Fujian Province, China [31]. Consequently, *P. incisi* holds significant promise for the biological control of *B. dorsalis* in this particular region of China [29]. Extensive research has been conducted on various aspects of this species, encompassing studies of fitness [32–34], field applicability [29, 35], biology [36–38], behavior [39], molecular characterization [40], and mitochondrial genome analysis [41]. The effectiveness of parasitoids as biocontrol agents relies on their ability to seek out hosts using chemical cues [42]. Therefore, it is crucial to comprehend the chemosensory system of *P. incisi* in order to enhance the efficiency of the biological control measures against *B. dorsalis* within agricultural ecosystems.

This study analyzed the antennal transcriptome of *P. incisi* and identified olfactory gene clusters through bioinformatic analysis. The expression patterns of olfactory genes in different tissues were investigated using real-time quantitative PCR (qRT-PCR). Phylogenetic trees were constructed to predict evolutionary relationships among the chemosensory genes of hymenopterans. This study provides the basis for understanding the function of these genes and the host-seeking behavior of *P. incisi*.

Materials and methods

Insect rearing and sample collection

A strain of *B. dorsalis* and a strain of *P. incisi* were obtained from ripe guava fruits collected in Zhangzhou City, Fujian Province, China, in 2004, and were established at the Institute of Beneficial Insects, Fujian Agriculture and Forestry University [31]. *Bactrocera dorsalis* were allowed to oviposit in perforated plastic bottles, and the eggs were then collected and transferred to a tray containing milled feed for larval development, following the previously described [43]. Adult flies were fed a diet of yeast extract and sugar (1:3, wt/wt) and provided with water. Adult parasitoids were maintained in "Hawaii-type" cages (30 × 30 × 30 cm) and given honey and water as food. To prevent superparasitism, two plates (each containing approximately 1000 larvae) were placed in a cage containing 1000 *P. incisi* adults (500 females and 500 males) and left for 24 h. *Bactrocera dorsalis* and *P. incisi* were reared under a 12 h light, 12 h dark cycle at 25 ± 1 °C and a relative humidity of 65 ± 5% [29].

For tissue expression profiling, around 200 male and 200 female *P. incisi* adults were dissected on ice under a light microscope. The following body parts were collected: antennae (200 pairs), heads without antennae (100), thoraces without legs and wings (100), abdomens (50), and legs (200). In total, three biological replicates were collected, transferred to 1.5 mL centrifuge tubes. The samples were then frozen in liquid nitrogen and stored at − 80 °C until further use.

RNA extraction, cDNA library construction, and Illumina sequencing

Total RNA was extracted from the antennae of female and male adults (20 mg) using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. The extracted RNA samples were then treated with RNase-free DNase I (TaKaRa, Dalian, Liaoning, China) to remove any contaminating DNA. The concentration and purity of the RNA samples were determined using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). In addition, the integrity of the RNA was assessed by performing 1% agarose gel electrophoresis. The total RNA extracted from the antennae was subsequently frozen in liquid nitrogen and stored at − 80 °C for further use. Alternatively, the RNA samples were transported on dry ice to Sangon Biotech (Shanghai, China) for transcriptome sequencing using the Illumina Hiseq™ platform.

The construction of the cDNA library for each sample was performed using the NEB Next Ultra RNA Library Prep Kit for Illumina (New England Biolabs, Ipswich, MA, USA) following the manufacturer's protocol. To summarize the procedure, 10 ug of total RNA from each tissue was used to isolated mRNA using oligo (dT) magnetic beads. The isolated mRNA was then fragmented by incubating with the fragmentation buffer supplied with the kit at 94 °C for 5 min. Next, first-strand cDNA was synthesized using random hexamers and the fragmented mRNA as a template. Subsequently, second-strand cDNA was carried out using a combination of buffer, dNTPs, RNase H, and DNA polymerase I. After performing end-repair, A-tailing, and adaptor ligation, the resulting products were amplified by PCR. The amplified DNA fragments were purified using the QIA Quick PCR Purification Kit (Qiagen, Valencia, CA, USA) to obtain the final sequencing library. The cDNA library was then subjected to sequencing using the Illumina Hiseq™ platform.

De novo assembly and gene annotation

Illumina Hiseq™ raw data files were analyzed with CASAVA and converted to raw reads through base calling. The raw sequencing reads underwent trimming using Trimmomatic, where adaptor sequences, reads with a percentage of unknown nucleotides higher than 10%, and low-quality reads (containing more than 50% bases with Q-value ≤ 20) were removed. Then, *de novo* transcriptome assembly was performed using three modules within Trinity: Inchworm, Chrysalis, and Butterfly [44]. Redundant sequences were removed, and the longest transcript was designated as unigene.

Unigenes were annotated using BLAST alignment with an E-value cutoff of 1.0×10^{-5} against seven databases: NCBI non-redundant (NCBI-NR), NCBI nucleotide (NCBI-NT), Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), Protein Family (Pfam), EuKaryotic Ortholog Groups/Clusters of Orthologous Groups (KOG/COG), and SwissProt [45]. The BLAST alignment was performed through the BLAST website (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Additional parameters were customized according to the system. Based on the best alignment scores, the directions of the unigene sequence were determined, and protein function annotation was carried out. Blast2GO was used for the annotation of Gene Onotology (GO) terms and unigenes [46].

To identify candidate chemosensory genes, transcriptome data from the antennae of male and female adults were screened. Unigenes were annotated by performing BLASTx against NCBI-NR sequences with an E-value cutoff of $< 1.0 \times 10^{-5}$. Unigenes encoding odorant-binding proteins (OBPs), gustatory receptors (GRs), olfactory receptors (ORs), ionotropic receptors (IRs), chemosensory proteins (CSPs), and sensory neuron membrane proteins (SNMPs) were selected as putative chemosensory genes. All candidate genes were manually verified using BLASTx against the NR database. Relevant parameters such as sequence length, species with the highest homology, gene name, number of entries, E-value, and gene sequence homology were recorded. Open reading frames (ORFs) were predicted using the ORF finder tool (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). Signal peptides were predicted using SignalP 5.0 server (https://services.healthtech.dtu.dk/service.php?SignalP-5.0).

Chemosensory gene verification and bioinformatic analysis

The conserved domains and ORFs of candidate chemosensory genes (OBPs, ORs, SNMPs, GRs, and IRs) were manually validated. Amino acid sequences were obtained through BLASTx alignment and coding region prediction. Orthologs were identified, and the degree of homology was calculated using BLASTx (http://blast.ncbi.nlm.nih.gov/blast.cgi). Nucleotide sequences were translated using Expasy (https://web.expasy.org/translate/). The theoretical isoelectric point (pI) and molecular weight (Mw) of the deduced proteins were estimated using ProtParam (https://web.expasy.org/protparam/). Protein domains were predicted using EMBL-EBI services (https://pfam.xfam.org/search#tabview=tab1). Transmembrane domains (TMDs) were identified using TMHMM version 2.0 (https://services.healthtech.dtu.dk/service.php?TMHMM-2.0) [47]. All OBP genes reported in this study have complete ORFs. The nucleotide sequences have been deposited in GenBank under accession numbers OP484699 to OP484711.

Phylogenetic analysis

To validate the annotation of candidate genes and identify homologous sequences, we conducted a phylogenetic analysis involving *P. incisi*, other hymenopteran species, and model insects. GenBank was searched using BLAST to find sequences with the highest homology to the candidate genes. Evolutionary trees were constructed based on nucleic acid sequences from *Aphidius gifuensis* [48], *Cephus cinctus* [49], *Diachasma alloeum* [50], *Fopius arisanus* [51], *Microplitis demolitor* [52], *Microplitis mediator* [53], *Chelonus insularis* [54], *Cotesia glomerata* [55] (Hymenoptera: Braconidae), and *Nasonia vitripennis* (Hymenoptera: Pteromalidae) [56]. Multiple alignments were performed using MAFFT (https://mafft.cbrc.jp/alignment/server/index.html), with the "auto" strategy and customized parameters. Sequences edited was done using BioEdit. The best-fit partition model (Edge-linked) was selected using ModelFinder based on the Akaike information criterion. Maximum likelihood phylogenies were inferred using IQ-TREE under the selected model (Table S1) using the "auto" option for model selection (http://iqtree.cibiv.univie.ac.at/, accessed on 18 October 2021) with 20,000 ultrafast bootstraps and approximate Bayes test [57]. A circular phylogenetic tree was generated and color-coded using iTOL tools (https://itol.embl.de/) and annotated using Adobe Illustrator CC 2018 (Adobe, CA, USA).

Quantitative real-time PCR (qRT-PCR)

Quantitative real-time PCR (qRT-PCR) was used to evaluate the expression levels of candidate OBPs in different tissues of both sexes. Total RNA was extracted from the antennae, head, thorax, abdomen, and legs using aforementioned protocol. Subsequently, cDNA was synthesized from the total RNA using the FastKing gDNA Dispelling RT SuperMix

Kit (TIANGEN, Beijing, China) according to the following steps: 10 µg of total RNA from each tissue was utilized for mRNA isolation, and reverse transcriptase (FastKing-RT Enzyme) was used for cDNA synthesis at 42 °C for 15 min. The resulting cDNA was then stored at − 20 °C until further analysis. Each cDNA sample was diluted to a concentration of 200 ng/µL using nuclease-free water. Gene-specific primers were designed with the assistance of Oligo 7.0 software and are listed in Supplementary Table S2. The lengths of the PCR products ranged from 80 to 150 bp. The gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH), with GenBank Accession OP593307, is a well-known housekeeping gene often used as a normalizer for the expression levels of target genes in different tissues, including antennae, heads, thorax, abdomens and legs of various insects such as *Spodoptera frugiperda* [58], *Trichogramma japonicum* [18], *Aenasius bambawalei* [19], *Myzus persicae* [59]. To construct a relative standard curve, templates were diluted into a series of five two-fold dilutions (1:1, 1:2, 1:4, 1:8, and 1:16). The amplification efficiency (E) and R^2 value of each primer pair were calculated using the slope of the standard curve. The amplification efficiency was calculated as follows: E=[10^(-1/slope)-1] ×100%. Only primers with amplification efficiencies between 90% and 110% were used for subsequent data analysis [60].

RT-qPCR was performed using a QuantStudio 3 PCR system (Applied Biosystems, Foster City, CA, USA) in 96-well reaction plates. The reactions were conducted in a 20 µL mixture containing 10 µL of PerfectStart Green qPCR SuperMix (+Dye II) (TRANS, Beijing, China), 0.4 µL of each primer (10 µM), 2 µL of cDNA (200 ng/µL), and 7.2 µL of nuclease-free water. A negative control using sterile water was included. The reaction conditions were as follows: one cycle at 94 °C for 30 s, followed by 40 cycles at 94 °C for 5 s and 60 °C for 30 s. After 40 cycles, melting curves were analyzed at a temperature range of 60–95 °C to verify the presence of a single gene-specific peak for each gene, without any primer dimer peaks. All reactions were performed independently and in triplicate three times. The relative levels of gene expression among the different samples were measured using the $2^{-\Delta\Delta Ct}$ method [61]. The data are presented as mean ± standard error. Statistical comparison of gene expression was performed using one-way analysis of variance followed by Tukey's honestly significant difference test. P-values less than 0.05 were considered statistically significant. The statistical analysis was performed using SPSS version 21.0 and GraphPad Prism version 9.0.0 (Inc., La Jolla, CA, USA). The figures were created using Adobe Illustrator CC 2018 (Adobe, CA, USA).

Results

Transcriptome sequence and assembly

The transcriptome analysis of *P. incisi* adults antennae involved the use of Illumina sequencing, which generated a total of 268,697,486 raw reads. After Trinity assembly, 261,177,254 clean reads were obtained, which were further assembled into 159,379 transcripts. These transcripts comprised 64,515 unigenes, ranging in length from 201 bp to 24,806 bp, with an N50 length of 2347 bp. The mean length of transcripts was 1637 bp, while the average length of unigenes was 876 bp. It is worth mentioning that 21,440 unigenes, accounting for 33.23% of the total, had a length exceeding 1000 bp. The number of transcripts decreased as the length increased, as depicted in Figure S1. A summary of the sequencing and assembly process is provided in Table S3.

Functional annotation and expression level of *P. incisi* antennae transcriptome

Functional annotation of unigenes involved selecting the proteins with the highest similarity. Out of the 64,515 unigenes, a total of 19,243 (29.83%) had homologous sequences in NCBI-NR database, 12,147 (18.83%) in NCBI-NT, 8,667 (13.43%) in GO, 7,218 (11.19%) in KOG, 6,791 (10.53%) in PFAM, 6,088 (9.44%) in CDD, and 3,738 (5.79%) in KEGG (Table S4). The homologous sequences were determined based on their alignment scores (E-value) in the NR database. The analysis revealed a significant number of homologous genes in Hymenoptera. Among the species with the highest homology, *D. alloeum* had the highest percentage (56.72%), followed by *F. arisanus* (14.51%), *B. dorsalis* (2.82%), *Lasius niger* (Hymenoptera: Formicidae) (2.23%), *Cotesia congregate* (Hymenoptera: Braconidae) (1.26%), and *M. demolitor* (1.07%). The respective number of matched unigenes for these species was 10,914, 2,793, 543, 429, 243, and 205 (Figure S2).

The functional characterization of the transcriptome was performed using GO enrichment analysis. A total of 8,667 unigenes were successfully annotated, and they were found to be enriched in biological processes (816,342), cellular components (192,348), and molecular functions (98,682) (Figure S3).

In the transcriptome of the antennae, a total of 3,738 unigenes were annotated in the KEGG database. These unigenes exhibited enrichment in various categories, including cellular processes (1,040), environmental information processing (1,107), genetic information processing (1,076), metabolism (2,111), organizational systems (1,950), signal transduction (935), translation (474), endocrine system

(459), and transport and catabolism (430 unigenes) (Figure S4).

In the KOG database, a total of 7,218 unigenes were annotated and categorized into 25 different categories. Among these categories, the four most significant ones, each with more than 500 unigenes, were T, R, K and O (Figure S5).

Identification of OBPs

Thirteen putative OBPs were identified through a comparative analysis of the antennal transcriptome of *P. incisi* using BLASTx. The degree of homology to NCBI database sequences from *P. incisi* and other species ranged from 40.32 to 99.32%. Out of the thirteen identified OBPs, all had complete ORFs and encoded 103 to 167 amino acids. Notably, two OBPs (1 and 13) lacked a signal peptide (Table 1). Among the thirteen OBPs, three (5, 6, and 10) exhibited typical characteristics, while five (1, 4, 8, 11, and 12) posessed a minus-C motif, and the remaining five (2, 3, 7, 9, and 13) displayed plus-C motif.

A BLAST search against the NCBI database revealed that the candidate OBPs shared significant similarities with amino acid sequences from other insect species such as *A. gifuensis*, *C. cinctus*, *D. alloeum*, *F. arisanus*, *M. demolitor*, and *M. mediator*. To further analyze the relationships among these OBPs, a phylogenetic tree was constructed using the maximum likelihood method with 20,000 bootstrap replicates. Clustering was determined based on bootstrap values, with a cutoff of 50% cutoff. The OBP sequences were divided into distinct clusters based on their clustering patterns. Notably, OBP-1 and OBP-6 from *P. incisi* clustered together with OBP-9 and OBP-4 from *C. cinctus*, respectively. Furthermore, OBP-3, OBP-4, and OBP-5 from *P. incisi* clustered with OBP69a-like, OBP69a-like, and OBP83a-like from *F. arisanus*, respectively (Fig. 1).

Tissue- and sex-specific expression of OBPs

We performed qRT-PCR to examine the expression profiles of the 13 OBPs genes in different tissues. The results showed a diverse distribution pattern of these OBPs among adult wasps. Specifically, seven OBPs exhibited high expression in the antennae. Among them, four OBPs (3, 6, 8, and 12) demonstrated notably elevated expression in the antennae of male adults, displaying a distinct expression profile compared to other tissues. This finding suggests that these OBPs, which are highly expressed in the male antennae, might be associated with mating behavior [62]. On the other hand, three OBPs (1, 7, and 13) showed high expression in the antennae of female adults and may be involved in host-seeking and selecting suitable spawning sites [63]. OBP5 showed prominent expression in the male legs. However,

Table 1 BLASTx alignment of odorant-binding protein genes of *Psyttalia incisi* and other insect species

Gene name	ORF (bp)	CompleteORF	Signal peptide	Transmembrane helix	Best alignment score				
					Name	Accession number	Species	*E*-value	Homology (%)
OBP1	501	YES	NO	2	general odorant-binding protein 69a	XP_015122793.1	*Diachasma alloeum*	9.00E-10	90.20%
OBP2	420	YES	1–20	0	general odorant-binding protein 83a	XP_015117724.1	*Diachasma alloeum*	1.00E-32	41.84%
OBP3	441	YES	1–23	0	general odorant-binding protein 69a-like	XP_011301198.1	*Fopius arisanus*	2.00E-98	93.84%
OBP4	330	YES	1–23	0	general odorant-binding protein 69a-like	XP_011301198.1	*Fopius arisanus*	1.00E-69	74.66%
OBP5	420	YES	1–20	0	general odorant-binding protein 83a-like	XP_011299167.1	*Fopius arisanus*	2.00E-94	97.12%
OBP6	447	YES	1–23	1	general odorant-binding protein 83a	XP_011212472.1	*Bactrocera dorsalis*	6.00E-88	99.32%
OBP7	426	YES	1–22	1	general odorant-binding protein 69a	XP_011304865.1	*Fopius arisanus*	4.00E-86	97.16%
OBP8	312	YES	1–26	0	general odorant-binding protein 12	AKM45830.1	*Bactrocera dorsalis*	1.00E-68	99.04%
OBP9	387	YES	1–19	0	general odorant-binding protein 69a	XP_015125081.1	*Diachasma alloeum*	6.00E-18	40.32%
OBP10	408	YES	1–34	1	general odorant-binding protein 56d	XP_015111205.1	*Diachasma alloeum*	1.00E-55	74.26%
OBP11	369	YES	1–24	0	general odorant-binding protein 84a	XP_011206667.1	*Bactrocera dorsalis*	2.00E-70	97.56%
OBP12	474	YES	1–22	0	putative odorant-binding protein A10 isoform X2	XP_011208559.1	*Bactrocera dorsalis*	1.00E-68	97.30%
OBP13	504	YES	NO	1	odorant-binding protein	THK33210.1	*Diachasma alloeum*	1.00E-05	57.69%

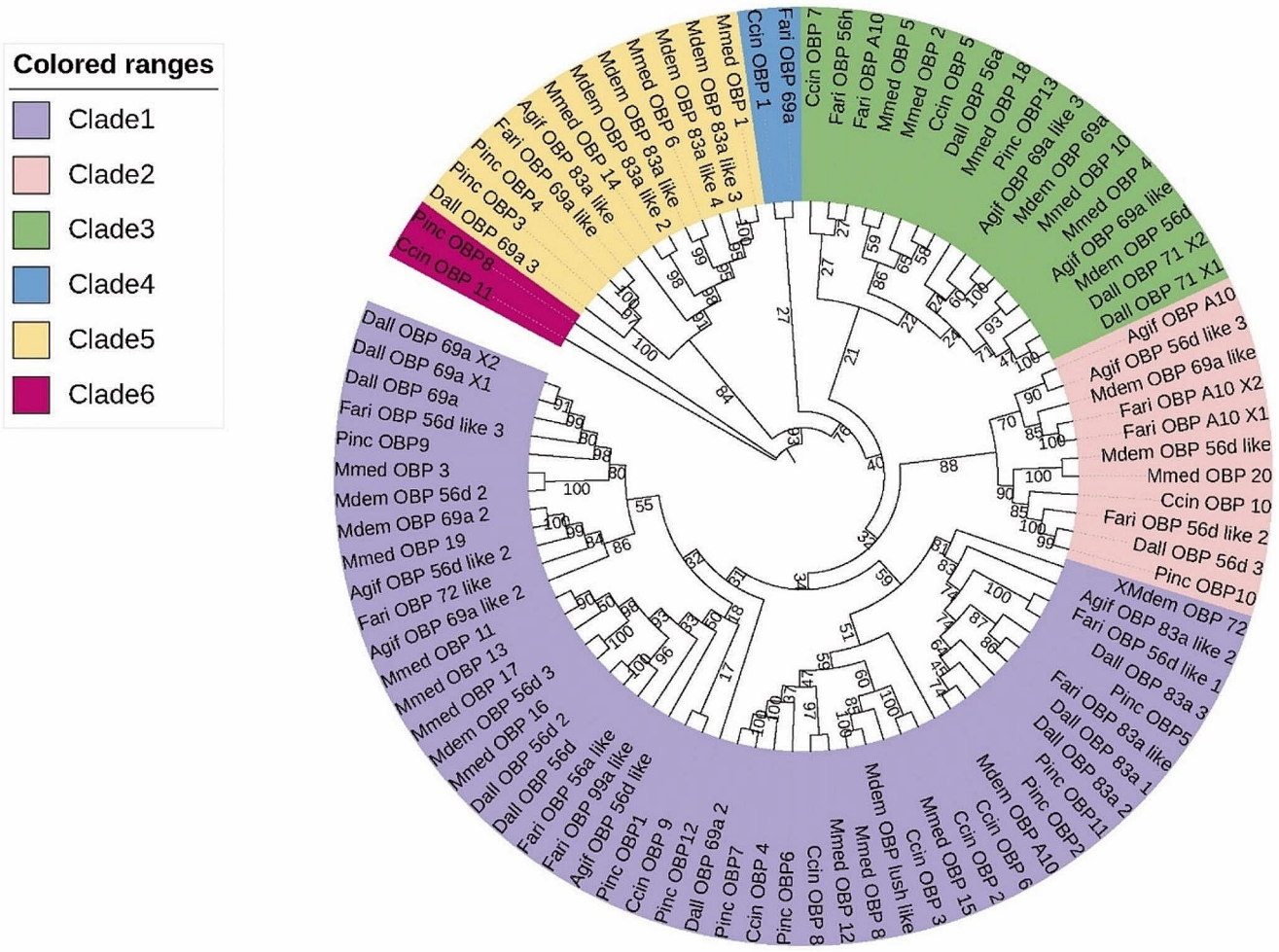

Fig. 1 Phylogenetic tree of *P. incisi* OBPs. The tree was constructed using IQ-TREE with the Maximum-likelihood method. The values on phylogenetic tree branches were confidence when replicates were 20,000

five OBPs (2, 4, 9, 10, and 11) exhibited significantly higher expression in the adult abdomen, indicating that OBPs are also expressed in non-olfactory organs, indicating a potential role beyond olfaction (Fig. 2).

Figure 2. Relative expression levels of candidate odorant-binding protein (OBP) genes of *Psyttalia incisi* adults in different tissues by qRT-PCR. Expression was normalized to the housekeeping gene GAPDH using the $2^{-\Delta\Delta Ct}$ method. Data are mean ± SEM. Different letters (a–e) indicate significant differences in means ($p < 0.05$) between males and females by one-way analysis of variance.

Identification of GRs

The transcriptome analysis identified seven candidate GR transcripts (GenBank Accessions OP535007 to OP535013) with 2 to 7 transmembrane domains and a degree of homology ranging from 79.10 to 92.68%. Among them, five GRs (2, 4–7) had complete ORFs. The BLASTx alignment revealed that three GRs (3, 5, and 7) clustered with GRs associated with sugar detection (Table S5). The phylogenetic tree constructed using the GR sequences included a putative sugar receptor and a carbon dioxide receptor. Furthermore, the phylogenetic analysis demonstrated that three GRs (3, 5, and 7) clustered with gustatory (sugar) receptor genes, while two GRs (2 and 4) clustered with CO_2 receptor genes (Fig. 3).

Identification of ORs

We identified 55 putative OR genes (54 typical ORs and one atypical odorant receptor coreceptor [Orco]) from the transcriptome and genome of *P. incisi* (Table S6) (GenBank Accessions OP956154 to OP956208). The candidate Orco shared a high sequence identity with other conserved insect co-receptors. Compared to typical ORs, Orco was highly conserved in insects, with homology ranging from 50 to 99%. Additionally, amino acid sequence analysis revealed

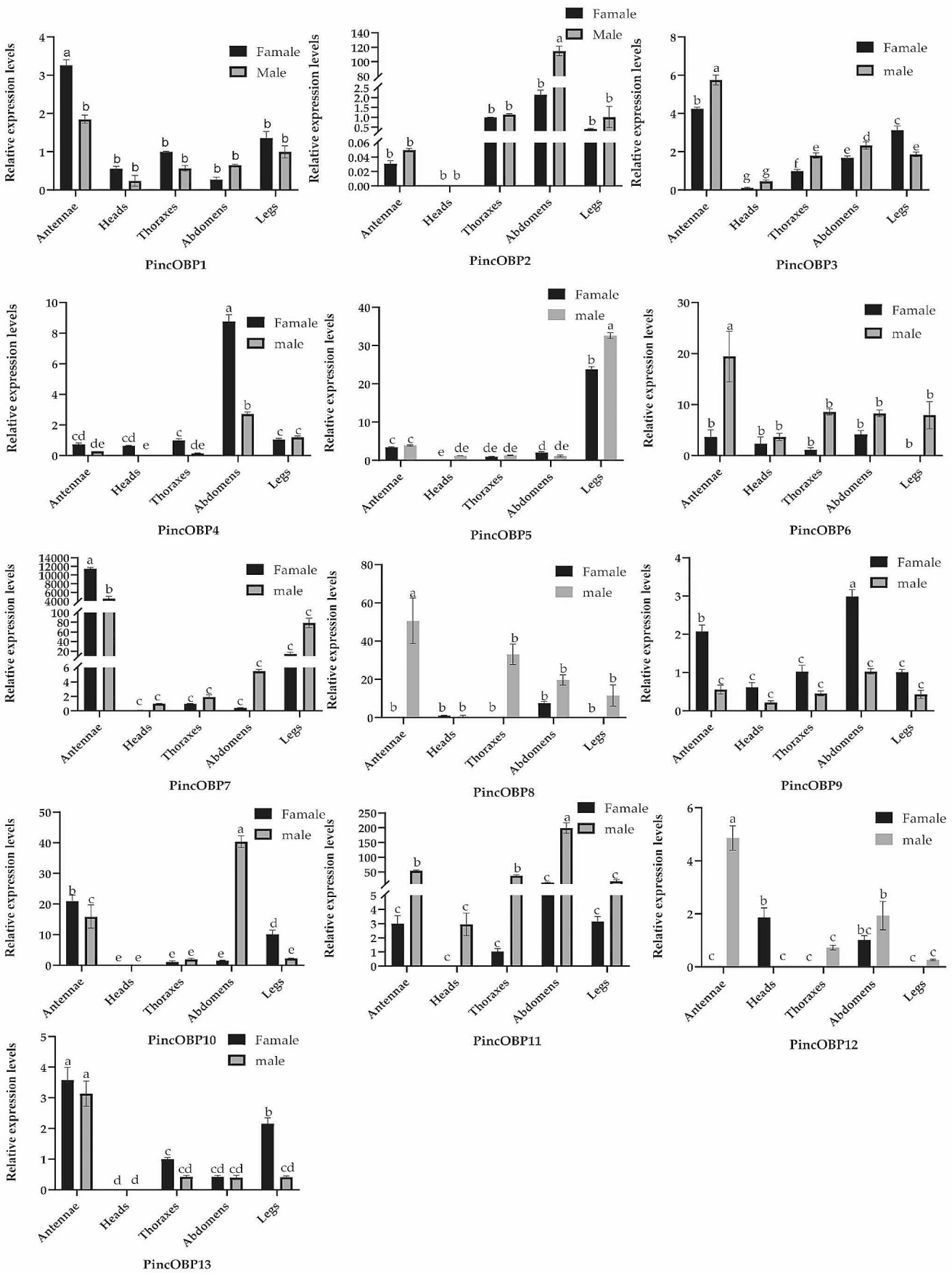
Relative expression levels
Famale
Male
male
Antennae
Heads
Thoraxes
Abdomens
Legs
PincOBP1
PincOBP2
PincOBP3
PincOBP4
PincOBP5
PincOBP6
PincOBP7
PincOBP8
PincOBP9
PincOBP10
PincOBP11
PincOBP12
PincOBP13

Fig. 2 Relative expression levels of candidate odorant-binding protein (OBP) genes of *Psyttalia incisi* adults in different tissues by qRT-PCR. Expression was normalized to the housekeeping gene GAPDH using the $2^{-\Delta\Delta Ct}$ method. Data are mean ± SEM. Different letters (**a**–**e**) indicate significant differences in means ($p < 0.05$) between males and females by one-way analysis of variance

a highly conserved region at the C-terminal [64]. The ORs were named following the convention "ORx," where the numbers x were assigned in ascending order of coding region length. Alignment of the ORs against the NR database showed homology ranging from 42.72 to 95.74%, and the presence of ORFs (324–1539 bp) encoding 107 to 512 amino acids. Based on sequence characteristics of ORs, the prediction of 0 to 7 transmembrane domains was made, of which four ORs (36, 42, 48, and 49) possessed complete ORFs and exhibited the classical feature of having seven transmembrane domains, characteristic of insect ORs (Table S6). Phylogenetic analysis showed that Orco clustered with *C. cinctus* Orco. No *P. incisi*-specific OR family expansion was observed in the phylogenetic tree (Fig. 4).

Identification of IRs

The transcriptome analysis identified 10 *P. incisi* IRs (GenBank Accessions OP558961 to OP558970) with zero to four transmembrane domains (Table S7). Among them, seven IRs (2, 4, 6, 7, 8, 9, and 10) had complete ORFs, encoding proteins with 361 to 731 amino acids. When performing a BlastX homology comparison, nine *P. incisi* IRs (1–7, 9, and 10) exhibited over 83% homology with the IR gene of *D. alloeum*, while *P. incisi* IR-8 showed the highest homology (85%) with the IR gene of *Meteorus pulchricornis*. The IRs from eight different species were classified into subfamilies (Fig. 5). Previous studies have indicated that IR8a and IR25a act as co-receptors for IRs [65]. In our analysis, IR-3, 9, and 10 clustered together with the coreceptor IR25a, with a bootstrap support value of 79, 27, and 65, respectively (Fig. 5). Similarly, two IRs genes were found in *C. cinctus, C. insularis*, *N. vitripennis*, and *C. glomerata*, while one homolog of IR25a was found in the wasps *A. gifuensis* and *D. alloeum*. Additionally, *P. incisi* IR-8 exhibited a match with an N-methyl-D-aspartic acid receptor.

Identification of SNMPs

BLASTx and cluster analysis identified two candidate SNMP transcripts (GenBank Accessions OP558971 and OP558972) (Table S8). The ORFs of SNMP-1a and 1b were found to be 669 bp and 630 bp in length, respectively, indicating that both proteins were not encoded by full-length transcripts. These transcripts exhibited a degree of homology of 86.60% and 96.17% with *D. alloeum*, respectively, and no transmembrane domains were found. Phylogenetic analysis showed that SNMP1a and SNMP1b clustered together with the SNMP1 group and showed a closer relationship to *D. alloeum* SNMPs (Fig. 6).

Discussion

The rapid development of genomics and transcriptomics has enabled the identification and characterization of olfactory genes in various parasitoid species, including *A. bambawalei* [19], *F. arisanus* [51], *M. mediator* [53], *N. vitripennis* [56], *E. formosa* [60], *C. vestalis* [66], *D. longicaudata* [67], and *Zele chlorophthalmus* [68] (Hymenoptera: Braconidae). The olfactory system plays a crucial role in host-seeking and parasitism for parasitoids. In insects, the antennae serve as the primary olfactory organ and contain a high density of chemosensory receptors [69] Therefore, sequencing the antennal transcriptome is essential for the identification of olfactory genes and serves as the foundation for understanding host-seeking behavior.

The present study involved sequencing and analyzing the antennal transcriptome of the fruit fly parasitoid *P. incisi*. A total of 64,515 unigenes were identified, with 58.26% of them being longer than 300 bp, demonstrating the high quality and depth of the transcriptome. A homology search using BLASTx against the NR database revealed that the identified *P. incisi* unigenes shared 56.72% and 14.15% identity with *D. alloeum* and *F. arisanus*, respectively (Figure S2), which is potentially due to their common affiliation with the Braconidae family. The number of unigenes annotated in the GO database (8,667) was lower than that in the NCBI-NR database (19,243). GO analysis indicated that *P. incisi* unigenes were mainly associated with binding and catalytic activities, in line with a previous study [70]. In addition, among the annotated unigenes, 7,218 and 3,738 were identified in the KOG and KEGG databases, respectively. KOG and KEGG analyses showed that a significant enrichment of unigenes involved in signal transduction (Figures S4 and S5), suggesting that potential paralogy or common ancestry with olfactory genes [71].

The transcriptome analysis revealed the presence of 87 chemosensory genes in *P. incisi*, including 13 OBPs, 55 ORs, 7 GRs, 10 IRs, and 2 SNMPs. The expression patterns of OBPs were further assessed using qRT-PCR. The number of OBP genes in *P. incisi* was similar to that in *F. arisanus* (13) [51] and *D. alloeum* (14) [50], but lower compared to *C. vestalis* (74) [72], *A. bambawalei* (54) [19], and *M. mediator* (20) [53]. OBP-3, OBP-4, and OBP-5 were found to cluster with OBP69a-like, OBP69a-like, and OBP83a-like genes from *F. arisanus*, respectively (Fig. 1), suggesting a possible functional similarity among these genes.

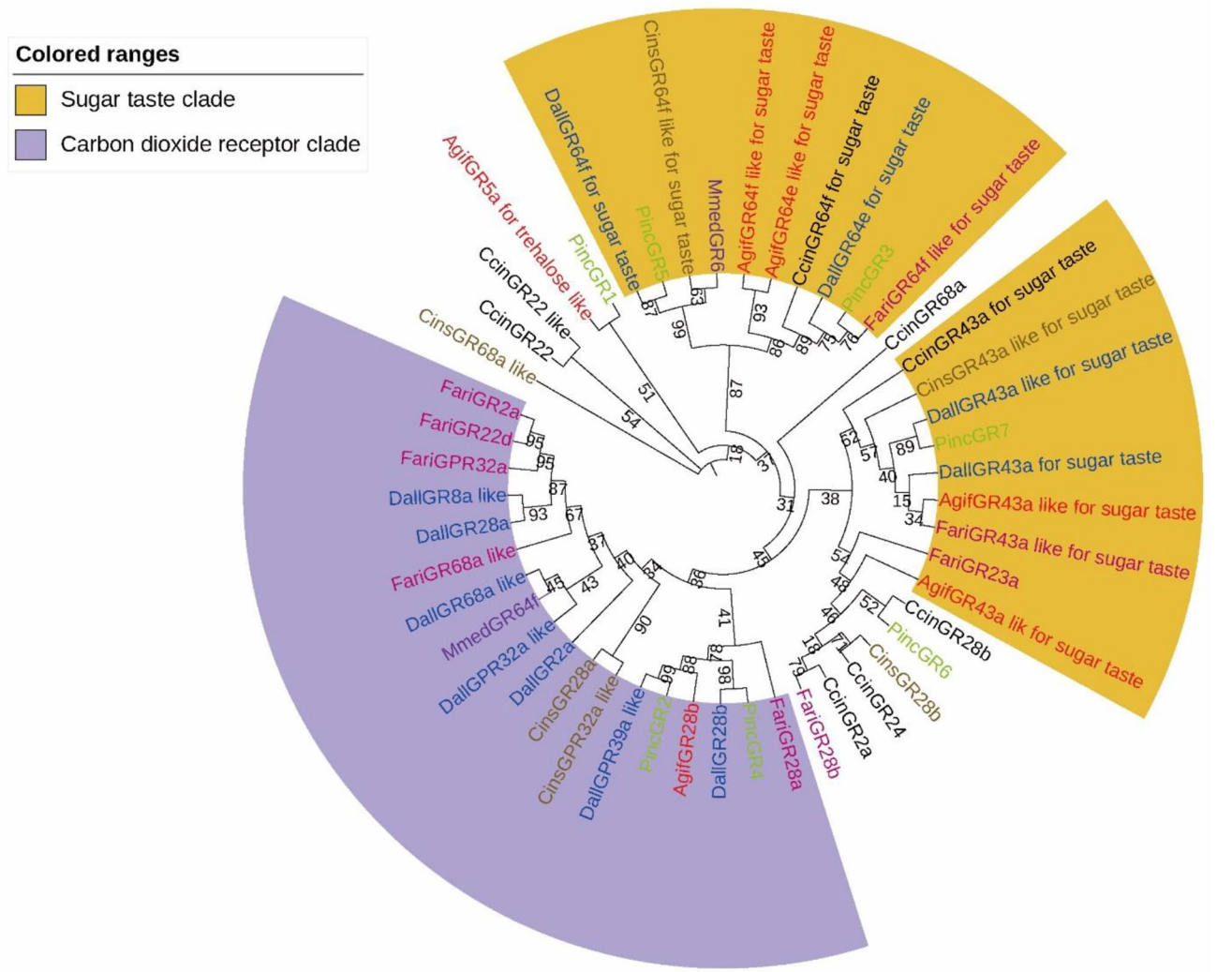

Fig. 3 Phylogenetic tree of *P. incisi* GR genes. Molecular phylogeny comparing PincGRs with gustatory receptors (GRs) from 6 other insect species: A total of 7 GRs (PincGR1-7) from *P. incisi* (Pinc) and others from *F. arisanus* (Fari), *D. alloeum* (Dall), *C. cinctus* (Ccin), *M. mediator* (Mmed), *C. insularis* (Cins) and *A. gifuensis* (Agif)

The RT-qPCR analysis showed that seven OBPs (1, 3, 6, 7, 8, 12, and 13) exhibited high expression levels in the antennae, suggesting their potential involvement in recognizing host odors or pheromones. This finding is consistent with the gene expression profiles observed in other insects like *A. bambawalei* [19] and *E. formosa* [60]. In addition, OBP-1, OBP-7, and OBP-13 showed high expression specifically in the female antennae (Fig. 2), suggesting their potential roles in mate and host finding. Moreover, the highly expressed OBP genes in the antennae may also play a role in recognizing herbivore-induced plant volatiles [63]. Interestingly, five OBPs (2, 4, 9, 10, and 11) exhibited significantly higher expression levels in the adult abdomen, suggesting their potential involvement in detecting sex pheromones, as demonstrated in *Sclerodermus* sp [73]. . Moreover, the expression of OBP-5 was found to be higher in male legs compared to other body parts, which is consistent with the tissue-specific expression of OBP2 in *Plutella xylostella*, suggesting a potential involvement in chemotaxis [74]. Several *P. incisi* OBPs were found to be exclusively or predominantly expressed in the antennae, highlighting their specific roles in olfactory perception. Furthermore, the expression of certain OBP genes exhibited sexual dimorphism, indicating functional differences between males and females.

The number of GRs in the antennae varies considerably among species—33 in *F. arisanus* [51], 23 in *Z. chloroph-thalmus* [68], and seven in *P. incisi*—presumably because the antennae are not the major olfactory organ. This variability may reflect differences in the ability to detect sugars, carbon dioxide, and pheromones. The phylogenetic analysis indicates that GR2 and GR6 may be involved in carbon dioxide sensing, while GR3, GR5, and GR7 may be

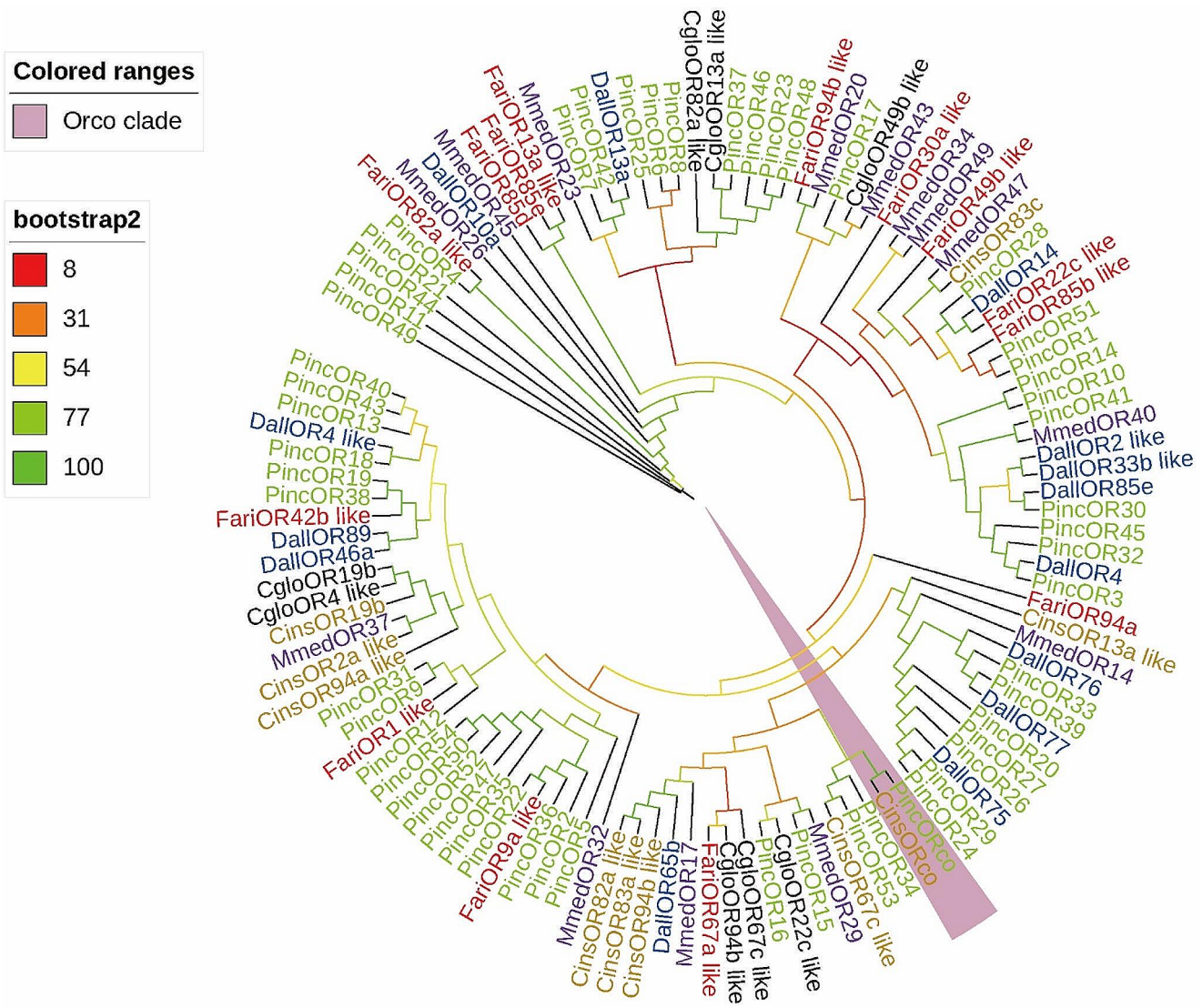

Fig. 4 Phylogenetic tree of *P. incisi* ORs. Molecular phylogeny comparing PincORs with odorant receptors (ORs) from 5 other insect species: A total of 55 ORs (PincOR1-54 and PincOrco) from *P. incisi* (Pinc) and others from *F. arisanus* (Fari), *D. alloeum* (Dall), *M. mediator* (Mmed), *C. insularis* (Cins) and *Cotesia glomerata* (Cgol). The color of the branches refers to the bootstrap values

implicated in sugar detection. Other GRs may be involved in different chemosensory processes. Since gustatory sensilla are mainly distributed in mouthparts (proboscises and labial palps), antennae, wings, legs, and ovipositor [75], it is necessary to identify other GR genes from the transcriptome of these additional tissues to better understand chemotaxis.

ORs are important chemoreceptors that primarily detect sex pheromones and other odorants [4]. The number of ORs in *P. incisi* was much lower (55) compared to *F. arisanus* (157) [51], *Meteorus, pulchricornis* (99) [76], and *N. vitripennis* (269) [56], but higher than *C. vestalis* (9) [63]. Variations in the number of OR genes could be attributed to differences in sequencing methods and depth. Through nucleic acid sequence analysis, several OR and Orco genes were identified. Phylogenetic analyses showed that most OR sequences clustered with those of *D. alloeum* and *F. arisanus*. The Orco receptors are conserved among insects [64]. One Orco sequence clustered with the highly conserved Orco of *C. insularis*, suggesting that they may have a similar role in odorant detection.

IR genes were first identified in the genome of *D. melanogaster*, and these genes are abundantly expressed in sensory neurons without ORs and GRs, playing a role in detecting various odor combinations [3]. The number of IRs in the antennae of *P. incisi* was similar to *C. cinctus* (16) [49] and *Macrocentrus cingulum* (13) [77]. IR sequences clustered into five groups, including IR3 and IR9, while IR10 clustered with the coreceptor IR25a, indicating that IRs within each group may have a similar function in the antennae.

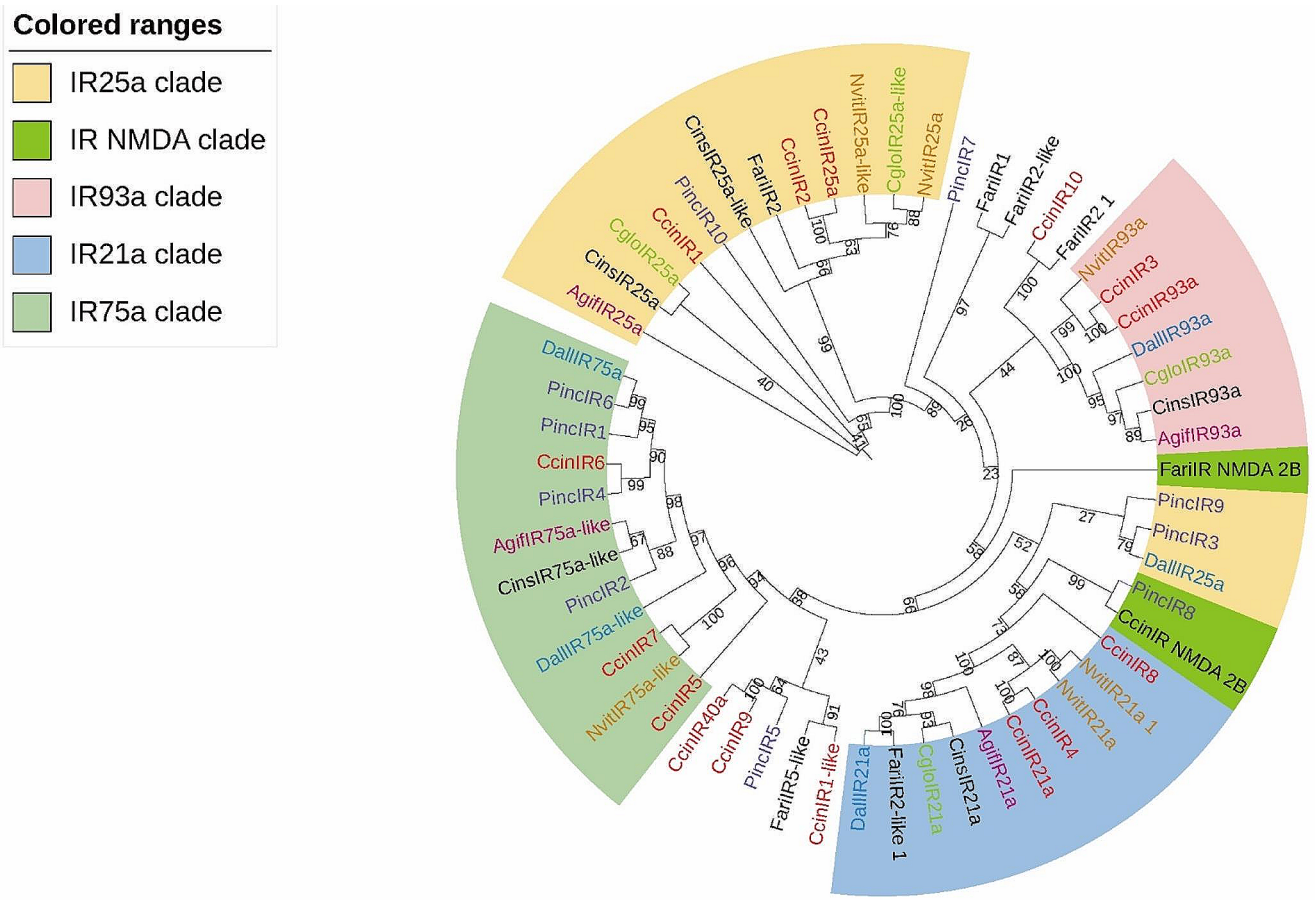

Fig. 5 Phylogenetic tree of *P. incisi* IR genes. A total of 10 IRs (PincIR1–10) from *P. incisi* (Pinc) and others from *F. arisanus* (Fari), *D. alloeum* (Dall), *C. cinctus* (Ccin), *C. insularis* (Cins), A. gifuensis (Agif), *N. vitripennis* (Nvit) and *C. glomerata* (Cglo)

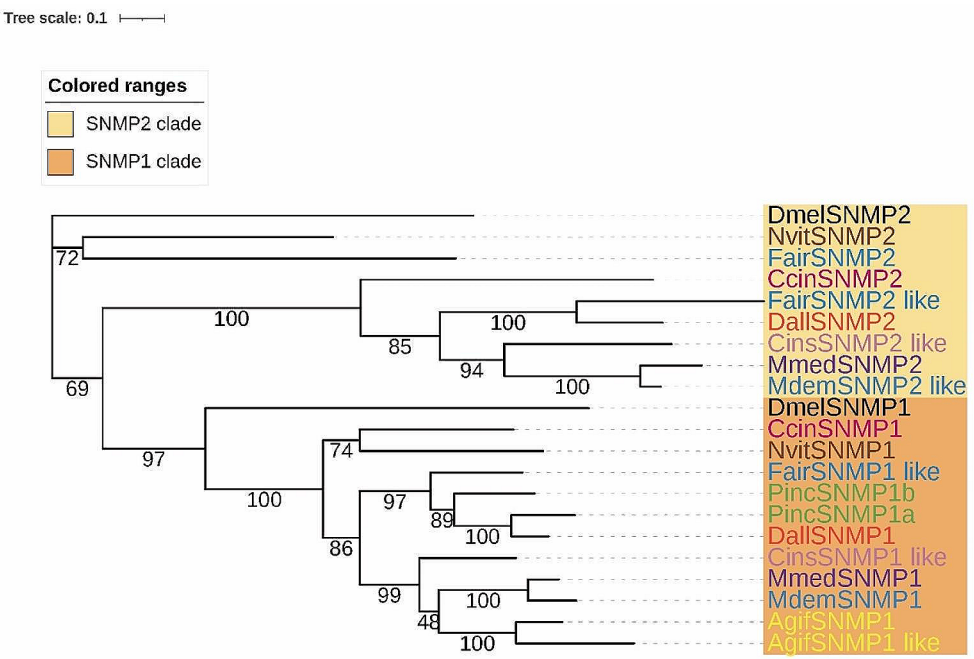

Fig. 6 Phylogenetic tree of *P. incisi* SNMP genes. A total of 2 SNMPs (PincSNMP1a, PincSNMP1b) from *P. incisi* (Pinc) and others from *F. arisanus* (Fari), *D. alloeum* (Dall), *C. cinctus* (Ccin), *C. insularis* (Cins), *A. gifuensis* (Agif), *N. vitripennis* (Nvit), *M. mediatorand* (Mmed) and *M. demolitor* (Mdem). The scale bar represents the 0.1 amino acid substitutions per site

SNMPs are classified as SNMP1 or SNMP2. SNMP1 is expressed in the dendritic membranes of olfactory receptor neurons, while SNMP2 is expressed in Sertoli cells or lymph [78]. In this study, two SNMPs were identified, and phylogenetic analysis showed that SNMP1a and SNMP1b clustered together with the SNMP1 group.

CSPs are involved in various such as chemosensory function, lipid transport, immunity, insecticide resistance, and xenobiotic degradation. While no CSP genes were found in *P. incisi*, they have been identified in other body parts such as antennae, head, thorax and wings in other species [79]. The number of chemosensory genes can vary depending on the species, body part, and testing method used.

In this study, we sequenced and assembled the transcriptome of *P. incisi* antennae and functionally annotated a total of 64,515 unigenes. Among these, 87 chemosensory genes were identified, including 13 OBPs, 55 ORs, 7 GRs, 10 IRs, and 2 SNMPs. We compared these genes with sequences from other insects, which enhanced our understanding of the olfactory systems in parasitoids and provided important insights into exploring the binding characteristics of OBPs in *P. incisi*. However, further investigations into olfactory mechanisms in *P. incisi* should be conducted using alternative methods such as protein expression profiling, fluorescence polarization competition, and molecular docking.

Conclusion

Eighty-seven chemosensory genes, including 13 OBPs, 55 ORs, 7 GRs, 10 IRs, and 2 SNMPs, were identified in the antennal transcriptome of *P. incisi.* These genes were further studied by phylogenetic and bioinformatics methods. The expression patterns of OBPs were also examined through qRT-PCR. This study significantly contributed to our understanding of *P. incisi*'s the chemosensory system. Moreover, it provided a foundation for developing attractants to monitor the population dynamics of parasitoid in the field and improve the efficacy of biological control for *B. dorsalis*.

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Author contributions Methodology, D.Y., Q.J., J.Y. and P.C.; performed the experiments, D.Y., L.J., G.Y., X.H. and K.X.; analyzed the data, D.Y., L.J., Y.H. and D.L.; drafted the manuscript, D.Y. and P.C.; revised the manuscript, D.Y., J.Y. and P.C. All the authors read and approved the publication. All authors have read and agreed to the published version of the manuscript.

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Data availability The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

Declarations

Competing interests The authors declare no competing interests.

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

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