Transcriptome and Expression Patterns of Chemosensory Genes in Antennae of the Parasitoid Wasp Chouioia cunea

Yanni Zhao¹, Fengzhu Wang¹, Xinyue Zhang¹, Suhua Zhang², Shilong Guo¹, Gengping Zhu¹, Qiang Liu¹, Min Li¹*

¹ Tianjin Key Laboratory of Animal and Plant Resistance, Tianjin Normal University, 300387, Tianjin, China,
² Natural Enemy Breeding Center of Luohe Central South Forestry, 462000, Henan, China

* skylm@mail.tjnu.edu.cn

Abstract

Chouioia cunea Yang is an endoparasitic wasp that attacks pupae of Hyphantria cunea (Drury), an invasive moth species that severely damages forests in China. Chemosensory systems of insects are used to detect volatile chemical odors such as female sex pheromones and host plant volatiles. The antennae of parasite wasps are important for host detection and other sensory-mediated behaviors. We identified and documented differential expression profiles of chemoreception genes in C. cunea antennae. A total of 25 OBPs, 80 ORs, 10 IRs, 11 CSP, 1 SNMPs, and 17 GRs were annotated from adult male and female C. cunea antennal transcriptomes. The expression profiles of 25 OBPs, 16 ORs, and 17 GRs, 5 CSP, 5 IRs and 1 SNMP were determined by RT-PCR and RT-qPCR for the antenna, head, thorax, and abdomen of male and female C. cunea. A total of 8 OBPs, 14 ORs, and 8 GRs, 1 CSP, 4 IRs and 1 SNMPs were exclusively or primarily expressed in female antennae. These female antennal-specific or dominant expression profiles may assist in locating suitable host and oviposition sites. These genes will provide useful targets for advanced study of their biological functions.

Introduction

Insects use chemoreception when searching for food, oviposition sites, and mates, as well as for social communication [1]. Chemoreception refers to the classical senses of smell (olfaction, the detection of volatile chemical stimuli) and taste (gustation, or ‘contact chemoreception’ for the detection of dissolved or solid chemicals) [2, 3]. It is often stated that “insects live in a chemical world” with chemical messages from host plant volatiles, pheromones, and predator odors captured by chemoreceptors and translated into physiological signals (chemical and electric) that modify behavior [4]. Chemoreceptors are mainly located on antennae, maxillary palps, and labial palps. Antennae are the principal location of insect olfactory receptors [5].

During the last 30 years, knowledge of the molecular and cellular basis of insect chemoreception has greatly expanded. Several multi-gene families encode proteins with crucial roles in
chemoreception systems. This list includes both receptor and non-receptor proteins. The receptors located mainly on the antennae, and to a lesser extent on other sensory appendages, are encoded by three large gene families [6]: odorant receptors (ORs), gustatory receptors (GRs), and ionotropic receptors (IRs) [7–9]. The ORs and GRs belong to the same receptor superfamily [10]. The ORs detect volatile chemicals (odors) while the GRs are responsible for contact chemoreception and detection of carbon dioxide [6, 9]. IRs belong to an ancient family of chemosensory receptors that are relatives of ionotropic glutamate receptors (iGluR), and are divided into two subfamilies, the conserved “antennal” IRs and the species-specific “divergent” IRs [11, 12]. The IRs have been identified in several insect species from different orders [13–17]. However, IRs have only been functionally characterized in Drosophila. Benton et al. [7] demonstrated that several Drosophila IRs are expressed at the ciliated endings of some antennal receptor neurons. The ectopic expression of some IRs in specific sensillae triggers novel odor-evoked responses, suggesting a functional connection between IRs and odor perception [7].

The non-receptor proteins are encoded by three gene families, the odorant binding proteins (OBPs), chemosensory proteins (CSPs) and sensory neuron membrane proteins (SNMP). The OBPs are small (10 to 30 kDa) soluble proteins that are highly abundant in the sensillum hemolymph [18–19]. OBPs bind odor molecules, most of which are hydrophobic, and transport them through the hydrophilic environment in the sensillum to the membrane-bound receptor. Additionally, OBPs may filter or purify odorants, act as activator factors of ORs (after conformational change), or as carriers expressed in non-olfactory tissues. Insect CSPs, which are also called OS-D like proteins [20] or sensory appendage proteins (SAPs) [21], represent a novel group of olfactory proteins involved in insect olfaction. CSPs have shown broad expression profiles in chemosensory tissues, including antennae [22–27], maxillary palps [28], labial palps [28–29], and the proboscis [30]. However, these proteins are also found in non-chemosensory organs, such as legs [31, 32], wings [33, 34], and pheromone glands [25]. Insect CSPs have multiple functions in insect chemoreception, growth and development. In different species, variable expression of the genes occurs depending on, for instance, sex, tissue, or life stage [24, 35]. The sensory neuron membrane proteins (SNMP) are expressed in pheromone-responding neurons in Drosophila and Lepidoptera and are, in some cases, important for proper pheromone responses [36–38].

Transcriptomes have been used to identify chemosensory genes based on next-generation sequencing data in species for which complete genomic sequence is unavailable. High-throughput transcriptomic approaches are more efficient for large-scale gene discovery, compared to conventional homology-cloning methods, and are especially useful for the identification of highly diverse gene families [39]. Transcriptomes assembled from high-throughput sequencing data have been used to identify protein families involved in chemoreception in many pest insects, such as Anopheles gambiae [40], Manduca sexta [41], Cydia pomonella [13], Helicoverpa armigera [42], Agrilus planipennis [43], Aphis gossypii [44], Spodoptera littoralis [45], Ips typographus and Dendroctonus ponderosae [46]. However, identification of chemosensory proteins among natural insect enemies/predators (e.g. parasitoids) is much more limited. Up to now, only a few species, Nasonia vitripennis [47], Cotesia vestalis [48] and Microplitis mediator [49] have been studied.

Parasitic wasps (parasitoids) serve as important natural agents that play an important role in the biological control of insect pests [50]. The success of parasitic wasps in suppressing pest populations depends on their ability to locate hosts in a complex chemical environment [50, 51]. Like most insects, parasitic wasps locate their hosts by foraging and reproduction occurs through a series of behavioral activities, regulated mainly by chemoreception [52–54]. The identification of chemosensory genes in parasitoid wasps is crucial, both to address the mechanisms controlling intraspecific or interspecific chemical communication and for potential
genetic manipulation of parasitoid behavioral responses via modification of their ability to discriminate the chemical cues used in biological control strategies [49].

The parasitoid wasp *Chouioia cunea* Yang (Hymenoptera: Eulophidae), is an endoparasitic chalcid wasp, native to China, that parasitizes the fall webworm, *Hyphantria cunea* Drury, [55]. *H. cunea* is a worldwide pest that has invaded China that utilizes > 175 different plant species in 49 families and 108 genera as acceptable hosts [56, 57]. *C. cunea* also parasitizes other Lepidoptera defoliators, including *Clostera anachoreta* F., *Micromilalopha troglodyta* (Graeser) (Notodontidae), *Stilpnotia salicis* (L.), *S. candida* Staudinger, *Ivela ochropoda* Fabricius (Lymantriidae) and *Clania variegeta* Snelleny (Psychidae) [55].

*C. cunea* are small, with adults on the scale of 1.1–1.5 mm long. As many as 365 adult wasps can be reared from a single *H. cunea* pupa with a high percentage (98–99%) of females [58]. In China, *C. cunea* has shown great promise for reducing *H. cunea* populations [56, 58, 59–61]. While previous research with *C. cunea* has focused primarily on ecology, behavior and anatomy [61], there is no information regarding its chemosensory abilities. This study investigated the antennal chemosensory gene families expressed in *C. cunea* via transcriptomic analysis using a next-generation sequencing (NGS) 454 GS FLX platform. Identification of members of the primary chemosensory families (including OBPs, GRs, ORs, IRs, CSPs and SNMPs) from female and male *C. cunea* antennae will permit a better understanding of the molecular basis of *C. cunea* chemoreception. Using RT-PCR and real-time quantitative-PCR (RT-qPCR), we screened many antennae-specific or enriched chemosensory genes from the assembled antennal transcriptomes that may have important functions in *C. cunea* chemoreception. This information could lead to the identification of targets for novel control strategies and an improved understanding of how *C. cunea* recognizes, locates and parasitizes hosts.

**Materials and Methods**

**Ethics statement**

*Antheraea pernyi* and *C. cunea* are common insects and are not included in the "List of Endangered and Protected Animals in China". All operations were performed according to ethical guidelines in order to minimize pain and discomfort to the insects.

**Insect rearing and tissue collection**

*C. cunea* were obtained in 2012 from the Natural Enemy Breeding Center of Luohe Central South Forestry (Henan, China). The tussah, *Antheraea pernyi* Guerin-Meneville (Lepidoptera: Saturniidae), an alternate host of *C. cunea*, was obtained from the Benxi Tussah Breeding Base (Liaoning, China). To obtain several generations of *C. cunea*, adult wasps were placed with *A. pernyi* pupae in erlenmeyer flasks and the openings sealed with cotton wool. They were maintained at 25°C, 75% RH, and a 14:10 light:dark cycle and incubated until the adults emerged (17–20 days). Emerged adult wasps were collected within 24 h. Parts of *C. cunea* (antenna, heads without antennae, thoraxes, and abdomens) were excised from 1-day-old male and female wasps, immersed in RNA Later (Ambion, AM7020) and collected in Eppendorf tubes. The tubes contained either 50 antennae, 50 heads, 50 thoraxes, or 50 abdomens and each tube constituted a unit sample. A total of 1500 females and 1500 males were sampled. All tubes were stored at -20°C until processing.

**RNA preparation and cDNA library construction**

A total of 500 antennae from each sex were pooled for total RNA extraction using TRIZol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s instructions. RNA
was quantified using a NanoDrop spectrophotometer (Thermo Scientific, Wilmington, DE, USA) and quality checked using electrophoresis through a 1.1% agarose gel. Approximately 500 ng messenger RNA was further purified from 50 μg total RNA using a PolyATract mRNA Isolation System III (Promega, Madison, WI, USA). The mRNAs were then sheared into approximately 800 nucleotide lengths via RNA Fragmentation Solution (Autolab, Beijing, China) at 70 uC for 30 sec, then cleaned and condensed using an RNeasy MinElute Cleanup Kit (Qiagen, Valencia, CA, USA). The first-strand cDNA was synthesized using N6 random primers and MMLV reverse transcriptase (TaKaRa, Dalian, China). Then, the second strand cDNAs were synthesized using secondary strand cDNA synthesis enzyme mixtures (Autolab, Beijing, China). The cDNAs with the desired length were purified using a QIAquick PCR Puri-
fication Kit (Qiagen, Valencia, CA, USA) and eluted with 10 μl elution buffer. After blunting and appending with a poly-A tail at the 3’ end according to Roche Rapid Library Preparing protocols (Roche, USA), the purified cDNAs were linked to GS-FLX Sequencing Adaptors (Roche, USA). Finally, cDNAs shorter than 500 bp were removed using AMPure Beads accord-
ing to the manufacturer’s instructions (Beckman, USA) prior to cDNA library preparation.

454 de novo transcriptome assembly and analysis
Pyrosequencing of the cDNA library was performed using a 454 GS-FLX sequencer (Roche, IN, USA) according to the manufacturer’s instructions. All raw reads were processed to remove low quality and adaptor sequences. Cleaned reads shorter than 60 bases were discarded based on the assumption that these reads represent sequencing artifacts [62]. Sequence reads were then clustered and assembled using the Trinity short reads assembling program (version: 2013-08-14)(http://sourceforge.net/projects/trinityrnaseq/files/PREV_CONTENTS/previous releases/) [63, 64] with a minimum sequence overlap of 49 nt and a minimum percentage overlap identity of 80%. The resulting contigs and singletons >100 bp in length were retained as unigenes and annotated as described below. Following assembly, homology searches of all unigenes were performed using the BLASTx and BLASTn programs against the NCBI non-redundant protein (nr) and nucleotide sequence (nt) databases [65]. Matches with an E-value less than 1.0E-5 were considered significant [66]. Gene names were assigned to each unigene based on the best BLASTx hit with the highest score value.

Gene Ontology terms were assigned using Blast2GO [67] through the BLASTx program with an E-value less than 1.0E-5. WEGO software [68] was used to assign each GO ID to the related ontology entries. The longest open reading frame (ORF) for each unigene was deter-
mined with the ORF finder tool (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi).
Sequence and phylogenetic analysis

The putative N-terminal signal peptides and the most likely cleavage sites were predicted using SignalP V3.0 (http://www.cbs.dtu.dk/services/SignalP/). Sequence alignments were performed using ClustalX 2.1 [71] with default gap penalty parameters of gap opening 10 and extension 0.2, and were edited using GeneDoc 2.7.0. A neighbor-joining tree [72] was constructed using MEGA 5.0 [73] with a p-distance model and a pairwise deletion of gaps. The bootstrap support of tree branches was assessed by re-sampling amino acid positions 1000 times.

RT-PCR and RT-qPCR analysis

Samples of 400 male antennae, 400 female antennae, 200 male heads, 200 female heads, 100 male thoraxes, 100 female thoraxes, 100 male abdomens, and 100 female abdomens were used for RNA extraction using TRIzol reagent. Before transcription, total RNA was treated with RQ1 RNase-Free DNase (Promega, Madison, USA) to remove residual genomic DNA. First-strand cDNA was synthesized using a TransScript RT reagent Kit (TransGen, Beijing, China). An equal amount of cDNA (200 ng) was used as templates for RT-PCR and RT-qPCR. The specific primer pairs used for RT-PCR and RT-qPCR were designed with Primer 5 (http://frodo.wi.mit.edu/). GADPH was used as the control gene to test the integrity of the cDNAs. Primers used for this study are shown in S1 Table. The RT-PCR was performed using the following conditions: 94°C for 30 sec, followed by 35 cycles of 94°C for 30 sec, 60°C for 20 sec, 72°C for 40 sec, and a final extension for 3 min at 72°C. PCR products were analyzed on 1.2% agarose gels and visualized after staining with ethidium bromide. To evaluate reproducibility, amplification of each target gene was performed three times with three biological samples. RT-qPCR analysis was conducted using an ABI 7500 Real-Time PCR System (Applied Biosystems, Carlsbad, CA). The reference gene GADPH was used to normalize target gene expression and for correcting for sample-to-sample variation. Each RT-qPCR reaction was conducted in a 20 μL reaction containing 10 μL of TransStart TopGreen qPCR SuperMix (TransGen, Beijing, China), 0.4 μL of each primer (10μM), 1 μL of sample cDNA, and 8.2 μL of sterilized H2O. The RT-qPCR cycling parameters were: 94°C for 30 sec, followed by 40 cycles of 94°C for 5 sec, 55°C for 15 sec and 72°C for 10 sec. The PCR products were heated to 95°C for 15 sec, cooled to 60°C for 1 min, heated to 95°C for 30 sec and cooled to 60°C for 15 sec to measure the dissociation curves. Negative controls without either template or transcriptase were included in each experiment. To check reproducibility, each RT-qPCR reaction for each sample was performed in three technical replicates using three biological replicates. The comparative \( 2^{-\Delta\Delta CT} \) method was used to calculate the relative quantification between tissues [74]. Comparative analyses of each target gene among the various tissues were determined using a one-way nested analysis of variance (ANOVA), followed by Tukey’s honestly significant difference (HSD) test using SPSS Statistics 18.0 (SPSS Inc., Chicago, IL, USA). When applicable, values are presented as the mean ± SE. To explore the pairwise relationship of RPKM value and quantitative Real-Time PCR, correlation analyses were calculated using the Pearson test in SPSS Statistics 18.0.

Results

454 sequencing and de novo assembly

Two non-normalized cDNA libraries for male and female C. cunea antennae were constructed. Sequencing using a 454 GS FLX platform yielded a total of 87,079,732 and 84,677,350 raw reads for the male and female antennae samples respectively. After eliminating adaptor sequences, contaminating sequences and low quality sequences, 79,802,284 (Total Clean Nucleotides (nt): 7,182,205,560) and 82,208,700 (Total Clean Nucleotides (nt): 7,398,783,000)
clean reads were obtained from male and females respectively and used in transcriptome assembly. All clean reads from male and female antennae were assembled to generate 39,309 (mean length 1005 bp) and 71,399 (mean length 794 bp) unigenes. We assembled all clean reads from male and female antennae together and generated 50,061 unigenes (mean length 1116 bp). Among these unigenes, 15,293 were Distinct Clusters (30.5%) and 34,768 were Distinct Singletons (69.5%). The assembled unigene lengths ranged from 200 bp to 17,890 bp. The size distribution of the assembled unigenes is shown in Fig 1.

Homology search of *C. cunea* antennal unigenes with other insect species

We searched for homologs in other insect species using the BLASTx and BLASTn programs with an E-value cut-off of 1E-5. The results indicated that 25,721 of the 50,061 unigenes (51.4%) had BLASTx hits in the non-redundant (nr) databases and that 21,809 (43.6%) had BLASTn hits in the non-redundant nucleotide sequence (nt) databases. Some unigenes are homologous to genes from other species with best BLASTx hits to hymenopteran insect genes (24,497 of the 25,721 nr-hits), which included 19,916 unigenes most homologous to *N. vitripennis*. The second highest hits corresponded to 902 unigenes homologous with *Megachile rotundata* sequences. The other unigenes were homologous to genes from *Harpegnathos saltator* (536 hits), *Camponotus floridanus* (506 hits), *Bombus impatiens* (506 hits), *Acromyrmex echinatior* (482 hits), and *Apis florea* (446 hits) (Fig 2).

Functional annotation of the *C. cunea* antennal unigenes

A total of 10,385 male antennal unigenes and 14,900 female antennal unigenes were annotated with the resulting functional groups classified via GO analyses [75] into one of three groups: biological process, cellular components or molecular functions (Fig 3). The abundance of annotated transcripts for each GO category was similar between the male and female antennal transcriptomes (Fig 3). Among the biological process category, cellular and metabolic processes were the two largest groups in both data sets. The molecular function category was mainly comprised of sequences involved in binding and catalytic activities. Under the cellular component category, cell and cell part were the most abundant GO terms (Fig 3). These GO annotations provide insights into the global gene expression profile for male and female antenna of *C. cunea*.

Candidate odorant binding proteins in the *C. cunea* antennae

In the *C. cunea* antennal transcriptomes, a total of 25 OBP genes were annotated based on tBLASTn results with an E-value of 1E-5 or lower (S2 Table). The nucleotide sequences of the OBP genes identified are listed in S3 Table. Among the 25 OBP genes, all have full-length ORFs that ranging in size from 330–681 bp. A neighbor-joining tree of OBPs from three hymenopteran insects showed that most *C. cunea* OBPs, with the exception of CcOBP14 and CcOBP15, which had high bootstrap support (98), did not form monophyletic groups (S1 Fig). RPKM analysis revealed that 12 OBP genes (CcOBP1- CcOBP12) are relatively abundant in the male and female antennal transcriptomes with value > 100 (S2 Table). RT-PCR analyses indicated 15 OBP genes (CcOBP1, CcOBP2, CcOBP4, CcOBP8, CcOBP9, CcOBP11, CcOBP13, CcOBP17, CcOBP18, CcOBP19, CcOBP20, CcOBP22, CcOBP23, CcOBP24 and CcOBP25) were uniquely or primarily expressed in the male and female antennae (Figs 4–6).

Real-time quantitative PCR (RT-qPCR) analysis was also performed to compare the accurate quantitative expression levels of these OBP genes among different tissues between the sexes. The OBPs had a wide range of expression patterns (Fig 7): Eight genes, OBP1, OBP8,
Fig 1. The size distribution of the assembled unigenes from C. cunea male and female antennal transcriptomes.

doi:10.1371/journal.pone.0148159.g001
OBP9, OBP11, OBP13, OBP19, OBP22 and OBP24 had significantly greater difference levels of mRNA expression in female than in male antennae \( (p < 0.05) \). OBP20 and OBP23, in contrast had significantly greater difference expression in male antennae than in female antennae \( (p < 0.05) \). The expression levels of OBP2, OBP4, OBP17, OBP18 and OBP25 were similar in the antennae of two sexes. Ten OBPs (OBP3, OBP5, OBP6, OBP7, OBP10, OBP12, OBP14, OBP15, OBP16 and OBP21) were expressed at different levels in all tested tissues. Some OBPs
Fig 4. *C. cunea* OBPs transcript levels in different body parts as evaluated by RT-PCR. GADPH was used as a control for the integrity of each cDNA template. MA: male antennae; FA: female antennae; MH: male heads; FH: female heads; MT: male thoraxes; FT: female thoraxes; MB: male abdomen; FB: male abdomen. Black graphic indicates robust amplification defined as a clearly detectable amplimer in the agarose gel, gray graphic indicates faint amplification, “-” indicates no amplification. Antennae specific or enriched genes are labeled with a capital letter “A”. doi:10.1371/journal.pone.0148159.g004

| Gene | Male | Female |
|------|------|--------|
|      | MA   | MH     | MT     | MB   | FA  | FH  | FT  | FB  |
| OBP1 | 0    | 0      | 0      | 0    | 0   | 0   | 0   | A   |
| OBP2 | 0    | 0      | 0      | 0    | 0   | 0   | 0   | A   |
| OBP3 | 0    | 0      | 0      | 0    | 0   | 0   | 0   | A   |
| OBP4 | 0    | 0      | 0      | 0    | 0   | 0   | 0   | A   |
| OBP5 | 0    | 0      | 0      | 0    | 0   | 0   | 0   | A   |
| OBP6 | 0    | 0      | 0      | 0    | 0   | 0   | 0   | A   |
| OBP7 | 0    | 0      | 0      | 0    | 0   | 0   | 0   | A   |
| OBP8 | 0    | 0      | 0      | 0    | 0   | 0   | 0   | A   |
| OBP9 | 0    | 0      | 0      | 0    | 0   | 0   | 0   | A   |
| OBP10| 0    | 0      | 0      | 0    | 0   | 0   | 0   | A   |
| OBP11| 0    | 0      | 0      | 0    | 0   | 0   | 0   | A   |
| OBP12| 0    | 0      | 0      | 0    | 0   | 0   | 0   | A   |
| OBP13| 0    | 0      | 0      | 0    | 0   | 0   | 0   | A   |
| OBP14| 0    | 0      | 0      | 0    | 0   | 0   | 0   | A   |
| OBP15| 0    | 0      | 0      | 0    | 0   | 0   | 0   | A   |
| OBP16| 0    | 0      | 0      | 0    | 0   | 0   | 0   | A   |
| OBP17| 0    | 0      | 0      | 0    | 0   | 0   | 0   | A   |
| OBP18| 0    | 0      | 0      | 0    | 0   | 0   | 0   | A   |
| OBP19| 0    | 0      | 0      | 0    | 0   | 0   | 0   | A   |
| OBP20| 0    | 0      | 0      | 0    | 0   | 0   | 0   | A   |
| OBP21| 0    | 0      | 0      | 0    | 0   | 0   | 0   | A   |
| OBP22| 0    | 0      | 0      | 0    | 0   | 0   | 0   | A   |
| OBP23| 0    | 0      | 0      | 0    | 0   | 0   | 0   | A   |
| OBP24| 0    | 0      | 0      | 0    | 0   | 0   | 0   | A   |
| OBP25| 0    | 0      | 0      | 0    | 0   | 0   | 0   | A   |
| GADPH| 0    | 0      | 0      | 0    | 0   | 0   | 0   | A   |

Fig 5. *C. cunea* ORs transcript levels in different body parts as evaluated by RT-PCR. GADPH was used as a control for the integrity of each cDNA template. MA: male antennae; FA: female antennae; MH: male heads; FH: female heads; MT: male thoraxes; FT: female thoraxes; MB: male abdomen; FB: male abdomen. Black graphic indicates robust amplification defined as a clearly detectable amplimer in the agarose gel, gray graphic indicates faint amplification, “-” indicates no amplification. Antennae specific or enriched genes are labeled with a capital letter “A”. doi:10.1371/journal.pone.0148159.g005

| Gene | Male | Female |
|------|------|--------|
|      | MA   | MH     | MT     | MB   | FA  | FH  | FT  | FB  |
| ORs  |      |        |        |      |     |     |     |     |
| Orco | 0    | 0      | 0      | 0    | 0   | 0   | 0   | A   |
| OR1  | 0    | 0      | 0      | 0    | 0   | 0   | 0   | A   |
| OR2  | 0    | 0      | 0      | 0    | 0   | 0   | 0   | A   |
| OR3  | 0    | 0      | 0      | 0    | 0   | 0   | 0   | A   |
| OR4  | 0    | 0      | 0      | 0    | 0   | 0   | 0   | A   |
| OR5  | 0    | 0      | 0      | 0    | 0   | 0   | 0   | A   |
| OR6  | 0    | 0      | 0      | 0    | 0   | 0   | 0   | A   |
| OR7  | 0    | 0      | 0      | 0    | 0   | 0   | 0   | A   |
| OR8  | 0    | 0      | 0      | 0    | 0   | 0   | 0   | A   |
| OR9  | 0    | 0      | 0      | 0    | 0   | 0   | 0   | A   |
| OR10 | 0    | 0      | 0      | 0    | 0   | 0   | 0   | A   |
| OR11 | 0    | 0      | 0      | 0    | 0   | 0   | 0   | A   |
| OR12 | 0    | 0      | 0      | 0    | 0   | 0   | 0   | A   |
| OR13 | 0    | 0      | 0      | 0    | 0   | 0   | 0   | A   |
| OR70 | 0    | 0      | 0      | 0    | 0   | 0   | 0   | A   |
| OR71 | 0    | 0      | 0      | 0    | 0   | 0   | 0   | A   |
| GADPH| 0    | 0      | 0      | 0    | 0   | 0   | 0   | A   |
OBP7, OBP10, OBP12, OBP14 and OBP15) had relatively high expression in the head. OBP21 had significantly greater difference expression in male abdomen, a non-olfactory tissue than other tested tissues (p < 0.05) (Fig 7). Pearson correlation coefficient analyses showed a significant positive correlation between qPCR results and RPKM values (Female: Pearson coefficient (r) = 0.918, p = 0.00 < 0.05; Male: Pearson coefficient = 0.743, p = 0.00 < 0.05) (S2 Table and Fig 7).

Candidate odorant receptors in the C. cunea antennae

In the C. cunea antennal transcriptomes, a total of 80 OR genes (79 typical ORs and one atypical coreceptor Orco) were annotated based on the tBLASTn results with an E-value of 1E-5 or lower. Eight of the putative OR sequences had fully intact ORFs encompassing start and stop codons with lengths ranging from 1167–1440 bp (S4 Table). The C. cunea OR nucleotide sequences are listed in S3 Table. A neighbor-joining tree of ORs from three hymenopteran
Fig 7. *C. cunea* OBPs transcript levels in different tissues as measured by RT-qPCR. MA: male antennae; FA: female antennae; MH: male head; FH: female head; MT: male thorax; FT: female thorax; MB: male abdomen; FB: female abdomen. The glyceraldehyde-3-phosphate dehydrogenase (GADPH) was used to normalize transcript levels in each sample. The standard error is represented by the error bar, and the different letters (a, b, c) above each bar denote significant differences (p<0.05).

doi:10.1371/journal.pone.0148159.g007
insects showed that most ORs from *C. cunea* did not form monophyletic groups. The lone exceptions were CcOR25 and CcOR48, which formed monophyletic groups with strong bootstrap support (100). The olfactory co-receptor family is highly conserved across the three hymenopteran species (S2 Fig). RPKM analyses revealed that Orco was the most highly expressed of the 80 CcORs, with RPKM values of 9.2183 and 30.2326 in male and female antennae, respectively. The other 79 typical ORs, however, had relatively low expression levels (RPKM values ranging from 0 to 10.6553) as compared with the Orco, OBP, and CSP sequences. CcOrco and CcOR1 through CcOR13 were selected for additional RT-PCR and quantitative RT-qPCR analyses to assess their expression in different tissues (antenna, head, thorax, and abdomen) of males and females. RPKM values suggested that all 14 ORs were more abundant in the female antenna compared to male. Two ORs, CcOR70 and CcOR71 were also chosen as representative OR with male dominant expression (S4 Table).

The RT-PCR and RT-qPCR results indicated that most of the OR genes (15 ORs: CcOrco, CcOR1-5, CcOR7-13, CcOR70-OR71) were exclusively or primarily expressed in the antennae (Figs 5 and 8). Furthermore, expression of 14 of these ORs (CcOrco, CcOR1-OR5 and CcOR7-OR13) was significantly greater difference in the antennae of the female wasps than in male wasps (p < 0.05). Consistent with the RNA sequencing data, CcOR70 and CcOR71 were expressed at significantly greater difference in males than in females (p < 0.05). CcOR6 was expressed at varying levels in the other tested tissues. Pearson correlation coefficient analyses showed significant positive correlation of qPCR results and RPKM values (Female: Pearson coefficient (r) = 0.715, p = 0.03 < 0.05; Male: Pearson coefficient = 0.797, p = 0.00 < 0.05). These RT-qPCR results are consistent with RPKM values (Fig 8 and S4 Table).

**Candidate gustatory receptors (GRs) in *C. cunea* antennae**

In the present study, we identified 17 GR genes, albeit none encompassing complete ORFs (S5 Table). The nucleotide sequences of the putative CcGR transcripts are listed in S3 Table. A neighbor-joining tree of GRs from four hymenopteran species showed that most GRs from *C. cunea* did not form monophyletic group, exceptions included two monophyletic branches comprised of CcGR3/CcGR14 and CcGR2/CcGR16 (S3 Fig). RT-PCR and RT-qPCR results revealed that most of the GRs (CcGR1, CcGR2, CcGR4-CcGR6, CcGR8, CcGR9, CcGR11, CcGR12, CcGR14, CcGR15 and CcGR17) were exclusively or primarily expressed in the antennae (Figs 6 and 9). The expression of 8 GRs (CcGR1, CcGR2, CcGR4, CcGR5, CcGR9, CcGR12, CcGR14 and CcGR15) in the antennae of female wasps was significantly greater difference than in the antennae of male wasps (p < 0.05), whereas CcGR6, CcGR8 and CcGR17 genes were expressed at similar levels in females and males. CcGR3, CcGR7, CcGR10, CcGR13 and CcGR16 were also expressed in the heads of males and females at different levels. CcGR11 and CcGR14 were also expressed in the male abdomens. Pearson correlation coefficient analyses showed a significant correlation of qPCR results and RPKM values (Female: Pearson coefficient (r) = 0.999, p = 0.00 < 0.05; Male: Pearson coefficient = 0.795, p = 0.00 < 0.05) (Fig 9 and S5 Table).

**Candidates for other chemosensory genes**

We identified 11 putative CSP genes in *C. cunea* antennae, five of which encompass full-length ORFs (S6 Table). The nucleotide sequences of these transcripts are listed in S3 Table. A neighbor-joining tree of CSPs from four hymenopteran species suggested orthologous sequences in *C. cunea*. However, a number of the CcCSPs formed monophyletic groups: CcCSP4/CcCSP8 (bootstrap: 80), CcCSP11/CcCSP6 (bootstrap: 100) and CcCSP3/CcCSP5 (bootstrap: 100) (S4 Fig). We conducted RT-PCR and quantitative RT-PCR analyses using different tissues...
Fig 8. C. cunea ORs transcript levels in different tissues as measured by RT-qPCR. MA: male antennae; FA: female antennae; MH: male head; FH: female head; MT: male thorax; FT: female thorax; MB: male abdomen; FB: female abdomen. The glyceraldehyde-3-phosphate dehydrogenase (GADPH) was used to normalize transcript levels in each sample. The standard error is represented by the error bar, and the different letters (a, b, c) above each bar denote significant differences (p<0.05).

doi:10.1371/journal.pone.0148159.g008
Fig 9. *C. cunea* GRs transcript levels in different tissues as measured by RT-qPCR. MA: male antennae; FA: female antennae; MH: male head; FH: female head; MT: male thorax; FT: female thorax; MB: male abdomen; FB: female abdomen. The glyceraldehyde-3-phosphate dehydrogenase (GADPH) was used to normalize transcript levels in each sample. The standard error is represented by the error bar, and the different letters (a, b, c) above each bar denote significant differences (p<0.05).

doi:10.1371/journal.pone.0148159.g009
(antenna, head, thorax, and abdomen) of adult males and females to assess the expression of CcCSP1—CcCSP5. CcCSP1 and CcCSP3 were exclusively or primarily expressed in the antennae (Figs 6 and 10). Furthermore, the expression of CcCSP3 in the antennae of female wasps was significantly greater difference than in the antennae of male wasps, while CSP1 in the antennae of male wasps was significantly greater difference than in the antennae of female wasps (p < 0.05). CcCSP2, CcCSP4 and CcCSP5 were expressed at varying levels in the other tissues tested. Pearson correlation coefficient analyses showed a significant positive correlation between qPCR results and RPKM values (Female: Pearson coefficient (r) = 0.984, p = 0.03 < 0.05; Male: Pearson coefficient = 0.993, p = 0.01 < 0.05) (Fig 10 and S6 Table).

In addition, we identified 10 putative IRs including three full length ORFs from the C. cunea antennal transcriptomes (S7 Table). The nucleotide sequences of the CcIR transcripts are listed in S3 Table. A neighbor-joining tree of IRs from two hymenopteran and one dipteran species suggested potential orthologs to the most of CcIR sequences (S5 Fig). Similar to the ORs, RPKM value analysis revealed that all CcIRs had relatively low expression levels (RPKM value ranged from 0 to 33) compared with the OBPs and CSPs (S7 Table). As before, we conducted RT-PCR and quantitative RT-PCR analyses using different tissues (antenna, head, thorax, and abdomen) of adult males and females to assess the expression of CcIR8a, CcIR75q2, CcIR64a, CcIR93a and CcIR1. The RT-PCR and RT-qPCR results showed that four IRs (CcIR8a, CcIR75q2, CcIR64a and CcIR1) were exclusively or primarily expressed in the antennae (Figs 6 and 11). The expression of CcIR8a, CcIR75q2, CcIR64a and CcIR1 in female antennae was significantly greater difference than in male antennae (p < 0.05). CcIR93a was also expressed in female head. Pearson correlation coefficient analyses showed a significant positive correlation between qPCR results and RPKM values (Female: Pearson coefficient (r) = 0.997, p = 0.00 < 0.05; Male: Pearson coefficient = 0.978, p = 0.04 < 0.05) (Fig 11 and S7 Table). We also identified a single full length ORF (1050 bp) encoding a putative SNMP gene, CcSNMP1 (S7 Table), the nucleotide sequences of which SNMP genes that were identified from the C.
cunea antennal transcriptomes is listed in S3 Table. It has fully intact ORF with a length of 1050 bp, and RPKM values of 0.3906 and 3.7699 in male and female antennae, respectively, suggest relatively low expression. Both RT-PCR and RT-qPCR showed that SNMP1 is exclusively expressed in the antennae (Figs 6 and 11) with significantly greater difference levels observed in female antennae than in male antennae (p<0.05).

Discussion

The molecular basis of chemoreception in parasitoids is not well understood compared to pest insects. In the present study, we generated antennal transcriptomes of male and female C. cunea using next-generation sequencing technology. We identified 144 chemosensory genes encoding members of six protein families, including 25 OBPs, 80 ORs, 10 IRs, 11 CSP, 1 SNMPs, and 17 GRs. However, transcripts present in low abundance and those that were too divergent to be identified using a BLAST search may have been overlooked in transcriptome analysis [76]. Thus, it is unlikely that the identified genes represent the total number of the related chemosensory genes in C. cunea. However, this is the first comprehensive characterization of chemosensory genes in C. cunea. Our findings provide insight into the molecular mechanisms of chemoreception in C. cunea, and identify potential molecular targets for possible use in biological control strategies. C. cunea is an endoparasitic chalcid wasp that parasitizes the pupa of the fall webworm. Inside its host pupa, C. cunea develops from the egg to the pre-oviposition adult stage. The adult wasps mate inside the host pupa, and then chew a hole in the host pupal shell. The other wasps exit the pupa using this same hole. Thus, the adult females can parasitize new hosts soon after their ‘emergence’ [58]. In this study, the wasps we collected were emerged adult wasps within 24 h from the host pupa. So the emerging wasps had completed mating. We assume that, these chemosensory genes with female antennal-specific or dominant expression profiles may play important roles in locating suitable hosts and oviposition sites. This however, should be verified in further experiments.
In this study, Pearson correlation coefficient analyses showed significant positive correlation between qPCR results and RPKM values of all of the tested chemosensory genes. It shows that qPCR results agree well with the RPKM analyses and indicates that RNAseq count data on steady state transcript levels [77]. And it also suggests that the results of RNAseq count data are confident. A total of 25 OBPs were identified in the antennal transcriptome of C. cunea. The number of C. cunea OBPs identified was smaller than that identified from the genomes of B. mori (44) [78], A. gambiae (57) [79], D. melanogaster (51) [80], and A. ipsilon (33) [81]. There may be OBP genes that are not yet identified in C. cunea antennae due to low expression levels. Moreover the lab-reared wasps had no exposure to host insect odors or plant odors in natural surroundings. There may be OBP genes not yet identified due to this lack of exposure [49]. Many OBPs in insects have a wide range of expression patterns among different tissues of males and females [48, 76, 81]. Here, 15 OBPs had obvious antennal-specific expression profiles. Based on the different expression profiles of these OBPs in male and female antennae, we suggest that these male or female antennae-enriched OBPs may play a role in sex-specific behaviors. A total of eight OBPs had obvious female antennal-specific expression. These genes may play important roles in locating suitable hosts and oviposition sites. The remaining 10 OBPs were expressed at different levels in a variety of tissues. These may be related to other functions [82]. A total of 4 OBPs had relatively high expression in the head. This expression may be in the maxillary or labial palps; however, this should be experimentally verified. One OBP (OBP21) was highly expressed in a non-olfactory body section (abdomen of the male) and may be a carrier of ligands other than odorants [83]. Future functional investigation of these genes is warranted to determine their specific roles.

Insect olfactory receptors (ORs) are the most important components in sex pheromone and general odorant detection [81]. In this study, a total of 80 ORs were identified in the antennal transcriptome of C. cunea (79 typical ORs and the atypical co-receptor Orco). The number of ORs identified in C. cunea is fewer than in A. mellifera (170) [84] and migratory locust (142) [85], but was much greater than in the parasitoids M. mediator (60) [49] and C. vestalis (6) [48]. Most ORs in insects are extensively expressed in the antennae [86]. In our study, most of the tested ORs showed an antennal-specific expression profile. Expression of a majority of the examined ORs (14/16) was much higher in the antennae of female wasps than in male antennae. The ORs with antennal-specific or dominant expression profiles may play crucial roles in the olfactory chemoreception of wasps. These female antennal-specific or dominant expression genes may play crucial roles in locating suitable hosts and oviposition sites. We also identified several ORs expressed in non-chemosensory tissues, similar to the findings in other studies [81, 87–89]. The expression of ORs in non-olfactory tissues suggests that they may have physiological functions in other organs. Unlike other studies that showed Orco expression in other tissues [88, 90], Cc Orco had a clear antennal-specific expression pattern. This is consistent with studies in Agrotis ipsilon [81]. The OR tree showed that the Orco are highly conserved (S2 Fig). Based on the analysis of selection pressure, CcOrco appears to be primarily negatively selected [91], which likely explain the conservation of CcOrco.

GRs are generally expressed in gustatory receptor neurons (GRNs) within gustatory organs [55] and are crucial for responses to soluble taste and contact pheromones [11, 56]. However, some GRs are also expressed in antennal dendrites and respond to carbon dioxide, potentially implicating them in olfaction [92, 93]. GRs are more highly conserved in sequence and structure than ORs [94, 95], a feature that has been suggested to be due to the comparatively smaller search space among cues associated with GRs than ORs. Because GR expression levels are quite low and mainly expressed in gustatory organs [96, 97], only 17 putative GR-encoding transcripts were identified from C. cunea antennae. However, this number is greater than that in M. mediator (2), which was the first report of GR genes in wasp antennae.
[49]. Similar to ORs, *C. cunea* GRs that were identified in the antennal transcriptome were primarily expressed in the antennae. Expression of eight CcGRs in the antennae of female wasps was much higher than in the antennae of male wasps. It is will be interesting to determine if these GRs function in locating oviposition sites. Another five GRs were expressed at varying levels in other tested tissues. These proteins may have multiple functions in insect chemoreception, growth, and development.

Chemosensory proteins (CSPs) represent a more recently discovered soluble carrier proteins that probably function in a manner similar to OBPs in insect chemoreception [98]. These proteins have broad expression profiles in chemosensory tissues, including antennae [24, 25, 28, 26], maxillary palps [29], labial palps [29, 30], and proboscis [31]. However, these proteins are also found in non-chemosensory organs, such as legs [32, 33], wings [34, 56], and pheromone glands [26]. We identified 11 CSPs in *C. cunea*. In our study, two of the five tested CSPs showed an obvious female antennal-specific expression profile. These CSPs may play important roles in odorant detection.

Insect chemosensory ionotropic receptors (IRs) belong to an ancient chemosensory receptor family that was first discovered in *D. melanogaster*. They are expressed in sensory neurons that respond to different odorants but do not express either ORs or gustatory receptors (GRs) [7]. At present, 66 IRs have been identified in *D. melanogaster* [7, 12], 17 IRs in the *Spodoptera littoralis* [16, 99], 21 IRs in *Manduca sexta* [100], 15 IRs in *Cydia pomonella* [13] and 12 IRs in *Helicoverpa armigera* [17] have been identified. Because of IRs have relatively well conserved sequence homology across insect orders compared to other chemosensory genes, the CcIRs were named based on homology to similar genes in other species. The neighbor-joining tree for IRs from four hymenopteran species showed that most IRs clustered with their respective orthologs. It also showed that the IRs are highly conserved. The RPKM value analysis revealed that all the CcIRs had relatively low expression levels compared with the OBPs and CSPs. Four of the five CcIRs tested by RT-PCR and RT-qPCR had female antennal-specific expression profiles, suggesting that these genes may play roles in locating suitable hosts and oviposition sites.

Insect SNMPs are two trans-membrane domain-containing proteins that may play significant roles in insect chemoreception [18, 29, 101]. Two SNMP subfamilies, SNMP1 and SNMP2, have been identified in insects; however, these subfamilies show different expression profiles in the antennae sensilla: SNP1 proteins are detected in pheromone-sensitive olfactory receptor neurons (ORNs) [102–104]; however, SNP2 proteins are expressed in the supporting cells [99, 100]. In the present study, we identified one SNMP gene, CcSNMP1 that was antennal-specific. The expression of CcSNMP1 in the antennae of female wasps was much higher than in the antennae of males. In certain insects, a high level of antenna-specific expression has been observed for SNMP1, whereas SNMP2 is expressed at various levels in a variety of tissues [19, 105]. However, there are few studies of SNMPs in Hymenoptera. We speculate that the level of SNMP2 expression in the antenna of *C. cunea* may be too low, and thus undetectable based on the transcriptome analysis.

In this study, we identified and annotated several groups of chemosensory genes in the antennae of *C. cunea*. An expression profile analysis revealed that some chemosensory genes are uniquely or primarily expressed in female antennae. One possible explanation for the observed female-biased expression genes is that female *C. cunea* have large numbers of sensilla trichodea present on their antennae that are much more than the male [106]. These female antennal-specific or dominant expression OBPs, CSPs, ORs, GRs, IRs, and SNMPs may be important for locating suitable hosts and oviposition sites. The biological functions of these genes and their products are still poorly known but our results should help pave the way for future studies of this nature.
Supporting Information

S1 Fig. Neighbor-joining tree of candidate OBPs from C.cunea (red), Microplitis mediator (green) and Nasonia vitripennis (blue). The protein names and sequences of OBPs that were used in this analysis are listed in S8 Table. Bootstrap supports are given at the node. The values greater than or equal to 50 were shown on the tree. (TIF)

S2 Fig. Neighbor-joining tree of candidate ORs from C.cunea (red), Microplitis mediator (green) and Nasonia vitripennis (blue). The protein names and sequences of ORs that were used in this analysis are listed in S9 Table. Bootstrap supports are given at the node. The values greater than or equal to 50 were shown on the tree. (TIF)

S3 Fig. Neighbor-joining tree of candidate GRs from C.cunea (red), Microplitis mediator (green), Sclerodermus sp. (purple) and Nasonia vitripennis (blue). The protein names and sequences of GRs that were used in this analysis are listed in S10 Table. Bootstrap supports are given at the node. The values greater than or equal to 50 were shown on the tree. (TIF)

S4 Fig. Neighbor-joining tree of candidate CSPs from C.cunea (pink), Solenopsis invicta (green), Camponotus japonicus (orange) and A. cerana (blue). The protein names and sequences of CSPs that were used in this analysis are listed in S11 Table. Bootstrap supports are given at the node. The values greater than or equal to 50 were shown on the tree. (TIF)

S5 Fig. Neighbor-joining tree of candidate IRs from C.cunea (red), M. mediator (blue), and Drosophila melanogaster (green). The protein names and sequences of IRs that were used in this analysis are listed in S12 Table. Bootstrap supports are given at the node. The values greater than or equal to 50 were shown on the tree. (TIF)

S1 Table. Primers used for RT-PCR and RT-qPCR analysis of olfactory genes of the C. cunea. (DOC)

S2 Table. List of OBP genes in C.cunea antennae. (DOCX)

S3 Table. The nucleotide sequences of 25 OBPs, 11 CSPs, 80 ORs, 10 IRs, 1 SNMPs and 17 GRs of C.cunea identified in present study. (DOC)

S4 Table. List of OR genes in C.cunea antennae. (DOCX)

S5 Table. List of GR genes in C.cunea antennae. (DOCX)

S6 Table. List of CSP genes in C.cunea antennae. (DOCX)

S7 Table. List of IR and SNMP genes in C.cunea antennae. (DOCX)
S8 Table. The protein names and sequences of OBPs that were used in phylogenetic tree analysis. (DOCX)

S9 Table. The protein names and sequences of ORs that were used in phylogenetic tree analysis. (DOCX)

S10 Table. The protein names and sequences of GRs that were used in phylogenetic tree analysis. (DOCX)

S11 Table. The protein names and sequences of CSPs that were used in phylogenetic tree analysis. (DOCX)

S12 Table. The protein names and sequences of IRs that were used in phylogenetic tree analysis. (DOCX)

Author Contributions
Conceived and designed the experiments: ML YNZ. Performed the experiments: YNZ FZW XYZ. Analyzed the data: YNZ SLG GPZ. Contributed reagents/materials/analysis tools: QL SHZ. Wrote the paper: ML YNZ. Bioinformatics analysis and qRT-PCR: YNZ.

References
1. Schoonhoven LM, van Loon JJA, Dicke M. Insect-plant biology. 2nd ed. New York: Oxford University Press; 2005.
2. Barbarossa IT, Muroni P, Dardani M, Casula P, Angiolo AM. New insight into the antennal chemosensory function of *Opis concolor* (Hymenoptera, Braconidae). Ital J Zool. 1998; 65: 367–70.
3. Su CY, Muenz K, Carlson JR. Olfactory perception: receptors, cells, and circuits. Cell. 2009; 139: 45–59. doi: 10.1016/j.cell.2009.09.015 PMID: 19804753
4. Jacquin-Joly E, Merlin C. Insect olfactory receptors: contributions of molecular biology to chemical ecology. J Chem Ecol. 2004; 30(12): 2359–2397. PMID: 15724962
5. Hallem E, Ho M, Carlson JR. The molecular basis of odor coding in the *Drosophila* antenna. Cell. 2004; 117(7): 965–79. PMID: 15210116
6. Vosshall LB, Stocker RF. Molecular architecture of smell and taste in *Drosophila*. Annu Rev Neurosci. 2007; 30: 505–533. PMID: 17506643
7. Benton R, Vannice KS, Gomez-Diaz C, Vosshall LB. Variant ionotropic glutamate receptors as chemosensory receptors in *Drosophila*. Cell. 2009; 136: 149–162. doi: 10.1016/j.cell.2008.12.001 PMID: 19135896
8. Clyne P, Warr C, Freeman M, Lessing D, Kim J, Carlson JR. A novel family of divergent seven-transmembrane proteins: candidate odorant receptors in *Drosophila*. Neuron. 1999; 22: 327–338. PMID: 1069338
9. Robertson HM, Kent LB. Evolution of the gene lineage encoding the carbon dioxide receptor in insects. J Insect Sci. 2009; 9: 1–14.
10. Robertson HM, Warr CG, Carlson JR. Molecular evolution of the insect chemoreceptor gene superfamily in *Drosophila melanogaster*. PNAS. 2003; 100: 14537–14542. PMID: 14608037
11. Abuin L, Bargenton B, Ulbrich MH, Isacoff EY, Kellenberger S, Benton R. Functional architecture of olfactory ionotropic glutamate receptors. Neuron. 2011; 69: 44–60. doi: 10.1016/j.neuron.2010.11.042 PMID: 21220098
12. Croset V, Rytz R, Cummins SF, Budd A, Brawand D, Kaeussmann H, et al. Ancient protostome origin of chemosensory ionotropic glutamate receptors and the evolution of insect taste and olfaction. Plos Genet. 2010; 6: e1001064. doi: 10.1371/journal.pgen.1001064 PMID: 20808886
13. Bengtsson JM, Trona F, Montagné N, Anfora G, I gnell R, Witzgall P, et al. Putative chemosensory receptors of the codling moth, *Cydia pomonella*, identified by antennal transcriptome analysis. PLoS ONE. 2012; 7(2): e31620. doi: 10.1371/journal.pone.0031620 PMID: 22363688

14. Li ZQ, Zhang S, Luo JY, Wang SB, Wang CY, Lv LM, et al. Identification and expression pattern of candidate olfactory genes in Chrysoperla sinica by antennal transcriptome analysis. Comp Biochem Phys D. 2015; 15: 28–38.

15. Wu ZZ, Zhang H, Wang ZB, Bin SY, He HL, Lin JT. Discovery of Chemosensory Genes in the Oriental Fruit Fly, *Bactrocera dorsalis*. PLoS ONE. 2015; 10(6): e0129794. doi: 10.1371/journal.pone.0129794 PMID: 26070069

16. Olivier V, Monsenpes C, Francois MC, Poivet E, Jacquin-Joly E. Candidate chemosensory ionotropic receptors in a Lepidoptera. Insect Mol Biol. 2011; 20: 189–199. doi: 10.1111/j.1365-2583.2010.01057.x PMID: 21091811

17. Gu XC, Zhang YN, Kang K, Dong SL, Zhang LW. Antennal Transcriptome Analysis of Odorant Receptor Genes in the Red Turpentine Beetle (RTB), *Dendroctonus valens*. PLoS ONE. 2015; 10(5): e0125159. doi: 10.1371/journal.pone.0125159 PMID: 25938508

18. Sánchez-Gracia A, Vieira FG, Rozas J. Molecular evolution of the major chemosensory gene families in insects. Heredity. 2009; 103:208–216. doi: 10.1038/hdy.2009.55 PMID: 19436326

19. Vogt RG. Biochemical diversity of odor detection: OBPs, ODEs and SNMPs. In: Blomquist GJ, Vogt RG, editors. Insect pheromone biochemistry and molecular biology. San Diego: Academic Press; 2003. pp. 391–445.

20. McKenna MP, Hekmat-Scafe DS, Gaines P, Carlson JR. Putative *Drosophila* pheromone-binding proteins expressed in a subregion of the olfactory system. J Biol Chem. 1994; 269: 16340–16347. PMID: 7545907

21. Pikielny C, Hasan G, Rouyer F, Rosbash M. Members of a family of *Drosophila* putative odorant-binding proteins are expressed in different subsets of olfactory hairs, Neuron. 1994; 12(1): 35–49. PMID: 8206941

22. Robertson HM, Martos R, Sears CR, Todres EZ, Walden KKO, Nardi JB. Diversity of odorant binding proteins revealed by an expressed sequence tag project on male *Manduca sexta* moth antennae. Insect Mol Biol. 1999; 8: 501–518. PMID: 10620045

23. Angeli S, Ceron F, Scaloni A, Monti M, Monteforti G, Minnocci A, et al. Purification, structural characterization, cloning and immunocytochemical localization of chemoreception proteins from *Schistocerca gregaria*. Eur J Biochem. 1999; 262: 745–754. PMID: 10411636

24. Liu R, He X, Lehane S, Lehane M, Hertz-Fowler C, Berriman M, et al. Expression of chemosensory proteins in the tsetse fly *Glossina morsitans morsitans* is related to female host-seeking behavior. Insect Mol Biol. 2012; 21: 41–48. doi: 10.1111/j.1365-2583.2011.01114.x PMID: 22074189

25. Vogt RG, Franci, G, Nagnan-Le Meillour P. Functional and expression pattern analysis of chemosensory proteins expressed in antennae and pheromonal gland of *Mamestra brassicae*. Chem Senses. 2001; 26: 833–844. PMID: 11555479

26. González D, Zhao Q, McMahen C, Velasquez D, Haskins WE, Sponsel V, et al. The major antennal chemosensory protein of red imported fire ant workers. Insect Mol Biol. 2009; 18: 395–404. doi: 10.1111/j.1365-2583.2009.00883.x PMID: 19523071

27. Maleszka R, Stange G. Molecular cloning, by a novel approach, of a cDNA encoding a putative olfactory protein in the labial palps of the moth *Cactoblastis cactorum*. Gene. 1997; 202: 39–43. doi: 10.1016/S0378-1119(97)00432-5 PMID: 927543

28. Jin X, Brandazza A, Navarrini A, Ban L, Zhang S, Steinbrecht RA, et al. Expression and immunolocalisation of odorant-binding and chemosensory proteins in locusts. Cell Mol Life Sci. 2005; 62: 1156–1166. PMID: 15928808

29. Nagnan-Le Meillour P, Cain AH, Jacquin-Joly E, Francois MC, Ramachandran S, Maida R, et al. Chemosensory proteins from the proboscis of *Mamestra brassicae*. Chem Senses. 2000; 25: 541–553. PMID: 11015326

30. Nomura A, Kawasaki K, Kubo T, Natori S. Purification and localization of p10, a novel protein that increases in nymphal regenerating legs of *Periplaneta americana* (American cockroach). Int J Dev Biol. 1992; 36: 391–398. PMID: 1445782

31. Kitabayashi AN, Arai T, Kubo T, Natori S. Molecular cloning of cDNA for p10, a novel protein that increases in the regenerating legs of *Periplaneta americana* (American cockroach). Insect Biochem Mol Biol. 1998; 28: 785–790. PMID: 9807224
33. Ban L, Scaloni A, Brandazza A, Angeli S, Zhang L, Yan Y, et al. Chemosensory proteins of Locusta migratoria. Insect Mol Biol. 2003; 12: 125–134. PMID: 12653934
34. Zhou SH, Zhang J, Zhang SG, Zhang L. Expression of chemosensory proteins in hairs on wings of Locusta migratoria (Orthoptera: Acrididae). J Apol Entomol. 2008; 132: 439–450.
35. Guo W, Wang X, Ma Z, Xue L, Han J, Yu D, et al. CSP and takeout genes modulate the switch between attraction and repulsion during behavioral phase change in the migratory locust. PLoS Genet. 2011; 7(2): e1001291. doi: 10.1371/journal.pgen.1001291 PMID: 21304893
36. Benton R, Vannice KS, Vossall LB. An essential role for a CD36-related receptor in pheromone detection in Drosophila. Nature. 2007; 450:289–293. PMID: 17943085
37. Vogt RG, Miller NE, Litvack R, Fandino RA, Sparks J, Staples J, et al. The insect SNMP gene family. Insect Biochem Mol Biol. 2009; 39:448–456. doi: 10.1016/j.ibmb.2009.03.007 PMID: 19364529
38. Jin X, Ha TS, Smith DP: SNMP is a signaling component required for pheromone sensitivity in Drosophila. Proc Natl Acad Sci USA. 2011; 108: 7449–7454. doi: 10.1073/pnas.1017963108 PMID: 21498690
39. Wanner KW, Nichols AS, Allen JE, Buenger PL, Garcynski SF, Linn CE, et al. Sex pheromone receptor specificity in the European corn borer moth, Ostrinia nubilalis. PLoS One. 2010; 5: e8685. doi: 10.1371/journal.pone.0008685 PMID: 20084265
40. Pitts RJ, Rinker DC, Jones PL, Rokas A, Zwiebel LJ. Transcriptome profiling of chemosensory appendages in the malaria vector Anopheles gambiae reveals tissue- and sex-specific signatures of odor coding. BMC Genomics. 2011; 12: 271: doi: 10.1186/1471-2164-12-271 PMID: 21619637
41. Grosse-Wilde E, Kuebler LS, Bucks S, Vogel H, Wichler D, Hansson B S. Antennal transcriptome of Manduca sexta. Proc Natl Acad Sci USA. 2011; 108: 7449–7454. doi: 10.1073/pnas.1017963108 PMID: 21498690
42. Liu Y, Gu S, Zhang Y, Guo Y, Wang G. Candidate olfaction genes identified within the Helicoverpa armigera antennal transcriptome. PLoS ONE. 2012; 7(10): e48260. doi: 10.1371/journal.pone.0048260 PMID: 23102222
43. Mamidala P, Wijeratne AJ, Wijeratne S, Poland T, Qazi SS, Doucet D, et al. Identification of odorant binding proteins and chemosensory proteins between two wingless morphs and a winged morph of the cotton aphid Aphis gossypii Glover. PLoS ONE. 2013; 8(9): e73524. doi: 10.1371/journal.pone.0073524 PMID: 24073197
44. Poivet E, Galliot A, Montagné N, Glaser N, Legeai F, Jacquin-Joly E. A comparison of the olfactory gene repertoires of adults and larvae in the noctuid moth Spodoptera littoralis. PLoS ONE. 2013; 8(4): e60263. doi: 10.1371/journal.pone.0060263 PMID: 23565215
45. Andersson MN, Grosse-Wilde E, Keeling CI, Bengtsson JM, Yuen MM, Li M, et al. Antennal transcriptome analysis of the chemosensory gene families in the tree killing bark beetles, Ips typographus and Dendroctonus ponderosae (Coleoptera: Curculionidae: Scolytinae). BMC Genomics. 2013; 14: 198. doi: 10.1186/1471-2164-14-198 PMID: 23517120
46. Roberton HM, Gadau J, Wanner KW. The insect chemoreceptor superfamily of the parasitoid jewel wasp Nasonia vitripennis. Insect Mol Biol. 19 Suppl. 2010; 1: 121–136.
47. Nishimura O, Brilliola C, Yazawa S, Maffei ME, Arimura G. Transcriptome pyrosequencing of the parasitoid wasp Cotesia vestalis: genes involved in the antennal odorant-sensory system. PLoS ONE. 2012; 7(11): e50664. doi: 10.1371/journal.pone.0050664 PMID: 23226348
48. Wang SN, Peng Y, Lu ZY, Dhilloo KH, Gu SH, Li RJ, et al. Identification and Expression Analysis of Putative Chemosensory Receptor Genes in Microplitis mediator by Antennal Transcriptome Screening. Int J Biol Sci. 2015; 11(7): 737–751. doi: 10.7150/ijbs.11786 PMID: 26078716
49. Godfray HCJ. Parasitoids: Behavioral and Evolutionary Ecology. New Jersey: Princeton University Press; 1994.
50. Vinson SB. The general host selection behavior of parasitoid Hymenoptera and a comparison of initial strategies utilized by larvaphagous and oophagous species. Biol Control. 1998; 11: 96–103.
51. Vet LEM, Dicke M. Ecology of Infochemical Use by Natural Enemies in a Tritrophic Context. Annu Rev Entomol. 1992; 37: 141–171.
52. Turlings TC, Tumlinson JH, Lewis WJ. Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. Science. 1990; 250: 1251–3. PMID: 17929213
53. de Moraes CM, Lewis WJ, Pare PW, Albom HT, Tumlinson JH. Herbivore-infested plants selectively attract parasitoids. Nature. 1998; 393: 570–3.
55. Yang ZQ. A new genus and species of Eulophidae (Hymenoptera: Chalcidoidea) parasitizing Hyphantria cunea (Druy) (Lepidoptera: Arctiidae) in China. Entomotaxonomia. 1989; 11(1–2): 117–130 (in Chinese and English).

56. Qu BX, Li HH, Ao XY, Ren XL, Guo BM, Wang WP. Forecasting study of Hyphantria cunea. Journal of Northwestern College of Forestry. 1987; 2(2): 41–49 (in Chinese).

57. Wang ZP. The Integrated Control Techniques of the Fall Webworm. China: Liaoning Science and Technology Press; 1995. (in Chinese).

58. Yang ZQ. Anatomy of internal reproductive system of Chouioia cunea (Hymenoptera, Chalcidoidea, Eulophidae). Scientia Silvae Sinicae. 1990a; 31(1): 23–26 (in Chinese).

59. Yang ZQ. An effective parasitoid of the fall webworm—Chouioia cunea Yang (Hymenoptera: Eulophidae) from China. Forest Pest and Disease. 1990b; 2, 17 (in Chinese).

60. Yang ZQ, Wei JR, Wang XY. Mass rearing and augmentative releases of the native parasitoid Chouioia cunea for biological control of the introduced fall webworm Hyphantria cunea in China. Bio Control. 2006; 51(4): 401–418.

61. Meyer E, Aglyamova GV, Wang S, Buchanan-Carter J, Abrego D, Colbourne JK, et al. Sequencing and de novo analysis of a coral larval transcriptome using 454 GSFlx. BMC Genomics. 2009; 10: 219. doi: 10.1186/1471-2164-10-219 PMID: 19435504

62. Grabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I, et al. Full length transcriptome assembly from RNA-seq data without a reference genome. Nat Biotechnol. 2011; 29: 644–652. doi: 10.1038/nbt.1883 PMID: 21572440

63. Haas BJ, Papanicolaou A, Yassour M, Grabherr M, Blood PD, Bowden J, et al. De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. Nat Protoc. 2013; 8: 1494–1512. doi: 10.1038/nprot.2013.084 PMID: 23845962

64. Altschul SF, Madden TL, Schäffer AA, Zhang JH, Zhang Z, Miller W, et al. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 1997; 25: 3389–3402. PMID: 9254694

65. Conesa A, Gotz S, Garcia-Gómez JM, Terol J, Talón M, Robles M. Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. Bioinformatics. 2005; 21: 3674–3676. PMID: 16081474

66. Ye J, Fang L, Zheng HK, Zhang Y, Chen J, Zhang ZZ, et al. WEGO: a web tool for plotting GO annotations. Nucleic Acids Res. 2006; 34: W293–W297. PMID: 16845012

67. Mortazavi A, Williams BA, McCue K, Schaeffer L, Wold B. Mapping and quantifying mammalian transcriptomes by RNA-Seq. Nat Methods. 2008; 5: 621–628. doi: 10.1038/nmeth.1226 PMID: 18516045

68. Li RQ, Yu C, Li YR, Lam TW, Yi SM, Kristiansen K, Wang J. SOAP2: an improved ultrafast tool for short read alignment. Bioinformatics. 2009; 25: 1966–1967. doi: 10.1093/bioinformatics/btp336 PMID: 19497893

69. Corcoran JA, Jordan MD, Thrimawithana AH, Crowhurst RN, Newcomb RD. The Peripheral Olfactory Repertoire of the Lightbrown Apple Moth, Epiphyas postvittana. PLoS ONE. 2015; 10(5): e0121504. doi: 10.1371/journal.pone.0121504 PMID: 25856077

70. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 1997; 25: 4876–4882. PMID: 9396791

71. Saitou N, Nei M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol Biol Evol. 1987; 4: 406–425. PMID: 3447015

72. Tamura K, Dudley J, Nei M, Kumar S. MEGA4: Molecular evolutionary genomics analysis (MEGA) software version 4.0. Mol Biol Evol. 2007; 24: 1596–1599. PMID: 17488738

73. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(–Delta Delta C(T)) method. Methods. 2001; 25: 402–408. PMID: 11846609

74. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, et al. Gene ontology: tool for the unification of biology. Nat Genet. 2000; 25: 25–29. PMID: 10802651

75. Li X, Ju Q, Jie W, Li F, Jiang X, Hu J, et al. Chemosensory Gene Families in Adult Antennae of Anomala corpulenta Motschulsky (Coleoptera: Scarabaeidae: Rutelinae). PLoS ONE. 2015; 10(4): e0121504. doi: 10.1371/journal.pone.0121504 PMID: 25856077

76. Motschulsky (Coleoptera: Scarabaeidae: Rutelinae). PLoS ONE. 2015; 10(4): e0128596. doi: 10.1371/journal.pone.0128596 PMID: 26017144
78. Gong DP, Zhang HJ, Zhao P, Xia QY, Xiang ZH. The odorant binding protein gene family from the genome of silkworm, *Bombyx mori*. BMC Genomics. 2009; 10: 332. doi: 10.1186/1471-2164-10-332 PMID: 19624863

79. Xu PX, Zwiebel LJ, Smith DP. Identification of a distinct family of genes encoding atypical odorant-binding proteins in the malaria vector mosquito, *Anopheles gambiae*. Insect Mol Biol. 2003; 12: 549–560. PMID: 14986916

80. Hekmat-Scafe DS, Scafe CR, McKinney AJ, Tanouye MA. Genome-wide analysis of the odorant-binding protein gene family in *Drosophila melanogaster*. Genome Res. 2002; 12: 1357–1369. PMID: 12213773

81. Gu SH, Zhou JJ, Wang GR, Zhang YJ, Guo YY. Sex pheromone recognition and immunolocalization of three pheromone binding proteins in the black cutworm moth *Agrotis ipsilon*. Insect Biochem Mol Biol. 2013; 43: 237–251. doi: 10.1016/j.ibmb.2012.12.009 PMID: 23298680

82. Zhou JJ, Field LM, He XL. Insect odorant-binding proteins: Do they offer an alternative pest control strategy? Outlooks on Pest Management. 2010; 21: 31–34.

83. Bigiani A, Mucignat-Caretta C, Montani G, Tirindelli R. Pheromone reception in mammals Rev. Physiol Biochem Pharmacol. 2005; 154: 1–35

84. Robertson HM, Wanner KW. The chemoreceptor superfamily in the honey bee, *Apis mellifera*: expansion of the odorant, but not gustatory, receptor family. Genome Res. 2006; 16: 1395–1403. PMID: 17065611

85. Wang ZF, Yang PC, Chen DF, Jiang F, Li Y, Wang XH, et al. Identification and functional analysis of olfactory receptor family reveal unusual characteristics of the olfactory system in the migratory locust. Cell Mol Life Sci. 2015; 72(23): 4429–43 doi: 10.1007/s00018-015-2009-9 PMID: 26265180

86. Vosshall LB, Amrein H, Morozov PS, Rzhetsky A, Axel R. A spatial map of olfactory receptor expression in the *Drosophila* antenna. Cell. 1999; 99: 725–36. PMID: 10098877

87. Zhang YN, Jin JY, Jin R, Xia YH, Zhou JJ, Deng JY, et al. Differential Expression Patterns in Chemosensory and Non-Chemosensory Tissues of Putative Chemosensory Genes Identified by Transcriptome Analysis of Insect Pest the Purple Stem Borer *Sesamia inferens* (Walker). PLoS One. 2013; 8: e69715. doi: 10.1371/journal.pone.0069715 PMID: 23894529

88. Pitts RJ, Liu C, Zhou X, Malpartida JC, Zwiebel LJ. Odorant receptor-mediated sperm activation in disease vector mosquitoes. PNAS. 2014; 111: 2566–71. doi: 10.1073/pnas.1322923111 PMID: 24550284

89. Andresson MN, Videvall E, Walden KK, Harris MO, Robertson HM, Loftstedt C. Sex- and tissue-specific profiles of chemosensory gene expression in a herbivorous gall-inducing fly (Diptera: Cecidomyiidae). BMC Genomics. 2014; 15: 501. doi: 10.1186/1471-2164-15-501 PMID: 24948464

90. Abdel-latief M. A family of chemoreceptors in *Tribolium castaneum* (Tenebrionidae: Coleoptera). PLoS One. 2007; 2: e1319. PMID: 18091992

91. Zhao YN, Liu Q, Li M. Identification and evolution of the Or83b olfactory gene of Chouioia cunea Yang. Chinese Journal of Applied Entomology. 2014; 51(4): 1026–1034. (in Chinese with English summary).

92. Montell C. A Taste of the Drosophila Gustatory Receptors. Curr Opin Neurobiol. 2010; 19: 345–353.

93. Fiala A. Olfaction and olfactory learning in Drosophila: recent progress. Curr Opin Neurobiol. 2007; 17: 720–726. doi: 10.1016/j.conb.2007.11.009 PMID: 18242976

94. McBride CS, Arguello JR, O’Meara BC. Five Drosophila genomes reveal nonneutral evolution and the signature of host specialization in the chemoreceptor superfamily. Genetics. 2007; 177: 1395–1416. PMID: 18039874

95. Gardiner A, Barker D, Butlin RK, Jordan WC, Ritchie MG. Drosophila chemoreceptor gene evolution: selection, specialization and genome size. Mol Ecol. 2008; 17: 1648–1657. doi: 10.1111/j.1365-294X.2008.03713.x PMID: 18371013

96. Scott K, Brady R, Cravchik A, Morozov P, Rzhetsky A, Zuker C, et al. A chemosensory gene family encoding candidate gustatory and olfactory receptors in *Drosophila*. Cell. 2001; 104: 661–73. PMID: 11257221

97. Clyne PJ, Warr CG, Carlson JR. Candidate taste receptors in *Drosophila*. Science. 2000; 287: 1830–4. PMID: 10710312

98. Wanner KW, Willis LG, Theilmann DA, Isman MB, Feng QL, Plettner E. Analysis of the insect os-d-like gene family. J Chem Ecol. 2004; 30: 889–911. PMID: 15274438

99. Poivet E, Gallet A, Montagné N, Glaser N, Legeai F, Jacquin-Joly E. A Comparison of the Olfactory Gene Repertoires of Adults and Larvae in the Noctuid Moth *Spodoptera littoralis*. PLoS ONE. 2013; 8 (4): e60263. doi: 10.1371/journal.pone.0060263 PMID: 23655215
100. Koenig C, Hirsh A, Bucks S, Kliner C, Vogel H, Shukla A, et al. A Reference Gene Set For Chemosensory Receptor Genes of *Manduca Sexta*. Insect Biochem Molec. 2015; 66: 51–63. doi: 10.1016/J.Ibmb.2015.09.007

101. Zhang J, Liu Y, Walker WB, Dong SL, Wang GR. Identification and localization of two sensory neuron membrane proteins from *Spodoptera litura* (Lepidoptera: Noctuidae) Insect Sci. 2015; 22(3): 399–408. doi: 10.1111/1744-7917.12131 PMID: 24757100

102. Rogers ME, Steinbrecht RA, Vogt RG. Expression of SNMP-1 in olfactory neurons and sensilla of male and female antennae of the silkmoth *Antheraea polyphemus*. Cell Tissue Res. 2001; 303: 433–446. PMID: 11320659

103. Forstner M, Gohl T, Gondesen I, Raming K, Breer H, Krieger J. Differential expression of SNMP-1 and SNMP-2 proteins in pheromone-sensitive hairs of moths. Chem Senses. 2008; 33: 291–299. doi: 10.1093/chemse/bjm087 PMID: 18209018

104. Gu SH, Yang RN, Guo MB, Wang GR, Wu KM, Guo YY, et al. Molecular identification and differential expression of sensory neuron membrane proteins in the antennae of the black cutworm moth *Agrotis ipsilon*. J Insect Physiol, 2013; 59: 430–443. doi: 10.1016/j.jinsphys.2013.02.003 PMID: 23454276

105. Zhang J, Liu Y, Walker WB, Dong SL, Wang GR. Identification and localization of two sensory neuron membrane proteins from *Spodoptera litura* (Lepidoptera: Noctuidae). Insect Sci. 2014; doi: 10.1111/1744-7917.12131

106. Xu XR, Qi JY, Lv SS, Xu Z, Wang Y, Sun SH, et al. Antennal Sensilla of *Chouioia cunea* Yang (Hymenoptera: Eulophidae) Observed with Scanning Electron Microscopy. J Shenyang Agr Uni. 2013; 44 (1): 42–46