The whole body transcriptome of *Coleophora obducta* reveals important olfactory proteins

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ABSTRACT

Background: The tiny casebearer moth *Coleophora obducta*, an important defoliator of *Larix* spp., is a major threat to ecological security in north China. Studies have shown that *C. obducta* is strongly specific to host plants; it is unable complete its life cycle without *Larix* spp. The sex pheromones of *C. obducta* Z5-10:OH have been elucidated; and eight types of antennae sensilla, have been detected, indicating that an exploration of its olfactory proteins is necessary, due to the general lack of information on this topic.

Methods: We investigated the whole body transcriptome of *C. obducta*, performed a phylogenetic analysis of its olfactory proteins and produced expression profiles of three pheromone-binding proteins (*CobdPBPs*) by qRT–PCR.

Results: We identified 16 odorant binding proteins, 14 chemosensory proteins, three sensory neuron membrane proteins, six odorant degrading enzymes, five antennal esterases, 13 odorant receptors, seven ionotropic receptors and 10 gustatory receptors, including three PBPs and one odorant co-receptor. Additionally, three putative pheromone receptors, two bitter gustatory receptors and five functional ionotropic receptors were found by phylogenetic analysis. The expression profiles of three PBPs in males and females showed that all of them exhibited male-specific expression and two were expressed at significantly higher levels in males. These data provide a molecular foundation from which to explore the olfactory recognition process and may be useful in the development of a new integrated pest management strategy targeting olfactory recognition of *C. obducta*.

INTRODUCTION

The olfactory insect-plant chemical communication system is central and significant to survival and propagation due to its essential function in growth and development e.g., eating, orientation, searching for hosts, copulation and oviposition (*Jones et al., 2011*; *Syed & Leal, 2008*; *Zwiebel & Takken, 2004*). To achieve integrated pest management, the molecular mechanisms of the olfactory recognition system have come under scrutiny.

Figure 1 The figure of *Coleophora obducta* and damaged leaves of *Larix gmelini* (A). Old sheath on the leaf of *Larix gmelini* (B). Blue boxes in (A) and (B) are new and old sheath, respectively.

Full-size ▧ DOI: 10.7717/peerj.8902/fig-1

The tiny casebearer moth *Coleophora obducta* (Meyrick) (Lepidoptera: Coleophoridae) is an important defoliator of larch that exclusively destroys the leaves of *Larix* spp., including *Larix gmelini*, *Larix principisrupprechtii*, *Larix olgensis* and *Larix kaempferi*, which are widely distributed in far eastern Russian, Japan, Korea and Liaoning, Jilin, Heilongjiang, Inner Mongolia, Hebei and the Henan province in China. *C. obducta* is 8–10 mm long, including 2–3 mm wing over the abdomen (Fig. 1) (*Li, 2003*; *Yang, 1984*). In northeast China, especially the Greater Khingan Mountains, *L. gmelini*, which makes up half of the trees in the forest, has been damaged by *C. obducta* since 1956. From 1956 to 1990, there were four 10-year fastigium cycles (*Yushan, 2004*), so *C. obducta* is the main defoliator of *L. gmelini* in northeast China. The larvae remain on the leaves for the first two instars; they then produce and wear a sheath that damages larch leaves. The oldest instar larvae cause the fastest and greatest damage by eating four needles in 1 day (*Bao, Yang & Zhao, 1990*). When there is an outbreak, the damage, which is similar to fire disastersn larch forests, seriously affects the growth and development of trees and may lead directly to the death of a large number of trees. As a result the ecological value of a forest is weakened. Because the local tree species, *L. gmelini* has important ecological value in northeast China, *C. obducta* has been deemed the main menace to north China's ecological security, especially in the Greater Khingan Mountains (*Bao, Yang & Zhao, 1990*) (Fig. 1).

After a long period of coevolution between insects and plants, a complex chemical information network has been gradually established. These chemicals are responsible for many insect behaviors and physiological reactions, coordinating utrition among plants, herbivorous insects and their natural enemies (*Du & Yan, 1994*). Previous studies have

shown that *C. obducta* cannot complete its lifecycle without *Larix* spp., due to its strong specificity for host plants (*Shu et al., 2003*), so the chemical communication and messaging between *C. obducta* and larch trees is interesting. Eight types of *C. obducta* sensillum, placodea, basiconica, coeloconica, styloconica, trichodea, squamiformia, furcatea of sensillum and Bohm bristles, have been found in antennae; they function as chemoreceptors and as gustatory and mechanosensory receptors (*Yang, Yan & Liu, 2009*). Z5-10:OH (*cis*-5-decene-1-alcohol), the sex pheromone of *C. obducta*, was shown to be a strong attractant for male moths. Z5-10:AC and Z5-12:OH were also found to be strong and weak inhibitors, respectively, of this pheromone (*Clearwater et al., 1991*). An optimal dose of 100 µg of pheromone was developed for field monitoring (*Chu & Zhang, 1995*); however, the trapping effect is not efficient at high population densities (*Chen et al., 2002*; *Liu et al., 2008*). Thus, we investigated the olfactory recognition system to clarify the sex pheromone recognition process.

In the first step of olfactory recognition, the perireceptor event, mostly hydrophobic olfactory chemical molecules (pheromones and odors) are transformed into water-soluble molecules and transported from the external environment to the membranes of chemosensing neurons (*Zhou, 2010*). This is performed by odorant binding proteins (OBPs) and chemosensory proteins (CSPs), which are small soluble proteins that are highly concentrated in the lymph of chemosensilla (*Ban et al., 2002*; *Fleischer et al., 2018*; *Kaissling, 2001*; *Leal et al., 2005*; *Pelosi et al., 2006*; *Vieira & Rozas, 2011*; *Vogt & Riddiford, 1981*). Soluble OBPs have a conserved pattern of six cysteines that form three disulfide bridges (*Leal, Nikonova & Peng, 1999*). Pheromone binding proteins (PBPs) are members of a subfamily of OBPs (*Zhou, 2010*) that bind to pheromone compounds, participate in the pheromone recognition process and exhibit biased expression in antenna, such as in *Eogystia hippophaecolus, Sesamia nonagrioides* and *Helicoverpa assulta* (*Glaser et al., 2013*; *Hu et al., 2016a*; *Li et al., 2015*). CSPs have only four cysteines are smaller than OBPs (*Pelosi et al., 2006*), and bind to various odors (*Ban et al., 2002*; *Briand et al., 2010*; *Jacquinjoly et al., 2001*; *Lartigue et al., 2002*). Since the development of genomic and transcriptomic sequencing techniques, OBPs and CSPs have been widely investigated (*Pelosi et al., 2018*). In the insect chemosensory system, OBPs and CSPs function in the detection and recognition of environmental chemical stimuli. OBPs and CSPs also have different functions in non-sensory organs, including the solubilization of nutrients, pheromone delivery, development and insecticide resistance (*Pelosi et al., 2018*). OBPs are thought to be involved in the conveyance of odors to odorant receptors (ORs) for specific signal transduction of behaviorally active odors (*Venthur & Zhou, 2018*).

In insects, chemoreception is mediated by transmembrane receptors, including ORs, ionotropic receptors (IRs), gustatory receptors (GRs) and sensory neuron membrane proteins (SNMPs), which recognize and discriminate between different kinds of semiochemicals and environmental odors (*Carraher et al., 2015*; *Clyne et al., 1999*; *Fleischer et al., 2018*; *Leal, 2013*; *Vosshall et al., 1999*; *Wicher, 2015*). Among these, ORs have been most extensively studied. ORs transmit chemical signals through heteromeric complexes with an Orco-receptor (Orco), which functions as a nonselective cation channel (*Sato et al., 2008*; *Wicher et al., 2008*). ORs perceive most food odors

(*Ronderos & Smith, 2009*), while pheromone receptors (PRs) bind pheromones and their complexes (*Chang et al., 2015*; *Leal, 2013*). ORs can raise the specificity and sensitivity of odorant recognition. When *CsupPR4* and *CsupPR6* were co-expressed with *CsupPBP4*, the sensitivity of the reaction with (Z)-11-hexadecenal was significantly enhanced (*Chang et al., 2015*). Many kinds of odors, including acids, aldehydes and even humidity, are perceived by IRs (*Ronderos & Smith, 2009*). GRs perceive nucleotides, sugars, amino acids, salts, CO_2, acidic pH conditions and multifarious bitter compounds (*Liman, Zhang & Montell, 2014*). The main function of ORs and GRs is as ligand-gated ion channels in the perception of pheromones and environmental chemicals. They are also involved in photoreception and thermosensation and they have non-sensory roles (*Richard, 2015*). SNMPs are part of the CD36 protein family, which participates in pheromone recognition (*Vogt et al., 2009*) and is conserved throughout holometabolous insects (*Jiang et al., 2016*; *Vogt et al., 2009*). Depending on the functional groups of different odors, which may be plant volatiles, pheromones, aldehydes, alcohols, or esters, degradation involves specific enzymes in odor degradation pathways, including multi-functional odorant-degrading enzymes (ODEs), pheromone degrading enzymes (PDEs) and antennal esterases (AES) and all of them are belong to carboxylesterases (CXEs). The subgroup of CXEs uses the Oakeshott classification system (*Chertemps et al., 2015*).

In this study, we examined the whole body transcriptome of *C. obducta*, identified olfactory proteins and evaluated the phylogenetic relationships between *C. obducta* and other species. We also explored the expression profiles of three PBPs in *C. obducta* males and females. The identified olfactory proteins provide a molecular foundation from which to explore the olfactory recognition process and to develop a new integrated pest management strategy targeting olfactory recognition in *C. obducta*.

MATERIALS AND METHODS

Ethics statement

The tiny casebearer moth *C. obducta* is a Chinese forestry pest and collection of it is permitted by the leader of Xinganmeng forestry bureau, Yinghua Lu and member Tianhua Zhen. It is not in "List of Endangered and Protected Animals in China". For reduction ache and discomfort to them, all operations were implemented on the basis of ethical guidelines.

Insect collection

Artificial rearing *C. obducta* need *L. gmelini* to feed, pupate, eclosion, oviposition, environment to mating and overwintering. We are exploring it, but it's hard to control. So we collected spoiling *L. gmelini* braches with pupa and mature larva of *C. obducta* on leaves, putted the braches in bucket with clean water and fed in insect cage outdoors during end of May to end of June 2019 in Wuchagou, Xinganmeng, China, then collected the adult *C. obducta* from the cage during that period every day. In this way we collected hundreds of *C. obducta*, which are too tiny and not enough to construct antennal transcriptome and extract antennal RNA. All bodies of *C. obducta* were stored in RNAlater (Ambion, Austin, TX, USA), then deposited at −80 °C.

cDNA library construction and illumina sequencing

We extracted total RNA from whole body of *C. obducta* males and females utilization TRIzol reagent (Ambion, Austin, TX, USA) and the RNeasy Plus Mini Kit (No. 74134; Qiagen, Hilden, Germany) according to the manufacturer's instructions. NanoDrop2008 (Thermo, Waltham, MA, USA) and agarose gel electrophoresis examined density and quality of RNA. Half RNA of male and female bodies with three biological replicates were used to construct three cDNA libraries respectively. Construction cDNA libraries and Illumina sequencing of samples were implemented at Majorbio Corporation (Shanghai, China). Using TruSeq RNA Sample Preparation Kit v2-Set A (No. RS-122-2001; Illumina, San Diego, CA, USA) was to perform purification and fragmentation of mRNA samples. The first-strand cDNA was synthesized by utilization random hexamer primers, then using RNase H, dNTPs, buffer and DNA polymerase I at 16 °C for 1 h to synthesize the second-strand cDNA. After end repair, A-tailing and the ligation of adaptors, the products were amplified by PCR and quantified precisely by the Qubit DNA Br Assay Kit (Q10211; Invitrogen, Carlsbad, CA, USA). cDNA libraries were obtained after they were purified by the MinElute Gel Extraction Kit (Cat No. 28604; Qiagen, Hilden, Germany). On the HiSeq2500 platform three cDNA libraries were sequenced.

Assembly and functional annotation

All low quality and adaptor sequences in all raw reads were removed by Trimmomatic (http://www.usadellab.org/cms/index.php?page=trimmomatic) to get clean reads. Clean reads assembly was implemented by Trinity (Version: r2019-07-31) with the default parameters. The largest alternative splicing variants in the Trinity results were unigenes. The annotation of unigenes was in six databases which include NCBI non-redundant protein sequences (Nr), Protein family (Pfam), Clusters of Orthologous Groups of proteins (COG), Swiss-Prot, Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO). We performed search against the Nr database (http://www.ncbi.nlm.nih.gov/genbank/), Swiss-Prot (http://www.uniprot.org/) and COG (http://www.ncbi.nlm.nih.gov/COG/) with an *E* value cutoff of 1.0E−5 in Diamond (v0.8.37.99) to annotate and classify putative protein sequences. KEGG database (http://www.genome.jp/kegg/pathway.html) were searched by Kobas (2.1.1) with default parameters. Pfam (http://pfam.sanger.ac.uk/) were searched by HMMER 3.0 package (*Finn, Clements & Eddy, 2011*) with default parameters. BLAST2GO was used to obtain Gene Ontology (GO) annotation of assembled unigenes with an default parameters (*Götz et al., 2008*). Using the NCBI ORF Finder tool (http://www.ncbi.nlm.nih.gov/gorf/gorf.html) explored the longest complete open reading frames (ORFs) of unigenes. The FPKM (fragments per kilobase per million reads) represent expression levels (*Mortazavi et al., 2008*), which was calculated by RSEM (RNA-Seq by Expectation–Maximization) (Version: v1.2.6) with default parameters (*Bo & Dewey, 2011*).

Identification of chemosensory genes

Candidate unigenes involved in *C. obducta* olfaction from Nr database were identified by Diamond (v0.8.37.99) based on the available sequences of OBPs, CSPs, SNMPs, ODEs,

ORs, GRs and IRs proteins from insecta species. All putative OBPs, CSPs, SNMPs, ORs, GRs, IRs and ODEs were examined by tBLASTn online manually to assess the Diamond results. In File S1, the nucleic acid sequences of all chemosensory genes were listed.

Sequence and phylogenetic analysis

The N-terminal signal peptides of OBPs and PBPs were checked by SignalP4.0 (http://www.cbs.dtu.dk/services/SignalP/). All sequences to construct phylogenetic tree except *C. obducta* were obtained from NCBI protein database. Utilization Muscle method implemented in Mega v6.0 software package carried out amino acid sequence alignment (*Tamura et al., 2011*). The phylogenetic trees were constructed using the neighbor-joining (NJ) method (*Saitou & Nei, 1987*) with a pairwise deletion of gaps and *P*-distances model implemented in Mega v6.0 and colored in FigTree (Version 1.4.2). The reliability of the tree structure and node support was assessed by bootstrap analysis with 1,000 replicates. Considering higher reliability of tree, we eliminated binding proteins with less than 122 amino acids and membrane proteins with less than 333 amino acids. The phylogenetic tree of *OBPs* was based on two amino acid sequences of GOBPs, 9 OBPs (except *CobdOBP1* and 2) and 3 PBPs of *C. obducta*, all Lepidoptera PBPs, 15 of *Apis mellifera*, 23 of *Bombyx mori*, 28 of *Tribolium castaneum*, 21 of *Dendrolimus kikuchii*, 11 of *Heliothis virescens* and 14 of *C. suppressalis*. CSPs tree was based on seven of *C. obducta* (except *CobdCSP1, 4, 7, 8, 10, 11, 12*) 20 of *D. melanogaster*, 17 of *T. castaneum*, 15 of *E. hippophaecolus*, 14 of *Ostrinia furnacali* and 14 of *D. kikuchii*. The phylogenetic analyses of ORs were based on six ORs of *C. obducta* (except *CobdOR2, 5, 6, 7, 8, 9, 10*), 33 of *D. kikuchii*, 4 Lepidoptera PRs, 17 of *Tenebrio molitor*, 7 of *D. mauritiana, 41 of Manduca sexta* and all 39 of insect Orco. ODEs tree were based on all ODEs and CXEs of *C. obducta* (except *CobdCXE4*) and all known CXEs of *Plodia interpunctella* (*Liu et al., 2019*), *Ectropis oblique* (*Sun et al., 2017b*), *Spodoptera littoralis* (*Durand et al., 2010*) and *Chertemps et al. (2015)*. IRs tree was based on all IRs of *C. obducta*, 35 of *D. melanogaster*, 7 of *E. hippophaecolus*, 3 of *H. armigera* and IRs of *M. sexta, B. mori, D. plexippus* and *H. Melpomene* (*Schooten et al., 2016*). GRs tree was based on five GRs of *C. obduct* (except *CobdGR1, 2, 3, 64, 43a*), 30 of *D. melanogaster*, 7 of *B. mori* and Xu used in *H. armigera* (*Xu et al., 2016*). SNMPs tree was constructed with 3 SNMPs of *C. obducta* and all known insect SNMPs. Accession number of all chemosensory protein sequences obtained from NCBI protein database in phylogenetic tree without reference was listed in File S2.

Expression analysis by fluorescence quantitative real-time PCR

Fluorescence quantitative real-time PCR was performed to verify the expression of candidate chemosensory genes. Total RNA of whole body of males and females were extracted following the methods described above. cDNA was synthesized from total RNA using the PrimeScriptRT Reagent Kit with gDNA Eraser to remove gDNA (No. RR047A; TaKaRa, Shiga, Japan). Gene-specific primers were designed using Primer3 (http://bioinfo.ut.ee/primer3-0.4.0/) (File S3). *Lymantria dispar* β-actin was set as

Table 1 Number and length of unigenes.

Quality indexs	Transcriptome 1	Transcriptome 2	Transcriptome 3
Raw reads	44,539,192	49,142,628	43,896,156
Clean reads	44,072,110	48,571,928	43,368,300
Q20 (%)	97.71	97.55	97.45
Q30 (%)	93.84	93.48	93.27
GC content (%)	47.83	47.64	48.11
Total transcripts number	96,657		
Total unigenes number	52,354		
Largest length (bp)	19,273		
Average length (bp)	900		
N50	1,533		

reference gene (File S3). The fluorescence quantitative real-time PCR (qRT–PCR) analysis was conducted using the Bio-Rad CFX96 PCR System (Hercules, CA, USA). SYBRPremix ExTaq^{TM} II (No. RR820A; TaKaRa, Shiga, Japan) was used for the PCR reaction under a three-step amplification. Each PCR reaction was conducted in a 25 ml reaction mixture containing 12.5 µl of SYBR Premix Ex Taq II, one ml of each primer (10 mM), two µl of sample cDNA (2.5 ng of RNA) and 8.5 µl of dH$_2$O (sterile distilled water). The qRT–PCR cycling parameters were as follows: 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s, 60 °C for 30 s and 65 °C to 95 °C in increments of 0.5 °C for 5 s to generate the melting curves. To examine reproducibility, each qRT–PCR reaction for each tissue was performed in three biological replicates and three technical replicates, in which each biological replication was with 20 individuals. Negative controls without either template were included in each experiment. Bio-Rad CFX Manager (version 3.1.1517.0823) was used to normalize expression based on ΔΔCt values, with *CobdPBP3* of male in analyze mode as control sample and the $2^{-\Delta\Delta CT}$ method was used (the amplification efficiency for three genes was equal to 100%) (*Livak & Schmittgen, 2001*). A Chi-square test was using to compare the expression level of male and female adult in SPSS Statistics 22.0. Values are presented as means ± SE.

RESULTS

Transcriptome sequencing and sequence assembly

We generated 44.07, 48.57 and 43.37 million clean reads from cDNA libraries of three biological repeats of *C. obducta* whole body (half males and females). The q20 quality scores were 97.71%, 97.55% and 97.45% respectively. The q30 quality scores were 93.84%, 93.48% and 93.27%, respectively (Table 1). After splicing and assembly, 96,657 transcripts, 52,354 unigenes, with a N50 of 1,533 bp, an average length of 900 bp and a maximal length of 19,273 bp were obtained (Table 1; Fig. 2A). The raw reads of three *C. obducta* transcriptome have been deposited in the NCBI SRA database under the accession number PRJNA587422.
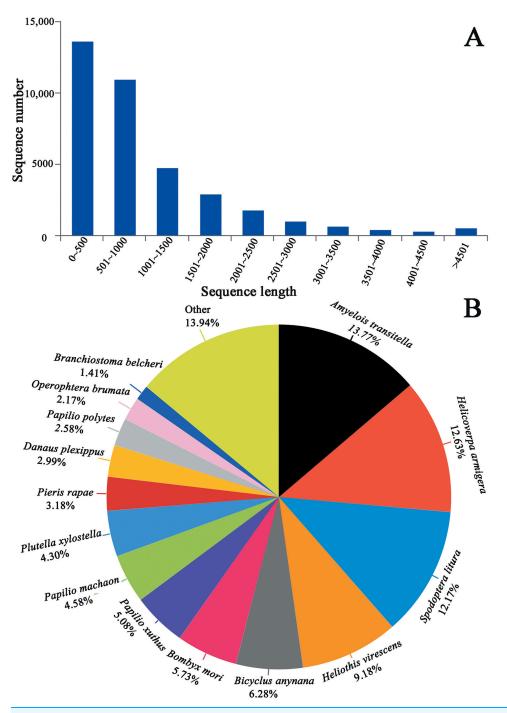
Figure 2 Length distribution of unigene and BLASTx unigenes with other species in the whole body transcriptome of *C. obducta* (A) length distribution of unigenes; (B) BLASTx analysis of identified unigenes with known homologs from other species. Full-size ☑ DOI: 10.7717/peerj.8902/fig-2

Homology analysis and gene ontology annotation

There were 17,176 (46.52%) unigenes that were obtained through annotation with the Nr database (Table 2). Of those, 12,206 (33.06%) aligned to the Swiss-Prot database, 12,674 (34.32%) aligned to the protein family (Pfam) database, 9,741 (26.38%) aligned to GO

Table 2 Functional annotation of unigenes using various public protein databases.

Annotated in databases	Number of unigenes	Percentage
Nr	17,176	46.00
SwissProt	12,206	33.06
Pfam	12,674	34.32
COG	2,937	7.95
GO	9,741	26.38
KEGG	9,192	24.89
Annotated in all databases	17,821	48.26
Annotated in at least one databases	36,925	70.50

database and 2,937 (7.95%) aligned to the COG database (Table 2). In all, 17,821 (48.26%) unigenes were annotated in six databases. Interestingly, 2,001, 88, 155, 4 and 9 unigenes were uniquely annotated to the Nr, Swiss-Prot, Pfam, COG and KEGG databases, respectively (Table 2). The greatest number of sequences (13.77%) matched genes from *Amyelois transitella*, followed by 12.63% from *H.armigera*, 12.17% from *Spodoptera litura*, 9.18% from *Heliothis virescens* and 6.28% from *Bicyclus anynana*. A BLASTx search found that 32.03% were similar to nine species and 13.94% of unigenes were similar to other species (Fig. 2B). There were 40,565 unigenes that were categorized as functional groups by gene ontology (GO) annotation. In the *C. obducta* transcriptome, the ontology category with the most annotations was cellular component (39.96%, 16,209 gene numbers), followed by biological process (33.32%, 13,515 gene numbers) and molecular functions (26.72%, 10,841 gene numbers). In the cellular component category, the terms membrane, cell and cell part were the most representative. In the biology process category, cellular process, metabolic process and single-organism process were the most enriched terms. Binding, catalytic activity and transporter activity were the most abundant molecular functions terms (Fig. 3A). In total, 1,492 unigenes were classified into 24 COG categories. The major COG category was with 210 unigenes relating to storage and processing (14.08%), followed by cellular processes and signaling (180 unigenes, 12.06%) and poorly characterized (153 unigenes, 10.25%) (Fig. 3B). To elucidate active biosynthesis pathways in *C. obducta*, annotation of Nr data with the Kyoto Encyclopedia of Genes and Genomes (KEGG) database discovered that 14,419 gene numbers were assigned to six main categories. The highest number of KO identifiers were involved in human diseases (2,488 unigenes), followed by organismal systems (1,927), metabolism (1,840), cellular processes (1,008), genetic information processing (368) and environmental information processing (309). Signal transduction (1,321), cancer overview (854), transport and catabolism (759), the endocrine system (702), the immune system (637) and translation (630) were largest number of KO identifiers in pathways (Fig. 3C).

Nonreceptor olfactory gene families
Odorant binding proteins
We identified 16 unigenes encoding putative OBPs in *C. obducta*, including two general odorant binding proteins (GOBPs) and three PBPs. Only *CobdOBP11* was a full-length
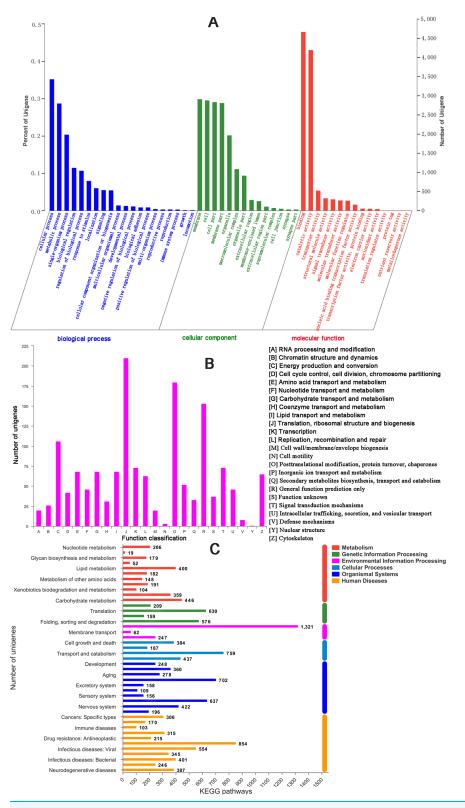
Figure 3 GO gene function classification, KOG and KEGG function classification. (A) GO classification. (B) KOG function classification and (C) KEGG function classification.

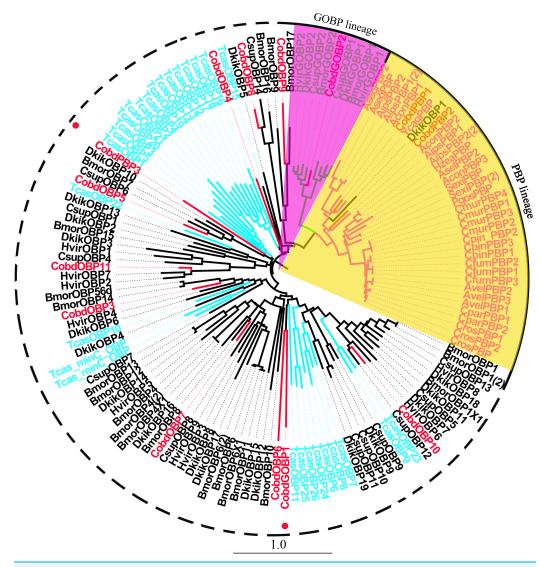Full-size ⬚ DOI: 10.7717/peerj.8902/fig-3

Figure 4 **Neighbor-joining phylogenetic tree of odorant binding proteins (OBPs).** The NJ phylogenetic analysis of OBPs of *C. obducta* (*CobdOBP*, red) was performed with reference OBPs of Lepidoptera (black) and Coleoptera (celeste). The yellow and purple fill area refer to PBP and GOBP lineage respectively. The scale bar represents 1.0 substitutions per site.

Full-size ☑ DOI: 10.7717/peerj.8902/fig-4

gene with a complete ORF, with signal peptides and a length >400 bp (File S4). The FPKM of *CobdOBPs* showed that *CobdOBP4, CobdGOBP1, CobdGOBP2, CobdOBP11, CobdOBP1, CobdOBP8* and *CobdPBP1* exhibited the highest expression; the top three FPKMs of *CobdOBP4, CobdGOBP1* and *CobdGOBP2* were 197.37, 178.96 and 86.05, respectively. *CobdPBP1* was exhibited the highest expression with an FPKM of 23.86, followed by *CobdPBP3* and *CobdPBP2* with FPKM values of 9.08 and 5.00, respectively. In the phylogenetic tree (Fig. 4; File S2), the distinct *PBPs* clade included *CobdPBP1, CobdPBP2, DkikOBP1* and all other *PBPs*; however, *CobdPBP3* was not in the PBP lineage. The GOBP clade included *CobdGOBP2* and all other GOBPs except *CobdGOBP1*. By fluorescence quantitative real-time PCR, we verified the expression of three PBPs in
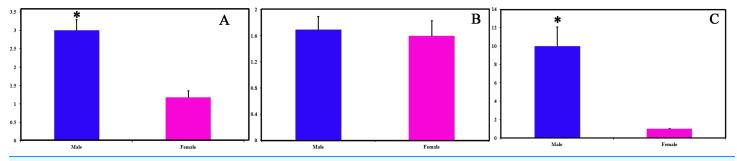
Figure 5 **Pheromone binding protein (PBPs) transcript levels of *C. obducta* in male and female.** (A) CobdPBP1; (B) CobdPBP2; (C) CobdPBP3; Actin was used as the reference gene to normalize target gene expression. The standard errors are represented by the error bars, asterisk above the bars denote significant differences at $p < 0.05$.

Full-size 🖼 DOI: 10.7717/peerj.8902/fig-5

adult males and females and observed higher expression levels in males than females in three PBPs. Moreover, we detected significantly higher expression levels of *CobdPBP1* and *CobdPBP3* in males than in females ($p < 0.05$). *CobdPBP3* in males exhibited the highest expression of the remaining PBPs investigated in males and females. The expression of *CobdPBP2* did not differ between males and females obviously (Fig. 5).

Chemosensory proteins

A total of 14 unigenes encoding putative CSPs were identified (Table 1). The FPKM of *CobdCSP* showed that *CobdCSP14, CobdCSP3, CobdCSP4, CobdCSP10, CobdCSP1, CobdCSP6* and *CobdCSP5* exhibited the highest expression in antenna; the top three FPKM of *CobdCSP14, CobdCSP3* and *CobdCSP4* were 379.12, 370.89 and 92.22, respectively. However, the FPKM of the other seven *CobdCSP* ranged from 1.43 to 12.72 (File S4). Based on the neighbor-joining tree of *CSPs* (Files S2 and S5), the Dipteran (*D. melanogaster*) clade was labeled with green circles; however the CSPs of Coleoptera (*T. castaneum*) were divided into five clades.

Sensory neuron membrane proteins

We identified three SNMPs unigenes. The FPKM of *CobdSNMPs* showed that *CobdSNMP3* was much higher expressed than *CobdSNMP1* and *CobdSNMP2* (File S4). In the phylogenetic tree of SNMPs (Files S2 and S6), three clades of SNMPs (SNMP1, SNMP2 and SNMP3) were revealed. The clades of SNMP1, SNMP2 and SNMP3 was labeled with blue, green and black circle.

Odorant degrading enzymes

Six putative odorant degrading enzymes (ODEs) and five putative antennal esterases (CXE) were identified. Nine of these, excluding *CobdCXE4* and *CobdCXE5*, the other nine hadORFs of approximately 1,600 bp. The FPKM of *CobdCXEs* showed that *CobdCXE2, CobdODE3* and *CobdODE4* were the highest expression in the transcriptome; the FPKM of the other eight ranged from 2.86 to 9.72 (File S4). Based on the neighbor-joining tree of ODEs and CXEs (Fig. 6; File S2) and the classification system described in *Chertemps et al. (2015)*, we found that *CobdCXE1, CobdODE2* and *CobdODE6* belong to mitochondrial and cytosolic esterases (yellow area in phylogenetic tree), *CobdODE5* belongs to microsomal α-esterases (pink) *CobdODE4, CobdODE1, CobdCXE3* and
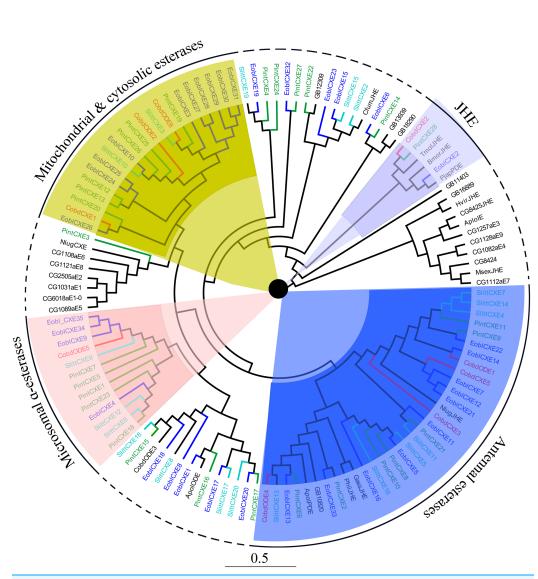
Figure 6 Neighbor-joining phylogenetic tree of carboxylesterases (CXEs). The NJ phylogenetic analysis of CXEs of *C. obducta* (*CobdODEs and CobdCXEs*, red) was performed with reference CXEs of *Plodia interpunctella* (*Liu et al., 2019*), *Ectropis oblique* (*Sun et al., 2017b*), *Spodoptera littoralis* (*Durand et al., 2010*) and *Chertemps et al. (2015)*. The pink, blue, gray and yellow fill area refer to microsomal α-esterases, antennal esterases, juvenile hormone esterases (JHE) and mitochondrial and cytosolic esterases, respectively. The scale bar represents 0.5 substitutions per site.

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CobdCXE5 belong to antennal esterases (blue) and *CobdCXE2* belongs to Lepidopteran juvenile hormone esterases (JHE) (gray).

Receptor encoding genes
Odorant receptors

A total of 13 ORs were identified. Among them, the full length genes of *CobdOR3*, *CobdOrco* and *CobdOR11* encoded more than 405 amino acids and had complete ORFs. Only two ORs of *C. obducta* were best matches with the same species and sequence (i.e., accession number), the best match for *CobdOR8* and *CobdOR10* was

Helicoverpa armigera AIG51872.1. *CobdOR10* and *CobdOR12* were the highest expression with a FPKM of 24.94 and 19.56, respectively; the other eleven *CobdOR*s had a FPKM ranging from 1.25 to 7.95 (File S4). In the neighbor-joining tree (Files S2 and S7), the Orco lineage (yellow filled area) included all known insect Orco, *CobdOrco*, *MsexOR2* and *DkikOR32*. *CobdPR1* (Cobd*OR1*), *CobdPR2* (*CobdOR3*) and *CobdPR3* (*CobdOR4*) formed a clade with a PR lineage, including *MsexPR*, *HarmPR*, *ObruPR*, *OnubPR*, *CmedPR1*, *CmedPR2* and three *MsexORs*.

Ionotropic receptors

A total of 7 IRs were identified in the transcriptome, including IR25a and IR93a. Among them, five were full length genes with complete ORFs longer than 1,500 bp. *CobdIR5, CobdIR4 and CobdIR25a* were the highest expression with a FPKM of 25.69, 23.73 and 12.86, respectively; the other four had a FPKM ranging from 1.6 to 8.22 (File S4). In the NJ tree (Fig. 7; File S2), most IRs were clustered with a known group; the IR62a group contained *CobdIR62a (CobdIR3)*, seven *HmelIR62a*, two *DpleIR62a* and two *BmorIR62a* and the IR25a clade contained *CobdIR25a, DmelIR25a, BmorIR25a, DpleIR25a* and *HmelIR25a*. The IR76b group contained *CobdIR76b (CobdIR5) DmelIR76b, BmorIR76b, DpleIR76b* and *HmelIR76b*, while the IR93a clade contained *CobdIR93a, DmelIR93a, BmorIR93a, DpleIR93a, HmelIR93a* and *HarmIRa*. The IR64a group included *CobdIR64a (CobdIR2), BmorIR64a, DpleIR64a* and *HmelIR64a*,the IR75p2 group included *CobdIR75p2 (CobdIR1), BmorIR75p2, DpleIR75p2* and *EhipIR75p2* and the IR68a group contained *CobdIR68a (CobdIR4), BmorIR68a, DpleIR68a* and *EhipIR68a*.

Gustatory receptors

We identified 10 putative GRs, including two GRs for sugar, *CobdGR64* and *CobdGR43a*, which did not have a full-length gene. Four (*CobdGR4, CobdGR5, CobdGR6 and CobdGR8*) were the best matches with *Athetis dissimilis*. *CobdGR1* and *CobdGR64* were the highest expression in males and females with a FPKM of 172.98 and 70.34, respectively; the other eight GRs had a FPKM ranging from 1.40 to 9.92 (File S4). In the phylogenetic tree (Fig. 8; File S2), the bitter lineage consisted of two subclades, one including *CobdGR4, HarmGR14, HarmGR78p* and *DmelGR66a* and the other containing *CobdGR5, BmorGR68* and *DmelGR33a*.

DISCUSSION

Olfactory proteins of 246 species of insects have been reported in the NCBI protein database, which includes 51 lepidopteran species (6th-August-2019). However, the olfactory proteins in lepidopteran species that are small and slender with lance-like wings and have larvae covered with a sheath, such as the casebearer moth Coleophoridae, have not been studied to date. We explored CSPs in the body transcriptome of *C. obducta*, based on the feasibility and necessity of exploring the chemical ecology of *C. obducta*. These data provide a direct molecular foundation for understanding olfactory protein function in chemosensory reception. They also show the important function of olfactory proteins in the casebearer moth and establish the groundwork for understanding the
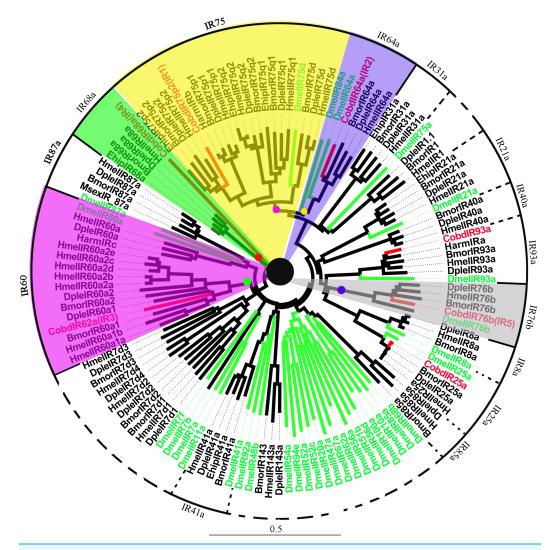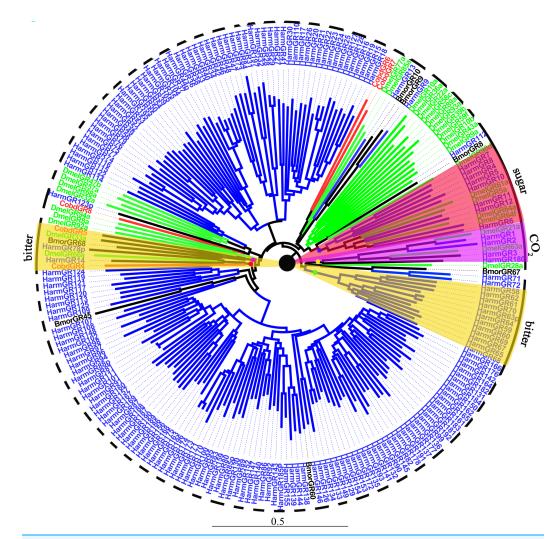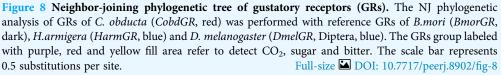
Figure 7 Neighbor-joining phylogenetic tree of ionotropic receptors (IRs). The NJ phylogenetic analysis of IRs of *C. obducta* (*CobdIR*, red) was performed with reference IRs of Lepidoptera, Diptera species. There are 14 subgroup of IRs in the tree. The scale bar represents 0.5 substitutions per site.

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molecular mechanisms of olfactory recognition and applying these data to *C. obducta* integrated pest management.

In terms of the number of olfactory proteins, *C. obducta* had similar amount OBPs compared with the whole body transcriptome of *Oedaleus infernalis* (*Zhang et al., 2018*). Compared with the insect antenna transcriptome (*Chang et al., 2017*; *Nie et al., 2017*; *Rojas et al., 2018*; *Sheng et al., 2017*; *Wang, Liu & Wang, 2017*; *Wu et al., 2017*; *Yang et al., 2017*, *2018*) (File S8), *C. obducta* had an intermediate number of OBPs. The number of olfactory proteins identified in the whole body transcriptome of *C. obducta* is smaller than that inmost other species based on analyses of insect antennal genes. This is probably due to the inclusion of multiple tissues (besides antenna) which would be expected to show a lower abundance of antennal proteins and the exclusion of genes with very low expression levels from our bioinformatics analysis. *C. obducta* is a specialist insect and

Figure 8 Neighbor-joining phylogenetic tree of gustatory receptors (GRs). The NJ phylogenetic analysis of GRs of *C. obducta* (*CobdGR*, red) was performed with reference GRs of *B.mori* (*BmorGR*, dark), *H.armigera* (*HarmGR*, blue) and *D. melanogaster* (*DmelGR*, Diptera, blue). The GRs group labeled with purple, red and yellow fill area refer to detect CO_2, sugar and bitter. The scale bar represents 0.5 substitutions per site. Full-size 🖼 DOI: 10.7717/peerj.8902/fig-8

polyphagous insects with huge expansions of genes associated with chemosensation compared with specialist insects have been identified (*Gouin et al., 2017*). Importantly, 74 CSPs were identified in the whole body transcriptome of *C. obducta*, including 3 PBPs, 1 Orco, 3 PRs, 3 SNMPs and 2 bitter and sugar GRs, which included most of the important olfactory genes. Thus, the preparation of a whole body transcriptome was a feasible way to search for most of the olfactory proteins in this tiny insect species, which cannot be reared artificially and for which sample collection is difficult.

OBPs are thought of as the first gate in the odorant recognition process with important biological functions, to bind and convey odors across the lymph in the sensillum (*Scaloni et al., 1999*). The ability of OBP native sensing units to detect odorants and eliminate important behaviors may be useful in the development of novel strategies for

insect population management, as well as other biotechnological applications (*Brito, Moreira & Melo, 2016*). It was recently demonstrated that OBPs can function as molecular recognition units in gas-phase biosensors (*Barbosa, Oliveira & Roque, 2018*) including OBP22 of *Aedes aegypti* (*Zhao et al., 2012*). We identified 16 putative OBPs including 2 GOBPs and 3 PBPs. However, from the results of the OBPs phylogenetic tree, determining whether *CobdPBP3* and *CobdGOBP1* belong to PBPs GOBPs, respectively, will require further study.The FPKM values of six *CobdOBPs* were higher than the FPKM values of the *CobdPBPs* in the whole body transcriptome. Considering that OBPs in insects have numerous non-olfactory functions, such as pheromone delivery, solubilization of nutrients, development and insecticide resistance (*Pelosi et al., 2018*), the strong expression of *CobdOBP4* s, *CobdGOBP1*, *CobdGOBP2*, *CobdOBP11, CobdOBP1* and *CobdOBP8* should make them good targets to determine their expression profiles and functions. Meanwhile, the eight OBPs found in *Ceracris kiangsu* revealed a clear divergence, indicating their varying functions (*Li, Jiang & Dong, 2018*). It has also been demonstrated that *Cves*OBP2 can bind the *Carpomya vesuviana* male-emitted odor (Z)-3-hexen-1-ol acetate (*Li et al., 2017*). Notably, most OBPs are expressed in antennae, which indicating the important functions of OBPs in antennal identification processes, such as in *C. kiangsu* (*Li, Jiang & Dong, 2018*), *H. assulta* (*Jin et al., 2015*) and *C. suppressalis* (*Xia et al., 2015*). *AlinOBP11* is expressed in the tarsal gustatory sensilla of *Adelphocoris lineolatus* (*Sun et al., 2017a*). Expression profiling showed that three *CobdPBPs* were more highly expressed in males than females and two exhibited significantly higher expression in males, indicating the sex biased expression of *CobdPBPs*, similar to *Conopomorpha sinensis* (*Li et al., 2018*); thus, these proteins may function in male binding of female-emitted pheromones. *CobdPBP3* exhibited the highest expression in males. The same expression profile was identified in of *E. hippophaecolus* for PBP1 (*Hu et al., 2018*), which is the main protein involved in binding sex-pheromone components during pheromone communication.

We identified 14 CSPs. The top two *Cobd*CSPs, *CobdCSP14* and *CobdCSP3* had FPKM values that were nearly double those of the top two *CobdOBPs* (379.12 and 370.89). OBPs and CSPs exhibit different expression patterns, OBPs are expressed in the antenna, while CSPs do not have a distinct expression preference (*Zhang et al., 2013*). *Anoplophora glabripennis* CSPs are not expressed in the antenna, but are highly expressed in the maxillary palps and propodeum (*Hu et al., 2016b*). The expression pattern of CSPs in *Empoasca onukii* showed that CSPs were highly expressed in the head and thorax (*Bian et al., 2018*). An analysis of the expression patterns of CSPs, indicated that exploration of the functions of *CobdCSP14* and *CobdCSP3* in antenna and other tissues will be important. Some CSPs were significantly expressed in antennae, including most of the CSPs of *Lobesia botrana* (*Rojas et al., 2018*) and MmedCSP2 and MmedCSP3 of *Microplitis mediator* (*Peng et al., 2017*). MmedCSP3 can bind insect odors and plant volatiles, as well as pheromone components of *Noctuidae*, Z11-16:Ald, Z11-16: OH and E11-14:Ac (*Peng et al., 2017*), illustrating the binding ability and functions of CSPs in olfactory recognition. CSPs of Diptera constitute an order-specific clade in

the CSP phylogenetic tree, which was the same as *Mamestra brassicae* (*Jacquinjoly et al., 2001*).

Three SNMPs were identified from the transcriptome. Both *CobdSNMP3* and *CobdSNMP2* were identified as nearly full-length genes and the annotation results suggested they were homologous to *Ostrinia nubilalis* sequences. Moreover, SNMPs are conserved throughout holometabolous insects (*Jiang et al., 2016*; *Vogt et al., 2009*); three lineages of SNMPs (SNMP1, SNMP2 and SNMP3) were obvious in the phylogenetic tree.

SixODEs and fiveCXEs were also identified. The biggest groups identified in *C. obducta*, the mitochondrial and cytosolic esterases of *CobdCXE1, CobdODE2* and *CobdODE6* and the antennal esterases of *CobdODE4, CobdODE1, CobdCXE3* and *CobdCXE5*, have also been found in *P. interpunctella* (*Liu et al., 2019*). *CobdODE5* is a microsomal α-esterases. Such enzymes are well known for their involvement in the detoxification of insecticides and xenobiotics and in the digestion of dietary esters (*Campbell et al., 2003*; *Gong et al., 2017*; *Yang et al., 2016*). The best characterized ODE in *D. melanogaster* is esterase 6, which degrades the major volatile, aggregation pheromone cis-vaccenyl acetate (*Chertemps et al., 2012*; *Mane, Tompkins & Richmond, 1983*) and various short chain fatty acid food esters (*Benton, 2007*; *Chertemps et al., 2015*).

ORs combine olfactory sensory neurons with binding proteins and they function in olfactory signal transduction, which also uses native sensing units to detect odors. In addition, the interactions between odorant molecules and ORs or OBPs are a source of inspiration for the design of peptides with tunable odorant selectivity (*Barbosa, Oliveira & Roque, 2018*; *Venthur & Zhou, 2018*). Insect olfactory receptors are dimers consisting of constant and variable regions (*Horsfield, Haase & Turin, 2017*). The constant region is a seven transmembrane helix spanning the membrane receptor Orco (formerly OR83b) (*Larsson et al., 2004*). *CobdOrco* in the Orco lineage of the OR tree demonstrated that we identified *C. obducta* Orco again. The expression of ORs show that 59 ORs of *Tessaratoma papillosa* were primarily expressed in the antennae (*Wu et al., 2017*) and that most ORs and two PRs of *Loxostege sticticalis* showed antenna-biased expression (*Wei et al., 2017*), suggesting their putative role in olfaction. It is also obvious that OR subtypes are expressed in different numbers of cells (*Fleischer et al., 2018*) and can be co-expressed, such as the six co-expressed ORs in the *A. gambia* genome (*Karner et al., 2015*). Insect ORs appear to be more specifically tuned to odorants than OBPs (*Fleischer et al., 2018*). In *B. mori*, the receptors for bombykol (the major component of the sex pheromone) and bombykal (the minor component of the sex pheromone) are *BmorOR1* and *BmorOR3*, respectively (*Große-Wilde, Svatoš & Krieger, 2006*; *Nakagawa et al., 2005*). *HvirOR13* and *HvirOR6* have also been identified as PRs for the major and minor sex pheromone constituents, respectively (*Große-Wilde et al., 2007*). Besides, *CobdPR1 (CobdOR1), CobdPR2 (CobdOR3)* and *CobdPR3 (CobdOR4)* are putative PRs in *C. obducta*; the functions of these receptors require further exploration. Identifying ORs, their ligands and key amino acid positions in the receptors (e.g., Ala195 in *AgamOR15* which functions as part of an inhibitor interaction site) (*Rahman & Luetje, 2017*), could serve as a foundation for the design of pest control agents for a given insect species.

In the antennae of *D. melanogaste*r, ionotropic glutamate receptors responsive to chemical compounds were identified and annotated as IRs (*Abuin et al., 2011*). The IR group was added and improved based on the genomic analysis of *Heliconius* IRs (*Schooten et al., 2016*). Overall, the amino acid sequence identities of *Drosophila* IRs range from 10% to 70%, suggesting functional diversity (*Richard et al., 2009*). Seven IR groups were identified in *C. obducta*, *CobdIR62a*, *CobdIR76b*, *CobdIR64a*, *CobdIR75p2*, *EhipIR68a*, *CobdIR25a* and *CobdIR93a*, with different functions. There are three subtypes of IRs, antennal IRs, divergent IRs and IR25a and IR8a, which are expressed, along with antennal IRs, in antennae, gustatory organs (e.g., the labellum) and coeloconic olfactory sensory neurons of the antenna respectively. IR25a and IR8a are co-expressed (*Croset et al., 2010*; *Abuin et al., 2011*; *Rytz, Croset & Benton, 2013*), but we did not identify *CobdIR8a*. IRs also have multiple functions and expression patterns that are general essential chemosensory cues for insects.

Ten GRs were detected in the transcriptome. GRs typically function in sensing sugar, CO_2 and bitter molecules (*Liman, Zhang & Montell, 2014*), they were also clustered in groups according to different functions in the phylogenetic tree. The type 2 bitter GRs of *H. armigera* were clustered together in the phylogenetic tree (*Xu et al., 2016*); two groups of bitter lineages were also identified. *CobdGR4* and *CobdGR5* were clustered with *DmelGR66a* and *DmelGR33a*, respectively, indicating *CobdGR4* and *CobdGR5* function as bitter sensors. *DmelGR21a* and *DmelGR63a* are required for responsiveness to CO_2 (*Jones et al., 2007*; *Kwon et al., 2007*). The sugar receptor lineages included *BmorGR8* and *BmorGR9* (*Koji, Kana & Kazushige, 2011*; *Zhang et al., 2011*) and *HarmGR8, HarmGR7, HarmGR4, HarmGR12, HarmGR10, HarmGR6* and *HarmGR5* (*Xu et al., 2016*), consistent with their function. We did not find that *CobdGRs* function in sugar and CO_2 sensing. Some GRs are important in pheromone detection, which is required for sexual behavior (*Joseph & Carlson, 2015*). For example, a previous study speculated that Gr39a participates in female pheromone detection and the authors demonstrated that the knockdown of Gr39a led to less courtship behavior in males (*Watanabe et al., 2011*). Thus, GRs-binding pheromones are usually considered olfactory signals (*Bray & Amrein, 2003*; *Jeong et al., 2013*; *Miyamoto & Amrein, 2008*; *Moon et al., 2009*).

CONCLUSIONS

We reported the whole body transcriptome of *C. obducta*; this is the first analysis of olfactory proteins in a Coleophoridae species. We identified 74 olfactory proteins, which will provide a foundation for exploring their functions in olfactory recognition process and system. We also explored the expression profiles of three *CobdPBPs*, which showed that all PBPs exhibited higher expression in males than females, consistent with the previously reported male-biased expression of PBPs. Future studies will explore the functions of the identified olfactory proteins in the antenna of *C. obducta*.

ACKNOWLEDGEMENTS

ADDITIONAL INFORMATION AND DECLARATIONS

Competing Interests

The authors declare that they have no competing interests.

Author Contributions

- Dongbai Wang conceived and designed the experiments, performed the experiments, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Jing Tao conceived and designed the experiments, authored or reviewed drafts of the paper, and approved the final draft.
- Pengfei Lu performed the experiments, analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.
- Youqing Luo conceived and designed the experiments, analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.
- Ping Hu conceived and designed the experiments, performed the experiments, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.

Field Study Permissions

The following information was supplied relating to field study approvals (i.e., approving body and any reference numbers):

The tiny casebearer moth *C. obducta* is a Chinese forestry pest and collection of *C. obducta* is permitted by the leader of Xinganmeng forestry bureau, Yinghua Lu, and member Tianhua Zhen.

Data Availability

The following information was supplied regarding data availability:

The sequence data is available at NCBI SRA: PRJNA587422.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.8902#supplemental-information.

Wang et al. (2020), *PeerJ,* DOI 10.7717/peerj.8902

20/27

REFERENCES

Abuin L, Bargeton B, Ulbrich MH, Isacoff EY, Kellenberger S, Benton R. 2011. Functional architecture of olfactory ionotropic glutamate receptors. *Neuron* **69(1)**:44–60 DOI 10.1016/j.neuron.2010.11.042.

Ban L, Zhang L, Yan Y, Pelosi P. 2002. Binding properties of a locust's chemosensory protein. *Biochemical & Biophysical Research Communications* **293(1)**:50–54 DOI 10.1016/S0006-291X(02)00185-7.

Bao W, Yang L, Zhao C. 1990. Study on the effective accumulated temperature of *Coleophora obducta*. *Journal of Northeast Forestry University* **18(4)**:118–123.

Barbosa AJ, Oliveira AR, Roque AC. 2018. Protein-and peptide-based biosensors in artificial olfaction. *Trends in Biotechnology* **36(12)**:1244–1258.

Benton R. 2007. Sensitivity and specificity in drosophila pheromone perception. *Trends in Neurosciences* **30(10)**:512–519 DOI 10.1016/j.tins.2007.07.004.

Bian L, Li Z-Q, Ma L, Cai X-M, Luo Z-X, Chen Z-M. 2018. Identification of the genes in tea leafhopper, *Empoasca onukii* (Hemiptera: Cicadellidae), that encode odorant-binding proteins and chemosensory proteins using transcriptome analyses of insect heads. *Applied Entomology & Zoology* **53(1)**:93–105 DOI 10.1007/s13355-017-0533-9.

Bo L, Dewey CN. 2011. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC Bioinformatics* **12(1)**:323 DOI 10.1186/1471-2105-12-323.

Bray S, Amrein H. 2003. A putative drosophila pheromone receptor expressed in male-specific taste neurons is required for efficient courtship. *Neuron* **39(6)**:1019–1029 DOI 10.1016/S0896-6273(03)00542-7.

Briand L, Swasdipan N, Nespoulous C, Bézirard V, Blon F, Huet JC, Ebert P, Penollet JC. 2010. Characterization of a chemosensory protein (ASP3c) from honeybee (*Apis mellifera* L.) as a brood pheromone carrier. *European Journal of Biochemistry* **269**:4586–4596.

Brito NF, Moreira MF, Melo AC. 2016. A look inside odorant-binding proteins in insect chemoreception. *Journal of Insect Physiology* **95**:51–65 DOI 10.1016/j.jinsphys.2016.09.008.

Campbell PM, De Q. Robin GC, Court LN, Dorrian SJ, Russell RJ, Oakeshott JG. 2003. Developmental expression and gene/enzyme identifications in the alpha esterase gene cluster of Drosophila melanogaster. *Insect Molecular Biology* **12(5)**:459–471 DOI 10.1046/j.1365-2583.2003.00430.x.

Carraher C, Dalziel J, Jordan MD, Christie DL, Newcomb RD, Kralicek AV. 2015. Towards an understanding of the structural basis for insect olfaction by odorant receptors. *Insect Biochemistry and Molecular Biology* **66**:31–41 DOI 10.1016/j.ibmb.2015.09.010.

Chang H, Liu Y, Yang T, Pelosi P, Dong S, Wang G. 2015. Pheromone binding proteins enhance the sensitivity of olfactory receptors to sex pheromones in *Chilo suppressalis*. *Scientific Reports* **5(1)**:13093 DOI 10.1038/srep13093.

Chang X-Q, Nie X-P, Zhang Z, Zeng F-F, Lv L, Zhang S, Wang M-Q. 2017. De novo analysis of the oriental armyworm *Mythimna separata* antennal transcriptome and expression patterns of odorant-binding proteins. *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics* **22**:120–130 DOI 10.1016/j.cbd.2017.03.001.

Chen G, Xiong H, Shu C, Hao Y, Gao J, Sun J. 2002. Application of the sex attractant of *Coleophora obducta* in the monitoring of its occurrence period. *Chinese Forest Pests* **21**:23–25.

Chertemps T, François A, Durand N, Rosell G, Dekker T, Lucas P, Maïbèche-Coisne M. 2012. A carboxylesterase, Esterase-6, modulates sensory physiological and behavioral response dynamics to pheromone in *Drosophila*. *BMC Biology* **10(1)**:56 DOI 10.1186/1741-7007-10-56.

Chertemps T, Younus F, Steiner C, Durand N, Coppin CW, Pandey G, Oakeshott JG, Maïbèche M. 2015. An antennal carboxylesterase from *Drosophila melanogaster*, esterase 6, is a candidate odorant-degrading enzyme toward food odorants. *Frontiers in Physiology* **6**:315 DOI 10.3389/fphys.2015.00315.

Chu D, Zhang Q. 1995. Study on the application technology of sex attractant of *Coleophora obducta*. *Journal of Northeast Forestry University* **23(6)**:14–20.

Clearwater JR, Foster SP, Muggleston SJ, Dugdale JS, Priesner E. 1991. Intraspecific variation and interspecific differences in sex pheromones of sibling species in *Ctenopseustis obliquana* complex. *Journal of Chemical Ecology* **17(2)**:413–429 DOI 10.1007/BF00994342.

Clyne PJ, Warr CG, Freeman MR, Lessing D, Kim J, Carlson JR. 1999. A novel family of divergent seven-transmembrane proteins: candidate odorant receptors in *Drosophila*. *Neuron* **22(2)**:327–338 DOI 10.1016/S0896-6273(00)81093-4.

Croset V, Rytz R, Cummins SF, Budd A, Brawand D, Kaessmann H, Gibson TJ, Benton R. 2010. Ancient protostome origin of chemosensory ionotropic glutamate receptors and the evolution of insect taste and olfaction. *PLOS Genetics* **6(8)**:e1001064 DOI 10.1371/journal.pgen.1001064.

Du Y, Yan F. 1994. Mechanism of plant volatile secondary substances in the relationship between herbivorous insects parasitic plants and insect natural enemies. *Acta Entomologica Sinica* **37(2)**:233–250.

Durand N, Carot-Sans G, Chertemps T, Montagné N, Jacquin-Joly E, Debernard S, Maïbèche-Coisne M. 2010. A diversity of putative carboxylesterases are expressed in the antennae of the noctuid moth Spodoptera littoralis. *Insect Molecular Biology* **19(1)**:87–97 DOI 10.1111/j.1365-2583.2009.00939.x.

Finn RD, Clements J, Eddy SR. 2011. HMMER web server: interactive sequence similarity searching. *Nucleic Acids Research* **39(Suppl. 2)**:W29–W37 DOI 10.1093/nar/gkr367.

Fleischer J, Pregitzer P, Breer H, Krieger J. 2018. Access to the odor world: olfactory receptors and their role for signal transduction in insects. *Cellular and Molecular Life Sciences* **75(3)**:485–508 DOI 10.1007/s00018-017-2627-5.

Götz S, Garcíagómez JM, Terol J, Williams TD, Nagaraj SH, Nueda MJ, Robles M, Talón M, Dopazo J, Conesa A. 2008. High-throughput functional annotation and data mining with the Blast2GO suite. *Nucleic Acids Research* **36(10)**:3420–3435 DOI 10.1093/nar/gkn176.

Glaser N, Gallot A, Legeai F, Montagné N, Poivet E, Harry M, Calatayud PA, Jacquinjoly E. 2013. Candidate chemosensory genes in the stemborer *Sesamia nonagrioides*. *International Journal of Biological Sciences* **9(5)**:481–495 DOI 10.7150/ijbs.6109.

Gong Y-H, Ai G-M, Li M, Shi X-Y, Diao Q-Y, Gao X-W. 2017. Functional characterization of carboxylesterase gene mutations involved in *Aphis gossypii* resistance to organophosphate insecticides. *Insect Molecular Biology* **26(6)**:702–714 DOI 10.1111/imb.12331.

Gouin A, Bretaudeau A, Nam K, Gimenez S, Aury J-M, Duvic B, Hilliou F, Durand N, Montagné N, Darboux I, Kuwar S, Chertemps T, Siaussat D, Bretschneider A, Moné Y, Ahn S-J, Hänniger S, Grenet A-SG, Neunemann D, Maumus F, Luyten I, Labadie K, Xu W, Koutroumpa F, Escoubas J-M, Llopis A, Maïbèche-Coisne M, Salasc F, Tomar A, Anderson AR, Khan SA, Dumas P, Orsucci M, Guy J, Belser C, Alberti A, Noel B, Couloux A, Mercier J, Nidelet S, Dubois E, Liu N-Y, Boulogne I, Mirabeau O, Le Goff G, Gordon K, Oakeshott J, Consoli FL, Volkoff A-N, Fescemyer HW, Marden JH, Luthe DS, Herrero S, Heckel DG, Wincker P, Kergoat GJ, Amselem J, Quesneville H, Groot AT, Jacquin-Joly E, Nègre N, Lemaitre C, Legeai F, D'Alençon E, Fournier P. 2017. Two genomes

of highly polyphagous lepidopteran pests (*Spodoptera frugiperda*, Noctuidae) with different host-plant ranges. *Scientific Reports* **7(1)**:11816 DOI 10.1038/s41598-017-10461-4.

Große-Wilde E, Svatoš A, Krieger J. 2006. A pheromone-binding protein mediates the bombykol-induced activation of a pheromone receptor in vitro. *Chemical Senses* **31(6)**:547–555 DOI 10.1093/chemse/bjj059.

Große-Wilde E, Gohl T, Bouché E, Breer H, Krieger J. 2007. Candidate pheromone receptors provide the basis for the response of distinct antennal neurons to pheromonal compounds. *European Journal of Neuroscience* **25(8)**:2364–2373 DOI 10.1111/j.1460-9568.2007.05512.x.

Horsfield AP, Haase A, Turin L. 2017. Molecular recognition in olfaction. *Advances in Physics* **2(3)**:937–977 DOI 10.1080/23746149.2017.1378594.

Hu P, Tao J, Cui M, Gao C, Lu P, Luo Y. 2016a. Antennal transcriptome analysis and expression profiles of odorant binding proteins in *Eogystia hippophaecolus* (Lepidoptera: Cossidae). *BMC Genomics* **17(1)**:651 DOI 10.1186/s12864-016-3008-4.

Hu P, Wang J, Cui M, Tao J, Luo Y. 2016b. Antennal transcriptome analysis of the Asian longhorned beetle *Anoplophora glabripennis*. *Scientific Reports* **6(1)**:26652 DOI 10.1038/srep26652.

Hu P, Gao C, Zong S, Luo Y, Tao J. 2018. Phenomenon binding protein PBP1 of seabuckthorn carpenterworm enriched in male antenna, leg and external genitals binding with sex phenomenon and volatiles. *Frontiers in Physiology* **9**:447 DOI 10.3389/fphys.2018.00447.

Jacquinjoly E, Vogt RG, François MC, Nagnanle Meillour P. 2001. Functional and expression pattern analysis of chemosensory proteins expressed in antennae and pheromonal gland of *Mamestra brassicae*. *Chemical Senses* **26(7)**:833–844 DOI 10.1093/chemse/26.7.833.

Jeong YT, Shim J, Oh SR, Yoon HI, Kim CH, Moon SJ, Montell C. 2013. An odorant-binding protein required for suppression of sweet taste by bitter chemicals. *Neuron* **79(4)**:725–737 DOI 10.1016/j.neuron.2013.06.025.

Jiang X, Pregitzer P, Grosse-wilde E, Breer H, Krieger J. 2016. Identification and characterization of two sensory neuron membrane proteins (SNMPs) of the desert locust, *Schistocerca gregaria* (Orthoptera: Acrididae). *Journal of Insect Science* **16(1)**:33 DOI 10.1093/jisesa/iew015.

Jin Z, Bing W, Shuanglin D, Depan C, Junfeng D, Walker WB, Yang L, Guirong W. 2015. Antennal transcriptome analysis and comparison of chemosensory gene families in two closely related noctuidae moths, *Helicoverpa armigera* and *H. assulta*. *PlOS ONE* **10**:e0117054.

Jones PL, Pask GM, Rinker DC, Zwiebel LJ. 2011. Functional agonism of insect odorant receptor ion channels. *Proceedings of the National Academy of Sciences of the United States of America* **108(21)**:8821–8825 DOI 10.1073/pnas.1102425108.

Jones WD, Cayirlioglu P, Kadow IG, Vosshall LB. 2007. Two chemosensory receptors together mediate carbon dioxide detection in drosophila. *Nature* **445(7123)**:86–90 DOI 10.1038/nature05466.

Joseph RM, Carlson JR. 2015. Drosophila chemoreceptors: a molecular interface between the chemical world and the brain. *Trends in Genetics* **31(12)**:683–695 DOI 10.1016/j.tig.2015.09.005.

Kaissling K-E. 2001. Sensory transduction. *Integrative & Comparative Biology* **43(6)**:802 DOI 10.1093/icb/43.6.802.

Karner T, Kellner I, Schultze A, Breer H, Krieger J. 2015. Co-expression of six tightly clustered odorant receptor genes in the antenna of the malaria mosquito Anopheles gambiae. *Frontiers in Ecology and Evolution* **3**:26 DOI 10.3389/fevo.2015.00026.

Koji S, Kana T, Kazushige T. 2011. Sugar-regulated cation channel formed by an insect gustatory receptor. *Proceedings of the National Academy of Sciences of the United States of America* **108(28)**:11680–11685 DOI 10.1073/pnas.1019622108.

Kwon JY, Dahanukar A, Weiss LA, Carlson JR. 2007. The molecular basis of CO_2 reception in Drosophila. *Proceedings of the National Academy of Sciences of the United States of America* **104(9)**:3574–3578 DOI 10.1073/pnas.0700079104.

Larsson MC, Domingos AI, Jones WD, Chiappe ME, Amrein H, Vosshall LB. 2004. Or83b encodes a broadly expressed odorant receptor essential for Drosophila olfaction. *Neuron* **43(5)**:703–714 DOI 10.1016/j.neuron.2004.08.019.

Lartigue A, Campanacci V, Roussel A, Larsson AM, Jones TA, Tegoni M, Cambillau C. 2002. X-ray structure and ligand binding study of a moth chemosensory protein. *Journal of Biological Chemistry* **277(35)**:32094–32098 DOI 10.1074/jbc.M204371200.

Leal WS. 2013. Odorant reception in insects: roles of receptors, binding proteins, and degrading enzymes. *Annual Review of Entomology* **58(1)**:373–391 DOI 10.1146/annurev-ento-120811-153635.

Leal WS, Chen AM, Ishida Y, Chiang VP, Erickson ML, Morgan TI, Tsuruda JM. 2005. Kinetics and molecular properties of pheromone binding and release. *Proceedings of the National Academy of Sciences of the United States of America* **102(15)**:5386–5391 DOI 10.1073/pnas.0501447102.

Leal WS, Nikonova L, Peng G. 1999. Disulfide structure of the pheromone binding protein from the silkworm moth, *Bombyx mori. FEBS Letters* **464(1–2)**:85–90 DOI 10.1016/S0014-5793(99)01683-X.

Li H. 2003. Morphological study and redescription of *Coleophora sinensis* yang and *Coleophora obducta* (Meyrick) (Lepidoptera: Coleophoridae). *Entomotaxonomia* **25**:295–302.

Li P, Liu Y, Wang S, Sun H, Bai J, Peng G, Gong X. 2018. Sequence analysis and expression profiling of pheromone binding protein genes in the litchi fruit borer, *Conopomorpha sinensi* (Lepidoptera: Gracillariidae). *Acta Entomologica Sinica* **61**:527–536.

Li R, Jiang GF, Dong SY. 2018. A head transcriptome provides insights into odorant binding proteins of the bamboo grasshopper. *Genes & Genomics* **40**:991–1000 DOI 10.1007/s13258-018-0706-0.

Li Y, Zhou P, Zhang J, Yang D, Li Z, Zhang X, Zhu S, Yu Y, Chen N. 2017. Identification of odorant binding proteins in *Carpomya vesuviana* and their binding affinity to the male-borne semiochemicals and host plant volatiles. *Journal of Insect Physiology* **100**:100–107 DOI 10.1016/j.jinsphys.2017.05.013.

Li Z-Q, Zhang S, Luo J-Y, Wang C-Y, Lv L-M, Dong S-L, Cui J-J. 2015. Transcriptome comparison of the sex pheromone glands from two sibling *Helicoverpa* species with opposite sex pheromone components. *Scientific Reports* **5(1)**:9324 DOI 10.1038/srep09324.

Liman ER, Zhang YV, Montell C. 2014. Peripheral coding of taste. *Neuron* **81(5)**:984–1000 DOI 10.1016/j.neuron.2014.02.022.

Liu H, Lei X, Du L, Yin J, Shi H, Zhang T, Zhang Q. 2019. Antennae-specific carboxylesterase genes from Indian meal moth: identification, tissue distribution and the response to semiochemicals. *Journal of Stored Products Research* **84**:101528 DOI 10.1016/j.jspr.2019.101528.

Liu X, Jiang Z, Wu H, Zhang K, Lv Z, Zhang X. 2008. Monitoring and control of adult insect with sex attractant of Coleophora obducta. *Chinese Forest Pests* **27**:9–10.

Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* **25(4)**:402–408 DOI 10.1006/meth.2001.1262.

Mane SD, Tompkins L, Richmond RC. 1983. Male esterase 6 catalyzes the synthesis of a sex pheromone in *Drosophila melanogaster* females. *Science* **222(4622)**:419–421 DOI 10.1126/science.222.4622.419.

Miyamoto T, Amrein H. 2008. Suppression of male courtship by a *Drosophila* pheromone receptor. *Nature Neuroscience* **11(8)**:874–876 DOI 10.1038/nn.2161.

Moon SJ, Lee Y, Jiao Y, Montell C. 2009. A Drosophila gustatory receptor essential for aversive taste and inhibiting male-to-male courtship. *Current Biology* **19(19)**:1623–1627 DOI 10.1016/j.cub.2009.07.061.

Mortazavi A, Williams BA, McCue K, Schaeffer L, Wold B. 2008. Mapping and quantifying mammalian transcriptomes by RNA-seq. *Nature Methods* **5(7)**:621–628 DOI 10.1038/nmeth.1226.

Nakagawa T, Sakurai T, Nishioka T, Touhara K. 2005. Insect sex-pheromone signals mediated by specific combinations of olfactory receptors. *Science* **307(5715)**:1638–1642 DOI 10.1126/science.1106267.

Nie XP, Li QL, Xu C, Li DZ, Zhang Z, Wang MQ, Zhou AM, Li SQ. 2017. Antennal transcriptome and odorant binding protein expression profiles of an invasive mealybug and its parasitoid. *Journal of Applied Entomology* **142**:149–161 DOI 10.1111/jen.1241.

Pelosi P, Iovinella I, Zhu J, Wang G, Dani FR. 2018. Beyond chemoreception: diverse tasks of soluble olfactory proteins in insects. *Biological Reviews* **93(1)**:184–200 DOI 10.1111/brv.12339.

Pelosi P, Zhou JJ, Ban LP, Calvello M. 2006. Soluble proteins in insect chemical communication. *Cellular & Molecular Life Sciences* **63(14)**:1658–1676 DOI 10.1007/s00018-005-5607-0.

Peng Y, Wang S-N, Li K-M, Liu J-T, Zheng Y, Shan S, Yang Y-Q, Li R-J, Zhang Y-J, Guo Y-Y. 2017. Identification of odorant binding proteins and chemosensory proteins in Microplitis mediator as well as functional characterization of chemosensory protein 3. *PLOS ONE* **12(7)**:e0180775 DOI 10.1371/journal.pone.0180775.

Rahman S, Luetje CW. 2017. Mutant cycle analysis identifies a ligand interaction site in an odorant receptor of the malaria vector *Anopheles gambiae*. *Journal of Biological Chemistry* **292(46)**:18916–18923 DOI 10.1074/jbc.M117.810374.

Richard B. 2015. Multigene family evolution: perspectives from insect chemoreceptors. *Trends in Ecology & Evolution* **30(10)**:590–600 DOI 10.1016/j.tree.2015.07.009.

Richard B, Vannice KS, Carolina GD, Vosshall LB. 2009. Variant ionotropic glutamate receptors as chemosensory receptors in *Drosophila*. *Cell* **136(1)**:149–162 DOI 10.1016/j.cell.2008.12.001.

Rojas V, Jiménez H, Palma-Millanao R, González-González A, Machuca J, Godoy R, Ceballos R, Mutis A, Venthur H. 2018. Analysis of the grapevine moth *Lobesia botrana* antennal transcriptome and expression of odorant-binding and chemosensory proteins. *Comparative Biochemistry & Physiology Part D Genomics & Proteomics* **27**:1–12 DOI 10.1016/j.cbd.2018.04.003.

Ronderos D, Smith D. 2009. Diverse signaling mechanisms mediate volatile odorant detection in Drosophila. *Fly* **3(4)**:8–297 DOI 10.4161/fly.9801.

Rytz R, Croset V, Benton R. 2013. Ionotropic receptors (IRs): chemosensory ionotropic glutamate receptors in *Drosophila* and beyond. *Insect Biochemistry and Molecular Biology* **43(9)**:888–897 DOI 10.1016/j.ibmb.2013.02.007.

Saitou N, Nei M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* **4**:406–425.

Sato K, Pellegrino M, Nakagawa T, Nakagawa T, Vosshall LB, Touhara K. 2008. Insect olfactory receptors are heteromeric ligand-gated ion channels. *Nature* **452(7190)**:1002–1006 DOI 10.1038/nature06850.

Scaloni A, Monti M, Angeli S, Pelosi P. 1999. Structural analysis and disulfide-bridge pairing of two odorant-binding proteins from *Bombyx mori*. *Biochemical and Biophysical Research Communications* **266(2)**:386–391 DOI 10.1006/bbrc.1999.1791.

Schooten BV, Jiggins CD, Briscoe AD, Papa R. 2016. Genome-wide analysis of ionotropic receptors provides insight into their evolution in Heliconius butterflies. *BMC Genomics* **17(1)**:254 DOI 10.1186/s12864-016-2572-y.

Sheng S, Liao C-W, Zheng Y, Zhou Y, Xu Y, Song W-M, He P, Zhang J, Wu FA. 2017. Candidate chemosensory genes identified in the endoparasitoid *Meteorus pulchricornis* (Hymenoptera: Braconidae) by antennal transcriptome analysis. *Comparative Biochemistry & Physiology Part D: Genomics & Proteomics* **22**:20–31 DOI 10.1016/j.cbd.2017.01.002.

Shu C, Chen G, Xiong H, Ai Q, Fan Y, Zhan M. 2003. Research situation and development direction of *Coleophora obducta* in China. *Liaoning Forestry Science and Technology* **1**:29–31.

Sun L, Wang Q, Wang Q, Dong K, Xiao Y, Zhang YJ. 2017a. Identification and characterization of odorant binding proteins in the forelegs of *Adelphocoris lineolatus* (Goeze). *Frontiers in Physiology* **8**:735 DOI 10.3389/fphys.2017.00735.

Sun L, Wang Q, Wang Q, Zhang Y, Tang M, Guo H, Fu J, Xiao Q, Zhang Y, Zhang Y. 2017b. Identification and expression patterns of putative diversified carboxylesterases in the tea geometrid *Ectropis obliqua* Prout. *Frontiers in Physiology* **8**:1085 DOI 10.3389/fphys.2017.01085.

Syed Z, Leal WS. 2008. Mosquitoes smell and avoid the insect repellent DEET. *Proceedings of the National Academy of Sciences of the United States of America* **105(36)**:13598–13603 DOI 10.1073/pnas.0805312105.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* **28(10)**:2731–2739 DOI 10.1093/molbev/msr121.

Venthur H, Zhou J-J. 2018. Odorant receptors and odorant-binding proteins as insect pest control targets: a comparative analysis. *Frontiers in Physiology* **9**:69 DOI 10.3389/fphys.2018.01163.

Vieira FG, Rozas J. 2011. Comparative genomics of the odorant-binding and chemosensory protein gene families across the Arthropoda: origin and evolutionary history of the chemosensory system. *Genome Biology and Evolution* **3(Pt. 1)**:476–490 DOI 10.1093/gbe/evr033.

Vogt RG, Miller NE, Litvack R, Fandino RA, Sparks J, Staples J, Friedman R, Dickens JC. 2009. The insect SNMP gene family. *Insect Biochemistry and Molecular Biology* **39(7)**:448–456 DOI 10.1016/j.ibmb.2009.03.007.

Vogt RG, Riddiford LM. 1981. Pheromone binding and inactivation by moth antennae. *Nature* **293(5828)**:161–163 DOI 10.1038/293161a0.

Vosshall LB, Amrein H, Morozov PS, Rzhetsky A, Axel R. 1999. A spatial map of olfactory receptor expression in the *Drosophila* antenna. *Cell* **96(5)**:725–736 DOI 10.1016/S0092-8674(00)80582-6.

Wang B, Liu Y, Wang G-R. 2017. Chemosensory genes in the antennal transcriptome of two syrphid species, *Episyrphus balteatus* and *Eupeodes corollae* (Diptera: Syrphidae). *BMC Genomics* **18(1)**:586 DOI 10.1186/s12864-017-3939-4.

Watanabe K, Toba G, Koganezawa M, Yamamoto D. 2011. Gr39a, a highly diversified gustatory receptor in *Drosophila*, has a role in sexual behavior. *Behavior Genetics* **41(5)**:746–753 DOI 10.1007/s10519-011-9461-6.

Wei H-S, Li K-B, Zhang S, Cao Y-Z, Yin J. 2017. Identification of candidate chemosensory genes by transcriptome analysis in *Loxostege sticticalis* Linnaeus. *PLOS ONE* **12(4)**:e0174036 DOI 10.1371/journal.pone.0174036.

Wicher D. 2015. *Olfactory signaling in insects: progress in molecular biology and translational science*. Amsterdam: Elsevier.

Wicher D, Schäfer R, Bauernfeind R, Stensmyr MC, Heller R, Heinemann SH, Hansson BS. 2008. *Drosophila* odorant receptors are both ligand-gated and cyclic-nucleotide-activated cation channels. *Nature* **452(7190)**:1007–1011 DOI 10.1038/nature06861.

Wu Z-Z, Qu M-Q, Pu X-H, Cui Y, Xiao W-Y, Zhao H-X, Bin S-Y, Lin J-T. 2017. Transcriptome sequencing of *Tessaratoma papillosa* antennae to identify and analyze expression patterns of putative olfaction genes. *Scientific Reports* **7(1)**:3070 DOI 10.1038/s41598-017-03306-7.

Xia Y-H, Zhang Y-N, Hou X-Q, Li F, Dong S-L. 2015. Large number of putative chemoreception and pheromone biosynthesis genes revealed by analyzing transcriptome from ovipositor-pheromone glands of *Chilo suppressalis*. *Scientific Reports* **5(1)**:7888 DOI 10.1038/srep07888.

Xu W, Papanicolaou A, Zhang HJ, Anderson A. 2016. Expansion of a bitter taste receptor family in a polyphagous insect herbivore. *Scientific Reports* **6(1)**:23666 DOI 10.1038/srep23666.

Zhang Y-N, Jin J-Y, Jin R, Xia Y-H, Zhou J-J, Deng J-Y, Dong S-L, Renou M. 2013. 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* **8(7)**:e69715 DOI 10.1371/journal.pone.0069715.

Yang H, Cai Y, Zhuo Z, Yang W, Yang C, Zhang J, Yang Y, Wang B, Guan F. 2018. Transcriptome analysis in different developmental stages of *Batocera horsfieldi* (Coleoptera: Cerambycidae) and comparison of candidate olfactory genes. *PLOS ONE* **13(2)**:e0192730 DOI 10.1371/journal.pone.0192730.

Yang H, Yan S-C, Liu D. 2009. Ultrastructural observations on antennal sensilla of *Coleophora obducta* (Meyrick) (Lepidoptera: Coleophoridae). *Micron* **40(2)**:231–238 DOI 10.1016/j.micron.2008.08.002.

Yang L. 1984. The revision of species names of *Coleophora obducta* in China and the discussion of related issues. *Forest Science* **20**:160–164.

Yang S, Cao D, Wang G, Liu Y. 2017. Identification of genes involved in chemoreception in *Plutella xyllostella* by antennal transcriptome analysis. *Scientific Reports* **7(1)**:11941 DOI 10.1038/s41598-017-11646-7.

Yang Y, Zhang Y, Yang B, Fang J, Liu Z. 2016. Transcriptomic responses to different doses of cycloxaprid involved in detoxification and stress response in the whitebacked planthopper, *Sogatella furcifera*. *Entomologia Experimentalis et Applicata* **158(3)**:248–257 DOI 10.1111/eea.12406.

Yushan H. 2004. *Study on biological control technology of Coleophora obducta master.* Harbin: Northeast Forestry University.

Zhang H-J, Anderson A, Trowell S, Luo A, Xiang Z-H, Xia Q-Y. 2011. Topological and functional characterization of an insect gustatory receptor. *PLOS ONE* **6(8)**:e24111 DOI 10.1371/journal.pone.0024111.

Zhang Y, Tan Y, Zhou X-R, Pang B-P. 2018. A whole-body transcriptome analysis and expression profiling of odorant binding protein genes in *Oedaleus infernalis*. *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics* **28**:134–141 DOI 10.1016/j.cbd.2018.08.003.

Zhao X, Ashley GM, Garcia-Gancedo L, Jin H, Luo J, Flewitt AJ, Lu JR. 2012. Protein functionalized ZnO thin film bulk acoustic resonator as an odorant biosensor. *Sensors and Actuators B: Chemical* **163(1)**:242–246 DOI 10.1016/j.snb.2012.01.046.

Zhou JJ. 2010. Chapter ten—odorant-binding proteins in insects. *Vitamins & Hormones* **83**:241–272.

Zwiebel LJ, Takken W. 2004. Olfactory regulation of mosquito–host interactions. *Insect Biochemistry and Molecular Biology* **34(7)**:645–652 DOI 10.1016/j.ibmb.2004.03.017.