Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergikafkas.org
E-ISSN: 1309-2251

Kafkas Univ Vet Fak Derg
30 (2): 259-267, 2024
DOI: 10.9775/kvfd.2023.30981

Research Article

Antennal Transcriptome and Proteome Analysis of Olfactory Genes and Tissue Expression Profiling of Odorant Binding Proteins in *Wohlfahrtia magnifica*

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How to cite this article?
Li LF, Wu RTDW, Zhu LX, Meg SYC, Tu JY, Ge C, Qi WM, Er D: Antennal transcriptome and proteome analysis of olfactory genes and tissue expression profiling of odorant binding proteins in *Wohlfahrtia magnifica*. *Kafkas Univ Vet Fak Derg*, 30 (2): 259-267, 2024.
DOI: 10.9775/kvfd.2023.30981

Article ID: KVFD-2023-30981
Received: 20.10.2023
Accepted: 25.12.2023
Published Online: 02.01.2024

Abstract

Wohlfahrtia magnifica is a species of fly that parasitizes Bactrian camels. The adult flies lay their larvae near the vulva of the camels, and these larvae develop and cause damage to the vaginal tissues, resulting in vaginal myiasis. Olfactory organs play an important role in the identification and location of host, foraging, mating and oviposition behavior of *W. magnifica*. Olfactory genes were identified by antennal transcriptome analysis. Twenty-four odor-binding proteins (OBPs) and two chemosensory proteins (CSPs) were identified in the antenna transcriptome of *W. magnifica*, and then the phylogenetic analysis of the olfactory genes of *W. magnifica* and other species was carried out. RT-qPCR was used for the first time to analyze the expression profile of OBPs in the antenna tissues of the *W. magnifica*. In the tissue expression analysis of OBP genes, it was found that many of them showed obvious gender bias in antennae, indicating their different roles in identifying pheromones. These results will help to lay a foundation for the future research on the sense of smell of *W. magnifica* and help to better reveal the change of odor reception of *W. magnifica* and provide new ideas for the research on biological prevention and control of vaginal myiasis.

Keywords: Antenna, Olfactory genes, Transcriptome, *W. magnifica*

Introduction

Wohlfahrtia magnifica belongs to the order Diptera, family Sarcophagidae, genus Wohlfahrtia Brauer. *W. magnifica* is the main pathogen of traumatic myiasis of flies, which is widely distributed in many countries in Asia, Europe, Africa and Northern China [1-3]. In China, *W. magnifica* is the sole pathogen responsible for causing vaginal myiasis in Bactrian camels. Female flies produce larvae near the vulva of Bactrian camels, and the larvae of *W. magnifica* parasitize and develop gradually in the vagina of Bactrian camels. The labia of the sick camel is swollen and closed, often accompanied by blood outflow. Upon opening the labia, it becomes evident that the lesion contains larvae at different stages of development. If it is not treated in time, the presence of *W. magnifica* in the environment will produce maggots at the focus, and the physical trauma will increase, which can lead to paralysis or even death of

the host. At present, there are no effective prevention and control measures, and it is urgent to seek new prevention and control methods [4].

Highly developed sensory organs help the *W. magnifica* adapt to the complex and changeable environment, and play a key role in its life, such as identifying and locating the host, foraging, mating and spawning [5,6]. As the most important olfactory organ of the *W. magnifica*, the main task of antenna is to recognize and perceive pheromones. The recognition of odor molecules in insects is a complex process, involving a variety of proteins related to smell, such as odorant binding proteins (OBPs), chemosensory proteins (CSPs), olfactory receptors (ORs), gustatory receptors (GRs), ionotropic receptors (IRs) and sensory neuron membrane proteins (SNMPs) [7-10]. The task of identifying pheromones and odors is mainly accomplished by odorant binding proteins (OBPs) and chemosensory proteins (CSPs) [11]. OBPs are mainly expressed in antenna and are small water-soluble acidic proteins. They exhibit a conserved structure comprising six cysteine residues that form three intertwined disulfide bridges. Typically, OBPs consist of around 120 to 150 amino acids and exhibit a mass of approximately 14 kDa [12-15]. CSPs are present at high concentrations in chemosensory sen-silla lymphs and are broadly expressed in non-sensory tissues. CSPs are soluble small molecule proteins with four cysteines that form two disulfide bridges [16,17]. It is considered that insect OBPs can bind and transport hydrophobic odorant molecules across the hydrophilic sensillum lymph to corresponding receptors on olfactory sensory neurons. Further, OBP-odorant complexes (or odorant itself) activate receptors (ORs or IRs) to stimulate a cascade of reaction, which converts chemical signals into electric signals and eventually lead to specific behaviors [18,19].

The OBPs are subdivided into three subfamilies: pheromone-binding proteins (PBPs), general odorant-binding proteins (GOBPs) and antennal-binding proteins (ABPs). PBPs are expressed in long sensillum trichodea and show a male antennae-biased expression pattern. PBPs are involved in the detection of female sex pheromones, playing a crucial role in mate location and mating behavior [20]. GOBPs, including GOBP1 and GOBP2, are usually distributed in sensillum basiconica [21]. GOBPs are thought to bind general odorants such as host plant volatiles and other environmental chemical cues. However, a few studies reported that GOBPs may also be involved in sex pheromone detection [22]. Research on olfactory-related proteins, including OBPs, has been conducted in various insect species. Over 150 species of OBPs have been identified in 35 species of lepidoptera insects alone [23]. With the deepening of their research, many insects have carried out research on the types, functions and olfactory mechanisms of olfactory related proteins. Olfactory-related proteins of insects are potential targets for the control of *W. magnifica* in the future [24-27].

In this study, we sequenced the antenna transcriptome of *W. magnifica*, analyzed and identified the genes encoding olfactory protein, and measured the transcription expression of important OBP genes in the antenna tissues of male and female adults of *W. magnifica* by fluorescence quantitative real-time PCR. The research aimed to identify and compare the olfactory genes of *W. magnifica* by analyzing the antenna transcriptome. This analysis provides a foundation for further understanding the underlying molecular mechanisms involved in olfactory perception in *W. magnifica*. Knowing these information can provide target genes for the biological control of myiasis, and then provide a good start for the integrated management of *W. magnifica* [28,29].

Material and Methods

Ethical Statement

All experimental procedures were approved by the Animal Protection and Use Committee of Inner Mongolia Agricultural University and strictly followed animal welfare and ethical guidelines.

Source of *W. magnifica* and Its Antenna Collections

In this study,we used the *W. magnifica* raised in the laboratory. The populations were kept in a laboratory insect rearing box (aluminium alloy frame, 120 mesh nylon sand mesh cover, space volume: 35 cm x 35 cm x 35 cm). The laboratory rearing conditions were kept at 25°C, relative humidity was 50%~60%, and the illumination period was L: D=15:9 h. For the transcriptome sequencing analysis, the hatched adults were immediately separated by sex. Both of 50 pairs of antennae of 3-day-old female and male *W. magnifica* were collected by dissecting microscope, and immediately frozen in liquid nitrogen and stored at −80°C until RNA was isolated, and further use.

RNA Extraction, CDNA Library Construction and Illumina Sequencing

In this study, total RNA was extracted from the male and female antenna tissue samples using a Total RNA Extractor kit from TaKaRa (Dalian, China). The concentration of RNA was measured using a Qubit 2.0 fluorometer. The integrity of the RNA and the presence of genomic DNA contamination were assessed using agarose gel electrophoresis. After the RNA extraction, library construction was performed. The constructed library was quantified using a Qubit 2.0 fluorometer and diluted to a concentration of 1 ng/L. The sequencing of the library was carried out by Sheng gong Bioengineering (Shanghai) Company.

Transcriptome Data Analysis and Gene Functional Annotation

The quality of the original sequencing data was evaluated by FastQC, and then the quality was cut by Trimmomatic, and relatively accurate and effective data were obtained. Trinity was used to assemble the clean data of all samples from scratch, and the assembly results were optimized and evaluated. The transcript was compared with databases such as CDD, KOG, COG, NR, NT, PFAM with NCBI Blast+, and its functional annotation information was obtained. Using KAAS to get KEGG annotation information of transcript. The CDS prediction was made according to the transcript and database Blast comparison results and Trans Decoder. Use ggplot2 software package v.3.5 for data visualization and drawing.

Annotation, Sequence Alignment and Phylogenetic Analysis of Olfactory Genes

All candidate genes of OBP and CSP were manually checked by BLASTx and BLASTn software of NCBI Online. ORF Finder online software is used to predict the open reading frame of genes. The candidate OBPs and CSPs were searched for the presence of N-terminal signal peptides using SignalP5.0. Using the nucleotide sequences of OBP and CSP as queries (BLASTx) in GenBank database, the sequences of different insect species were retrieved from GenBank database and used to construct phylogenetic trees. DNAMAN software was used for multiple alignment of amino acid sequences. Edit phylogenetic tree with MEGA software. The expression level is displayed as TPM value (the expression amount obtained after homogenization of gene length and sequencing depth), which is calculated by Saimon 0.8.2.

Analysis of Antenna Expression of OBP Genes

The relative expression level of OBP genes in male and female antennae was analyzed by fluorescence quantitative real-time PCR. Total RNA extraction is the same as above. Primer 3 plus designed specific primers. RT-qPCR data were analyzed by $2^{-(\Delta\Delta Ct)}$ method to calculate the relative expression level, and graph-pad was used to draw the result graph. SPSS 23.0 was used for one-way ANOVA, and Tukey method was used to test the significance of the difference ($P<0.05$).

Results

Transcriptome Assembly and Annotation

In order to identify the olfactory genes, the antennae of male and female adults of *W. magnifica* were sequenced by Illumina HiSeq, and 61,868,724 and 68,888,578 original readings were obtained from the antennae of the male and female *(Table 1)*. The raw data obtained by sequencing contains low-quality sequences with connectors.In order to ensure the quality of information analysis, the original data must be filtered.By removing the low-quality and trimmed readings with the length less than 20 nt, 59,685,374 and 65,806,910 male and female clean readings were obtained. The percentage of Q30 bases exceeded 94.81% and the GC content was 40.64-40.98% *(Table 2)*. After filtering out adapters and low-quality original sequences and assembling the readings from male and female antennae into a single transcription group, assembled into 153,779 unigenes with a total length of 76,933334bp, and 568 N50 500.29bp unigenes with a length between 201bp and 29622bp were obtained, of which 12605 unigenes with a length exceeding 1000bp accounted for 8.19% of all unigenes *(Table 3)*.

In total, 99085 unigenes from *W. magnifica* (64.43% of 153779 unigenes) were annotated in at least one of the data-

Table 1. *Sample statistics of Original Data Information*

Parameter		Male Antenna	Female Antenna
Total Reads Count (#)		61868724	68888578
Total Bases Count (bp)		9280308600	10333286700
Average Read Length (bp)		150	150
Q10 Bases	Count (bp)	9277656490	10329662377
	Ratio (%)	99.97%	99.96%
Q20 Bases	Count (bp)	9120877203	10129798266
	Ratio (%)	98.28%	98.03%
Q30 Bases	Count (bp)	8850535758	9796998975
	Ratio (%)	95.37%	94.81%
N Bases	Count (bp)	2652110	3624323
	Ratio (%)	0.03%	0.04%
GC Bases	Count (bp)	3803323380	4199868275
	Ratio (%)	40.98%	40.64%

Table 2. *Sample statistics of QC data information*

Parameter		Male Antenna	Female Antenna
Total Reads Count (#)		59685374	65806910
Total Bases Count (bp)		8771287389	9708026321
Average Read Length (bp)		146.96	147.52
Q10 Bases	Count (bp)	8770976692	9707581265
	Ratio (%)	100.00%	100.00%
Q20 Bases	Count (bp)	8698509459	9622028526
	Ratio (%)	99.17%	99.11%
Q30 Bases	Count (bp)	8507137252	9396553581
	Ratio (%)	96.99%	96.79%
N Bases	Count (bp)	310697	445056
	Ratio (%)	0.00%	0.00%
GC Bases	Count (bp)	3589396721	3946965602
	Ratio (%)	40.92%	40.66%

Table 3. *Summary of assembled transcript and unigenes*

Parameter	No.	Length Range/bp							
		>=500bp	>=1000bp	N50	N90	Max. Length	Min. Length	Total Length	Average Length
Transcript	262628	77017	32060	800	255	29622	201	155409503	591.75
Unigene	153779	36021	12605	568	240	29622	201	76933334	500.29

Table 4. *Functional annotation of the unigenes in different Databases*

Database	Number of Genes	Percentage (%)
Annotated in CDD	6793	4.42
Annotated in PFAM	8126	5.28
Annotated in KEGG	4320	2.81
Annotated in KOG	8246	5.36
Annotated in Swissprot	13165	8.56
Annotated in GO	11516	7.49
Annotated in NR	37603	24.45
Annotated in NT	93604	60.87
Annotated in at least one database	99085	64.43
Annotated in all database	1536	one
Total genes	153779	100

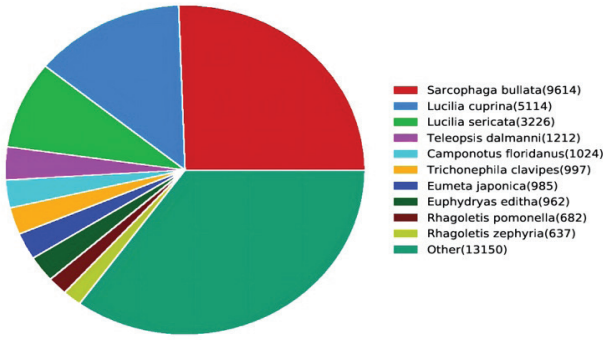

Fig 1. Pie chart of homologous species distribution

bases searched (NT, NR, PFAM, KOG, Swiss-Prot, KEGG, CDD and GO databases) *(Table 4)*. Homology searches againstthe Nr database showed that the *W. magnifica* antennal tran-scriptome shared the greatest homology with sequences from *Sarcophage bullate* (25%), followed by *Lucilia cuprina* (13.6%) and *Lucilia sericate* (8.6%) *(Fig. 1)*. Of the 153,779 unigenes, 11,516 (7.49%) correspond to at least one specific term in "biological process", "cellular component" and "molecular function". In the molecular function category, 6687 (58.1%) and3985 (34.6%) unigenes were linked to binding and catalytic activity. In terms of the biological process, 9696 (84.2%), 7453 (64.7%) unigenes were related to cellular processesand metabolic processes. In the cellular component category, 9683 (84.1%) unigenes were assigned to the terms cell and cell part *(Fig. 2)*.

Identification of Candidate Odor Binding Proteins

In the study, a total of 24 candidate OBP transcripts were identified in the antennal transcriptome of both male and female *W. magnifica* flies. These OBP gene sequences were found to be over 240 bp in length and possessed complete open reading frames (ORFs). Among them, 17 kinds of protein were identified as PBPs. These PBPs were found to be male-specific and associated with pheromone-sensitive neurons, suggesting their potential role in olfactory perception and mate recognition. To determine the homology of these OBP sequences, the BLASTx analysis was performed. The results revealed that Wmag36544 exhibited 96.34% orthology with the OBP sequence from *Sarcophaga caerulescens*, indicating a high level of

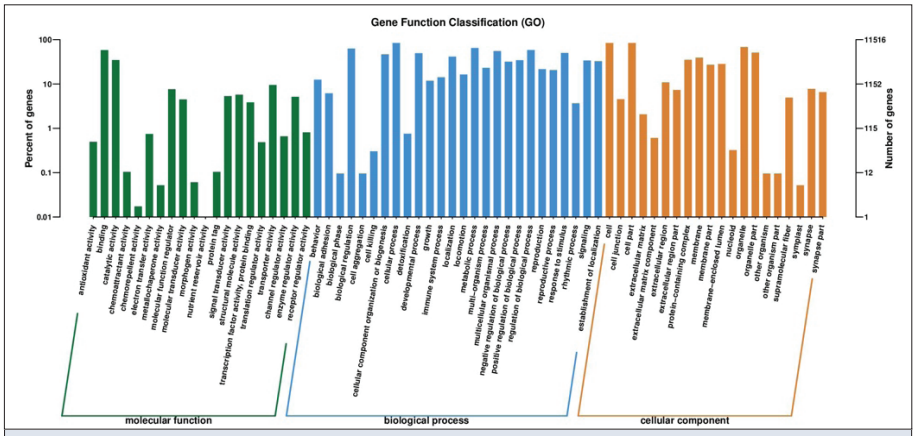

Fig 2. Gene function classification (GO)

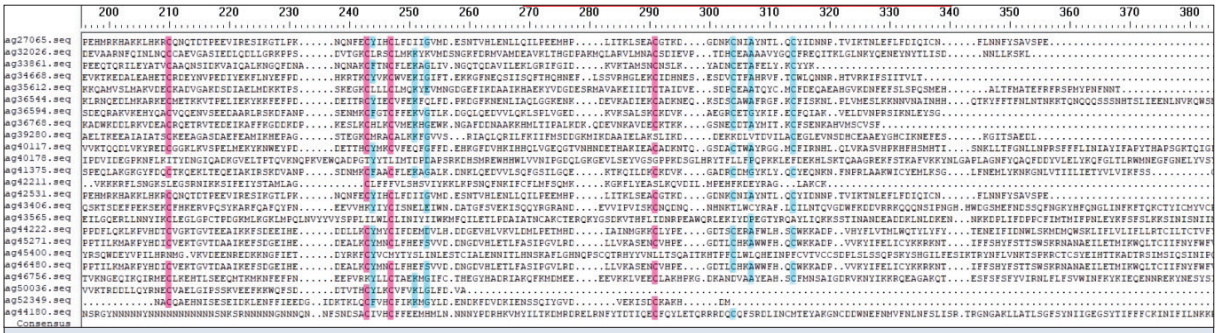

Fig 3. Amino acid sequence comparison of 24 odor-binding proteins of *W. magnifica*

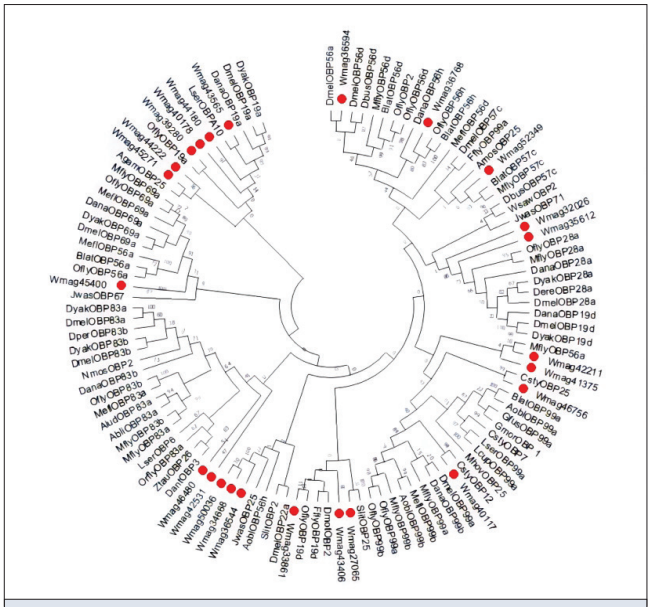

Fig 4. Neighbor-Joining method phylogenetic tree of odorant binding proteins of *W. magnifica* and other insects. Include those from the Zeugodacus cucurbitae (melon fly), Bactrocera dorsalis (oriental fruit fly), Drosophila busckii, Bactrocera latifrons, Anastrepha ludens (Mexican fruit fly), Drosophila ananassae, Cephus cinctus (wheat stem sawfly) OBPs. Marked a specific gene. Evaluate node support through 1000 boot replications

similarity between the two sequences. Additionally, the orthologs of other OBP sequences showed more than 80% similarity with their respective counterparts in other species.

According to the TPM value (measured as the proportion of a transcript in the RNA pool), 13 transcripts encoding OBPs were highly expressed in the male antenna and 11 transcripts encoding OBPs were highly expressed in the female antenna (TPM>100). Among these transcripts, Wmag45271 exhibited the highest expression level in the male antenna, indicating it was highly expressed in males. On the other hand, Wmag43565 showed the highest expression level in the female antenna, indicating it was highly expressed in females. Interestingly, Wmag52349 demonstrated the lowest expression level in both the female and male antennae, suggesting it had relatively low expression in both sexes.

The results of the amino acid sequence alignment revealed that all 19 OBPs identified in the study possessed six conserved cysteine sites. Additionally, there were three amino acid residues located between the second and third cysteine, which is a characteristic feature of typical OBPs. The other four OBPs (Wmag42211, Wmag40178, Wmag44180, Wmag45400) were found to lack the first cysteine site *(Fig. 3)*. Among the OBPs, only seven of them (Wmag36768, Wmag44222, Wmag34668, Wmag3659, Wmag27065, Wmag43565, and Wmag50036) exhibited the presence of signal peptides, indicating their potential role in secretion or membrane targeting. Phylogenetic tree shows the evolutionary relationship between insect OBP genes *(Fig. 4)*. Based on the phylogenetic tree analysis, the OBPs clustered together based on their respective subfamilies, forming eight distinct branches corresponding to different functional groups. In the phylogenetic tree, Wmag40117 is 99% similar to *Calliphora stygia* OBP25, and Wmag40117 is 98% similar to *Calliphora stygia* OBP12. These findings suggest a close evolutionary relationship between these OBP sequences.

Identification of Candidate Chemosensory Proteins

Two candidate CSPs were identified in male and female antenna transcriptome, which were named Wmag44066 and Wmag72266 respectively. The two putative CSP genes are both over 470 bp in length and have complete ORFs, among which Wmag72266 contains signal peptide. By performing a BLASTx analysis to assess gene homology, it was determined that the two CSP sequences exhibited approximately 80% similarity to CSP sequences from other species, indicating their conservation across different organisms. Significant differences were observed in the abundance of these CSP transcripts between the sexes. According to the TPM (Transcripts Per Million) values, the expression of Wmag44066 in the male antenna was higher (TPM>200) compared to its expression in the female antenna (TPM<50). This suggests a potential sex-specific role for Wmag44066 in chemosensory processes. The two CSPs have four highly conserved cysteine residues, which are consistent with the “CSP sequence motif” C1-X6-8-C2-X16-21-C3-X2-C4, where X represents any amino

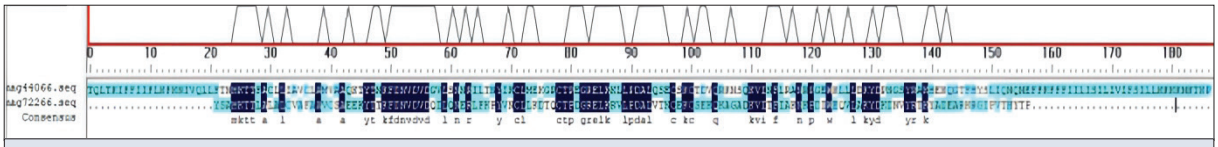

Fig 5. Amino acid sequence comparison of two typical chemosensory proteins of *W. magnifica*

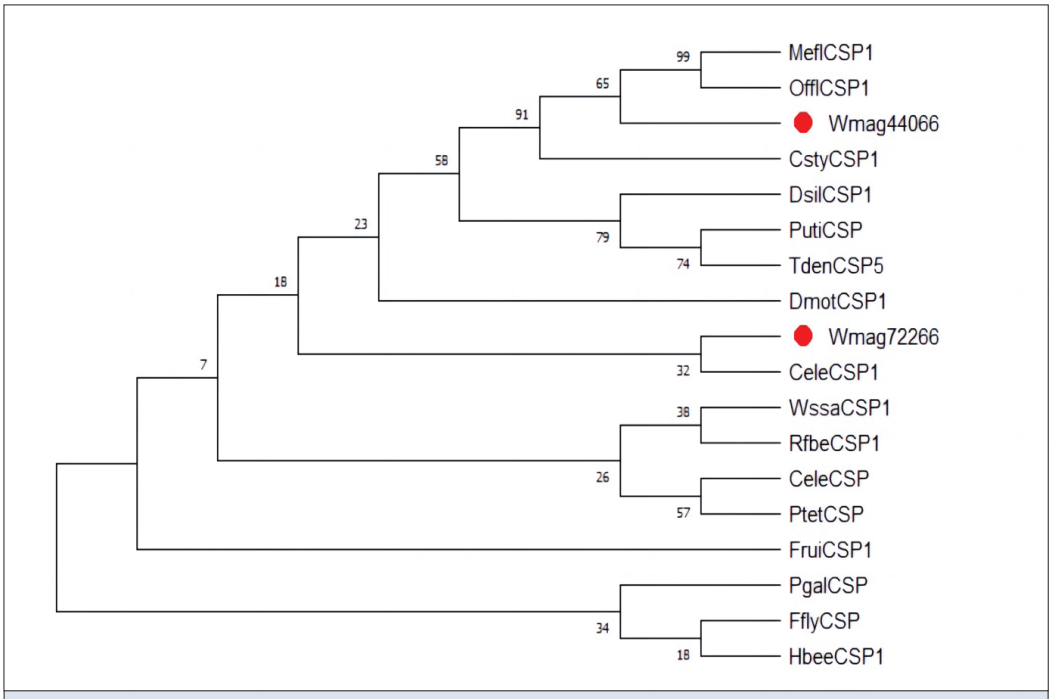

Fig 6. Neighbor-Joining method phylogenetic tree of chemosensitive proteins of *W. magnifica* and other insects. Include those from *Zeugodacus cucurbitae* (melon fly), Bactrocera dorsalis (oriental fruit fly), Drosophila melanogaster (fruit fly), *Plutella xylostella* (diamondback moth), *Cephus cinctus* (wheat stem sawfly) CSPs. Marked for specific genes. Node support was assessed by 1.000 guided copies

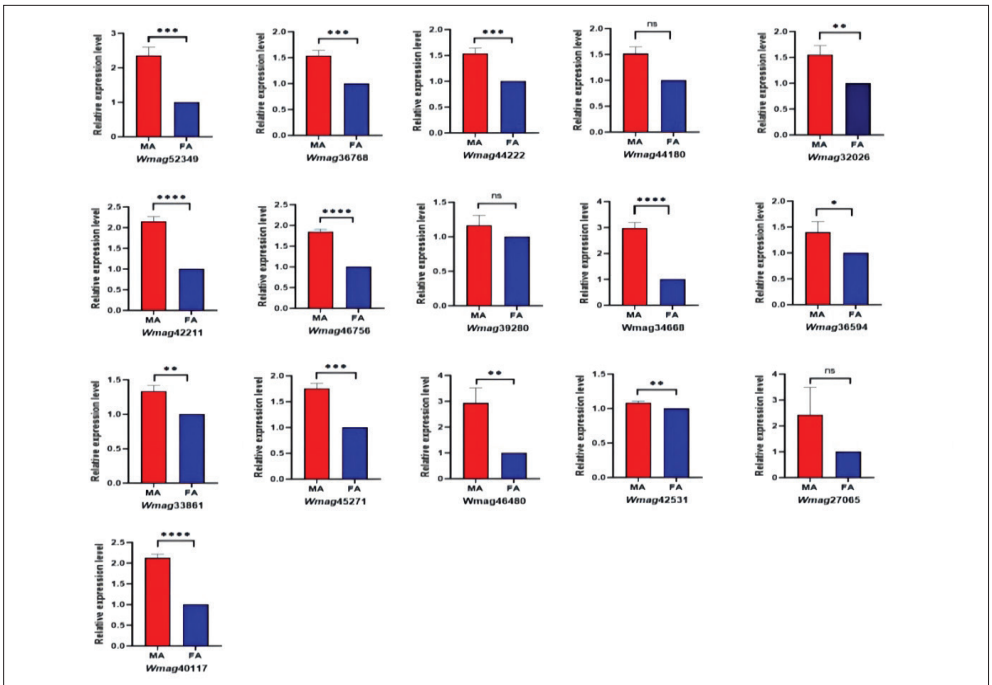

Fig 7. Expression profile of odor-binding protein in antenna tissue of *W. magnifica.* MA: male antennae; FA: female antennae. Asterisks above the bars indicate a significant difference (P<0.05, t-test); NS, no significant difference

acid, which is consistent with the structural characteristics of insect CSP gene *(Fig. 5)*. The phylogenetic tree analysis, which depicts the evolutionary relationship between chemosensory proteins in various insects, revealed that Wmag44066 and Wmag72266 occupied distinct positions in the tree *(Fig. 6)*. This indicates that these two CSPs have undergone divergent evolutionary paths and have unique evolutionary contexts within the chemosensory protein family.

Analysis of Antenna Tissue Expression of OBPs

In order to verify the expression profile of OBPs in the female and male antennae of *W. magnifica*, we carried out RT-qPCR on 16 kinds of OBPs. We compared the results of TPM and RT-qPCR, and found that the expression trend of most OBPs in male and female antennae was the same, which further proved the accuracy of transcriptome data. QPCR results showed that Wmag42531 and Wmag39280 were highly expressed in the antenna, indicating that OBPs may play a role in the combination and transmission of odor signals in the antenna. Moreover, significant gender-biased expression of several OBP genes was observed. Except for 3 OBP (Wmag44180, Wmag39280, Wmag27065), the other 13 OBPs were significantly different, and they were gender-biased in the antenna *(Fig.7)*. This gender bias indicates that these OBPs may have different functions in males and females, such as perception of the opposite sex or involvement in oviposition behavior.

Discussion

It is very important to understand how OBPs and CSPs participate in the process of insect olfactory perception and recognition of external environmental chemical stimuli for formulating effective pest management strategies [30,31]. Before this study, most of the research on *W. magnifica* focused on the field of morphology [32,33]. In this study, 24 OBPs and 2 CSPs were identified from the database of antenna transcriptome of adult *W. magnifica* by bioinformatics. These genes all play a key role in the olfactory perception of insects.Compared to the total number of insects in the world, OBPs have been identified in only a few species of insects to different degrees, such as *Drosophila melanogaster* (51 OBPs), *Anopheles gambiae* (57 OBPs) and *Parasitoid Wasp Nasonia vitripennis* (82 OBPs) [34-36]. In this study, 24 candidate OBPs were identified in *W. magnifica*, with most of them classified as PBP (pheromone binding proteins). The variation in the number of OBPs among different species suggests differences in olfactory discrimination and perception mechanisms among different insect species.

According to TPM value, there are also differences in the expression of antenna homologous genes between female and male *W. magnifica*. For example, Wmag43565 is abundantly expressed in both male and female antennae, but the expression level is higher in female antennae. This prompted further exploration of the expression profile of OBPs in the antenna. Through RT-qPCR analysis, it was discovered that Wmag45271 and Wmag46480 were highly expressed in the antenna of female flies. This suggests that these OBPs may play a crucial role in identifying the volatiles emitted by Bactrian camels or in locating suitable spawning sites. However, further functional verification is necessary to confirm these hypotheses. Notably, the expression level of Wmag45271 in male antennae was higher than that in female antennae, indicating its potential involvement in female sex pheromone recognition. Lepidoptera PBPs and GOBPs form a monophyletic lineage with a single ancestral origin. They have undergone divergence by gene duplication under different selection pressures [37]. Phylogenetic tree showed that Wmag40117 was 99% similar to *Calliphora stygia* OBP25, and Wmag40117 was 98% similar to *Calliphora stygia* OBP12. The evolutionary relationship of OBPs in insect specialization is further explained. Overall, the study's findings shed light on the expression patterns and potential functions of OBPs in *W. magnifica*, highlighting their role in olfactory perception and chemical communication in the species.

CSPs are widely expressed in insect antennae and other parts of the body, and participate in insect recognition pheromone perception and related behaviors [38,39]. In addition, CSPs has many functions. For example, CSPs may be involved in dissolving hydrocarbons in the stratum corneum to identify their symbiotic relationship [40]. This suggests that CSPs are important for maintaining specific ecological interactions. The competitive binding analysis of tryptophan fluorescence spectrum and molecular docking showed that some CSPs in *Bemisia tabaci* was very important to promote the transport of fatty acids, thus regulating some metabolic pathways of insect immune response [41]. These findings highlight the diverse roles of CSPs in insect physiology and behavior, showing their involvement in chemical communication, symbiosis, and immune response regulation.

In this study, two candidate CSP genes were identified. The number of CSPs is less than that of *Sclerodermus sp.* (10), *C. cunea* (11), *M. pulchricornis* (8) and *B. dorsalis* (4) [42-45]. According to TPM value, Wmag44066 is highly expressed in antenna, which may be related to chemical acceptance. Based on the TPM values, Wmag44066 was found to be highly expressed in the antennae, suggesting its potential involvement in chemical acceptance. Interestingly, the TPM value of Wmag44066 in male antennae was approximately four times higher than in female antennae, indicating its possible role in male *W. magnifica*'s recognition of female pheromones. On the other hand,

Wmag72266 was almost exclusively expressed in female antennae, suggesting its potential role in host location and oviposition behavior of female *W. magnifica*. Similar biased expression patterns have been observed in other species as well. The phylogenetic tree analysis revealed that Wmag44066 and Wmag72266 belong to different branches, indicating that they likely play different roles in recognition of pheromones and related behaviors. Overall, these findings suggest that the identified CSP genes in *W. magnifica* may have distinct functions in the perception and response to pheromones, potentially contributing to the species' mating behavior and host-seeking behavior.

In this study, we used the Illumina Hiseq™ 4000 high-throughput sequencing platform to sequence the transcriptome of the male and female antennae of *W. magnifica*. We identified 26 olfactory related genes and obtained the expression of 16 OBP genes in male and female antennae by RT-qPCR experiment, which confirmed the accuracy of our transcriptome data. It enables us to predict their functions and provides insights into the mechanism of *W. magnifica* looking for hosts. These studies also provide a molecular basis for the development of attractants and repellents for *W. magnifica* and provide a new idea for biological control of *W. magnifica*.

Declarations

Availability of Data and Materials: The datasets generated and/or analysed during the study are available from the corresponding authors and can be provided upon request.

Acknowledgments: We thank the Plan(Science and Technology Cooperation) Project of 2023 autonomous region major research and development and achievements transformation, Science and Technology Agency of Inner Mongolia Autonomous Region (2023KJHZ0003) project and all partners and laboratory members for their kind help.

Financial Support: The research was funded by the Plan (Science and Technology Cooperation) Project of 2023 autonomous region major research and development and achievements transformation, Science and Technology Agency of Inner Mongolia Autonomous Region (2023KJHZ0003) project.

Competing Interests: The authors declared that there is no conflict of interest.

Author Contributions: LFL performed the experiments, analysed the results, and drafted the manuscript. RW and LXZ assisted in the experimental design and summarized the experimental results. SM data interpretation and manuscript preparation. JYT and CG put forward valuable suggestions for the revision and improvement of the paper. DE and WMQ conceived and designed the study, revised the manuscript and funded the study. All authors have read and agreed to the published version of the manuscript.

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