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Transcriptome sequencing of *Antheraea pernyi* antennae for identification of olfactory-related genes

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Abstract

Background In insects, the olfactory system governs physiological and behavioral processes by detecting various odorous molecules. Despite its economic importance and adaptability, the olfactory mechanism of *Antheraea pernyi* remains insufficiently understood, limiting its potential for pest management and as a model organism. Hence, we aimed to conduct transcriptome sequencing to explore olfactory-related genes in the antennae, serving as the most important olfactory organ in adult *A. pernyi*.

Results Based on the datasets, 1184 differently expressed genes (DEGs), including 484 upregulated and 700 downregulated genes, were identified by comparing the transcriptome profiles of the male and female antennae of *A. pernyi*. Moreover, 20, 7, 30, 11, and 2 candidate genes encoding odorant-binding proteins (OBPs), chemosensory proteins (CSPs), odorant receptors (ORs), ionotropic receptors (IRs), and sensory neuron membrane proteins (SNMPs), respectively, involved in pheromone perception, odor binding, pesticide resistance, and growth and development regulation were screened, and most of which were expressed in both male and female antennae while the expression levels of these candidate genes varied significantly between males and females. Multiple sequence alignment indicated that the six OBPs exhibited typical characteristics, containing six conserved Cys residues with the sequence of C1-$X_{26\text{-}30}$-C2-X_3-C3-$X_{41\text{-}42}$-C4-$X_{8\text{-}10}$-C5-X_8-C6. All CSPs followed a highly conserved pattern with four Cys residues arranged with an exact spacing of C1-X_6-C2-$X_{18\text{-}19}$-C3-X_2-C4. Different numbers of transmembrane domains were found in ORs, IRs, and SNMPs. In addition, several DEGs involve signal transduction underlying chemoreception were also identified from the transcriptome data, including guanine nucleotide-binding protein (G protein), cGMP-dependent protein kinase (PKA), calmodulin-A (CaM-A), mitogen-activated protein kinase 1 (MAPK1), and phospholipase D2 (PLD2).

Conclusion This study enriches the olfactory gene database of *A. pernyi*, providing insights into olfactory mechanisms crucial for mating and pest control, with implications for enhancing breeding strategies and ensuring the sustainability of the silk industry. These findings may serve as a theoretical foundation for a better understanding of the olfactory mechanisms of *A. pernyi*.

Keywords *Antheraea pernyi*, Antennae, Transcriptome, Olfactory-related genes, Bioinformatics analysis

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Introduction

The olfactory system in insect antennae is essential for regulating physiological and behavioral actions by recognizing chemical odor molecules in the environment that are related to multiple olfactory proteins, including odorbinding proteins (OBPs), chemosensory proteins (CSPs), odorant receptors (ORs), gustatory receptors (GRs), ionotropic receptors (IRs), and sensory neuron membrane proteins (SNMPs) [1].

Lepidopteran OBPs can be divided into three categories: pheromone-binding proteins (PBPs), general odorant-binding proteins (GOBPs), and antennal-binding proteins (ABPs). Based on the number of conserved Cys residues, OBPs are divided into the following categories: classical subgroups possessing six highly conserved Cys residues with a three-residue gap between the second and third Cys residues and an eight-residue gap between the fifth and sixth Cys residues; and nonclassic subgroups with either more or fewer than six Cys sites [2]. Both OBPs and CSPs are soluble proteins with the N-terminal signal peptide removed during processing [3] and play similar roles in binding and transferring chemical pheromones [4]. CSPs contain four conserved Cys residues that form two disulfide bonds to maintain their structural stability [5]. ORs, GRs, and IRs belong to the chemoreceptor family and have different numbers of transmembrane domains in insects. ORs and GRs exhibit a similar membrane topology to the intracellular N-termini and extracellular C-termini [6], while a contrasting structure exists in IRs [7]. SNMPs are highly homologous to the human proteinCD36 which exhibits a large extracellular loop containing several Cys residues [8].

With the advancements in high-throughput sequencing technology, RNA-seq has been widely used to mine olfactory-related genes in the antennae of multiple lepidopteran species, with 71, 42, 171, 157, 85, and 88 genes identified in *Spodoptera frugiperda* [9], *Histia Rhodope* [10], *Lobesia botrana* [11], *Spodoptera exigua* [12], *Dendrolimus houi* [13], and *Dendrolimus kikuchii* [13], respectively. *Antheraea pernyi* is a typical lepidopteran insect that is important for the silk industry and accounts for a high proportion of agricultural economic output. Unlike the domesticated silkworm, *Bombyx mori*, *A. pernyi* lives in the wild and can adapt to harsh environments, resulting in a relatively sensitive olfactory system for feeding, mating, oviposition, and detecting harmful odor molecules. As an emerging model organism [14], the olfactory system of *A. pernyi* can be used as a reference for the prevention and control of agricultural and forestry lepidopteran pests. Hence, a comprehensive understanding of the olfactory mechanism would be beneficial in elucidating the biological characteristics of *A. pernyi* as an economically viable insect.

In this study, transcriptome sequencing was performed for *A. pernyi* antennae to identify the olfactory-related genes encoding OBPs, CSPs, SNMPs, ORs, GRs, and IRs, which differential expression analyses were carried out between the male and female antennae. Then bioinformatics analysis was conducted for further characterizing these genes. Overall, the results will enrich the gene datasets related to *A. pernyi* olfaction and provide a theoretical foundation for further research into the mechanisms underlying chemoreception in *A. pernyi*.

Materials and methods

Collection of antennae samples

Adult *A. pernyi* (Xuanda strain) used for sample preparation was obtained from the Shandong Sericulture Research Institute (Yantai, China). The antennae samples were collected at night after the moths emerging from their cocoons. Newly emerged male and female moths were separated into two groups (referred to as M and F, respectively; Fig. 1A). Each group consisted of 150 moths and was set up with three biological replicates (referred to as M1, M2, M3 and F1, F2, F3, respectively); thus, 50 individuals were included in each replicate. The antennae samples were collected separately from each replicate and then stored at −80 °C for further use.

RNA extraction and library construction

Total RNA was extracted from the antennae samples using TRIzol reagent (Invitrogen, USA) according to the manufacturer's instructions. RNA purity and quantity were analyzed using a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA). RNA integrity was evaluated using an Agilent 2100 Bioanalyzer (Agilent Technologies, USA). Sequencing library was constructed separately for each replicate using the VAHTS Universal V5 RNA-seq Library Prep Kit following the manufacturer's protocol.

RNA-Seq and data analysis

Illumina sequencing was also performed separately for each replicate by OE Biotech Co., Ltd. (Shanghai, China) with 150-bp paired-end reads generated on an Illumina Novaseq 6000 platform. The clean reads were obtained by filtering the low-quality reads by processing the raw reads in FASTQ format using a FASTQ preprocessor [15] and then mapping to the reference genome database of *A. pernyi* (https://bigd.big.ac.cn/gwh/, accession number: GWHABGR00000000) [16] using HISAT2 [17]. The FPKM measure [18] was used to calculate the expression of each gene, for which read counts were determined by HTSeq-count [19]. The biological duplication of samples was evaluated via principal component analysis (PCA) using R (v 3.2.0) (https://

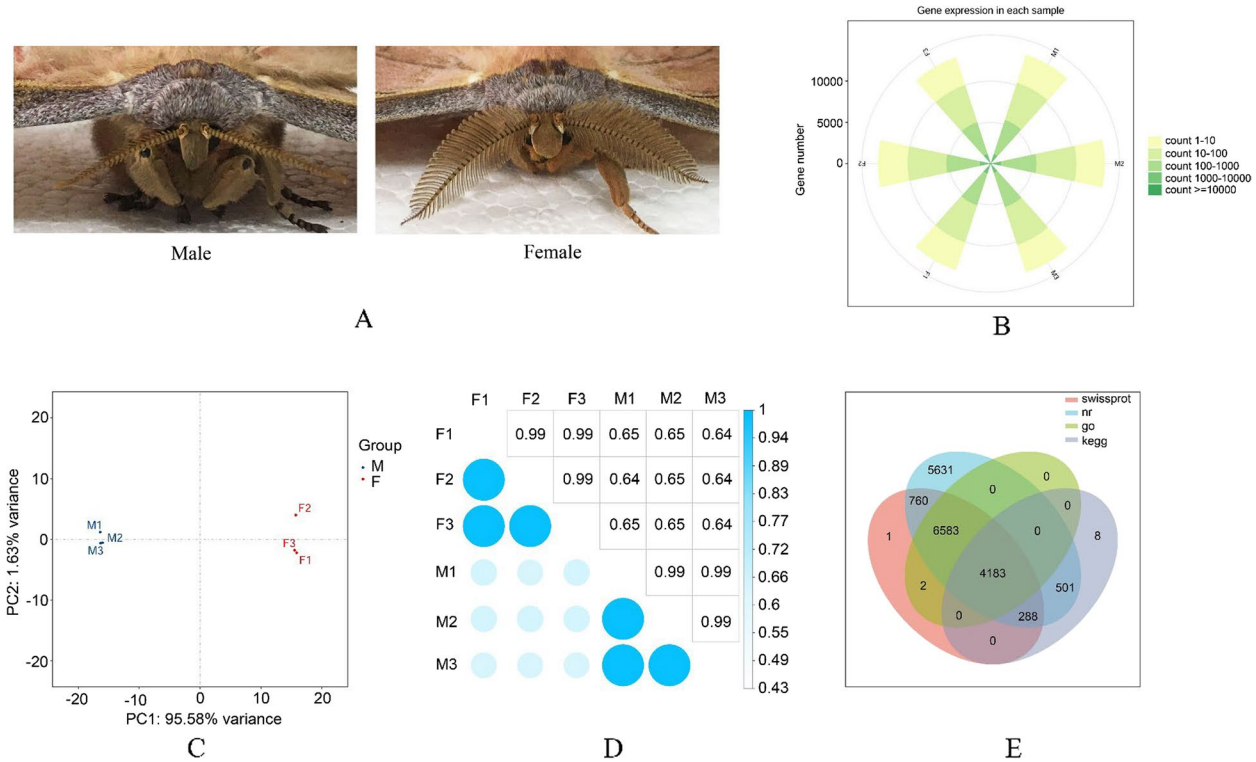


Fig. 1 Statistical analysis of the transcriptomic data of *A. pernyi* antennae. **A** Male and female antennae of *A. pernyi* moth. **B** Number of identified genes from each sample in the M and F group. **C** PCA of the samples from the M and F group. **D** Correlation test between the samples in the M and F group. **E** Number of identified genes separately annotated in Swiss-Prot, Nr, GO, and KEGG databases. M and F in (**B**), (**C**), and (**D**) represent the male and female groups, respectively

cloud.r-project.org/). Gene annotation was performed by aligning the gene sequence with the Swiss-Prot database and the NCBI non-redundant (NR) database to obtain the gene symbol and description, respectively, with a threshold of < 1e-5. GO classification was performed using the mapping relation between Swiss-Prot and GO term. The genes were mapped to the KEGG database to annotate potential pathways.

Analysis of DEGs

DESeq2 software was used for differential expression analysis [20]. Genes were considered significantly differentially expressed when foldchange ≥ 2 or ≤ 0.5 with a Q value < 0.05 was noted upon comparison of the two groups. The expression patterns of DEGs in different groups and samples were determined by hierarchical cluster analysis using R (v 3.2.0) (https://cloud.r-project.org/). GO function and KEGG pathway enrichment analyses of DEGs were conducted to screen for significantly enriched terms.

Identification and bioinformatics analysis of olfactory-related genes

Olfactory-related genes encoding OBPs, PBPs, ABPs, GOBPs, CSPs, OLRs, ORs, IRs, GRs, and SNMPs were screened from the transcriptome data of *A. pernyi* antennae. SignaIP 5.1, an online software package (http://www.cbs.dtu.dk/services/SignalP/), was used to predict the signal peptides. Sequence alignment was performed using ClustalX2 software (http://www.clustal.org/clustal2/), and the alignment results were analyzed using GeneDoc software (https://genedoc.software.informer. com/). Transmembrane (TM) domain prediction was performed using the online Deep TMHMM tool (https://services.healthtech.dtu.dk/service.php?DeepTMHMM-1.0) with default parameters. Phylogenetic analysis was performed in MEGA 7.0 software and the tree were constructed using neighbor-joining method with n = 1000 bootstrap replicates [21].

Verification of RNA-Seq data using qRT-PCR

Total RNA used for RNA-Seq was applied for cDNA synthesis, and subsequent qRT-PCR validation was performed on a CFX96 Real-Time PCR Detection System

(Bio-Rad, USA) with a 20-μL reaction system using TB Green® Premix Ex Taq™ II (Takara, China) following the manufacturer's protocol. The reaction procedure was as follows: pre-denaturation at 95 °C for 30 s, followed by 40 cycles of denaturation at 95 °C for 5 s, and annealing/extension at 60 °C for 30 s. All reactions were performed in triplicate. The relative transcript level of each gene was quantified using the C_t value obtained from the reaction. The housekeeping gene *Apactin1* of *A. pernyi* was used as a normalized internal control. Primers (Table S1) for the selected genes were designed based on their coding sequences reported in the online genome database of *A. pernyi* [16].

Results

Statistical analysis of A. pernyi antennae transcriptome data

The transcriptome data of *A. pernyi* antennae from the M and F groups generated 279.29 M clean reads after filtering for quality from 279.58 M raw reads, with the Q30 base ranging from 96.98% to 97.33% and the average GC content of 37.89% (Table 1). The match ratio ranged from 64.37% to 77.50%, as observed upon aligning the clean reads of each sample to the reference genome (Table 1). After assembling the short-read sequences, 13,869, 13,838, 13,855 and 13,556, 13,651, 13,513 genes were produced in each sample of the M and F groups, respectively, and 16,458 genes were obtained after removing redundant entries from all samples (Fig. 1B, Table S2). High intragroup repeatability and significant intergroup differences were observed using PCA and correlation tests (Fig. 1C & D). A total of 9874, 14,934, 9666, and 4503 genes were annotated separately in the Swiss-Prot, Nr, GO, and KEGG databases, respectively (Table 1, Fig. 1E).

Table 1 Statistical analysis of the transcriptome data of *A. pernyi* antennae

Items	Number
Total raw reads (M)	279.58
Total raw bases (G)	41.91
Total clean reads (M)	279.29
Total clean bases (G)	41.87
Q30 (%)	96.98 ~ 97.33
Average GC content (%)	37.89
Total mapped reads (%)	64.37 ~ 77.50
Total genes	16,458
Genes annotated in SwissProt	9874
Genes annotated in Nr	14,934
Genes annotated in GO	9666
Genes annotated in KEGG	4503

Differential expression analysis

Based on the screening criteria for significant differential expression, 1184 DEGs, including 484 upregulated (Table S3) and 700 downregulated genes (Table S4), were identified between the male and female antennae of *A. pernyi* using the M group as a control (Fig. 2A). The expression patterns of DEGs between each antennae sample in the M and F groups were determined using hierarchical cluster analysis (Fig. 2B). The results of GO enrichment analysis indicated that 715 DEGs were enriched in 1746 GO terms, which primarily involved integral component of membrane, plasma membrane, extracellular region, cytoplasm, multicellular organism development, proteolysis, metal ion binding, structural constituent of cuticle, and ATP binding (Fig. 2C, Table S5). KEGG enrichment analysis showed that 268 DEGs were enriched in 281 pathways, of which protein processing in endoplasmic reticulum, longevity-regulating pathway, peroxisome, glutathione metabolism, and metabolism of xenobiotics by cytochrome P450 were the most enriched (Fig. 2D, Table S6).

Screening and analysis of the expression patterns of olfactory-related genes

A total of 70 olfactory-related genes were screened based on gene annotations from the transcriptome data (Table 2). Among the 20 OBPs, 2 PBPs, 1 ABP, 8 OBPs, and 9 GOBPs were classified (Table 2). A total of 7, 30, 11, and 2 genes encoding CSPs, ORs, IRs, and SNMPs, respectively, were identified (Table 2). Hierarchical clustering analysis showed that most olfactory-related genes were expressed in both male and female antennae and that the expression levels of all these genes varied significantly between the two groups (Fig. 3). Several genes encoding ORs, including ApOR (GWHGABGR010755, GWHGABGR005934, GWHGABGR013978) and ApOR23a (GWHGABGR021059), were highly expressed in male antennae compared to those in females, whereas ApOR20 (GWHGABGR011421), ApOR (GWHGABGR021062), and ApOR30a (GWHGABGR011390) displayed high expression levels in female antennae (Fig. 3). In addition, a series of genes such as ApOBP31 (GWHGABGR003019), ApOBP32 (GWHGABGR003119), ApOBP38 (GWHGABGR019142), ApOR20 (GWHGABGR011405), ApIR75a (GWHGABGR012255), and ApIR3 (GWHGABGR014182) exhibited relatively low expression levels in the two groups (Fig. 3).

Sequence characteristics of the olfactory-related genes

Sequence analysis showed that the full length of the olfactory related genes ranged from 51 to 834 aa with 62 complete open reading frames and 16 out of the OBPs and CSPs with signal peptide cleavage site between 16 and 25 aa (Table 2). The results of amino acid multiple sequence alignment

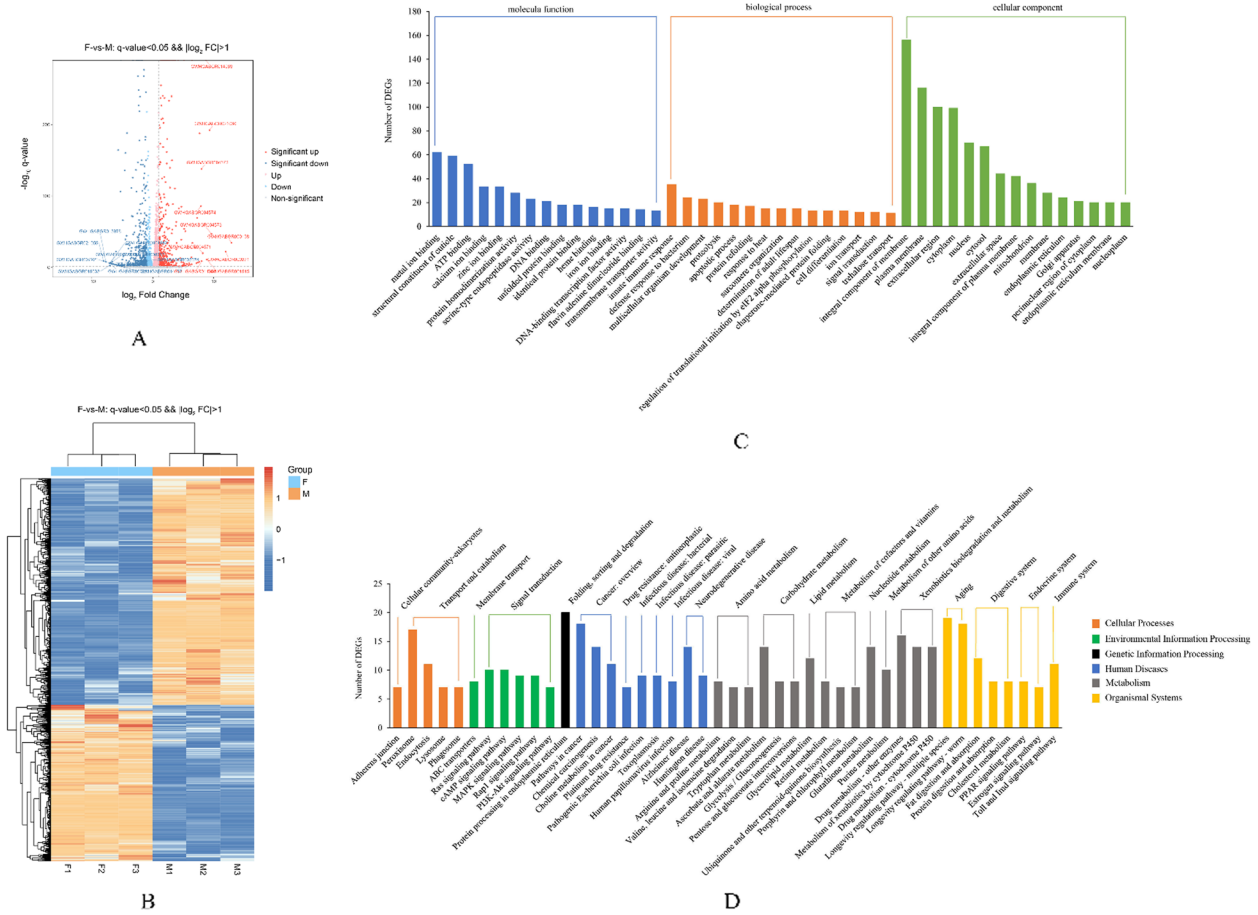


Fig. 2 Differential expression analysis between male and female antennae of *A. pernyi*. **A** DEGs are displayed in the volcano plot. The X- and Y-axes separately represent the fold changes in gene expression and the statistical significance of changes in gene expression. Different colored dots represent genes with different expression patterns. **B** Hierarchical cluster analysis of the DEGs. Different colors represent different levels of gene expression in each sample. The color gradient from red to blue represents the levels of expression of genes from high to low. **C** GO enrichment analysis of the DEGs. The top 15 GO terms with significant enrichment in cellular component, molecular function, and biological process are presented. **D** KEGG enrichment analysis of the DEGs. The top 45 pathways with significant enrichment are provided

indicated low homology among the OBPs, several of which, including ApPBP1 (GWHGABGR012197), ApPBP3 (GWHGABGR012195), ApABP (GWHGABGR018897), ApGOBP1 (GWHGABGR012186), ApOBP38 (GWHGABGR019142), and ApGOBP2 (GWHGABGR012194), exhibited typical characteristics comprising six conserved Cys residues, with the sequence of C1-$X_{26–30}$-C2-X_3-C3-$X_{41–42}$-C4-$X_{8–10}$-C5-X_8-C6, while ApOBP6 (GWHGABGR001129) and ApGOBP70 (GWHGABGR004634) missing C1 (Fig. 4A). In addition, all CSPs followed a highly conserved pattern with four Cys residues arranged with an exact spacing of C1-X_6-C2-$X_{18–19}$-C3-X_2-C4 (Fig. 4B). TM domain prediction showed that eight and seven TM domains were found in ApOR20 (GWHGABGR011405) and ApOR (GWHGABGR021060), respectively, whereas 0–6 domains were detected in other ORs. Among the IRs, three TM domains were observed in ApIR25a (GWHGABGR003112), ApIR93a (GWHGABGR005160), and ApIR4 (GWHGABGR005485), and four were identified in ApIR3 (GWHGABGR014182 and GWHGABGR014192). Besides, ApSNMP2 (GWHGABGR005182) was displayed with 2 TM domains.

Validation of reliability of RNA-Seq data using qRT-PCR

To validate the accuracy of RNA-Seq data, 14 candidates with seven upregulated and seven downregulated genes were selected from the DEGs for qRT-PCR analysis. The results showed expression patterns at the transcriptional level similar to those in the transcriptome of the selected genes (Fig. 5), further confirming the validity of the transcriptome data.

Table 2 Olfaction related genes identified in the adult antennal transcriptome of *A. pernyi*

Category	Genes	Description	Unigene ID in transcriptome	Complete ORF	Full length (aa)	Signal peptide (aa)
PBP	*ApPBP1*	Pheromone binding protein 1	GWHGABGR012197	Yes	165	21
	ApPBP3	Pheromone binding protein 3	GWHGABGR012195		164	22
ABP	*ApABPX*	Antennal binding protein X	GWHGABGR018897		135	20
OBP	*ApOBP6*	Odorant-binding protein 6	GWHGABGR001129		221	16
	ApOBP14	Odorant-binding protein 14	GWHGABGR014086		120	22
	ApOBP	putative odorant binding protein	GWHGABGR002800		230	24
		Odorant binding protein	GWHGABGR003018		294	No
	ApOBP16	Odorant binding protein 16	GWHGABGR006245	No	212	
	ApOBP31	Odorant binding protein 31	GWHGABGR003019	Yes	122	
	ApOBP32	Odorant binding protein 32	GWHGABGR003119		539	
	ApOBP38	Odorant binding protein 38	GWHGABGR019142		180	
GOBP	*ApGOBP67*	General odorant-binding protein 67-like	GWHGABGR003120		164	
	ApGOBP70	General odorant-binding protein 70	GWHGABGR004634		162	
	ApGOBP1	General odorant-binding protein 1	GWHGABGR012186		166	21
	ApGOBP2	General odorant-binding protein 2	GWHGABGR012194		166	25
	ApGOBP28a	General odorant-binding protein 28a-like	GWHGABGR014087		582	20
	ApGOBP19a	General odorant-binding protein 19a-like isoform X2	GWHGABGR014090	No	66	No
	ApGOBP72	General odorant-binding protein 72-like	GWHGABGR014091	Yes	84	
	ApGOBP19 d	General odorant-binding protein 19 d-like isoform X2	GWHGABGR014092		537	
	ApGOBP56a	General odorant-binding protein 56a	GWHGABGR017175		133	16
CSP	*ApCSP*	Chemosensory protein	GWHGABGR012364		125	20
			GWHGABGR001096		128	18
			GWHGABGR001110		122	17
	ApCSP9	Chemosensory protein 9	GWHGABGR001097		123	18
	ApCSP15	Chemosensory protein 15	GWHGABGR001108		122	18
	ApCSP7	Chemosensory protein 7	GWHGABGR001109		121	16
	ApCSP12	Chemosensory protein 12	GWHGABGR003779		128	No
OR	*ApOR23*	Olfactory receptor 23	GWHGABGR008832	No	169	/
	ApOR	Olfactory receptor-like receptor	GWHGABGR010415	Yes	301	
			GWHGABGR020491		187	
	ApOR20	Olfactory receptor 20	GWHGABGR011405		710	
			GWHGABGR011407		393	
			GWHGABGR011415		114	
			GWHGABGR011421		193	
	ApOR4	Odorant receptor 4-like	GWHGABGR020302		294	
			GWHGABGR008130		165	
			GWHGABGR012614		146	
	ApOR1	Odorant receptor 1-like	GWHGABGR008374		236	
			GWHGABGR010827		425	
	ApOR46a	Odorant receptor 46a-like	GWHGABGR010410		374	
			GWHGABGR016101		292	
	ApOR	Odorant receptor	GWHGABGR010755		348	
			GWHGABGR021060		510	

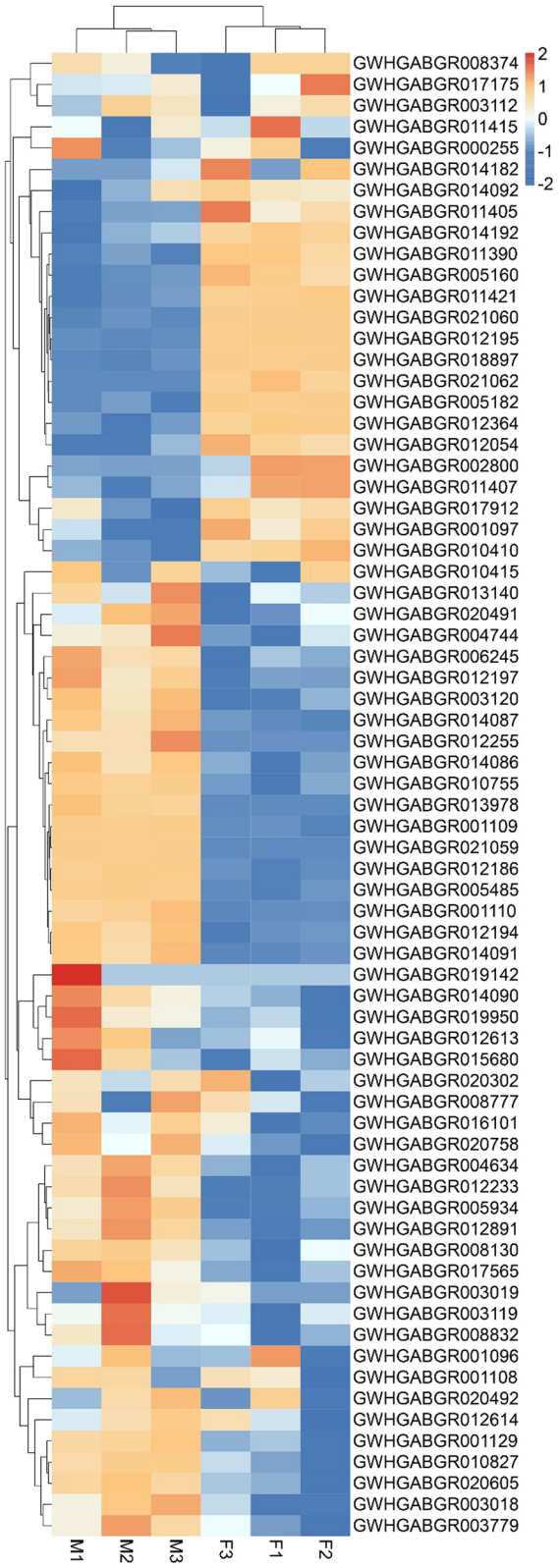
Table 2 (continued)

Category	Genes	Description	Unigene ID in transcriptome	Complete ORF	Full length (aa)	Signal peptide (aa)
			GWHGABGR021062		298	
			GWHGABGR005934	No	52	
			GWHGABGR020492		51	
			GWHGABGR013978		70	
	ApOR30a	Odorant receptor 30a-like	GWHGABGR011390	Yes	382	
	ApOR10a	Odorant receptor 10a-like	GWHGABGR012054		427	
	ApOR44	Odorant receptor 44	GWHGABGR012233	No	255	
	ApOR53	Odorant receptor 53	GWHGABGR012613	Yes	88	
	ApOR94b	Odorant receptor 94b	GWHGABGR012891		334	
	ApOR85e	putative odorant receptor 85e	GWHGABGR015680		104	
	ApOR85c	Odorant receptor 85c-like	GWHGABGR017565		396	
			GWHGABGR020758		224	
	ApOR2	Odorant receptor 2-like	GWHGABGR020605		307	
	ApOR23a	Odorant receptor 23a-like	GWHGABGR021059		158	
IR	*ApIR25a*	Ionotropic receptor 25a isoform X1	GWHGABGR003112		834	
			GWHGABGR019950		314	
	ApIR75a	Ionotropic receptor 75a-like	GWHGABGR004744		511	
			GWHGABGR012255		286	
	ApIR93a	Ionotropic receptor 93a	GWHGABGR005160	No	405	
	ApIR4	Ionotropic receptor 4	GWHGABGR005485	Yes	372	
	ApIR21a	Ionotropic receptor 21a	GWHGABGR008777		78	
	ApIR75 d	Ionotropic receptor 75 d	GWHGABGR013140		503	
	ApIR3	Ionotropic receptor 3	GWHGABGR014182		326	
			GWHGABGR014192		326	
	ApIR13	Ionotropic receptor IR13	GWHGABGR017912		350	
SNMP	*ApSNMP2*	Sensory neuron membrane protein 2	GWHGABGR000255		611	
			GWHGABGR005182		528	

Discussion

During olfactory recognition in insects, odorant molecules enter the olfactory sensilla in the antennae via micropores and interact with ORs or IRs. The molecules are transported via the sensilla lymph to the dendrite membrane upon binding with OBPs or CSPs, thus activating receptor neurons and converting chemical signals into electrical signals [3, 22]. Based on the antennal transcriptome data, 70 olfactory-related genes were identified in this study except the GRs involved in detecting sugars, bitter compounds, and carbon dioxide [23, 24], which may be attributed to that GRs are mainly expressed in the maxillary palps instead of antennae [25] and the moths no longer feeds during the adult stage of *A. pernyi* which primary physiological process is reproduction.

OBPs are key factors involved in odor reception in the olfactory system of insects and are mainly expressed in olfactory organs such as the antennae and labia [26]. As the first medium for insects to communicate with the external environment, OBPs carry hydrophobic odor molecules through the aqueous lymph to odor receptors, thereby guiding the biochemical



◀ **Fig. 3** Hierarchical clustering analysis of olfactory-related gene expression between *A. pernyi* male and female antennae. The color gradient from red to blue represents the levels of expression of genes from high to low

processes of insects to odors. PBPs are a subfamily of OBPs that selectively bind to sex pheromones in water-soluble hemolymph while filtering out other odor molecules [3]. In this study, the expression level of ApPBP1 (GWHGABGR012197) was higher in male antennae than that in females, whereas a contrasting trend was observed in ApPBP3 (GWHGABGR012195) expression (Figs. 3 & 5). This expression pattern corresponds to that of PBPs in males and females of *Spodoptera litura* [27]. In *S. litura*, the PBPs also exhibited a very different sex-biased expression in adult antenna with male-biased for SlitPBP1 which was supposed to play critical roles in the perception of female sex pheromones, while female-biased for SlitPBP3 with very weak binding affinities to sex pheromones [27]. Meanwhile, in *Agrotis ipsilon*, AipsPBP1 showed high binding affinities with the major female sex pheromone components Z7-12: Ac and Z9-14: Ac, while AipsPBP3 displayed low binding affinities with them [28]. We speculated that the identified PBPs from the transcriptome data may display sexual dimorphism in *A. pernyi*, and ApPBP1 with high expressions in male antennae may play essential roles in perceiving female sex pheromones, while ApPBP3 expressed highly in female antennae may have other functions. GOBPs predominantly perceive general odorants, such as plant volatiles or pheromones [29]. Among the identified GOBPs, ApGOBP19 d (GWHGABGR014092) and ApGOBP56a (GWHGABGR017175) were preferentially expressed in the female antennae, indicating that they may participate in recognizing and binding host plant odorants, thereby helping females select oviposition sites [30]. ABPs are specifically expressed in the antennae, yet they remain to be further functionally characterized [3]. Phylogenetic analysis showed that ApABPX (GWHGABGR018897) clustered with the ABPXs from *M. sexta* and *B. mori* (Fig. 6A), indicating a relatively close genetic relationship between these ABPs.

Although share certain similarities with OBPs in their roles, CSPs participate in the olfactory recognition process of insects by binding to non-volatile chemical pheromones [31]. Unlike the expression patterns of OBPs, CSPs are widely expressed in olfactory and non-olfactory sensory organs with multiple physiological functions involved in regulating reproduction, growth and development, and physiological rhythms [32]. In *Clostera restitura*, CrCSP1/2/3/4/5/6/7/8 regulate

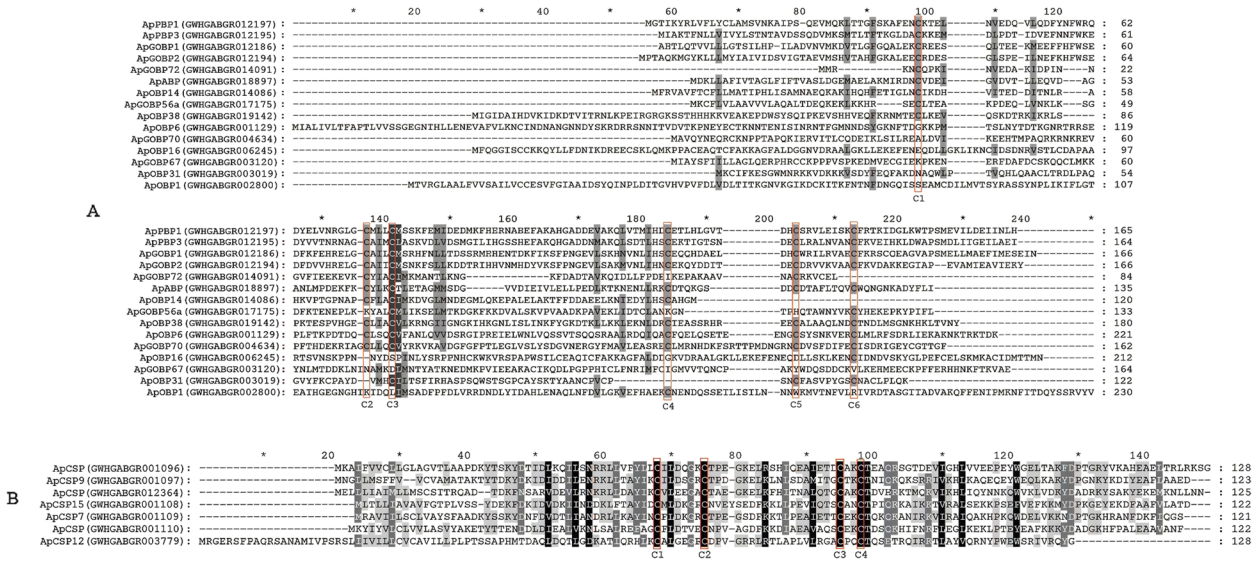


Fig. 4 Sequence alignment of the identified ApOBPs (**A**) and ApCSPs (**B**). The conserved Cys residues are highlighted by red wireframes

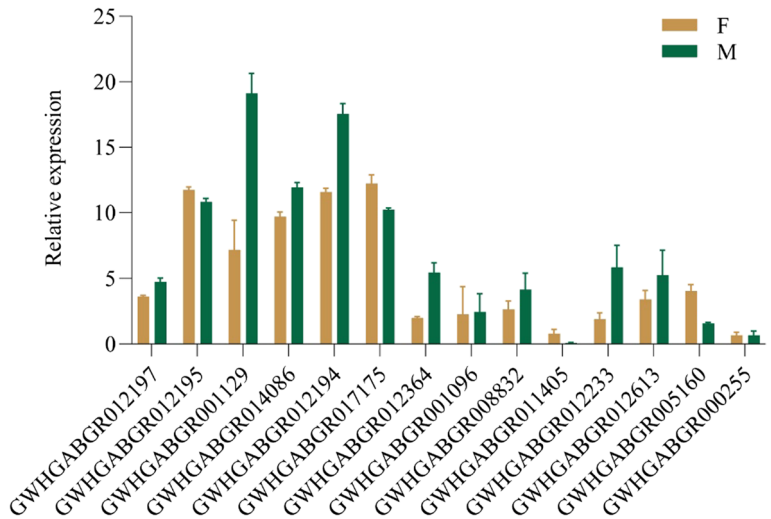


Fig. 5 Validation of transcriptome data by qRT-PCR. The X- and Y-axes separately represent the selected genes and the relative expression. The selected genes include pheromone binding protein 1 (GWHGABGR012197), pheromone binding protein 3 (GWHGABGR012195), odorant-binding protein 6 (GWHGABGR001129), odorant-binding protein 14 (GWHGABGR014086), general odorant-binding protein 2 (GWHGABGR012194), general odorant-binding protein 56a (GWHGABGR017175), chemosensory protein (GWHGABGR012364, GWHGABGR001096), olfactory receptor 23 (GWHGABGR008832), olfactory receptor 20 (GWHGABGR011405), odorant receptor 44 (GWHGABGR012233), odorant receptor 53 (GWHGABGR012613), ionotropic receptor 93a (GWHGABGR005160), sensory neuron membrane protein 2 (GWHGABGR000255)

growth, development, reproduction, and the perception of chemical molecules such as plant volatile substances and sex pheromones [33]. In *B. mori*, BmCSP1 is associated with insecticide resistance [34]. In this study, the close phylogenetic relationship between ApCSP (GWHGABGR001096) and CrCSP7, as well as between ApCSP15 (GWHGABGR001108) and CrCSP5 (Fig. 6B), may imply their possible roles in chemo sensing, odor binding, pheromone perception, and regulation of the lifecycle of *A. pernyi*. ApCSP (GWHGABGR012364) and ApCSP9 (GWHGABGR001097) were hypothesized to be associated with pesticide resistance, given their homology with BmCSP1 (Fig. 6B).

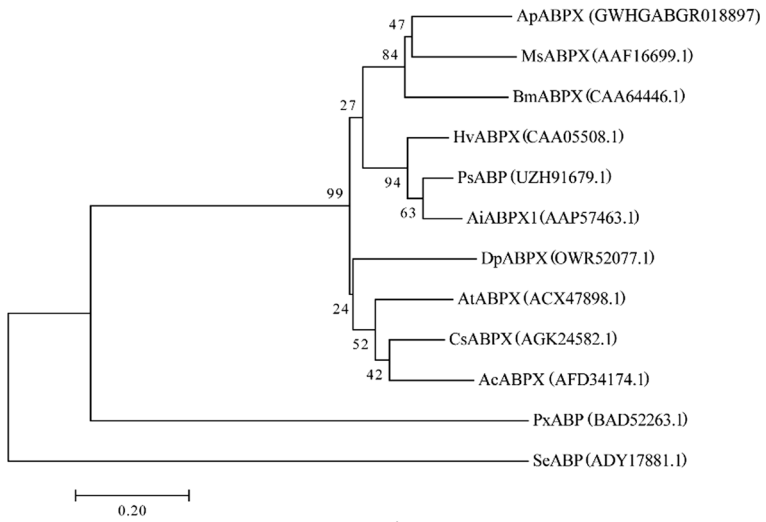
ORs are the main olfactory receptors distributed in the dendritic membrane of olfactory sensory neurons and play essential roles in detecting and recognizing a vast array of chemically diverse odorants in insects [35, 36]. In the current study, 30 ApORs were screened from the transcriptome. However, this number was lower than that in *Helicoverpa armigera* (81 ORs) [37], *Tuta absoluta* (58 ORs) [38], and *Phthorimaea operculella* (72 ORs) [39]. This discrepancy may be attributed to behavioral and ecological diversity among different lepidopteran species. Low sequence similarity was observed among ApORs (Fig. S1), suggesting their respective characteristics in recognizing different odors. Meanwhile, the identified ORs displayed different expression patterns between male and female *A. pernyi* (Fig. 3). In *A. polyphemus*, the OR (ApolOR1) is predominantly expressed in the pheromone-sensitive sensilla trichodea of the male antennae, and is concluded to be involved in perception of the sex pheromone [40]. In *H. armigera*, HarmOR56 expressed specifically in sensilla trichodea on female antennae is demonstrated to mediate oviposition deterrence by detecting oviposition-deterring pheromones [41]. We speculated that the ApORs with high expression levels in male antennae may be involved in the regulation of the male moths of *A. pernyi* recognizing and binding the female pheromones, while the individuals highly expressed in females were supposed to facilitate the female *A. pernyi* to recognize the odor molecules in the environment thus select oviposition sites. IRs are the second class of olfactory receptors that are mainly expressed in sensory neurons without ORs and GRs [42]. A total of 11 ApIRs were identified in *A. pernyi* antennae including two ApIR25as (GWHGABGR003112 and GWHGABGR019950) conserved among lepidopteran IR25as (Fig. S2) possessing an extracellular amino-terminal domain that is contained in ionotropic glutamate receptors [43], which was thought to act as co-receptors since IR25as are typically co-expressed along with other IRs in insects [44]. In addition, two genes (GWHGABGR000255 and GWHGABGR005182) encoding ApSNMP2 were identified, which may be involved in the recognition and transportation of lipophilic odor molecules, especially pheromones [45].

Meanwhile, based on KEGG enrichment analysis, a series of DEGs related to regulating signal transduction in Ras, Rap1, Calcium, cGMP-PKG, and cAMP signaling pathways which were believed to be involved in olfactory sensory transduction in both vertebrates and insects [46] were identified from the transcriptome data (Table S6, Fig. 7A), such as guanine nucleotide-binding protein (G protein, GWHGABGR007673), cGMP-dependent protein kinase (PKA, GWHGABGR009163), calmodulin-A (CaM-A, GWHGABGR011096), mitogen-activated protein kinase 1 (MAPK1, GWHGABGR010342), and phospholipase D2 (PLD2, GWHGABGR021202), and interactions were observed between these proteins according to the protein–protein interaction network exhibition (Fig. 7B). G proteins are key components of the GPCR signaling pathway and act as molecular switches inside the cells, which involves transmitting a variety of stimuli from extracellular to intracellular [47]. MAPK1, PKG, and PLD are the critical regulators of cell signal transduction. In addition, the cGMP-PKG signaling pathway plays important roles in regulating multiple behaviors, such as feeding, learning, and memory, in the insect nervous system [48, 49] and is involved in modulating the activity of the extracellular stimuli-responsive kinase pathway [50]. It has been demonstrated that CaM participating in the calcium signaling pathway could regulate the olfactory responses, including sensitization of ORs and odor localization by targeting co-receptors in *Drosophila melanogaster* [51]. The higher expression levels of the G protein, PKG, MAPK1, and PLD2 in male antennae and CaM-A in females (Fig. 7A) may indicate different capabilities in processing the transduction of different external stimuli into cells between male and female *A. pernyi*.

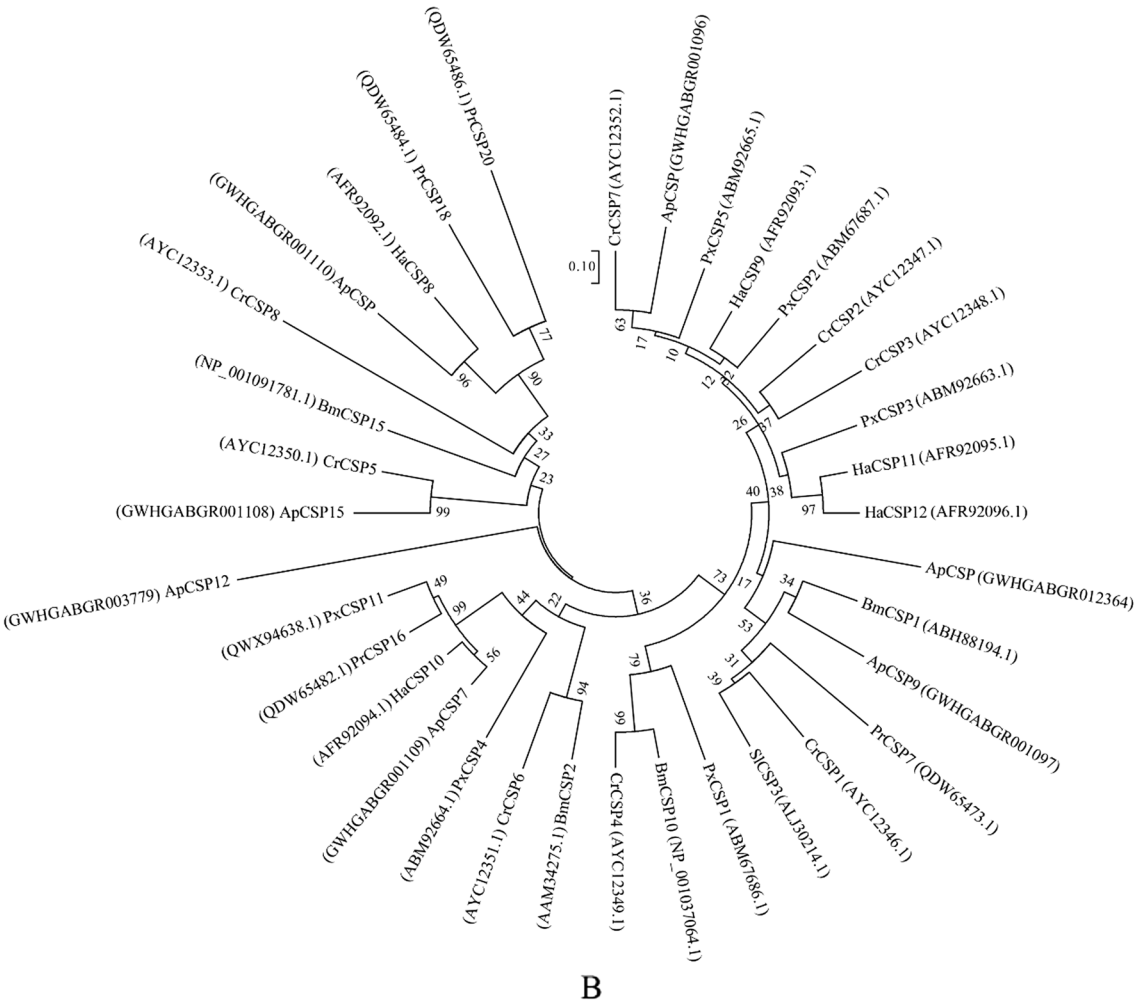
In this study, the number of identified olfactory-related genes may not represent the complete set of gene families involved in the olfactory recognition of *A. pernyi*. Certain genes may have been missed during the RNA-seq process due to low expression levels or lack of expression in the tested transcriptome. A few olfactory genes with significant differences with respect to the known query gene sequences may not have been detected by transcriptome analysis using homology search methods [52]. In

(See figure on next page.)

Fig. 6 Construction of phylogenetic trees for ApABPX and ApCSPs. **A** Phylogenetic analysis of ApABPX and selected ABPs from several lepidoptera insects including *Chilo suppressalis* (CsABPX), *Manduca sexta* (MsABPX), *Peridroma saucia* (PsABP), *Heliothis virescens* (HvABPX), *Agrotis ipsilon* (AiABPX1), *Argyresthia conjugella* (AcABPX), *Amyelois transitella* (AtABPX), *Spodoptera exigua* (SeABP), *Danaus Plexippus* (DpABPX), *Bombyx mori* (BmABPX), *Plutella xylostella* (PxABP). **B** Phylogenetic analysis of ApCSPs and selected lepidoptera insect CSPs including *Bombyx mori* (BmCSP1/2/10/15), *Clostera restitura* (CrCSP1/2/3/4/5/6/7), *Plutella xylostella* (PxCSP1/2/3/4/5/11), *Helicoverpa armigera* (HaCSP8/9/10/11/12), *Pieris rapae* (PrCSP7/16/18/20), *Spodoptera litura* (SlCSP3). Neighbor-joining tree is constructed using MEGA 7 software with 1000-fold bootstrap resampling. The numbers at the nodes of the branches represent the level of bootstrap support for each branch. The accession numbers of the ABPs and CSPs were marked in the brackets



A



B

Fig. 6 (See legend on previous page.)

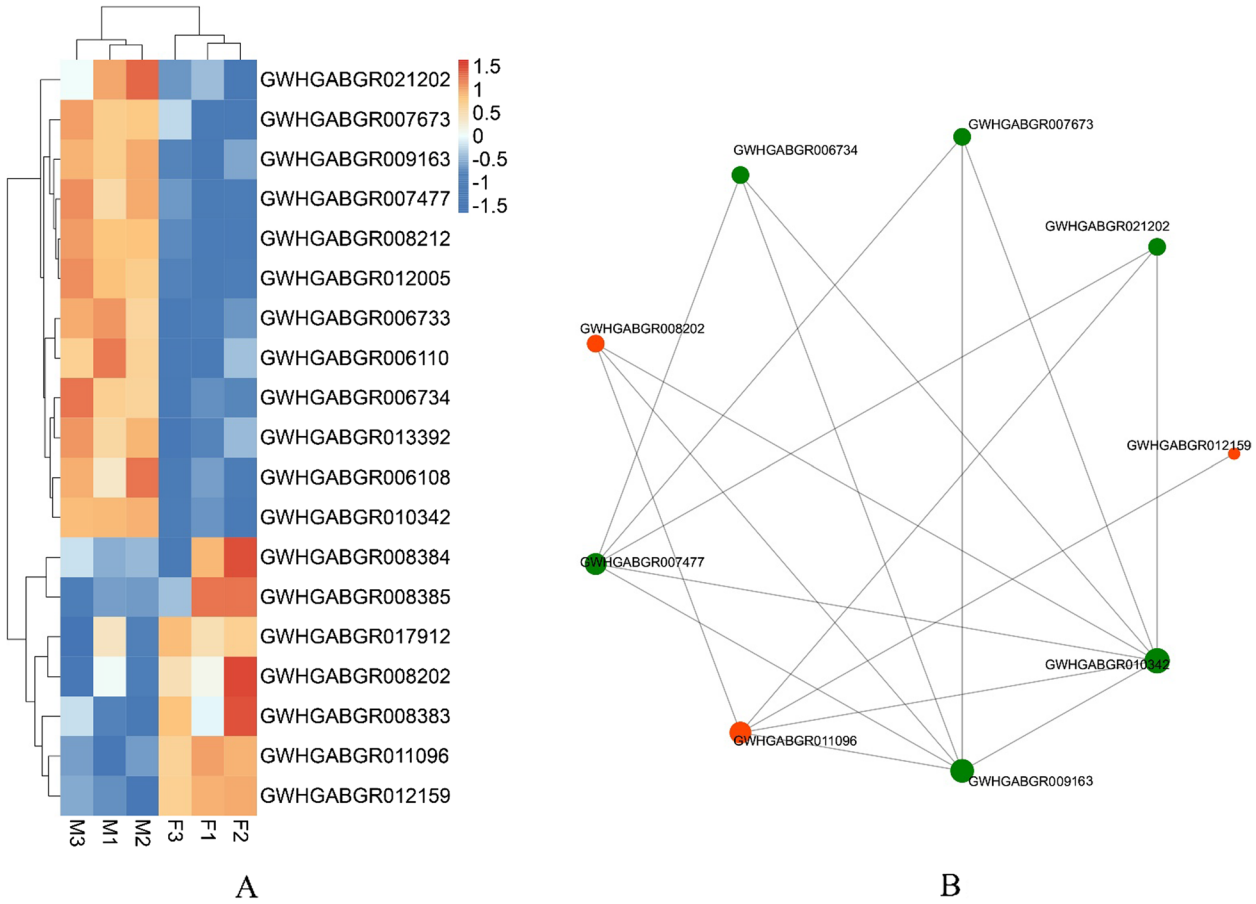


Fig. 7 Hierarchical clustering analysis (**A**) and the protein–protein interaction network (**B**) for the DEGs involved in olfactory transduction between male and female antennae of *A. pernyi*. The color gradient from red to blue in (**A**) represents the levels of expression of genes from high to low. The green and red dots in (**B**) separately represent the up-regulated and down-regulated genes

addition, *A. pernyi* live in wild and harsh condition, the environmental factors (such as temperature and humidity) or dietary factors (such as exposure to host plants) may also affect olfactory gene expression.

Recently, olfactory genes have been considered as important targets for pest control since their importance for insects in recognizing odors in the external environment [53]. In the future, further functional studies of the olfactory-related genes in the present study would provide references for investigating target genes for controlling the pests that cause damage to oaks, which are the primary source of food for *A. pernyi*. Meanwhile, the olfactory recognition by antennae between male and female moths is crucial for the courtship and mating behavior which promise the life circle completion thus ensure the silk industry continuity of *A. pernyi*. Overall, the findings of this study enrich the gene database related to the olfactory system, and also provide a foundation for better understanding the mechanisms underlying olfactory recognition, which could be further served as theoretical reference for well-breeding of *A. pernyi*.

Conclusions

By transcriptome sequencing for the adult *A. pernyi* antennae, we identified 70 olfactory-related genes including 20, 7, 30, 11, and 2 candidates encoding OBPs, CSPs, ORs, IRs, and SNMPs, respectively, which were suggested to involve pheromone perception, odor binding, pesticide resistance, and growth and development regulation. Six OBPs were categorized into classical subgroup containing six conserved Cys residues with the sequence of C1-X_{26-30}-C2-X_3-C3-X_{41-42}-C4-X_{8-10}-C5-X_8-C6. All CSPs followed the sequence pattern of C1-X_6-C2-X_{18-19}-C3-X_2-C4 with four highly conserved Cys residues. The

number of transmembrane domains were varied among ORs, IRs, and SNMPs. Meanwhile, several DEGs including G protein, PKA, CaM-A, MAPK1, and PLD2 were supposed to be involved in signal transduction underlying chemoreception of *A. pernyi* by differential expression analysis for the transcriptome profiles between the male and female antennae. In general, the results of the present study would provide theoretical basis for further elucidating the olfactory mechanisms in *A. pernyi*.

Abbreviations

OBPs	Odor-binding proteins
CSPs	Chemosensory proteins
ORs	Odorant receptors
GRs	Gustatory receptors
IRs	Ionotropic receptors
SNMPs	Sensory neuron membrane proteins
PBPs	Pheromone-binding proteins
GOBPs	General odorant-binding proteins
ABPs	Antennal-binding proteins
DEGs	Differentially expressed genes
GO	Gene Ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes
NR	NCBI non-redundant
TM	Transmembrane
PCA	Principal component analysis
G protein	Guanine nucleotide-binding protein
PKA	CGMP-dependent protein kinase
CaM-A	Calmodulin-A
MAPK1	mitogen-activated protein kinase 1
PLD2	Phospholipase D2

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12864-025-11698-4.

Supplementary Material 1.

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Not applicable.

Authors' contributions
XTL: Formal analysis; Project administration; Writing-original draft. SWM: Software; Data curation. XXZ: Investigation. XL: Supervision. LN: Resources; Visualization. GBW: Conceptualization; Funding acquisition; Methodology; Writing-review & editing.

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Data availability
The raw datasets generated during the current study are available in the Gene Expression Omnibus repository under accession number GSE283343.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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