Transcriptome analysis of the sex pheromone gland and pheromone biosynthetic pathway in *Eogystia hippophaecolus*

Ping Hu (✉ hupingcs@163.com)

GuangXi University <https://orcid.org/0000-0003-1713-063X>

Dongbai Wang

Xingan vocational and technical college

Chenglong Gao

Beijing forestry university

Pengfei Lu

Beijing forestry university

Jing Tao

Beijing forestry university

Youqing Luo

Beijing Forestry university

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Abstract

The moth *Eogystia hippophaecolus* (Hua et al.) is a major threat to sea buckthorn plantations in China. Specific and highly efficient artificial sex pheromone traps have been developed and used to control this pest species. However, the biosynthesis of sex pheromones Z7-14:Ac and E3-14:Ac remains poorly understood. Results We investigated the female pheromone gland transcriptome of *E. hippophaecolus* and identified two pheromone biosynthesis-activating neuropeptides (PBANs), two pheromone biosynthesis-activating neuropeptide receptors (PBANrs), five acetyl-CoA carboxylases (ACCs), six fatty acid synthases (FASs), 16 Acyl-CoA desaturases (DESSs), 26 reductases (REDs), 13 acetyltransferases (ACTs), one fatty acid transport protein (FATP), one acyl-CoA-binding protein (ACBP), and five elongation of very long-chain fatty acid proteins (ELOs) in pheromone biosynthesis pathways. Additionally, we identified 11 odorant-degrading enzymes (ODEs) and 16 odorant-binding proteins (OBPs), 14 chemosensory proteins (CSPs), two sensory neuron membrane proteins (SNMPs), three odorant receptors (ORs), seven ionotropic receptors (IRs), and six gustatory receptors (GRs). Conclusions 77 unigenes involved in female pheromone biosynthesis, 31 chemoreception proteins and 11 odorant degradation enzymes were identified, which provided insight into the regulation of the pheromone components and pheromone recognition in the sex gland, and knowledge pertinent to new integrated pest management strategy of interference pheromone biosynthesis and recognition.

Background

Pheromone trapping of moths and other insects based on species-specific sex pheromone-elicited sexual communication and reproduction behaviors is important for integrated pest management practices worldwide. In moths, sex pheromones are multicomponent blends of C10–C18 long-chain unsaturated alcohols, aldehydes, and acetate esters produced and released through a modified fatty acid biosynthesis pathway in sex pheromone glands (PGs) which situate in the intersegmental membrane between the 8-9th abdominal segments in females [1-3]. Adult male moth antennae perceive female sex pheromone components from afar specifically, and this allows them to successfully locate females to initiate mating [4]. Among Lepidoptera, pheromone components largely differ between species, but some share the same components in different combinations, as demonstrated in *Helicoverpa armigera* and *Helicoverpa assulta* that participate in the mutual sex pheromone components Z11-16:Ald and Z9-16:Ald, although the ratio of them is contrary entirely (7:100 in *H. assulta* and 100:7 in *H. armigera*) [5-7]. In moths, multiple varying combinations of reactions produce distinct species-specific pheromone mixtures among different species, due to management of relevant sex pheromone enzymes, leading to speciation via changes in mate recognition systems [7].

Sex pheromone biosynthesis makes use of a revised fatty acid biosynthesis pathway [1, 8, 9] which contains acetylation, desaturation, chain shortening, reduction, and oxidation by gland-specific enzymes processes, either separately or in combination variedly [1, 10]. Pheromone biosynthesis-activating neuropeptides (PBANs) was released from the subesophageal ganglion and transportation to the PG, where they bind to a pheromone biosynthesis-activating neuropeptide receptor (PBANr), which is the first

step of a characteristic moth pheromone biosynthetic pathway [11]. Then in turn activates functional group modification enzymes [1, 8, 12, 13] or acetyl-coenzyme A (CoA) carboxylase (ACC) [14]. The resulting signal triggers several enzyme pathways began by ACC that stimulates the conversion of acetyl-CoA to malonyl-CoA, leading to synthesize pheromone precursors (mostly 12, 14, 16, or 18 carbon saturated fatty acids) [10]. NADPH and Malonyl-CoA are catalyzed by fatty acid synthases (FASs) to generate most saturated palmitic acid (16:0) and stearic acid (18:0) precursors with 16 and 18 carbon atoms, respectively, without double bonds [15-17]. Fatty acid transport proteins (FATPs) then transport these precursors into the PG, and double bonds are introduced at peculiar locations ($\Delta 5$ [18], $\Delta 6$ [19], $\Delta 9$ [20, 21], $\Delta 10$ [22, 23], $\Delta 11$ [24, 25], and $\Delta 14$ [26]) by different desaturases (DESSs), many of which were demonstrated by clone and expression in many moth species functionally [14, 27], then β -oxidation shorten the chain [5]. The carboxyl carbon of formative unsaturated pheromone precursors with a specific chain length is modified by fatty acid reductase (FAR) enzymes to generate alcohols from fatty acyl precursors [28], which is oxidized to aldehydes in some species likely [29], or transformed to acetate esters (OAcS) by acetyltransferase (ACT) [30]. In addition to FATPs, acyl-CoA-binding proteins (ACBPs) have been demonstrated to work on production of the sex pheromone bombykol in *Bombyx mori* by *in vivo* RNA interference (RNAi) [28, 31]. Besides DES and FAR *B. mori* are showed that are responsible for pheromone production by the same way [32-34]. However, other critical enzymes listed above, such as ACC, PBAN, and FAS, have not been demonstrated at the enzymatic level in insects.

Males have developed a subtle antennal olfaction system that can trace exceptionally small amounts of sex pheromone components which delivered from females in long distance [35]. The first step of olfactory recognition process involves in the abundant odorant-binding proteins (OBPs) and chemosensory proteins (CSPs) in the lymph of sensilla and pheromones and plant volatiles were binding, transported and solvated by them [36-39]. Pheromone-binding proteins (PBP)s are a subfamily of OBPs [40] that bind to pheromone compounds and participate in the pheromone recognition process. After capturing odors, transport odors and OBP-odor complexes were transported to four membrane receptor types: odorant receptors (ORs), gustatory receptors (GRs), ionotropic receptors (IRs), and sensory neuron membrane proteins (SNMPs), which are situated in the outer dendrites of olfactory receptor neurons. Depending on the different pheromones functional groups, aldehydes, alcohols, or esters, degradation involves specific enzymes in odor degradation pathways, such as multi-functional odorant-degrading enzymes (ODEs), pheromone-degrading enzymes (PDEs), and antennal esterases (AES).

In 2003, the sea buckthorn carpenterworm *Eogystia hippophaecolus* (Hua et al.) (Lepidoptera: Cossidae) destroy 66,500 ha of sea buckthorn *Hippophae rhamnoides* L. (Rosales: Elaeagnaceae) in western and northern China that stems soil erosion and desertification [41], besides this pest species can destroy *Ulmus pumila* L. (Urticales: Ulmaceae) and two or three species in Rosaceae [42]. Due to *E. hippophaecolus* with sophisticated ecological and life history traits, such as larvae bore into trunks and roots, and accomplish one generation among 3-4 years, there is no highly effective method for preventing damage to larvae, which is deemed a key danger to sea buckthorn plantations in China [43]. Sex pheromones of *E. hippophaecolus* female PGs (*E*)-3-tetradecenyl acetate (E3-14:Ac) and (*Z*)-7-tetradecenyl acetate (Z7-14:Ac) have been identified [44], which have been developed specific and efficient artificial sex pheromone

traps [45]. And through the antennal transcriptome identified 137 olfactory genes including three PBPs, and found antennal biased expressed OBPs[46].

In the present study, we examined the female sex PG transcriptome of *E. hippophaecolus*, identified genes and enzymes related to pheromone biosynthesis, explored pheromone biosynthesis pathways, and identified putative genes related to chemoreception. Analysis of factors referred to the production of specific pheromones may provide insight into the regulation of the pheromone components, and hence the evolution of sexual communication in moth. Understanding interactions between pheromone biosynthesis and chemoreception genes in the sex PG and chemoreception genes in the antennae may reveal connections between pheromone biosynthesis in females and pheromone recognition in males, and provide knowledge pertinent to integrated pest management strategies.

Results

Transcriptome sequencing and sequence assembly

In total, we generated 28 million clean reads from a cDNA library of the female sex PG of *E. hippophaecolus*, of which 87.63% had q30 quality scores. After trimming adapters, removing low-quality raw sequences using Trimmomatic (<http://www.usadellab.org/cms/index.php?page=trimmomatic>), and splicing and assembly (using Trinity), we obtained 84,796 transcripts with an N50 of 2,021 bp and an average length of 1,068 bp, and 52,219 unigenes with an N50 of 1,410 bp (Figure 1). Raw reads have been deposited in the NCBI SRA database under GenBank accession number PRJNA549033.

Homology analysis and gene ontology annotation

In all, 20,363 unigenes (39.00%) were annotated with seven databases: 18,894 (92.79%) were aligned to the NCBI non-redundant protein sequences (Nr) database, 9,190 (45.13%) were aligned to the Swiss-Prot database, 12,341 (60.63%) were aligned to the protein family (Pfam) database, 11,905 (58.46%) were aligned to the euKaryotic Orthologous Groups (KOG) database, 4,557 (22.38%) were aligned to the Clusters of Orthologous Groups of proteins (COG) database, 10,304 (50.60%) were aligned to the Gene Ontology (GO) database, and 8,803 (43.23%) were aligned to the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Table 1). Interestingly, the greatest number of sequences matched *B. mori* (7,077), followed by *Danaus plexippus* (5,530), *Branchiostoma floridae* (520), *Papilio xuthus* (385), *Tribolium castaneum* (318), *Saccoglossus kowalevskii* (316), *Acyrtosiphon pisum* (299), *Solenopsis invicta* (210), and *Stegodyphus mimosarum* (166). For the remaining 20.59% of sequences, we detected matches with loci in other insects (Figure 2).

We used GO annotations to classify the 10,304 unigenes into functional groups using BLAST2GO based on *p*-values calculated by hypergeometric distribution tests and E-values $<1 \times 10^{-5}$. In the *E. hippophaecolus* transcriptome, the biological process category accounted for the majority of GO annotations, followed by cellular component and molecular function. In the molecular function category, genes expressed in the PG

were mostly related to binding, catalytic activity, and transporter activity, while cell part, cell, and organelle were the most enriched cellular component terms, and metabolic process was the most abundant molecular function subcategory (Figure 3a).

In total, 13,272 unigenes were classified into 25 KOG categories, and unigenes related to general function prediction only (2,712, 20.43%) were the major KOG category, followed by signal transduction mechanisms (1,904 unigenes, 14.35%) and post-translational modification, protein turnover, and chaperones (1,034 unigenes, 7.79%; Figure 3b).

Identification of putative genes related to chemoreception and sex pheromone biosynthesis

Homology searches identified members of gene subfamilies in the *E. hippophaecolus* PG transcriptome putatively involved in various biosynthetic processes, including pheromone production and chemoreception. A total of 77 genes potentially related to sex pheromone biosynthesis were identified, including two PBANs, two PBANrs, five ACCs, six FASs, 16 acyl-CoA desaturases (DESSs), 26 reductases (REDs) comprising 19 FARs and seven aldo-keto reductases, 13 ACTs, one FATP, one ACBP, and five elongation of very long-chain fatty acid proteins (ELOs; Table 2). We also identified 11 pheromone-degrading proteins including five ODEs and six AESs (Table 3). In addition, 31 putative chemoreception genes were identified, including 16 OBPs with three new genes, 14 CSPs including one new gene, two known SNMPs, three known ORs, seven IRs including four new genes, and six GRs with five new genes, based on comparison with the established antennal transcriptome of *E. hippophaecolus* (Table 3). The transcriptome data also contained genes encoding proteins directly related to pheromones, including PBP and PR proteins. Among the top 100 unigenes based on fragments per kilobase of transcript per million mapped reads (FPKM) values, EhipOBP17 was 36th and EhipCSP8, EhipCSP17, and EhipCSP6 were 72nd, 37th, and 20th, respectively (Additional file 1). All newly identified genes related to chemoreception are shown in bold font in Table 3. Sequences of all genes involved in pheromone biosynthesis and all new genes in chemoreception are listed in additional file 2.

Phylogenetic analyses

In order to assign putative functions to EhipDESSs, EhipREDs, EhipODEs, EhipIRs, and EhipGRs, phylogenetic analyses were conducted for each group. A phylogenetic tree of DES sequences revealed three Δ 11-desaturase orthologs including EhipDES3, and one Δ 9-desaturase (Figure 4). A phylogenetic tree of REDs revealed two reductases: a FAR (yellow in Figure 5) and an aldo-keto reductase (blue in Figure 5). Both trees support the same cladogenesis, but the aldo-keto reductase clade includes EhipRED6 and EhipRED17 (Figure 5). In the ODE tree, ODEs and six AESs are disturbed in a disorderly manner (Additional file 3). The IR75 clade is most obvious in the IR tree, which contains EhipIR13 and EhipIR14 (Figure 6), named EhipIR75p1-1(13) and EhipIR IR75p1-2(14), and IR is functionally divided into IR68a, IR60a, IR7d, IR25a, IR8a, IR31a, IR41a, IR21a, and IR85a.

Discussion

Overview of the PG transcriptome

The PG transcriptome of *E. hippophaecolus* was sequenced and analyzed. Among the identified 20,363 unigenes, only 50.60% were annotated to one or more GO terms, which indicated that massive *E. hippophaecolus* genes are either non-coding or share homology with genes not associated with GO terms, similar to the results of transcriptome analysis of the sex PG of *Spodoptera litura* [47], *H. armigera* and *H. assulta* [7], *Plutella xylostella* [48], *Ephestia cautella* [49], *Agrotis ipsilon* [50], *Lutzomyia longipalpis* [51], *Heliothis virescens* [52], and *Agrotis segetum* [53]. What's more, we identified 77 putative genes referred to sex pheromone biosynthesis in *E. hippophaecolus*, significantly more than the 55 identified in *S. litura*, [47], but fewer than the 87 in *P. xylostella* [48]. We also identified 31 chemoreception proteins in *E. hippophaecolus*, of which 13 were novel [46], and 11 ODEs. All in all, the results not merely offer a vital foundation for further elucidation of the molecular mechanisms of sex pheromone synthesis and metabolism, but also afford general perception into insect physiology and the exploitation of novel pest control strategies [54].

Genes involved in the sex pheromone biosynthesis pathway

Pheromone biosynthesis-activating neuropeptide receptor (PBANr)

In the PG of *E. hippophaecolus*, we identified two PBANr and two PBAN genes. Only four alternative splice transcripts of PBANr isoforms (A, B, and C) with diversities in C-terminal sequences have been reported for a single insect species [55]. PBAN receptor isoforms B and C have been characterized from *O. nubilalis* [56] and *H. virescens* [57] functionally, and isoforms C and B were identified in the PG of *E. hippophaecolus* in the present work, both presumably involved in pheromone production. Analysis of the PG transcriptomes of *A. segetum* [58] and *H. virescens* [52] revealed a G protein gamma subunit homolog, the diapause hormone receptor, that interacts with PBANr.

Acetyl-CoA carboxylase (ACC)

ACC catalyzing the production of malonyl-CoA from acetyl-CoA is the first committed and rate restrictive biosynthetic step in start pheromone biosynthesis [10, 59]. In the *E. hippophaecolus* PG, we identified five transcripts encoding ACCs expressed at relatively low levels.

Fatty acid synthase (FAS)

FAS is believed to facilitate the transformation of malonyl-CoA and NADPH to generate saturated fatty acids (16:acyl in *E. hippophaecolus*) [49]. We identified six FAS unigenes in the *E. hippophaecolus* PG,

among which EhipFAS3 has the longest open reading frame (ORF) and is expressed at distinct high levels (FPKM = 913), suggesting that it may be the main FAS.

Fatty acid transport protein (FATP)

The evolutionarily conserved membrane-bound proteins family FATPs promote the ingestion of extracellular long-chain fatty acids (LCFAs), and/or very LCFAs, and facilitate the ATP-dependent esterification of these fatty acids to their corresponding acyl-CoA derivatives [60]. In *E. hippophaecolus*, we identified one FATP, the function of which has been showed by *in vitro* expression and knockdown analysis in *B. mori* [60] and *Eilema japonica* [61].

Acyl-CoA desaturase (DES)

Moth sex pheromones typically include double bonds introduced by desaturases at specific locations in the fatty acyl carbon chains [25, 62, 63]. Newly in some Lepidoptera species, several reductase gene family have been discovered and functionally characterized, including *Heliothis virescens*, *Heliothis subflexa*, *H. armigera* and *H. assulta* [65], *Ostrinia scapularis* [64], *Yponomeuta evonymellus* (L.), *Yponomeuta padellus* (L.), and *Yponomeuta rorellus* (Hübner) [31], and *Ostrinia nubilalis* [66]. Sixteen putative sex pheromone components extracted from the *E. hippophaecolus* PG were unsaturated fatty acids with £16 carbons and acetate as the functional group. Notably, Z7-14:Ac and E3-14:Ac were previously identified as pheromones in *E. hippophaecolus* [44], and C16 fatty acids require β -oxidation to form short-chain C14 fatty acids, but β -oxidation enzymes were not identified in the *E. hippophaecolus* transcriptome. Female sex pheromones in the *E. hippophaecolus* PG have been identified as Z7-14:Ac and E3-14:Ac [44]. It is therefore rational to suggest that the saturated fatty acid precursor of *E. hippophaecolus* sex pheromones may be palmitic acid (16:0), which is desaturated by Δ 11-desaturase to constitute the precursor Z11-16:acyl-CoA for the production of two major components (Z7-14:OAc and E3-14:OAc; Figure 7). Other studies in Lepidoptera species indicate that a Δ 11-desaturase acting on palmitic acid leads to the production of the sex pheromone components [67, 68]. In the *E. hippophaecolus* PG transcriptome, we identified three Δ 11-desaturases, three Δ 9-desaturases, and one Δ 4-desaturase, and based on the DES phylogenetic tree, EhipDES3 is another Δ 11-desaturase named Ehip Δ 11DES-4.

Fatty acyl-CoA reductase (FAR)

Through a specific Δ 11 double bond brought in fatty acid precursors a fatty acyl-CoA precursor was formed, which is then shortened sequentially by β -oxidation for formation different shorter chain fatty acyl-CoA precursors [1] that are further lessened separately by FAR for formation corresponding fatty alcohols [31, 69]. We identified 19 FAR unigenes. Since the discovery of their ability to reduce fatty acids to alcohols in *B. mori* [19], these enzymes have also been functionally characterized in *Ostrinia* spp. [28, 66], *Yponomeuta* spp. [31], *Helicoverpa* spp., and *Heliothis* spp. [65]. FARs from *Spodoptera exigua* exhibit a preference for C14 and C16 fatty acid substrates [70].

Acetyltransferase (ACT)

In *E. hippophaecolus*, sex pheromone mixtures only contain acetates which are intermediates that are acetylated to acetate esters by ACTs [71]. In the present study, we identified 12 ACT unigenes. However, ACT enzymes that have not been verified functionally are postulated to be participated in the sex pheromone biosynthesis pathway. We identified only one ACBP gene, and its counterpart is expressed in the *B. mori* PG mainly, suggesting that ACBP takes vital effect on the production of sex pheromones adjusted by the neurohormone PBAN [72]. The proposed biosynthesis pathway for Z7-14:Ac and E3-14:Ac in *E. hippophaecolus* is shown in Figure 7.

Genes involved in chemoreception

Moth ovipositors have chemoreceptive sensilla [73, 74], and the sex PG expresses chemoreception proteins such as OBPs and CSPs that are postulated to facilitate the transportation of sex pheromones. OBPs are in the first gate of the odorant recognition process, which combine and transport especially for hydrophobic odorant molecules, including plant volatiles and pheromones across the lymph in the sensillum [75]. We identified 16 OBPs, including three new genes not previously identified in the antennae of *E. hippophaecolus*, in which EhipOBP2, EhipOBP4, EhipOBP5, and EhipOBP8 are expressed in male antennae, while EhipOBP1 and EhipOBP6 are expressed in the foot and external genitals, respectively [46]. EhipPBP3 was the second most abundant of the three PBPs in male antennae at both gene and protein expression levels [76]. The subfamily of OBPs, CSPs reveal diverse expression profiles with OBPs. OBP expression is antennae-biased, but CSPs are without obvious expression preference [77]. We identified 14 OBPs, including one new CSP not previously identified, in the antennae of *E. hippophaecolus* [46]. ORs couple binding proteins with olfactory sensory neurons and conduct olfactory signal transduction. In female and male antennae, we detected three known ORs, including the pheromone receptor EhipPR2 [46]. We identified seven IRs, including four novel genes [46]. Genome-wide analysis of IRs in *Heliconius* butterflies has been carried out [78], and the results of dendrogram analysis were consistent with their corresponding functions. Specifically, we identified EhipIR13 and EhipIR14 in the IR75 clade, which suggests that EhipIR13 and EhipIR14 are other types of *EhipIR75p1*, named EhipIR75p1-1(13) and EhipIR IR75p1-2(14). Notably *IR8a* and *IR25a* are considered function as IR co-receptors [79, 80], and also identified in the PG transcriptome. We also detected six GRs, including five new genes, and two known SNMPs [46] that are conserved throughout holometabolous insects [81, 82]. IRs are a conserved family of synaptic ligand-gated ion channels that evolved from ionotropic glutamate receptors (iGluRs) [79, 80], which are referred to both insects' smell and taste process [83, 84]. Mutations of *IR64a*, *IR84a*, *IR25a*, and *IR8a* in *Drosophila* restrain odorant-evoked neuronal responses [85, 86]. However, the specificity of ligand recognition by IRs is unclear [83]. Intriguingly, *Drosophila* IR94b is functionally involved in auditory system [87]. Five ODEs and six AES enzymes were identified in the PG transcriptome, all of which are new in *E. hippophaecolus* [46].

In summary, the female PG expresses OBPs, CSPs, ORs, IRs, GRs, SNMPs, and ODEs that together may mediate pheromone biosynthesis and recognition. Analysis of pheromone biosynthesis and chemoreception genes in the PG and antennae of *E. hippophaecolus* can reveal details of pheromone

biosynthesis, chemoreception, and transport and degradation mechanisms, which may elucidate connections between pheromone biosynthesis in females and pheromone recognition in male antennae.

Conclusion

Herein, we investigated the *E. hippophaecolus* sex PG transcriptome, in which we identified 77 unigenes involved in female pheromone biosynthesis, providing a foundation for further elucidation of the molecular mechanisms of sex pheromone biosynthesis. Additionally, 31 chemoreception proteins were identified, including 13 new genes, along with 11 novel odorant degradation enzymes, indicating that pheromone recognition may occur in the sex gland. The pheromone biosynthesis pathway and chemoreception results reveal putative connections between pheromone biosynthesis in females and pheromone recognition in male antennae, and provide knowledge that may be applicable for developing the future integrated pest management strategy of interference pheromone biosynthesis and recognition.

Methods

Ethics statement

The seabuckthorn carpenterworm *Eogystia hippophaecolus* (Lepidoptera: Cossidae) is a common forestry pest in China, which collections were made with the direct permission of Jianping forestry bureau. All operations were performed according to ethical guidelines in order to minimize pain and discomfort to the insects.

Insect and tissue collection

E. hippophaecolus were collected from damaging seabuckthorn forest by light during middle of June to end of July 2015 to 2016 in Jianping, Liaoning, China. Female sexual glands were excised and stored in RNAlater (Ambion, Austin, TX, USA) (Figure 8). Then all samples were taken back indoor and stored at -80°C.

cDNA library construction and Illumina sequencing

We extracted total RNA from sexual glands of *E. hippophaecolus* utilization TRIzol reagent (Ambion) and the RNeasy Plus Mini Kit (No. 74134; Qiagen, Hilden, Germany) abiding by the manufacturer's instructions. Using NanoDrop 8000 (Thermo, Waltham, MA, USA) detected RNA quantity. RNA of female sexual glands was used to construct three cDNA libraries respectively. Construction cDNA libraries and Illumina sequencing of samples were implemented at CapitalBio Corporation (Beijing, China). Using TruSeq RNA Sample Preparation Kit v2-Set A (No. RS-122-2001; Illumina, San Diego, CA, USA) performed purification and fragmentation of mRNA samples. The first-strand cDNA were 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

library were obtained after they were purified by the MinElute Gel Extraction Kit (Qiagen, Cat No. 28604). 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: r2018-07-31) with the default parameters. The largest alternative splicing variants in the Trinity results were unigenes.

The annotation of unigenes was in seven databases which include NCBI non-redundant protein sequences (Nr), Protein family (Pfam), Swiss-Prot, Kyoto Encyclopedia of Genes and Genomes (KEGG), and Gene Ontology (GO). For the putative protein sequences, we performed blastx search against the Nr database (<http://www.ncbi.nlm.nih.gov/genbank/>), Swiss-Prot (<http://www.uniprot.org/>), Groups of KEGG database (<http://www.genome.jp/kegg/pathway.html>) with an e-value cut-off of 1.0E-5. Pfam (<http://pfam.sanger.ac.uk/>) were searched by HMMER 3.0 package[88] with e-value of 1.0E-10. BLAST2GO was used to obtain Gene Ontology (GO) annotation of assembled unigenes for describing cellular component, molecular function, and biological process with an E value cut-off of 1.0E-5[89]. NCBI ORF Finder tool (<http://www.ncbi.nlm.nih.gov/gorf/gorf.html>) searched the longest ORF for each unigene. FPKM values (fragments per kilobase per million reads) was used to evaluate the expression levels [90], which was calculated by RSEM (RNA-Seq by Expectation-Maximization)(Version: v1.2.6)with default parameters[91].

Identification of candidate genes involved in *E. hippophaecolus* pheromone biosynthesis

Genes and enzymes involved in moth sex pheromone production have been reported previously, such as in *B. mori*[12, 60], *E. cautella*[49], *Chilo Suppressalis*[92], *H. virescens*[52], *A. ipsilon*[50], *S. litura*[47] and *P. xylostella*[48]. We focused our research on the target genes: (1) Pheromone biosynthesis activating neuropeptide (PBAN); (2) Acetyl-CoA carboxylase (ACC); (3)Fatty acid synthase(FAS); (4)Desaturase(DES); (5)Fatty acyl reductase(RED, contain FAR).

Identification of putative genes involved in fatty acid transport and pheromone degradation

A FATP was identified from the PG of *B. mori* [60]. We performed BLASTx to authenticate *E. hippophaecolus* FATP genes. Besides we focus on Acetyltransferase (ACT); Acyl-CoA binding protein(ACBP) and elongation of very long chain fatty acids protein(ELO) based on previous study. Since the sex pheromone *E. hippophaecolus* is comprised of Z7-14:Ac and E3-14:Ac, the earlier reports that esterases, for example odorant degrading enzyme(ODE) and antennal esterase(AES), may take vital effect on pheromone degradation[93, 94]. Therefore, we performed BLASTx to authenticate candidate esterase genes in the *E. hippophaecolus* pheromone gland NGS dataset.

Identification of putative genes involved in pheromone transport

With BLASTx, the available sequences of OBPs, CSPs, ORs, GRs, IRs, SNMPs and ODEs proteins from insect species were used as queries to authenticate candidate unigenes referred to olfaction in *E. hippophaecolus* from Nr, Pfam and Swiss-Prot database. All candidate OBPs, CSPs, ORs, GRs, IRs, and SNMPs were manually verified by tBLASTn in NCBI online evaluating the BLASTx results. Then compared the putative genes involved in pheromone transport in PG transcriptome with chemosensory genes in antennal transcriptome of *E. hippophaecolus*[46].

Phylogenetic analysis

Amino acid sequence alignment was implemented using Muscle method implemented in Mega v6.0 software package[95]. Using the neighbor-joining (NJ) method[96] with P-distances model and a pairwise deletion of gaps constructed the phylogenetic tree. The dependability of the tree structure and node support was valued by bootstrap analysis with 1000 replicates. The phylogenetic trees were colored and arranged in FigTree (Version 1.4.2). The phylogenetic analyses of DESs was based on 16 amino sequences of DESs of *E. hippophaecolus*, 21 DESs of *B. mori*, 4 of *A. ipsilon*, 9 of *A. segetum*, 8 of *H. armigera*, 5 of *O. nubilalis*, 7 of *O. furnacalis*, 2 of *O. latipennis* and 4 of *Drosophila melanogaster*. ODEs tree was based on 5 ODEs and 6 AESs of *E. hippophaecolus*, 23 AESs of *C. suppressalis*, 2 of *B. mori*, 12 AESs of *Cydia pomonella*, 10 ODEs of *Conogethes punctiferalis* and *Antheraea polyphemus*, 16 ODEs of *Sesamia inferens*, 7 ODEs of *Operophtera brumata*, and 2 of *D. melanogaster*. REDs tree were based on 19 FARs and 7 REDs of *E. hippophaecolus*, 2 REDs and 2 FARs of *D. melanogaster*, 10 REDs and 3 FARs of *A. ipsilon*, 5 FARs of *A. segetum*, 12 REDs and 15 FARs of *B. mori*, 1 REDs and 6 FARs of *H. armigera* and 9 FARs of *O. furnacalis*. IRs tree was constructed by 3 IRs of *H. armigera*, 7 IRs of *E. hippophaecolus*, 35 of *D. melanogaster* and Van Schooten used IRs of *M. sexta*, *B. mori*, *D. plexippus*, and *H. Melpomene*[78]. Accession number of chemosensory proteins in tree without reference was in additional file 4.

Abbreviations

CSPs: Chemosensory proteins; DEET: N,N-diethyl-meta-toluamide; GO: Gene Ontology; GPCRs: G protein-coupled receptors; GRs: Gustatory receptors; IRs: Ionotropic receptors; iGluRs: ionotropic glutamate receptors OBPs: Odorant binding proteins; ORFs: Open reading frame; ORs: Odorant receptors; Orco: Odorant receptor co-receptor; ORNs: Olfactory receptor neuron; PR: pheromone receptor; SNMPs: Sensory neuron membrane proteins. ODEs: odorant degrading enzymes. PBAN: pheromone biosynthesis activating neuropeptide, PBANr: pheromone biosynthesis activating neuropeptide receptor, ACC: acetyl-CoA carboxylase, FAS: fatty acid synthase, DES : Acyl-CoA desaturase, RED: reductase, FAR: fatty-acyl-CoA reductase , ACT: acetyltransferase, FATP: fatty acid transport protein, ACBP: acyl-CoA binding protein, ELO: elongation of very long chain fatty acids protein, PG: sex pheromone gland

Declarations

Ethics approval and consent to participate

The seabuckthorn carpenterworm *Eogystia hippophaecolus* (Lepidoptera: Cossidae) is a common forestry pest in China, which collections were made with the direct permission of Jianping forestry bureau. All operations were performed according to ethical guidelines in order to minimize pain and discomfort to the insects.

Consent to publish

All authors and organization with relationship with collection *Eogystia hippophaecolus* consent to publish the manuscript.

Availability of data and materials

All supporting data is included within the article and its additional files.

Competing interests

No competing interests and financial competing interests.

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Authors' contributions

Ping Hu carried out the molecular genetic studies, performed the sequence alignment, experiments and drafted the manuscript. Dongbai Wang and Chenglong Gao collected almost all samples and participated in some experiments. Chenglong Gao participated in all experiments. Pengfei Lu, Jing Tao and Youqing Luo designed and conceived of the study; Jing Tao and Youqing Luo also helped to draft the manuscript. All authors read and approved the final manuscript.

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Tables

Table 1 Functional annotation of unigenes using various public protein databases

Database	Number	Percentage
Nr	18894	92.79%
Pfam	12347	60.63%
Swissprot	9190	45.13%
COG	4557	22.38%
KOG	11905	58.46%
GO	10304	50.60%
KEGG	8803	43.23%
Total	20363	-

Table 2 Putative pheromone biosynthesis-related genes in the *E. hippophaecolus* pheromone gland

Number	Gene ID	Gene length (bp)	FPKM	Best Blast Match			
				Name	ACC. Number	Speceies	E-value
EhipPBAN1	c35789.graph_c0	325	0.430	Pheromone biosynthesis-activating neuropeptide diapause hormone- pheromone biosynthesis- activating neuropeptide	AAL05596.1	<i>Helicoverpa armigera</i>	2.00E- 38
EhipPBAN2	c36253.graph_c0	290	0.482	diapause hormone- pheromone biosynthesis activating neuropeptide	AOY34021.1	<i>Ostrinia nubilalis</i>	1.00E- 40
EhipPBANr-1	c4439.graph_c0	1516	0.676	Pheromone biosynthesis-activating neuropeptide receptor pheromone biosynthesis- activating neuropeptide receptor subtype C	ACQ90221.1	<i>Manduca sexta</i>	0
EhipPBANr-2	c30664.graph_c0	321	0.726	pheromone biosynthesis- activating neuropeptide receptor isoform B	ABU93813.1	<i>Heliothis virescens</i>	7.00E- 11
EhipACC1	c48404.graph_c0	622	0.300	Acetyl-CoA carboxylase acetyl-CoA carboxylase 1	NP_001009256.1	<i>Ovis aries</i>	4.00E- 137
EhipACC2	c25021.graph_c0	7711	21.823	acetyl-CoA carboxylase	ALS92678.1	<i>Helicoverpa armigera</i>	0
EhipACC3	c38636.graph_c0	420	0.644	acetyl coenzyme A carboxylase alpha	ABC96905.1	<i>Capra hircus</i>	7.00E- 81
EhipACC4	c35767.graph_c0	443	0.526	acetyl-CoA carboxylase 1	NP_001009256.1	<i>Ovis aries</i>	4.00E- 97
EhipACC5	c37234.graph_c0	316	0.442	acetyl-CoA carboxylase 1	XP_020740818.1	<i>Odocoileus virginianus</i>	1.00E- 68
EhipFAS1	c35372.graph_c0	647	0.432	Fatty acid synthase	KPJ07769.1	<i>Papilio machaon</i>	2.00E- 81
EhipFAS2	c36413.graph_c0	227	0.000	fatty acid synthase, isoform CRA_a	EDL34808.1	<i>Mus musculus</i>	4.00E- 43
EhipFAS3	c24943.graph_c1	6032	913.479	fatty acid synthase	AGR49310.1	<i>Agrotis ipsilon</i>	0
EhipFAS4	c10701.graph_c0	1171	0.876	PREDICTED: fatty acid synthase-like	XP_013134398.1	<i>Papilio polytes</i>	6.00E- 114
EhipFAS5	c39724.graph_c0	522	0.536	fatty acid synthase	NP_001272558.1	<i>Capra hircus</i>	6.00E- 85
EhipFAS6	c27600.graph_c0	1074	0.694	fatty acid synthase	ABB36643.1	<i>Capra hircus</i>	0
EhipDES1	c38302.graph_c0	215	0.000	Acyl-CoA desaturase Acyl-CoA desaturase 1	KPI92671.1	<i>Papilio xuthus</i>	2.00E- 34
EhipΔ11Des-1	c31444.graph_c0	1028	0.499	acyl-CoA Delta(11) desaturase-like	NP_001296508.1	<i>Bombyx mori</i>	0
EhipDES2	c44591.graph_c0	890	0.262	desaturase	AIM40222.1	<i>Cydia pomonella</i>	0
EhipΔ11DES-4(DES3)	c5434.graph_c1	547	7.924	desaturase	AIM40219.1	<i>Cydia pomonella</i>	4.00E- 85
EhipDES4	c20775.graph_c0	3494	2.291	desaturase	AAQ74260.1	<i>Spodoptera littoralis</i>	0
EhipDES5	c13452.graph_c0	1270	41.303	desaturase	ARD71185.1	<i>Spodoptera exigua</i>	4.00E- 173
EhipDES6	c13089.graph_c0	1447	1.407	Acyl-CoA desaturase	KPI99990.1	<i>Papilio xuthus</i>	0
EhipΔ4DES	c21169.graph_c0	1728	1.910	Sphingolipid delta(4)- desaturase DES1	JAA80499.1	<i>Pararge aegeria</i>	0
EhipΔ9DES-1	c19932.graph_c1	1167	379.279	acyl-CoA delta-9 desaturase	AAK94070.1	<i>Epiphyas</i>	2.00E-

						<i>postvittana</i>	43
EhipΔ9DES-2	c24745.graph_c1	5899	604.395	acyl-CoA-delta9-3a-desaturase	ABX71810.1	<i>Dendrolimus punctatus</i>	0
EhipΔ11DES-2	c5434.graph_c0	608	10.502	PREDICTED: acyl-CoA Delta(11) desaturase-like	XP_013195132.1	<i>Amyelois transitella</i>	1.00E-117
EhipΔ9DES-3	c19932.graph_c2	1299	489.669	acyl-CoA delta-9 desaturase	AAF81790.2	<i>Helicoverpa zea</i>	3.00E-166
EhipDES7	c5280.graph_c0	5138	1.660	acyl-CoA desaturase	NP_001272548.1	<i>Capra hircus</i>	0
EhipΔ11DES-3	c20663.graph_c0	2368	346.889	PREDICTED: acyl-CoA Delta(11) desaturase	XP_008550563.1	<i>Microplitis demolitor</i>	5.00E-109
EhipDES8	c51235.graph_c0	307	0.304	PREDICTED: fatty acid desaturase 2	XP_018892033.1	<i>Gorilla gorilla</i>	1.00E-54
EhipDES9	c24941.graph_c5	1877	37.385	desaturase	ARD71180.1	<i>Spodoptera exigua</i>	0
Fatty Acyl Reductase							
EhipFAR1	c23161.graph_c0	2228	13.602	Fatty Acyl Reductase-3	JAV01988.1	<i>Plutella xylostella</i>	1.00E-99
EhipFAR2	c22156.graph_c0	2008	1780.060	fatty-acyl-CoA reductase	AGD98718.1	<i>Bicyclus anynana</i>	3.00E-143
EhipFAR3	c20658.graph_c0	3456	1.767	fatty acyl-CoA reductase 12	AKD01790.1	<i>[Helicoverpa assulta]</i>	0
EhipFAR4	c10341.graph_c0	1284	1.016	fatty acyl reductase	ARD71198.1	<i>Spodoptera exigua</i>	0
EhipFAR5	c11193.graph_c0	1132	1.276	fatty-acyl CoA reductase II	ADD62442.1	<i>Yponomeuta padellus</i>	1.00E-98
EhipFAR6	c24306.graph_c2	1165	35.305	fatty-acyl CoA reductase 6	EHJ76493.1	<i>Danaus plexippus</i>	9.00E-58
EhipFAR7	c18110.graph_c0	1788	2.020	fatty acyl-CoA reductase 7	AKD01785.1	<i>Helicoverpa assulta</i>	0
EhipFAR8	c23034.graph_c0	2269	7.189	fatty acyl reductase	ARD71186.1	<i>Spodoptera exigua</i>	0
EhipFAR9	c24306.graph_c1	1229	38.766	fatty acyl-CoA reductase 4	AKD01782.1	<i>Helicoverpa assulta</i>	1.00E-158
EhipFAR10	c24663.graph_c0	7583	72.966	fatty acyl-CoA reductase 13	AKD01774.1	<i>Helicoverpa armigera</i>	0
EhipFAR11	c23247.graph_c1	1769	14.438	fatty-acyl CoA reductase 3	ADI82776.1	<i>Ostrinia nubilalis</i>	0
EhipFAR12	c9329.graph_c0	228	0.204	fatty acyl-CoA reductase 1	NP_001180219.1	<i>Apis mellifera</i>	4.00E-14
EhipFAR13	c22039.graph_c0	1284	4.813	fatty acyl-CoA reductase 1	NP_001299710.1	<i>Papilio xuthus</i>	0
EhipFAR14	c30760.graph_c0	469	0.497	fatty acyl-CoA reductase 10	AKD01788.1	<i>[Helicoverpa assulta]</i>	2.00E-58
EhipFAR15	c15554.graph_c0	510	1.188	fatty acyl-CoA reductase 6	AKD01767.1	<i>Helicoverpa armigera</i>	1.00E-10
EhipFAR16	c24624.graph_c1	1091	7.958	fatty-acyl CoA reductase 1	ADI82774.1	<i>Ostrinia nubilalis</i>	9.00E-143
EhipFAR17	c9053.graph_c0	1816	74.181	PREDICTED: very-long-chain enoyl-CoA reductase	XP_013134963.1	<i>Papilio polytes</i>	9.00E-175
EhipFAR18	c26609.graph_c0	822	0.680	fatty acyl reductase	ARD71192.1	<i>Spodoptera exigua</i>	5.00E-144
EhipFAR19	c24937.graph_c1	2074	18.488	fatty acid reductase	AFD04727.1	<i>Helicoverpa assulta]</i>	8.00E-152
Aldo-keto/Aldehyde Reductase							
EhipREDald1	c22250.graph_c0	1476	33.439	PREDICTED: aldo-keto reductase AKR2E4	XP_004928930.1	<i>Bombyx mori</i>	1.00E-175
EhipREDald2	c19531.graph_c0	1194	5.894	PREDICTED: aldo-keto reductase AKR2E4-like	XP_013143141.1	<i>Papilio polytes</i>	2.00E-172
EhipREDald3	c13706.graph_c0	1220	28.040	Aldehyde reductase-12	JAV02109.1	<i>Plutella</i>	1.00E-

						<i>xylostella</i>	163
EhipREDald4	c21883.graph_c0	1050	5.086	PREDICTED: aldo-keto reductase AKR2E4-like	XP_014367612.1	<i>Papilio machaon</i>	2.00E-147
EhipREDald5	c14137.graph_c0	1189	28.497	PREDICTED: aldose reductase-like isoform X1	XP_013174473.1	<i>Papilio xuthus</i>	0
EhipREDald6	c15484.graph_c0	1087	2.015	PREDICTED: aldose reductase-like	XP_013174394.1	<i>Papilio xuthus</i>	2.00E-169
EhipREDald7	c24729.graph_c1	1455	24.347	PREDICTED: aldo-keto reductase AKR2E4-like isoform X1	XP_004933321.1	<i>Bombyx mori</i>	0
Acetyl-CoA acetyltransferase							
EhipACT-1	c38955.graph_c0	316	0.442463	acetyl-CoA acetyltransferase, mitochondrial isoform X4	XP_020747028.1	<i>Odocoileus virginianus</i>	2.00E-41
EhipACT-2	c21157.graph_c0	2428	2.207457	PREDICTED: N-alpha-acetyltransferase 60 isoform X2	XP_011555279.1	<i>Plutella xylostella</i>	2.00E-165
EhipACT-3	c31920.graph_c0	966	0.627205	acetyl-CoA acetyltransferase, mitochondrial precursor	NP_001039540.1	<i>Bos taurus</i>	0
EhipACT-4	c23246.graph_c0	2892	143.1221	acetyltransferase 1	BAH03386.1	<i>Ostrinia scapulalis</i>	0
EhipACT-5	c13199.graph_c0	1822	41.52283	fatty alcohol acetyltransferase	AIN34682.1	<i>Agrotis segetum</i>	0
EhipACT-6	c23052.graph_c0	2375	10.53757	N-alpha-acetyltransferase 35, NatC auxiliary subunit	KPI97545.1	<i>Papilio xuthus</i>	0
EhipACT-7	c22060.graph_c0	2381	23.11712	fatty alcohol acetyltransferase	AIN34708.1	<i>Agrotis segetum</i>	0
EhipACT-8	c18248.graph_c0	1477	7.964045	N-acetyltransferase 9-like protein	KOB74382.1	<i>Operophtera brumata</i>	3.00E-44
EhipACT-9	c18773.graph_c0	1699	32.09487	N-acetyltransferase	JAA88866.1	<i>Pararge aegeria</i>	5.00E-118
EhipACT-10	c9805.graph_c0	1465	0.731701	N-acetyltransferase ESCO2	KPJ00801.1	<i>Papilio xuthus</i>	2.00E-142
EhipACT-11	c16633.graph_c0	1044	67.61795	N-alpha-acetyltransferase 10, NatA catalytic subunit	KPJ07254.1	<i>Papilio machaon</i>	5.00E-124
EhipACT-12	c17165.graph_c0	1570	37.04743	N-terminal acetyltransferase C complex catalytic subunit Mak3	JAA88748.1	<i>Pararge aegeria</i>	9.00E-98
EhipACT-13	c17529.graph_c0	1466	10.62102	N-acetyltransferase	EHJ68864.1	<i>Danaus plexippus</i>	5.00E-116
Fatty acid transport protein							
EhipFATP-1	c22320.graph_c0	3673	30.875	PREDICTED: long-chain fatty acid transport protein 4-like	XP_013181373.1	<i>Papilio xuthus</i>	0
Acyl-CoA binding protein							
EhipACBP-1	c16365.graph_c0	650	4.374	acyl-CoA binding protein-like	NP_001037023.1	<i>Bombyx mori</i>	4.00E-42
Elongation of very long chain fatty acids protein							
EhipELO1	c24384.graph_c0	4363	81.521	elongation of very long chain fatty acids protein AAEL008004-like	XP_022816647.1	<i>Spodoptera litura</i>	0
EhipELO2	c8605.graph_c0	1393	100.874	elongation of very long chain fatty acids protein AAEL008004-like	XP_022831965.1	<i>Spodoptera litura</i>	4.00E-139
EhipELO3	c25020.graph_c0	5370	30.610	elongation of very long chain fatty acids protein AAEL008004-like	XP_026485844.1	<i>Vanessa tameamea</i>	5.00E-176
EhipELO4	c23160.graph_c0	3422	16.544	elongation of very long chain fatty acids protein 7-like	XP_021181973.1	<i>Helicoverpa armigera</i>	0
EhipELO5	c23606.graph_c1	2514	8.167	elongation of very long chain fatty acids protein 6	XP_026490072.1	<i>Vanessa tameamea</i>	0

Table 3 Unigenes related to chemoreception in the *E. hippophaecolus* pheromone gland

					Best Blast Match			
Number	Gene ID	Gene length(bp)	ORF length(bp)	FPKM	Name	ACC. Number	Speceies	E- value
Odorant Binding Protein								
EhipOBP4	c25562.graph_c0	845	408	1.489	Odorant Binding Protein4	AOG12857.1	<i>Eogystia hippophaecolus</i>	1.00E- 71
EhipOBP13	c10691.graph_c0	999	687	3.440	Odorant Binding Protein13	AOG12868.1	<i>Eogystia hippophaecolus</i>	1.00E- 77
EhipOBP2	c18249.graph_c0	893	726	29.905	Odorant Binding Protein2	AOG12855.1	<i>Eogystia hippophaecolus</i>	5.00E- 163
EhipOBP25	c28284.graph_c0	719	453	0.778	odorant binding protein 6	ALZ45421.1	<i>Athetis dissimilis</i>	8.00E- 47
EhipOBP17	c8485.graph_c0	717	450	2648.035	Odorant Binding Protein17	AOG12872.1	<i>Eogystia hippophaecolus</i>	5.00E- 83
EhipOBP1	c14548.graph_c0	655	504	0.854	Odorant Binding Protein1	AOG12859.1	<i>Eogystia hippophaecolus</i>	5.00E- 104
EhipOBP9	c14205.graph_c0	1051	402	138.399	Odorant Binding Protein9	AOG12864.1	<i>Eogystia hippophaecolus</i>	2.00E- 89
EhipGOBP2	c31476.graph_c0	599	438	0.622	Genral Odorant Binding Protein2	AOG12862.1	<i>Eogystia hippophaecolus</i>	7.00E- 117
EhipOBP24	c13721.graph_c0	742	414	10.929	Odorant Binding Protein24	AOG12879.1	<i>Eogystia hippophaecolus</i>	4.00E- 93
EhipOBP19	c26667.graph_c0	618	471	1.207	Odorant Binding Protein19	AOG12874.1	<i>Eogystia hippophaecolus</i>	1.00E- 102
EhipOBP26	c8565.graph_c0	746	576	63.787	Odorant Binding Protein	JAI18067.1	<i>Epiphyas postvittana</i>	2.00E- 86
EhipOBP23	c27601.graph_c0	274	213	0.680	Odorant Binding Protein23	AOG12878.1	<i>Eogystia hippophaecolus</i>	7.00E- 60
EhipOBP8	c1156.graph_c0	234	150	0.797	Odorant Binding Protein8	AOG12863.1	<i>Eogystia hippophaecolus</i>	6.00E- 20
EhipPBP3	c6172.graph_c0	652	507	0.572	Pheromone binding protein 3	AOG12882.1	<i>Eogystia hippophaecolus</i>	6.00E- 112
EhipOBP7	c43627.graph_c0	202	147	0.461	Odorant Binding Protein7	AOG12861.1	<i>Eogystia hippophaecolus</i>	1.00E- 33
EhipOBP27	c43512.graph_c0	323	141	0.289	odorant- binding protein 14	ALS03862.1	<i>Ectropis obliqua</i>	2.00E- 30
Chemosensory protein								
EhipCSP4	c25191.graph_c0	461	369	504.782	chemosensory protein 4	AOG12888.1	<i>Eogystia hippophaecolus</i>	5.00E- 84
EhipCSP15	c32820.graph_c0	571	321	0.163	chemosensory protein15	AOG12899.1		1.00E- 71
EhipCSP19	c15890.graph_c1	608	363	283.623	chemosensory	BAV56810.1	<i>Ostrinia</i>	4.00E-

					protein 6		furnacalis	56
EhipCSP14	c5502.graph_c1	623	396	165.328	chemosensory protein14	AOG12898.1	<i>Eogystia hippophaecolus</i>	1.00E-86
EhipCSP3	c8449.graph_c0	570	372	124.365	chemosensory protein3	AOG12887.1	<i>Eogystia hippophaecolus</i>	2.00E-85
EhipCSP11	c32257.graph_c0	224	153	0.624	chemosensory protein11	AOG12895.1	<i>Eogystia hippophaecolus</i>	2.00E-48
EhipCSP8	c5518.graph_c0	656	387	1225.543	chemosensory protein8	AOG12892.1	<i>Eogystia hippophaecolus</i>	1.00E-86
EhipCSP16	c8553.graph_c0	1186	384	939.550	chemosensory protein16	AOG12900.1	<i>Eogystia hippophaecolus</i>	5.00E-85
EhipCSP17	c8432.graph_c0	686	384	2513.946	chemosensory protein17	AOG12901.1	<i>Eogystia hippophaecolus</i>	1.00E-87
EhipCSP13	c18139.graph_c0	861	366	9.290	chemosensory protein13	AOG12897.1	<i>Eogystia hippophaecolus</i>	2.00E-82
EhipCSP9	c23028.graph_c0	1400	435	72.606	chemosensory protein9	AOG12893.1	<i>Eogystia hippophaecolus</i>	7.00E-86
EhipCSP7	c19136.graph_c0	1192	336	2.634	chemosensory protein7	AOG12891.1	<i>Eogystia hippophaecolus</i>	1.00E-58
EhipCSP1	c14432.graph_c0	654	378	2921.079	chemosensory protein1	AOG12885.1	<i>Eogystia hippophaecolus</i>	8.00E-86
EhipCSP2	c17913.graph_c0	418	213	5.381	chemosensory protein2	AOG12886.1	<i>Eogystia hippophaecolus</i>	1.00E-33
EhipCSP6	c16159.graph_c0	1542	375	3977.722	chemosensory protein6	AOG12890.1	<i>Eogystia hippophaecolus</i>	6.00E-77
Odorant receptor								
EhipOR15	c49610.graph_c0	244	0	0.000	odorant receptor15	AOG12918.1	<i>Eogystia hippophaecolus</i>	2.00E-28
EhipOR32	c36330.graph_c0	335	270	0.417	odorant receptor 32	AOG12937.1	<i>Eogystia hippophaecolus</i>	1.00E-72
EhipPR2	c4712.graph_c0	273	186	0.000	pheromone receptor2	AOG12919.1	<i>Eogystia hippophaecolus</i>	3.00E-38
Ionotropic receptor								
EhipIR25a	c33519.graph_c0	326	180	0.429	ionotropic receptor 25a	AOG12847.1	<i>Eogystia hippophaecolus</i>	8.00E-67
EhipIR12	c50725.graph_c0	413	360	0.339	ionotropic receptor	AIG51922.1	<i>Helicoverpa armigera</i>	3.00E-63
EhipIR75p1-1(13)	c6710.graph_c0	874	768	0.587	ionotropic receptor 75p1	AMM70652.1	<i>Heliconius melpomene</i>	4.00E-124
EhipIR IR75p1-2(14)	c12066.graph_c0	777	594	1.020	ionotropic receptor	AIG51922.1	<i>Helicoverpa armigera</i>	3.00E-103
EhipIR76b	c50176.graph_c0	336	135	0.277	ionotropic receptor 76b	AOG12850.1	<i>Eogystia hippophaecolus</i>	8.00E-43
EhipIR15	c4645.graph_c0	260	132	0.000	ionotropic receptor	AIG51915.1	<i>Helicoverpa armigera</i>	7.00E-12
EhipIR8a	c50367.graph_c0	223	147	0.000	ionotropic receptor 8a	AOG12845.1	<i>Eogystia hippophaecolus</i>	1.00E-42
Gustatory receptor								
EhipGR 14	c27065.graph_c0	510	153	0.822	gustatory receptor 8	NP_001124344.1	<i>Bombyx mori</i>	8.00E-32
EhipGR15	c18526.graph_c0	1780	1401	3.102	gustatory receptor	AGA04648.1	<i>Helicoverpa armigera</i>	0
EhipGR16	c5431.graph_c0	338	285	0.814	Gustatory receptor 46	KOB66549.1	<i>Operophtera brumata</i>	2.00E-08
EhipGR7	c9964.graph_c0	382	171	0.488	gustatory receptor 7	AOG12966.1	<i>Eogystia hippophaecolus</i>	1.00E-32
EhipGR17	c8069.graph_c0	1011	858	0.645	gustatory receptor 7	DAA06374.1	<i>Bombyx mori</i>	2.00E-21

EhipGR18	c34087.graph_c0	285	225	0.491	gustatory receptor 3	AJD81596.1	<i>Helicoverpa assulta</i>	2.00E-44
Sensory neuron membrane protein								
EhipSNMP1	c9735.graph_c0	1792	1458	0.832	sensory neuron membrane protein 1	AOG12884.1	<i>Eogystia hippophaecolus</i>	0
EhipSNMP2	c21118.graph_c0	1791	1566	5.855	sensory neuron membrane protein 2	AOG12883.1	<i>Eogystia hippophaecolus</i>	0
Odorant degrading enzyme								
EhipODE1	c24925.graph_c1	5053	1668	10.713	odorant degrading enzyme CXE9	AI121983.1	<i>Sesamia inferens</i>	0
EhipODE2	c19816.graph_c0	938	354	13.465	ODE	JAP88603.1	<i>Conogethes punctiferalis</i>	2.00E-50
EhipODE3	c13543.graph_c0	1919	1635	3.789	Odorant degrading enzyme CXE20	KOB74700.1	<i>Operophtera brumata</i>	2.00E-152
EhipODE4	c22117.graph_c0	2481	1638	4.095	odorant degrading enzyme CXE10	AI121984.1	<i>Sesamia inferens</i>	0
EhipODE5	c21202.graph_c0	1919	1731	842.359	odorant degrading enzyme CXE5	AI121981.1	<i>Sesamia inferens</i>	0
Antennal esterase								
EhipAES1	c41817.graph_c0	484	171	0.193	Antennal esterase CXE19	KOB70665.1	<i>Operophtera brumata</i>	4.00E-88
EhipAES2	c24294.graph_c1	1760	900	13.445	antennal esterase CXE20	ADR64703.1	<i>Spodoptera exigua</i>	1.00E-160
EhipAES3	c19867.graph_c0	2110	1440	2.071	antennal esterase CXE14	AKP92859.1	<i>Cydia pomonella</i>	0
EhipAES4	c28951.graph_c0	431	297	0.649	antennal esterase CXE19	ACV60246.1	<i>Spodoptera littoralis</i>	1.00E-46
EhipAES5	c36194.graph_c0	492	480	0.189	antennal esterase CXE15	ACV60242.1	<i>Spodoptera littoralis</i>	6.00E-73
EhipAES6	c21538.graph_c0	2460	1602	5.026	antennal esterase CXE11	AEJ38206.1	<i>Spodoptera exigua</i>	0

Note: Unigenes with font bold are unknown genes in before antennal transcriptome of *E. hippophaecolus*.

Figures

Figure 1

Length distribution of unigenes assembled from the *E. hippophaecolus* sex pheromone gland transcriptome.

Figure 2

BLASTx analysis of identified unigenes with known homologs from other species.

Figure 3

GO gene function classification and KOG function classification. (a) GO classification. (b) KOG function classification.

Figure 5

Neighbor-joining phylogenetic tree of reductases (REDs).

Figure 6

Neighbor-joining phylogenetic tree of ionotropic receptors(IRs).

Figure 7

Biosynthesis pathway for Z7-14:Ac and E3-14:Ac in *E. hippophaecolus*.

Figure 8

Photograph showing the *E. hippophaecolus* female sex pheromone glands. The magnified view on the right shows the sclerotized ovipositor valves (1), the pheromone gland (2), and the sclerotized cuticle (3) that has been removed.

Supplementary Files

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- [Additionalfile3njAES.tif](#)
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