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ORIGINAL ARTICLE

Identification and expression pattern analysis of chemosensory receptor genes in the *Macrocentrus cingulum* (Hymenoptera: Braconidae) antennae

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Key words. Hymenoptera, Braconidae, *Macrocentrus cingulum*, cDNA library, odorant and ionotropic receptors, expression pattern

Abstract. *Macrocentrus cingulum* is an important polyembryonic endoparasitic wasp that attacks larvae of the Asian corn borer, *Ostrinia furnacalis* (Guenée) and the European corn borer, *O. nubilalis* (Hübner). Parasitoids use antennae as the main sensory organ to recognize herbivore-induced plant volatiles as host searching cues. The antennal olfaction proteins, odorant receptors (ORs) and ionotropic receptors (IRs) are involved in olfactory signal transduction pathway as a sensory neuron response. In the present study, we constructed a cDNA library from the male and female antennae for identifying the olfaction-related genes in *M. cingulum*. For that, we sequenced 3160 unique gene sequences and annotated them with gene ontology (GO), cluster of orthologous groups of proteins (COG), and KEGG ontology (KO). Through the homology search, we identified 9 odorant receptors (ORs), 3 ionotropic receptors (IRs) and 1 odorant binding protein (OBP) genes from the cDNA library sequences. Additionally, the expression patterns of these ORs and IRs in different tissues (antennae, heads, thoraxes, abdomens, and legs) were demonstrated by RT-PCR. The qualitative gene expression analyses showed that most of the OR genes were more highly expressed in female than male antennae; whereas IRs, unlike ORs, were more expressed in various male than females tissues. We are the first to report ORs and IRs in *M. cingulum*, which should help in deciphering the molecular basis of olfaction system in this wasp.

INTRODUCTION

Olfaction is an important sensory assessment of the environment, which is crucial to survival and reproduction, and which directly regulates the behavior of organisms. In insects, chemosensory receptors sense the alarm signals, host-plant cues and conspecific pheromones (Olivier et al., 2011). Volatile compounds are detected by olfactory receptors expressed in the dendritic membranes of olfactory sensory neurones (OSNs) that are housed in sensory hairs called olfactory sensilla, located on antennae, maxillary palps and even ovipositor sheaths (Bengtsson et al., 2012; Andersson et al., 2013). Olfactory receptors provide information on the chemical quality such as food sources or oviposition sites and also are used in intraspecific communication via pheromones (Grosse-Wilde et al., 2011). In almost all insect species, repertoires of several to several hundred of highly divergent odorant receptors (ORs) are responsible for detecting the myriad of volatile chemical signals in the environment (Su et al., 2009). Insect ORs are seven-transmembrane domain receptors with inverted membrane topology compared to vertebrate ORs, to which they are unrelated (Benton et al., 2006). ORs function with a conserved co-receptor protein named ORco, acting as an ion channel (Larsson et al., 2004). The gustatory receptors (GRs) are mostly expressed in gustatory receptor neurons (Anderson et al., 2013) and are structurally related to ORs, ionotropic receptors (IRs), and ionotropic glutamate receptors (Benton et al., 2009).

The number of insect OR genes varies from species to species due to their divergence with most insects having 50 to 200 such genes (Bengtsson et al., 2012). The first OR genes, identified with a genomic analysis of *Drosophila melanogaster* encoded seven transmembrane domains that were largely expressed in morphological and functional

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types of olfactory sensilla, especially trichoid and basiconic sensilla (Clyne et al., 1999; Gao & Chess, 1999; Vosshall et al., 1999; Couto et al., 2005). Insects respond to a variety of chemical cues with their highly diverse receptors (Howlett et al., 2012). In addition to receptor genes, other multigene families encode proteins such as odorant binding proteins (OBPs) and chemosensory proteins (CSPs), which are gene families with critical roles in taste and olfaction (Andersson et al., 2013). The small, water soluble OBPs within the lymphatic cavity of olfactory sensilla that facilitate ligand binding to ORs (Gong et al., 2009) but CSPs may help mediate binding between ligands and receptors (Gong et al., 2007).

Insect ionotropic receptor (IR) genes were discovered in *D. melanogaster* by bioinformatic analyses for insect-specific genes with enriched expression in OSNs (Benton et al., 2009). Apparently, IRs are related to ionotropic glutamate receptors (iGlurs), which are involved in synaptic signal transduction in both vertebrates and invertebrates (Bengtsson et al., 2012). IRs have atypical binding domains that are more conserved than ORs; thus it has been possible to identify several paralogous lineages among insects (Bengtsson et al., 2012). IR-induced responses appear to be conferred by assemblies of variable subunits in a heteromeric receptor, as up to five different IRs can be co-expressed in a single OSN (Benton et al., 2009). A functional complex is formed by two or more subunits of IRs, and its involved as a specific odor receptor (Bengtsson et al., 2012). Broadly expressed IR25a and IR8a in *D. melanogaster* function as co-receptors, similar to *OR83b* in OR complexes (Benton et al., 2009; Croset et al., 2010).

Olfaction is critical for insect survival as it plays an important role in locating food sources, mating partner, oviposition site, and prey or host (Liu et al., 2012; Nishimura et al., 2012; Ahmed et al., 2013). Parasitoid effectiveness depends on their searching ability to locate the host (Nordlund et al., 1988). *Macrocentrus cingulum* Brischke (Hymenoptera: Braconidae) is a polyembryonic endoparasitoid of the Asian corn borer, *Ostrinia furnacalis* (Guenée) (Lepidoptera: Crambidae) and the European corn borer, *O. nubilalis* (Hübner) (Edwards & Hopper, 1999; Hu et al., 2003). The native range of *M. cingulum* is distributed across Europe and throughout Asia, including Japan, Korea and China (Watanabe, 1967). Parasitoids use herbivore induced plant volatiles (HIPVs) and green leaf volatiles (GLVs) from infested plants by host insect as chemical cues (Ochieng et al., 2000; Shiojiri et al., 2000). *M. cingulum* has evolved an efficient olfaction system to locate its host (Ahmed et al., 2013). Female *M. cingulum* use host larval frass in stalk tunnels as host-searching cues (Parker, 1931). We constructed a cDNA library from the male and female antennae of *M. cingulum* and used it to identify olfaction-related genes and study olfactory signal transduction mechanisms. Sex-specific expression patterns of various tissues were analyzed for identified OR and IR genes.

MATERIALS AND METHODS

Insects

Specimens of *M. cingulum* were obtained from *O. furnacalis* larvae living on corn plants at the Langfang Experiment Station of the Institute of Plant Protection, Chinese Academy of Agricultural Sciences, China. The parasitoids emerged as mature larvae from the host larvae and pupated inside the silken cocoon. Adult parasitoid wasps were fed with 20% honey solution. A laboratory colony was maintained on host larvae of *O. furnacalis* that were reared on an artificial diet as described by Zhou et al. (1980). They were maintained in a room at 25°C with a 16L : 8D regime (Ahmed et al., 2013). Antennae, heads with maxillary palps (excluding antennae), legs, thorax, and abdomen of female and male individuals were dissected 1–3 days after eclosion and immediately frozen in liquid nitrogen, then stored at –80°C until RNA extraction.

Extraction of total RNA

The frozen antennae (100 pairs of each sex) or other tissues were homogenized with a liquid nitrogen cooled mortar and ground with a pestle into very fine dust. Homogenized tissues were covered with 1 mL of TriZol reagent (Invitrogen, Carlsbad, CA, USA) and total RNA were extracted following the manufacturer's instructions. After extraction, RNA integrity was verified by 1% agarose gel electrophoresis and quantity was assessed with a Nanodrop ND-1000 spectrophotometer (NanoDrop products, Wilmington, DE, USA).

Construction of cDNA library, gene identification and functional annotation

For the synthesis of the first-strand cDNA we used 1 μg of total RNA. The antennal cDNA library was constructed using the In-Fusion® SMARTer™ Directional cDNA Library Construction Kit (Clontech, Mountain, CA, USA), according to manufacturer's instructions. Sizes of cDNA inserts were determined using PCR. A pair of sequence-specific primers was designed based on the sequence of the pDNR-LIB plasmid. The clones that contained the cDNA insert longer than 400 bp were chosen and sequenced using ABI3730 sequencer (SANGON Sequencing Service, Shanghai, China). The nucleotide sequences were searched against the NCBI non-redundant protein database on a local server using the National Center for Biotechnology Information (NCBI) BLASTALL program (Altschul et al., 1997). Gene ontology (GO) annotation was performed by using Blast2GO (GO association done by a BLASTX against the NCBI NR database) (Conesa et al., 2005; Götz et al., 2008). Olfactory related genes were identified by homology searching. Duplicate genes were found by the alignment module of DNAMAN software (Lynnon Biosoft, San Ramon, CA, USA); MEGA 4.0 software was used to construct phylogenetic trees (Tamura et al., 2007). Also, unique gene sequences (unigenes) were aligned to the COG database (database of Clusters of Orthologous Groups of proteins; Tatusov et al., 2000) to find homologous genes, which facilitated more accurate function annotations. In addition, we used Kyoto Encyclopedia of Genes and Genomes (KEGG; Kanehisa Laboratories, Japan) ontology (KO) enrichment analyses to investigate biological functions.

RT-PCR for expression analysis

To explore the expression of the ORs/IRs identified from the antennal cDNA library analyses and to compare the differential expression pattern between the sexes, RT-PCR was conducted with cDNAs prepared from different tissues of male and female wasps. Independent triplicate individual samples of total RNA were isolated from the above mentioned tissues and correspond-

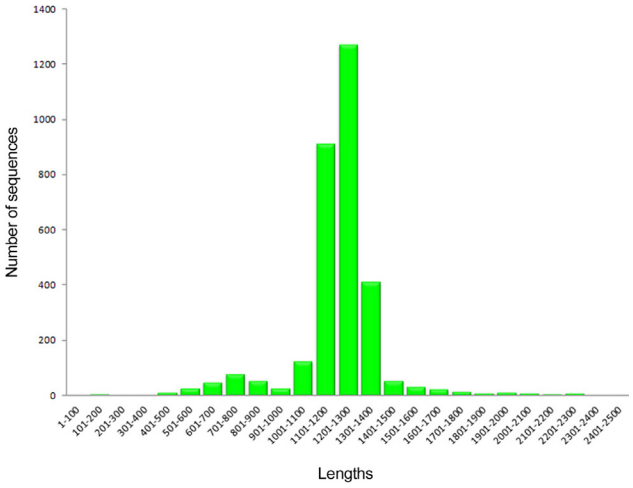

Fig. 1. Distribution of unigene sequences of cDNA library of *Macrocentrus cingulum* are shown in different length interval.

ing cDNAs were synthesized using the RT-for-PCR kit (Clontech, Mountain View, CA, USA) following the kit manual. *β-actin* was used as reference gene (accession no. EU585777.1) and it was used to select the cDNA templates on the PCR equipment. Primers that were designed manually or with the Primer 5 tool (http://frodo.wi.mit.edu/primer5/) to yield 100–200 bp product sequences are available in Table 1. Individual PCR reactions were repeated three times; controls consisted of no template PCR. The PCR conditions consisted of an initial 3-min step at 94°C, 30 cycles of 94°C for 30 s, 56, 57 or 59°C (depending on primers) for 30 s and 72°C for 3 min and finally 10-min step at 72°C. Products

Table 1. Primers for RT- PCR expression pattern analysis of ORs and IRs in *Macrocentrus cingulum.*

Primer name	Primer (5´to 3´)	Predicted Tm (°C)
McinOR1-Forward	CTGCTGCTAGGCAAGGATGAAT	60.1
McinOR1-Reverse	TGCCTCAGCGACAAGTGTTG	59.8
McinOR2-Forward	CCAATGCCTGCGCAAAAG	57.3
McinOR2-Reverse	GACTGCAGCATGTCGACTAGTTTC	62.0
McinOR3-Forward	GGCCAAAACAACAGACAGAACTC	60.2
McinOR3-Reverse	GCAGTAGCCAGGACTGAGCTTATAT	62.0
McinOR4-Forward	TGAGTGAACGATTTTCCATTTCC	56.6
McinOR4-Reverse	TCTTGGGTTGGCAAAGTCGTA	58.0
McinOR5-Forward	TGGACGACGGGTTTGCA	57.0
McinOR5-Reverse	CAACACAACGCTCACGAACAT	58.0
McinOR6-Forward	TCCGTGAGCCTGGTGATTATT	58.0
McinOR6-Reverse	GCGGTAAAGGGTGCAACATT	57.8
McinOR7-Forward	GCCTATATTGGCGGTGACGTT	60.0
McinOR7-Reverse	GCAGTTGAAATTGGCCACATAC	58.2
McinOR8-Forward	TGAGCAGTCATGCAGTCTGATG	60.1
McinOR8-Reverse	ATCCCAGGACGCAGAATGTAAG	60.1
McinOR9-Forward	CGGTGCTTTACTACTCGCTGTATC	62.0
McinOR9-Reverse	AAACTGCAACTCCCGTTGGT	57.8
McinIR1-Forward	CGTCACGGCGACAAAGATT	57.6
McinIR1-Reverse	AAGCGCGGCATTGAAGAG	57.3
McinIR2-Forward	GCGAAGAGCTTCCCCAAATT	57.8
McinIR2-Reverse	ACGTCCGAATCAATCTCGTCAT	58.2
McinIR3-Forward	TCCGATTTCACGCATCAGTTC	58.0
McinIR3-Reverse	CTCGTGGTGGGCATCACA	59.6
Mcinβactin-Forward	TATGTCGCCCTGGACTTTG	57.6
Mcinβactin-Reverse	TTGGTGATGATTCCGTGCT	55.4

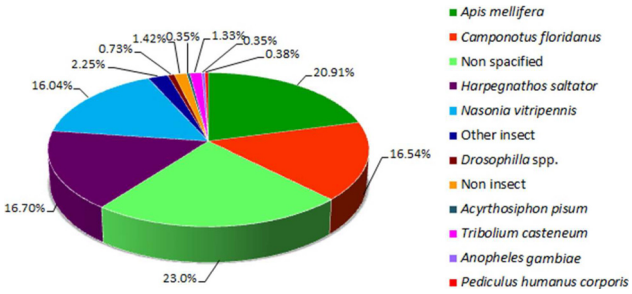

Fig. 2. Species distribution of homology search with the *Macrocentrus cingulum* unigenes against the Nr database. The species distribution is shown as a percentage of the total homologous sequences in the NCBI Nr protein database with an *E*-value < 10^{-5}.

were analyzed on a 1% agarose gel and visualized after staining with ethidium bromide.

RESULTS

Antennal cDNA library of *M. cingulum*

The antennal cDNA library was constructed successfully from female and male *M. cingulum*. Titer of this library was 1.6 × 10^6 pfu/mL; indicating a sufficient number of genes that were expressed. Genes, longer than ~0.4 kb were selected and sequenced from the library, which resulted in 3160 high quality unigenes for further analysis. Most of the unigenes ranged from 1000 bp to 1400 bp with a mean length of 1203 bp. The unigene length distributions are shown in Fig. 1.

Annotation of predicted proteins

To annotate the *M. cingulum* antennal unigenes, distinct sequences were searched by BLASTx against the NCBI non-redundant protein database (Nr) with a cut-off *E*-value of 10^{-5}. Of the total 3160 unigenes, 77.00% matched with known gene sequences; the other 23.00% were not similar to any sequence in the Nr database information at presents. The top BLAST hit results for each unique sequence are shown in Fig. 2. The unigenes from *M. cingulum* revealed that the highest number of genes, 661 (20.91%) are homologus to the honey bee (*Apis mellifera*) followed by 528 (16.70%) from the ponerine ant (*Harpegnathos saltator*), 523 (16.54%) from the Florida carpenter ant (*Camponotus floridanus*) and 507 (16.04%) from the jewel wasp (*Nasonia vitripennis*).

Functional annotation by GO, COG, and KEGG

International recommended gene function classification system of Gene Ontology (GO) covers three domains: biological process, cellular component and molecular function. The GO unigene annotation (WEGO software; Ye et al., 2006) allowed us to predict gene functions of both sexes of this species at the molecular level. The GO annotated unigenes were categorized into 46 functional groups. "Cell" and "Cellular process" are the two largest groups; containing 839 and 791 unigenes, respectively. Three smaller groups: viral reproduction, nucleic acid binding transcription activity and structural molecule activity; each had only one unigene (Fig. 3).

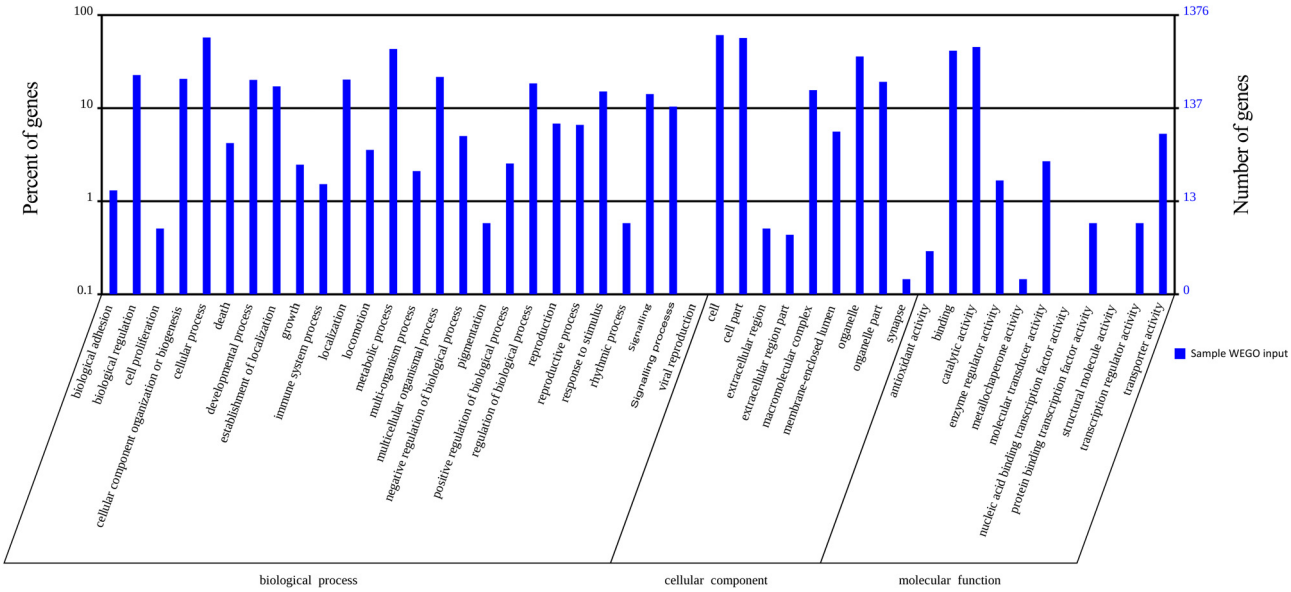

Fig. 3. The gene ontology (GO) distribution of the *Macrocentrus cingulum* unigenes were annotated by three categories: biological process, cellular component, and molecular function. The left and right Y-axis shows the percent and number of genes, and X-axis shows three categories.

After the unigenes were aligned with the COG database we found the following. In total, 1882 unigenes were annotated and classified into 25 clusters of orthologous groups (COG) (Fig. 4). The "General function prediction" cluster group is the largest (277 unigenes, 14.72%) among the functional classes, followed by "signal transduction mechanism" (232 unigenes, 12.33%), "post translational modification, protein turnover, chaperones" (210 unigenes, 11.16%), "cell motility" (5 unigenes, 0.27%) and "nuclear structure" (7 unigenes, 0.37%).

Annotation of a part of OR and IR genes

The unigenes with at least one match to ORs and IRs, and with an *E*-value of 1E-10^{-5} or lower were selectively annotated. After homology search with BLASTX, we obtained 9 OR and 3 IR genes (Table 2). A phylogenetic tree was built with the sequence alignment of the *M. cingulum* 9 OR to 65 OR sequences of others Hymenoptera species (Fig. 5). The ORs of *M. cingulum* are very divergent, and most of them (7 out of 9) have no homologues other hymenopteran species. McinOR2 is clustered with the MmedOR6 and MmedOR8 (*Microptilis mediator*), and NvitOR87 (*N. vitripennis*) with 70% bootstrap cutoff value. McinOR5 also clustered with NvitOR154 and MmedOR5 (Fig. 5) with the same bootstrap cutoff value (70%).

Expression pattern in different tissues

We conducted reverse transcription PCR (RT-PCR) analyses in different tissues (antenna, head, leg, thorax and abdomen) of adult males and females to assess the expression of *M. cingulum* OR1 to 9 and IR1 to 3. All the OR genes were expressed in the female antennae, although OR6 had a low expression. Only OR4 was not expressed in the male antennae, and OR2, OR8, and OR9 were expressed at low levels (Fig. 6). The following had low expressions in other tissues: male head (M.H: OR1, OR5); female head (M.H: OR1, OR4, OR5, OR9); female abdomen (F.Abd: OR1, OR3, OR4, OR8, and OR9); female leg (F.L: OR9). All

Table 2. The part of unigene from *Macrocentrus cingulum* with similarity to OR and IR genes.

Gene name	Length (bp)	TMHMM*	Accession no.	Homology	*E*-value
McinOR1	1163	3	KC887063	Odorant receptor 300 [*Nasonia vitripennis*]	2e-31
McinOR2	1239	2	KC887064	Odorant receptor 81 [*Nasonia vitripennis*]	6e-61
McinOR3	1184	5	KC887065	Odorant receptor 261 [*Nasonia vitripennis*]	8e-32
McinOR4	1275	3	KC887066	Odorant receptor 58 [*Apis mellifera*]	1e-24
McinOR5	1189	2	KC887067	Odorant receptor 202 [*Nasonia vitripennis*]	5e-34
McinOR6	1305	1	KC887068	Odorant receptor 82 [*Nasonia vitripennis*]	1e-44
McinOR7	1250	4	KC887069	Odorant receptor 60 [*Nasonia vitripennis*]	1e-24
McinOR8	1166	4	KC887070	Putative odorant receptor 13a [*Camponotus floridanus*]	9e-37
McinOR9	1140	4	KC887071	Olfactory receptor 10 [*Microplitis mediator*]	9e-48
McinIR1	1240	–	KC887072	Ionotropic receptor 8a [*Drosophila melanogaster*]	2e-14
McinIR2	1237	–	KC887073	Putative ionotropic receptor IR93a, partial [*Cydia pomonella*]	3e-35
McinIR3	1213	–	KC887074	Glutamate receptor, ionotropic kainate 2 [*Apis mellifera*]	4e-21

* Transmembrane Helices Hidden Markov Model (protein topology).

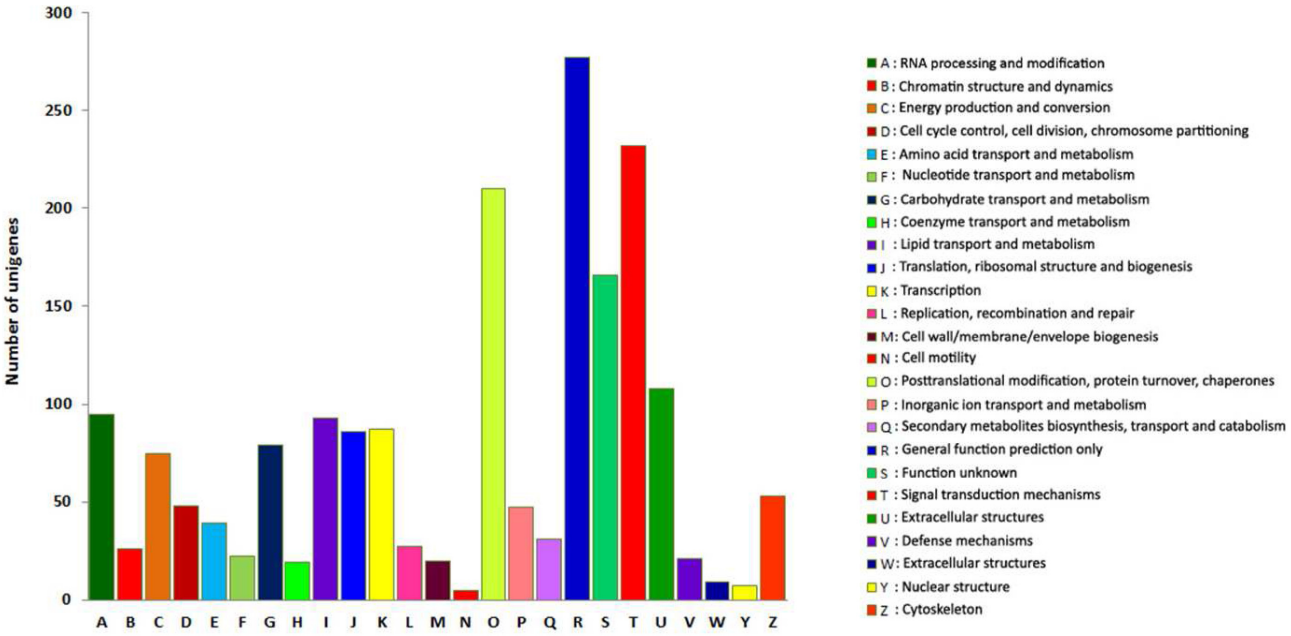

Fig. 4. The clusters of orthologous groups (COG) classification of the unigenes fell into 25 categories.

the IR genes (IR1, IR2, and IR3) expressed in the male antennae and only IR1 expressed (lightly) in the female antennae (Fig. 7). For the other tissues, only IR2 had a high expression, in this case in the male leg (M.L). Low IR gene expressions were found in other tissues: female thorax (F.T: IR2 and IR3); female abdomen (F.Abd: IR2); male thorax (M.T: IR3) (Fig. 7).

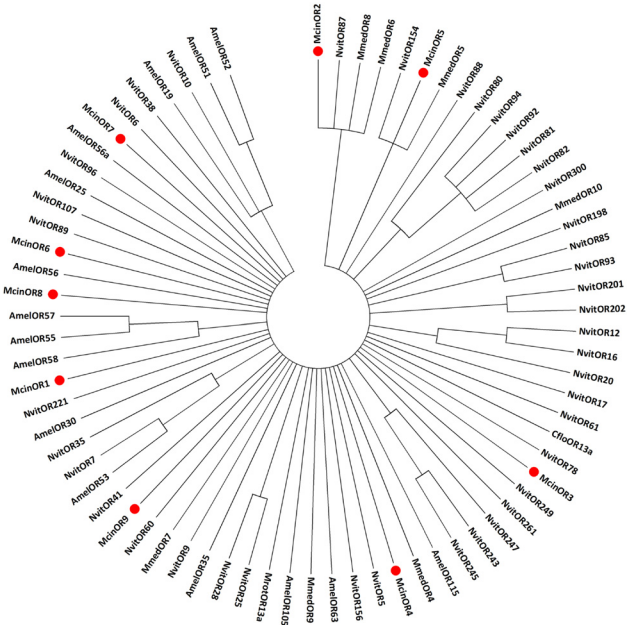

Fig. 5. Phylogenetic tree of annotated amino acids of ORs among *Macrocentrus cingulum* and other hymenoptera wasp. This tree was constructed from the multiple alignments using MEGA 4.0 software, generated with 1,000 bootstrap replicated samples using the neighbor-joining method, and presented with 70% cut-off bootstrap value. GenBank accession numbers of the 9 OR are listed in Table 2. Accession numbers of the other insect ORs are listed in Table S1.

DISCUSSION

We constructed a cDNA library from the antennae of the polyembryonic endoparasitoid, *M. cingulum* and using a bioinformatics approach we categorized the function of many of the genes. The BLASTx annotation of *M. cingulum* cDNA library unigene sequences revealed that 727 of 3160 unigenes (23.00%) did not have homologous sequences to other insect or non-insect species, which suggests that these genes may be insect specific. Additionally, we found that 1.42% of the genes were homologous to non-insect species but had no homology with genome sequences of *D. melanogaster*, *Bombyx mori* or *Acyrthosiphon pisum* (The International Aphid Genomics, 2010). Whether these sequences are *M. cingulum* specific or are non-coding genes needs to be further investigated.

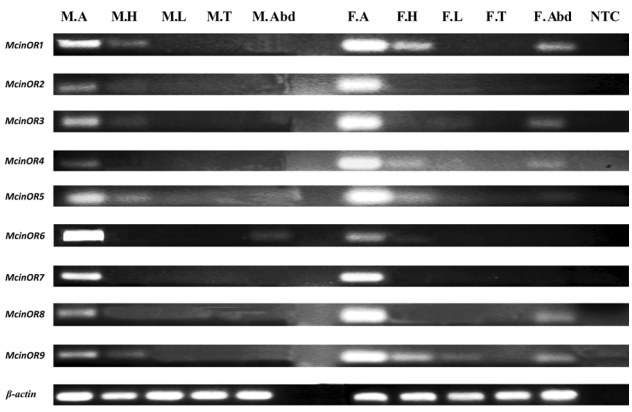

Fig. 6. Tissue and sex specific expression pattern of ORs genes in *Macrocentrus cingulum* adults. M.A – male antennae, M.H – male head, M.L – male leg, M.T – male thorax, M. Abd – male abdomen, F.A – female antennae, F.H – female head, F.L – female leg, F.T – female thorax, F. Abd – female abdomen, NTC – Non tissue control and *β actin* – reference gene of *M. cingulum*.

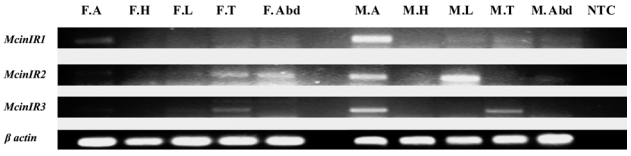

Fig. 7. Tissue and sex specific expression pattern of IRs genes in *Macrocentrus cingulum* adults. F.A – female antennae, F.H – female head, F.L – female leg, F.T – female thorax, F. Abd – female abdomen, M.A – male antennae, M.H – male head, M.L – male leg, M.T – male thorax, M. Abd – male abdomen, NTC – Non tissue control and *β actin* – reference gene of *M. cingulum*.

Only a few OR genes were identified with the homologue based strategy, with a focus on closely related species (Liu et al., 2012). The most abundant genes in the antennal cDNA libraries were those encoding ORs, suggesting their high expression in *M. cingulum* antennae, which was confirmed by RT-PCR (Fig. 6). The parasitoid wasp *N. vitripennis*, has 301 OR genes, of which 225 are intact genes and 76 are pseudogenes (Robertson et al., 2010). The honey bee *A. mellifera* genome analyses revealed a major expansion of the OR family to 170 genes (Robertson & Wanner, 2006). The silk moth, *B. mori* has approximately 60 ORs (Wanner et al., 2007; The International Silkworm Genome, 2008). *D. melanogaster* and *Anopheles gambiae*, have 62 and 79 ORs, respectively (Robertson & Wanner, 2006). Automated genome annotations of *C. floridanus* and *H. saltator* revealed about 100 ORs (Zhou et al., 2012). In this work we identified 9 OR genes in *M. cingulum* antennae. This is the first study of chemosensory receptors in *M. cingulum*. Many of the known ORs were not found in our EST data, for which we offer the following explanation: first, we only sequenced the antennal cDNA library of these wasps. Some ORs might be specifically expressed at different developmental stages or in other olfactory tissues. Second, limited ESTs that were sequenced, we reasoned that there are still some ORs not yet to be identified in this wasp. However, the number of identified ORs (9) in *M. cingulum* is higher than the six found for *Cotesia vestalis* from the transcriptome pyrosequencing (Nishimura et al., 2012) but less than *M. mediator*, in which 13 ORs were submitted to NCBI. The OR genes might enable the wasp to sense the wide range of pheromones, floral scents, HIPVs and other olfactory cues (Nishimura et al., 2012). Our study is the first to investigate the genetics of chemosensory receptors of *M. cingulum*, but for more comprehensive analysis, transcriptomic/genomic analyses of this species should be conducted.

All the 9 ORs were specifically expressed in antennae. Experiments were conducted to identify ORs with differential expression patterns between male and female because they might perform specific functions in each gender. The RT-PCR showed that only OR6 was more highly expressed in the male than female antennae but OR7 was expressed in the same level in both the male and female antennae, the other seven were highly expressed in the female than male antennae. The OR1, OR3, OR8, and OR9 were expressed in the female abdomen perhaps due to the presence of some sensilla in the ovipositor sheath. Also, OR1 was expressed at a low level in the male and female head.

IRs as chemosensory receptors have recently been discovered in *D. melanogaster* (Benton et al., 2009) followed by other species through genome annotation (Croset et al. 2010). In *D. melanogaster*, 66 IRs were identified (Benton et al., 2009) and 12 were reported in the antennae of *S. littoralis* (Olivier et al., 2011). In the *A. mellifera* and *N. vitripennis* genomes, 10 IRs genes were found for each (Croset et al., 2010), but the *Linepithema humile* genome had 32 IR genes (Smith et al., 2011). The two ants, *C. floridanus* and *H. saltator*, had 31 and 23 IR genes, respectively (Zhou et al., 2012). In the *M. cingulum* cDNA library we found only 3 IR genes, including one (IR93a) that appears to be orthologous to conserved IRs in other insect genomes that are expressed in olfactory organs. This is the first report of IRs in *M. cingulum*. Unlike ORs, the expression of the IRs was higher in the male than female antennae. The antenna specific IR1 was highly expressed in male antenna and IR3 was expressed in the antennae and thorax of both sexes. The IR2 was expressed in different tissues of male and female, but highly expressed in the male legs than antenna.

In summary, we conducted a preliminary study of olfactory gene expression analysis in *M. cingulum* through the construction and annotation of the antennal cDNA library. We identified for the first time 9 OR and 3 IR genes from *M. cingulum*. Our finding provides a foundational knowledge to understand the chemoreception mechanisms of this wasp. Future studies should characterize the function of these olfactory receptors with corn and *O. furnacalis* volatiles.

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Table S1. GenBank accession numbers of OR of different hymenopteran insects.

Species name	OR gene no.	GB accession no.
Apis melifera	AmelOR6	NP_001177432.1
	AmelOR19	NP_001229895.1
	AmelOR25	NP_001229896.1
	AmelOR30	NP_001229899.1
	AmelOR35	NP_001229900.1
	AmelOR51	NP_001229905.1
	AmelOR52	NP_001229906.1
	AmelOR53	NP_001229907.1
	AmelOR55	NP_001229908.1
	AmelOR56	NP_001229909.1
	AmelOR56a	XP_001122191.2
	AmelOR57	NP_001229910.1
	AmelOR58	NP_001229911.1
	AmelOR63	NP_001229913.1
	AmelOR105	NP_001229915.1
	AmelOR115	NP_001229918.1
Camponotus floridanus	CfloOR13a	XM_011265538.1
Microptilis mediator	MmedOR4	AGG17937.1
	MmedOR5	AGG17938.1
	MmedOR6	AGG17939.1
	MmedOR7	AGG17940.1
	MmedOR8	AGG17941.1
	MmedOR9	AGG17943.1
	MmedOR10	AGG17942.1
Megachile rotundata	MrotOR13a	XM_003700884.1
Nasonia vitripennis	NvitOR5	NP_001177431.1
	NvitOR7	NP_001177433.1
	NvitOR9	NP_001177435.1
	NvitOR10	NP_001177472.1
	NvitOR12	NP_001177468.1
	NvitOR16	NP_001177472.1
	NvitOR17	NP_001177473.1
	NvitOR20	NP_001177475.1
	NvitOR25	NP_001177480.1
	NvitOR28	NP_001177483.1
	NvitOR35	NP_001177486.1
	NvitOR38	NP_001177489.1
	NvitOR41	NP_001164391.1
	NvitOR60	NP_001177501.1
	NvitOR61	NP_001177502.1
	NvitOR78	NP_001177510.1
	NvitOR80	NP_001164396.1
	NvitOR81	NP_001164394.1
	NvitOR82	NP_001164395.1
	NvitOR85	NP_001164398.1
	NvitOR87	NP_001177513.1
	NvitOR93	NP_001177516.1