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Antennal transcriptome analysis and candidate olfactory genes in *Crematogaster rogenhoferi*

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Abstract

Crematogaster rogenhoferi (Hymenoptera: Formicidae), an omnivorous ant, is one of the dominant predatory natural enemies of a soft scale pest, *Parasaissetia nigra* Nietner (Homoptera: Coccidae), and can effectively control *P. nigra* populations in rubber forests. Olfaction plays a vital role in the process of predation. However, the information about the molecular mechanism of olfaction-evoked behaviour in *C. rogenhoferi* is limited. In this study, we conducted antennal transcriptome analysis to identify candidate olfactory genes. We obtained 53,892 unigenes, 16,185 of which were annotated. Based on annotations, we identified 49 unigenes related to chemoreception, including four odourant-binding proteins, three chemosensory proteins, 37 odourant receptors, two odourant ionotropic receptors and three sensory neuron membrane proteins. This is the first report on the molecular basis of the chemosensory system of *C. rogenhoferi*. The findings provide a basis for elucidating the molecular mechanisms of the olfactory-related behaviours of *C. rogenhoferi*, which would facilitate a better application of *C. rogenhoferi* as a biological control agent.

Introduction

Chemoreception plays an important role in the process of insect survival and reproduction, such as foraging, mating and ovipositing (Hildebrand, 1995; Birkett *et al.*, 2004; Richard and Hunt, 2013). Insects can perceive odour molecular information in the external environment and respond accordingly. Insect antennae are the principal chemosensory organs and have various sensilla on the flagellum. Olfactory genes located on the sensilla are used to sense various odourants (Hildebrand and Shepherd, 1997; Hallem *et al.*, 2006).

The recognition of odourants by insects is a very complex chain reaction. The lipophilic odourant molecule requires water-soluble carriers to be transported to receptive membranes. Binding proteins such as odourant-binding proteins (OBPs) and chemosensory proteins (CSPs) are responsible for the binding and transport of water-insoluble compounds through the sensillar lymph (Leal, 2013; Pelosi *et al.*, 2014). OBPs and CSPs are small, water-soluble proteins and characterised by the specific six and four α-helices domains, respectively. An inner hydrophobic pocket used for lipophilic ligand binding is established by the interconnection of disulphide bridges.

These proteins are secreted in large quantities by the supportive cells that surround the receptor neurons at the base of chemosensillar (Pelosi and Maida, 1995). The odourant receptor (OR), which is embedded in the dendrites of olfactory receptor neurons (ORNs), is associated with chemosensory signal transduction processes (Clyne *et al.*, 1999; Robertson *et al.*, 2010). A specific OR and an olfactory receptor coreceptor (Orco) are required to selectively bind stimulus chemicals and generate a series of action potentials. The function of specific ORs is responsible for various volatiles, for instance, the plant volatiles or insect pheromones. Orco, which is widely expressed in the majority of ORNs and highly conserved across insect orders, plays a role in ion channels. Ionotropic receptors (IRs), which were initially identified in *Drosophila melanogaster*, are a type of ligand-gated ion channels and characterised by three transmembrane domains (TMDs), though functional studies of IRs are limited (Benton *et al.*, 2009; Bengtsson *et al.*, 2012; Li *et al.*, 2015). Insect IRs can be divided into conserved 'antennal IRs', which have a function of odourant sensation, and 'divergent IRs', which may function in the process of taste (Croset *et al.*, 2010). Sensory neuron membrane proteins (SNMPs), the homologous genes of the CD36 family, are of importance in odour detection (Rogers *et al.*, 2001). This membrane protein is characterised by two-TMD and located on dendrite cilia (Benton *et al.*, 2007; Hu *et al.*, 2016). SNMPs can be divided into SNMP1 and SNMP2 subfamilies. The SNMP1 subfamily, which specifically expresses pheromone-specific olfactory sensory neurons in the insect antennae are mainly involved in pheromone sensing (Vogt *et al.*, 2009). The SNMP2 was expressed in the supporting cells with corresponding

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pheromone binding protein genes (Forstner *et al.*, 2008), while their function is still poorly understood.

Crematogaster rogenhoferi (Hymenoptera: Formicidae), an omnivorous ant, is widely distributed in South China. *C. rogenhoferi* has strong predation ability and plays a role in controlling pests, such as *Dendrolimus punctatus* Walker, *Xylotrechus quadripes* Chevrolat, *Anoplophora glabripennis* and *Zeuzera coffeae* Nietner (Huang *et al.*, 1984; Wei and Yu, 1998). *C. rogenhoferi* has also been identified as predatory natural enemy of *Parasaissetia nigra* Nietner (Homoptera: Coccidae), a major soft scale pest of rubber trees that has severely threatened the rubber industry in Hainan, China (Wang and Zhou, 2012; Chen *et al.*, 2014). In addition, *C. rogenhoferi* effectively reduces the population of symbiotic species of *P. nigra*, which slows the spread of *P. nigra* and reduces damage (Zhang *et al.*, 2017). The use of *C. rogenhoferi* is an effective way to biologically control pests.

Olfaction plays an important role in the process of predation, though the mechanism of chemical sensing and the genes involved in this process are still unclear. In this study, we obtained comprehensive information on the antennal transcriptome of *C. rogenhoferi* based on the Illumina Hiseq™ X Ten platform. In total, 49 genes related to chemoreception were identified, including four OBPs, three CSPs, 37 ORs, two IRs and three SNMPs. Our results are expected to expand our knowledge of the chemosensory genetic information of *C. rogenhoferi*.

Materials and methods

Insect samples and RNA extraction

C. rogenhoferi individuals were collected from rubber forests on the Danzhou campus of the Hainan University in Hainan Province. The collected worker ants were reared in a plastic box under conditions of 25 ± 1°C, 14:10 L:D cycle, and 65 ± 5% relative humidity. To prevent the escape, a layer of Vaseline was smeared around the edges of the container, and the container was placed in a shallow sink. Antennae were excised from about 150 worker ants using fine tweezer and immediately frozen in liquid nitrogen, and stored at −80°C until RNA isolation. The antennal sample was crushed in the hand mortar. Then total RNA was extracted using TRIzol reagent (Invitrogen, USA) according to the manufacturer's instructions. RNA degradation and purity were examined on 1% agarose gels, and RNA concentration and integrity were assessed using an RNA Nano 6000 Assay Kit for the Bioanalyzer 2100 system (Agilent Technologies, USA).

cDNA library construction and transcriptome sequencing

Sequencing libraries were constructed using NEBNext® Ultra™ RNA Library Prep Kit for Illumina (NEB, USA) following manufacturer's recommendations. Poly-T oligo-attached magnetic beads were utilised to isolate mRNA from the total RNA samples. The mRNA was fragmented randomly into short fragments using divalent cations under elevated temperature in NEBNext First Strand Synthesis Reaction Buffer (5×). The first-strand cDNA was synthesised by using the random hexamer primers, and the second-strand cDNA was synthesised by using buffers, RNaseH, dNTPs and DNA polymerase I. Then, the cDNA was purified using AMPure XP beads (Beckman Coulter, USA) and repaired at the ends. The polymerase chain reaction (PCR) was performed with Phusion High-Fidelity DNA polymerase (Thermo, USA), Universal PCR primers and Index (X) Primer. At last, the cDNA concentration was measured using a Qubit® RNA Assay Kit (Invitrogen, USA). An Agilent Bioanalyzer 2100 was used to assess the library quality. After the library was qualified, high-throughput sequencing using HiSeq x-ten, construction of the cDNA library and Illumina sequencing of the samples were performed at the Biomarker Technologies Company (Beijing, China).

Assembly and functional annotation

Clean reads were obtained by removing low-quality reads and reads containing adapter and ploy-N. Firstly, clean reads were assembled into contigs. Then the contigs were clustered and further assembled into transcripts based on the paired-end information and sequence similarity. Finally, TGI Clustering Tool was used to obtain unigenes from the potential transcript sequences (Pertea *et al.*, 2003). All unigenes were compared to the National Centre for Biotechnology Information (NCBI) non-redundant protein (NR), EuKaryotic Orthologous Groups of proteins (KOG) and Swiss-Prot databases by BLAST alignment with *E*-value less than 1.0×10^{-5} (Altschul *et al.*, 1997; Koonin *et al.*, 2004; Deng *et al.*, 2006). Amino acid sequences prediction was performed by HMMER software in the Pfam database (Eddy, 1998; Finn *et al.*, 2014). Gene ontology (GO) annotation was annotated by Blast2GO and GO functional classification of all unigenes was performed with WEGO software (Ashburner *et al.*, 2000). The involved pathways were identified according to the Kyoto Encyclopedia of Genes and Genome (KEGG) orthology terms, which is based on the annotation of sequences conducted on KEGG Automatic Annotation Server (Kanehisa *et al.*, 2007).

Olfactory gene identification

The NCBI-Blast network server (http://blast.ncbi.nlm.nih.gov) was used to calculate the similarity score. The putative signal peptides of CrogOBPs and CrogCSPs were predicted by SignalP 4.1 (http://www.cbs.dtu.dk/services/SignalP/). The TMDs of both CrogORs and CrogIRs were predicted with TMHMM Server Version 2.0 (http://www.cbs.dtu.dk/services/TMHMM). DNAMAN 6.0 software was employed for sequence comparison. A phylogenetic tree was constructed with MEGA5.2 software using the neighbour-joining method with 1000 bootstrap replications (Tamura *et al.*, 2011).

Results

Transcriptome sequencing

The eligible antennal RNA sample extracted from *C. rogenhoferi* was used to construct the sequencing library (Supplement material. fig. S1). Then the transcriptome sequencing was performed with HiSeq X-ten, and a total of 8.57 GB of transcriptome data was obtained. After data filtering, we acquired 28,755,562 clean reads. The Q30 percentages were calculated at 93.59%, and the GC content is ~41.74%. These data indicate that the sequencing was accurate and highly credible. The quality control assessment was performed to confirm that this library construction was qualified (Supplement material. fig. S2). The reads were assembled into 163,283 transcripts, with an average length of 2897 bp and an N50 length of 5046 bp using Trinity software. The transcripts were

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Table 1. Summary of annotations of the assembled *Crematogaster rogenhoferi* unigenes.

Database	Number of unigenes	Percentage
GO	7879	48.68
KEGG	5967	36.87
KOG	7365	45.51
Pfam	8353	51.61
Swissprot	6670	41.21
eggNOG	12,098	74.75
nr	16,074	99.31
All_	16,185	100

clustered and assembled into 53,892 unigenes, with a mean length of 904 bp and an N50 of 2145 bp.

Functional annotation and classification

All unigenes were aligned using the NR, Swiss-Prot, KEGG, KOG, GO and Pfam databases, with 16,185 unigenes annotated (table 1). A total of 16,074 (99.31%) of the unigenes had significant matches in the NR database: 6670 (41.21%) and 5967 (36.87%) were annotated in Swiss-Prot and KEGG, respectively. There were no significant matches in any of the databases for 37,707 unigenes.

NR database queries revealed that a high percentage of *C. rogenhoferi* sequences closely matched *Vollenhovia emeryi* (3214, 20.00%), *Wasmannia auropunctata* (1526, 9.49%), and *Solenopsis invicta* (1412, 8.78%) sequences (fig. 1A). Based on identity distribution, 34.73% of the unigenes had percentages ranging from 90 to 100% (fig. 1B). The *E*-value distribution of the top hits in the NR database showed that 35.84% of the mapped sequences were strongly homologous (lower than 1×10^{-50}), and 39.97% of the homologous sequences ranged between 1.0×10^{-10} and 1.0×10^{-50} (fig. 1C). KOG cluster analysis was used to predict and classify possible functions of the unigenes, and 7365 were categorised into 26 KOG categories (fig. 2). The clusteFr for 'General function prediction only' was the largest group (383, 13.85%), followed by 'Translation, ribosomal structure and biogenesis' (325, 11.75%).

Using Blast2GO and WEGO software, 7420 unigenes were categorised into 45 subcategories under three main GO categories: 'biological process', 'cellular component' and 'molecular function' (fig. 3). The 'cellular process' (3455, 46.56%) and 'metabolic process' (3422, 46.12%) terms were the most abundant in the 'biological process' category. A total of 904 unigenes are associated with 'response to stimulus', including 'response to chemical' (191, 2.57%) and 'chemosensory behaviour' (4, 0.05%), and 3928 are mainly related to 'binding', 114 (1.54%) of which are associated with 'odourant binding'.

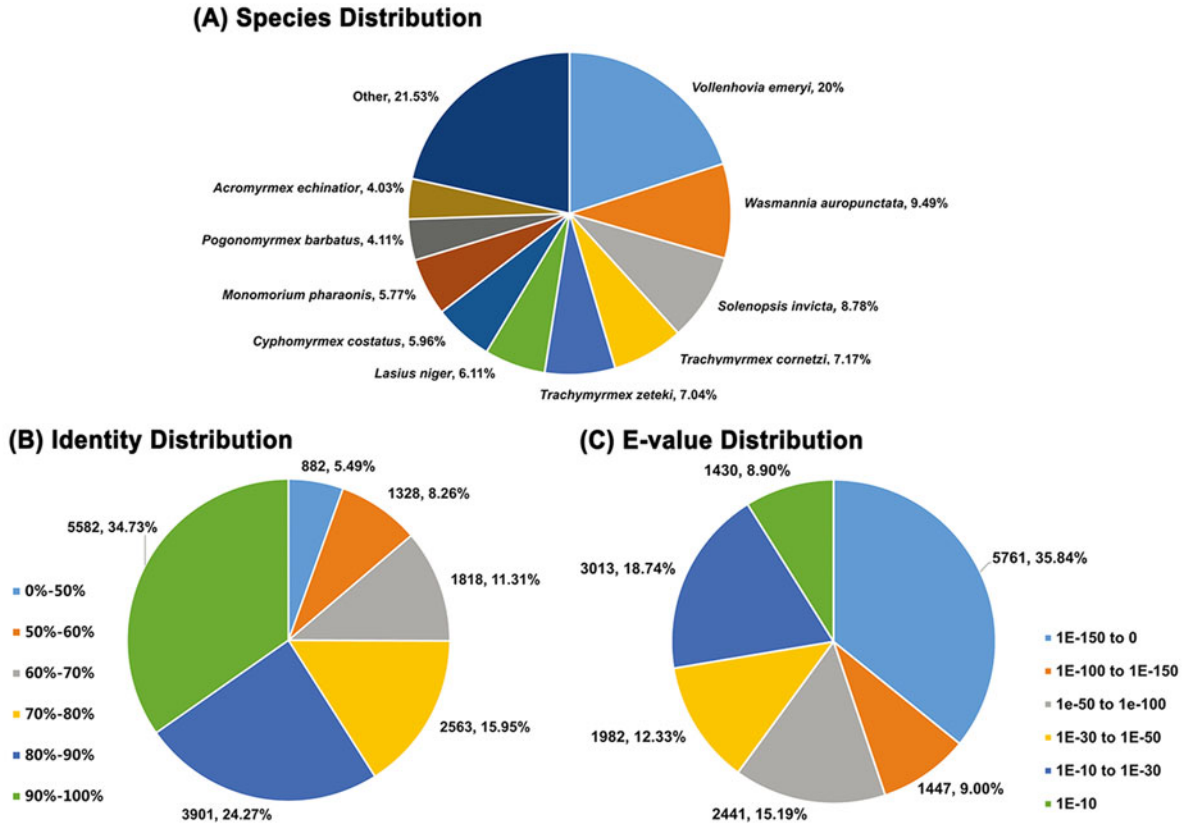


Figure 1. Characteristics of a homology search against the NR database. (A) Species distribution of BLASTX results. (B) Identity distribution of the best BLAST hits for each unigene. (C) *E*-value distribution of the best BLAST hits for each unigenes.

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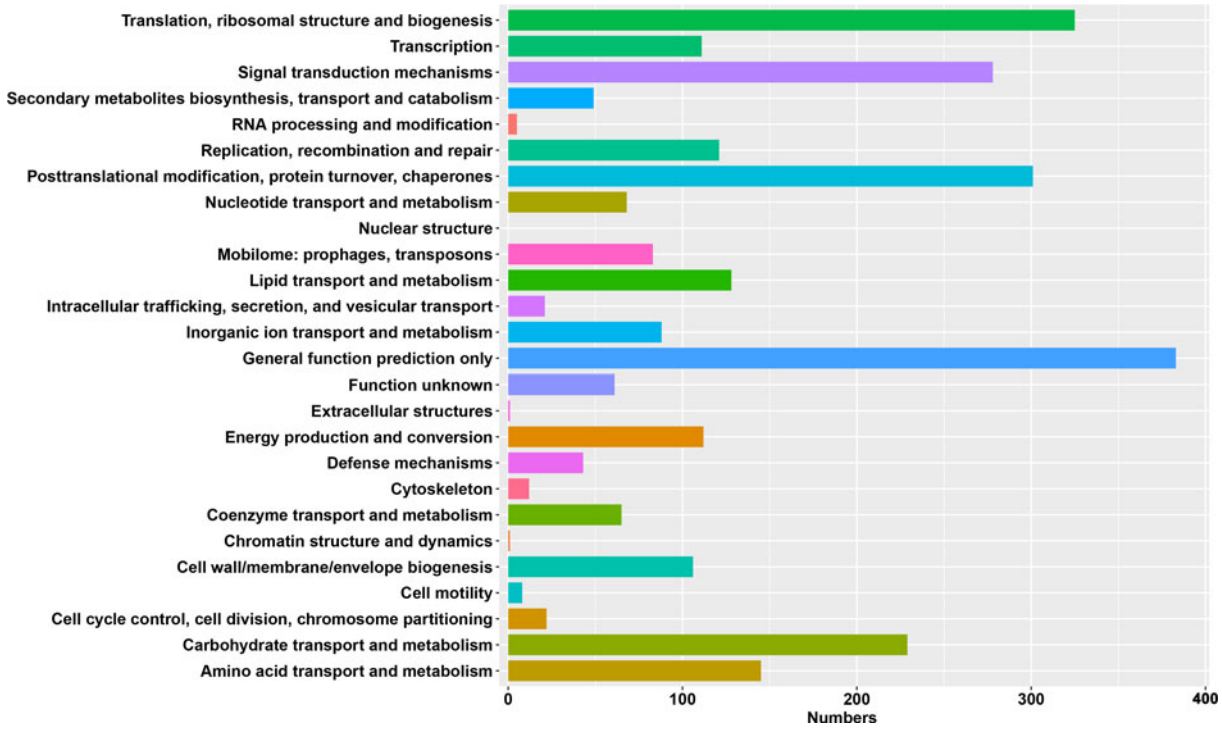


Figure 2. KOG functional classification of *Crematogaster rogenhoferi* transcriptome.

Pathway enrichment analysis was conducted by mapping all the unigenes to reference canonical pathways, and we mapped 5967 unigenes to 166 KEGG pathways. The pathway with the most gene enrichment was purine metabolism (117 1.96%), followed by endocytosis (110, 1.84%), ubiquitin-mediated proteolysis (108, 1.80%) and RNA transport (107, 1.79%).

Identification of OBPs and CSPs

Based on Nr and Pfam annotation, four OBP genes and three CSP genes, named *CrogOBPs* and *CrogCSPs*, were identified in *C. rogenhoferi*. All four *CrogOBPs* are full-length genes encoding complete open reading frames (ORFs) that encode proteins ranging in size from 133 to 146 amino acids. CrogOBPs were classified into the classic group with a typical six-cysteine signature: C_1-X_{15-39}-C_2-X_3-C_3-X_{21-44}-C_4-X_{7-12}-C_5-X_8-C_6. All CrogOBPs exhibit higher amino acid sequence identities with reported OBPs from other Myrmicinae species (table 2). CrogOBP1 shares the highest identity of 95.68% with GOBP from *Pogonomyrmex barbatus*, whereas CrogOBP3 shares the lowest identity of 61.81% with GOBP from *V. emeryi*. A phylogenetic tree based on the neighbour-joining method was constructed. The phylogenetic relationships indicated that these CrogOBPs are distributed in four subfamilies together with the OBPs of other Hymenoptera species (fig. 4). CrogOBP1 clustered into the OBP69 group and CrogOBP2 into the OBP56 group. CrogOBP3 clustered together with other OBP72, and CrogOBP4 sequences mostly clustered with OBP19a.

We also identified three unigenes with the potential to encode CSPs in the antennal transcriptome by annotation. All of them consisted intact ORFs and signal peptides. Alignment of the amino acid sequences of CrogCSPs revealed four conserved cysteine residues, which agrees with the strict structural characteristic model of C_1-X_6-C_2-X_{18}-C_3-X_2-C_4 of the CSP family. The phylogenetic relationships indicated that CrogCSP1, CrogCSP2 and CrogCSP3 all cluster with CjCSP6 and CjCSP7 from *Camponotus japonicus* (fig. 5).

Identification of candidate SNMPs

Three SNMPs were identified in the antennal transcriptome of *C. rogenhoferi*. *CrogSNMP1* and *CrogSNMP3* are predicted to have a partial-length ORF. CrogSNMP2 is a full-length gene encoding 520 amino acids. The identity values of these *CrogSNMPs* with SNMPs from other insects are higher than 83.00%. CrogSNMP1 shows similarity with SNMP of *Temnothorax curvispinosus*. CrogSNMP2 and CrogSNMP3 are similar to the SNMPs from *Acromyrmex echinatior* (table 2). The phylogenetic analysis revealed that three CrogSNMPs cluster with their SNMP orthologues into separate subclades (fig. 6).

Identification of candidate ORs and IRs

A total of 37 *CrogORs* were identified in the antennal transcriptome, 22 of which are full-length genes encoding 218–481 amino acids, with various numbers of TMDs ranging from three to eight. The identity values of these *CrogORs* with ORs from other Formicidae insects are higher than 72.00%. *CrogOR27* is hypothesised to function as a coreceptor and shares the highest identity of 97.00% with the conserved odourant receptor coreceptor (ORco) from *P. barbatus* (table 3). In addition, *CrogOR10*, *CrogOR20*, *CrogOR23* and *CrogOR24* had the best blast matches with the OR of *V. emeryi*, with identity values higher than 90.00%. Phylogenetic analysis revealed high divergence of OR genes, and all CrogORs were distributed in different branches of the phylogenetic tree (fig. 7).

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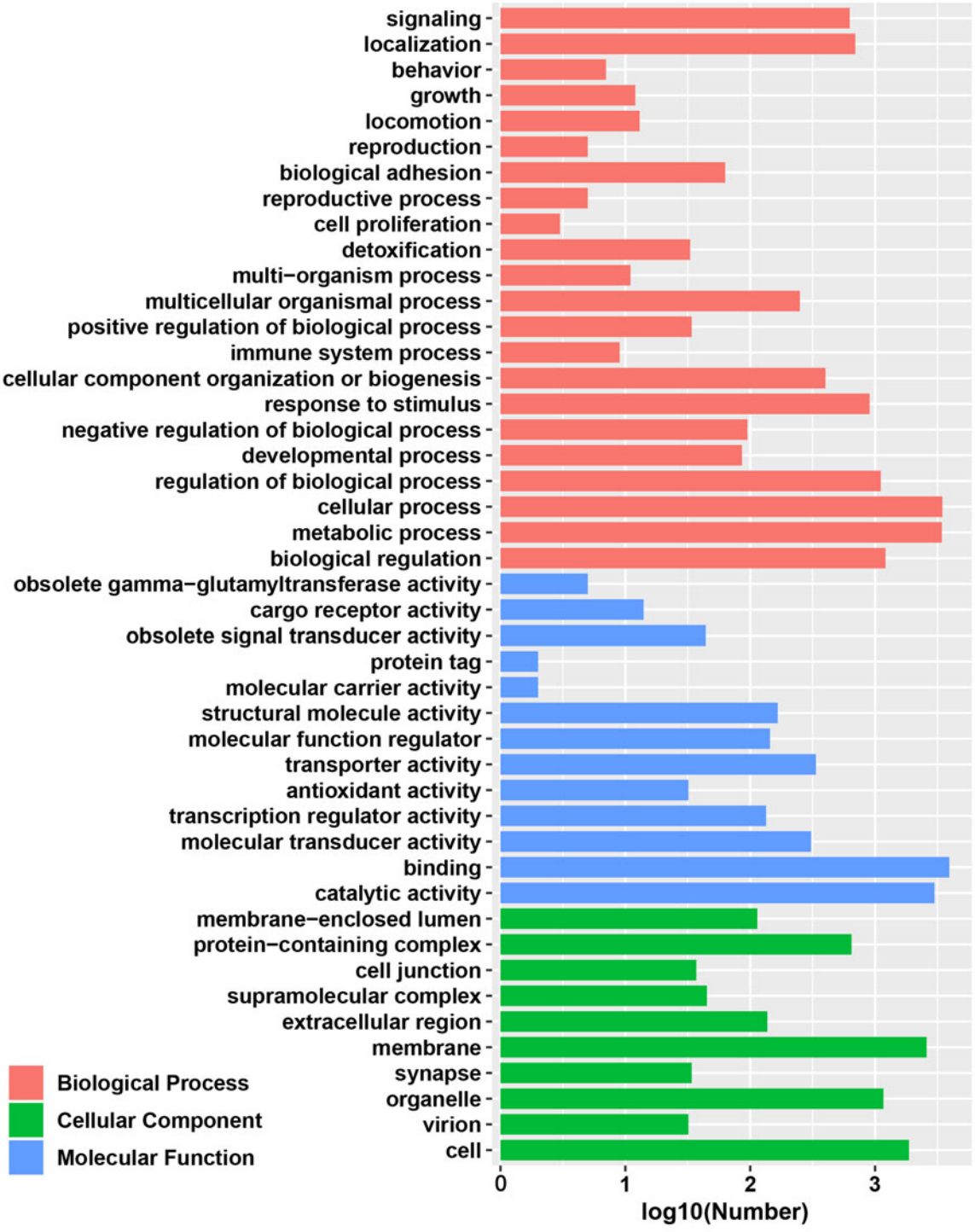


Figure 3. Gene ontology (GO) classification of unigenes.

We also identified two unigenes encoding full-length IRs. The ORF lengths of *CrogIR1* and *CrogIR2* are 2694 bp and 2808 bp, respectively. The identity values of *CrogIRs* are higher than 90.00%, and both *CrogIR1* and *CrogIR2* exhibit high similarities with the IRs from *T. curvispinosus* (table 3). In the phylogenetic tree, *CrogIR1* is closely related to *TcurlR25a,* and *CrogIR2* is closely related to *TcurlR25aX1* (fig. 8).

Discussion

Chemosensory systems play a central role in chemical communication, which is essential for ants to perceive their surroundings and critical to finding mates, food and oviposition sites. Insect antennae are the principal chemosensory organs and have various chemosensillae. Different olfactory protein families participate in different steps of peripheral olfactory signal

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Table 2. Sequence information of OBP, CSP and SNMP genes in *C. rogenhoferi.*

Unigene	Gene name	ORF	Signal peptide	Full length	Name	Species	Acc. no.	*E*-value	Identity
c10633.graph_c0	CrogOBP1	139	1–19	Yes	General odourant-binding protein 69a-like	*Pogonomyrmex barbatus*	XP_011637615.1	5.00×10^{-83}	95.68
c10639.graph_c0	CrogOBP2	133	1–17	Yes	General odourant-binding protein 56d	*Solenopsis invicta*	XP_025996520.1	5.00×10^{-55}	70.94
c10979.graph_c0	CrogOBP3	146	1–23	Yes	General odourant-binding protein 69a-like	*Vollenhovia emeryi*	XP_011875634.1	5.00×10^{-60}	61.81
c16482.graph_c0	CrogOBP4	144	1–22	Yes	General odourant-binding protein 19a	*Monomorium pharaonis*	XP_012526981.1	3.00×10^{-80}	77.78
c21804.graph_c0	CrogCSP1	112	1–20	Yes	Chemosensory protein	*Camponotus japonicus*	BAS29781.1	3.00×10^{-31}	53.4
c10634.graph_c0	CrogCSP2	120	1–20	Yes	Chemosensory protein	*Camponotus japonicus*	BAS29781.1	5.00×10^{-45}	65
c12243.graph_c0	CrogCSP3	118	1–20	Yes	Chemosensory protein	*Camponotus japonicus*	BAS29781.1	1.00×10^{-39}	60.2
c25498.graph_c0	CrogSNMP1	347	–	No	Ionotropic receptor 25a	*Temnothorax curvispinosus*	XP_024872632.1	0	93
c22440.graph_c0	CrogSNMP2	520	–	Yes	PREDICTED: sensory neuron membrane protein 1-like	*Acromyrmex echinatior*	XP_011061380.1	0	83
c21318.graph_c0	CrogSNMP3	56	–	No	Sensory neuron membrane protein 1-like isoform X1	*Acromyrmex echinatior*	XP_011061377.1	0	88

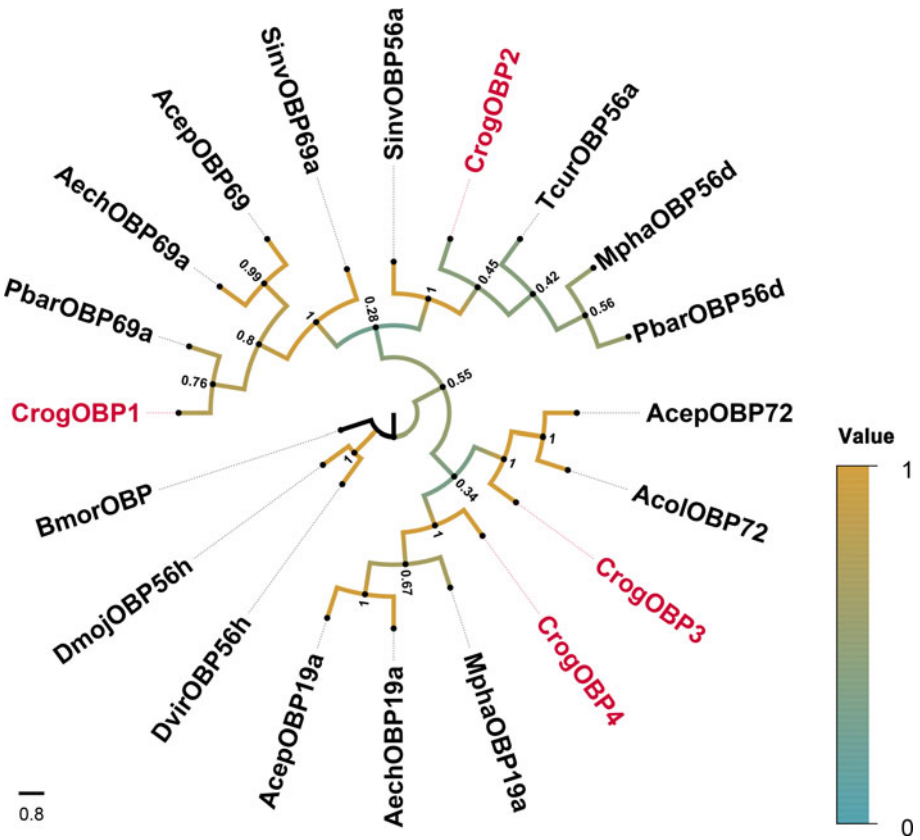


Figure 4. Phylogenetic relationships of candidate OBPs. *Atta cephalotes* (Acep), *A. colombica* (Acol), *Acromyrmex echinatior* (Aech), *Bombyx mori* (Bmor), *Pogonomyrmex barbatus* (Pbar), *Solenopsis invicta* (Sinv), *Temnothorax curvispinosus* (Tcur), *Monomorium pharaonic* (Mpha), *Drosophila virilis* (Dvir), *Drosophila mojavensi* (Dmoj). The tree was constructed in MEGA5.2 using the neighbour-joining method.

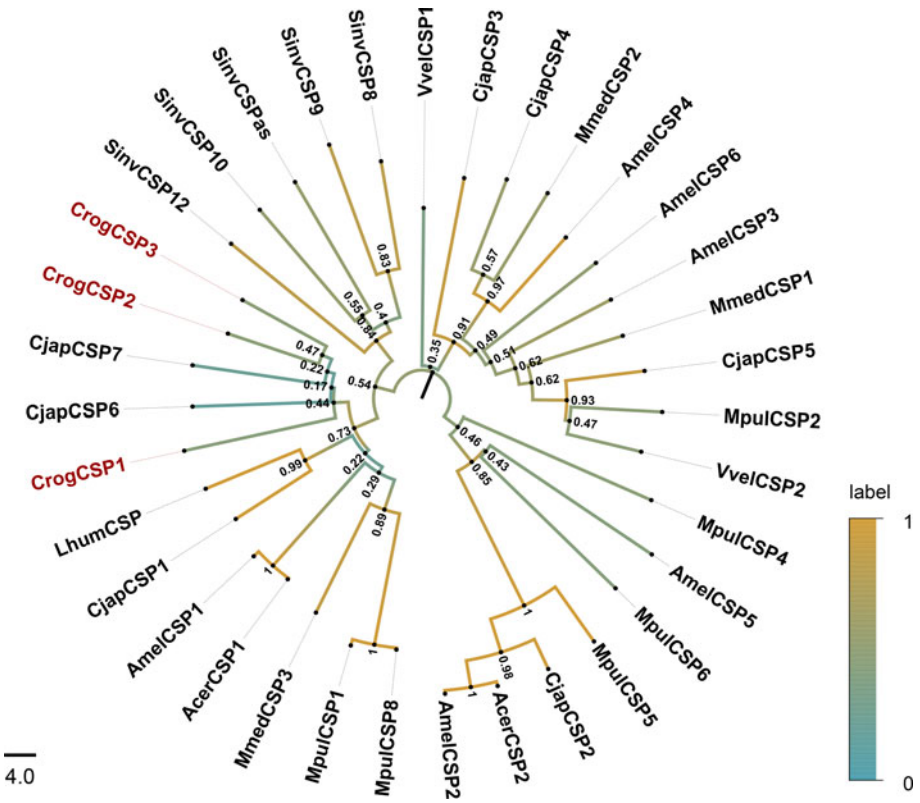


Figure 5. Phylogenetic relationships of candidate CSPs. *Meteorus_pulchricornis* (Mpul), *Camponotus_japonicus* (Cjap), *Apis_cerana_cerana* (Acer), *Apis_mellifera* (Amel), *Linepithema_humile* (Lhum), *Microplitis_mediator* (Mmed), *S._invicta* (Sinv), *Vespa_velutina* (Vvel). The tree was constructed in MEGA5.2 using the neighbour-joining method.

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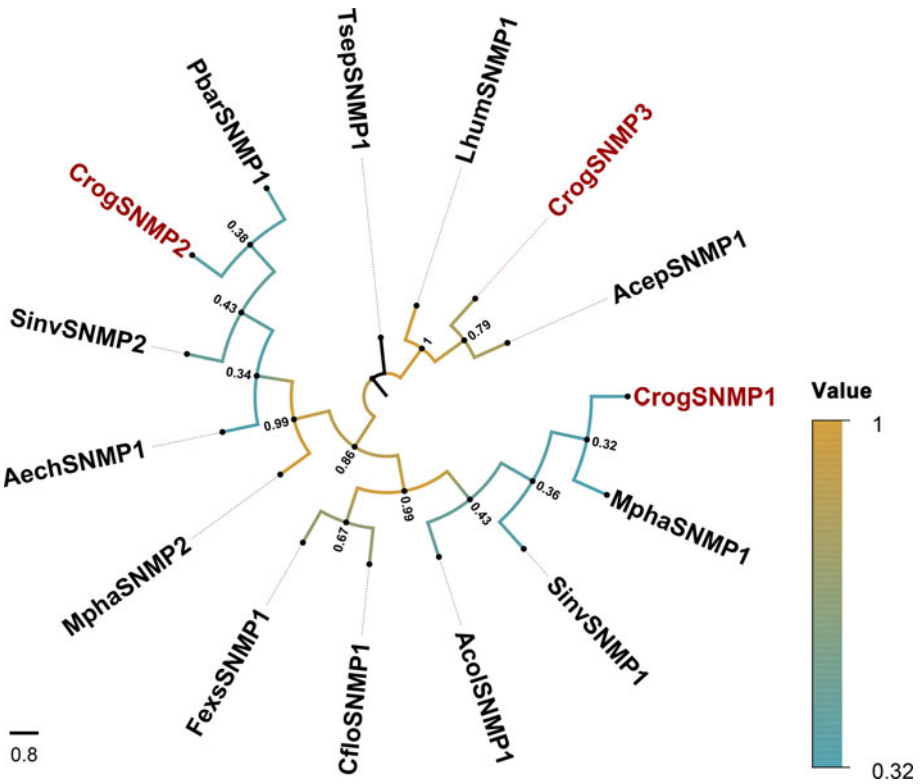


Figure 6. Phylogenetic relationships of candidate SNMPs. *A._echinatior* (Aech), *S. invicta* (Sinv), *P. barbatus* (Pbar), *A. cephalotes* (Acep), *L. humile* (Lhum), *Monomorium pharaonis* (Mpha), *A. colombica* (Acol), *Trachymyrmex_septentrionalis* (Tsep), *Camponotus floridanus* (Cflo), *Formica exsecta* (Fexs). The tree was constructed in MEGA5.2 using the neighbour-joining method.

transduction events, such as OBPs, CSPs, ORs, IRs and SNMPs. In this study, the antennal transcriptomic data sets of *C. rogenhoferi* were explored. We obtained more than 8.57 GB of clean reads, which were assembled into 53,892 unigenes. We identified four OBPs, three CSPs, 37 ORs, two IRs and three SNMPs, and the chemosensory genes in *C. rogenhoferi* were investigated first time. Our findings provide a basis for elucidating the molecular mechanisms of olfactory-related behaviours in ants.

Insects use both OBPs and CSPs to facilitate the transport of odourant molecules through the sensillar lymph and serve as the liaison between the external environment and ORs (Calvello *et al.*, 2005; Gong *et al.*, 2009; Gu *et al.*, 2011). These two protein families are thought to be the first interactants with odourant semiochemicals and pose an important discriminatory filter during the perception of chemosensory stimuli (Pelosi *et al.*, 2014). Some studies have revealed that most insect genomes contain 40–55 OBPs and 4–8 CSPs. The first ant OBP, gp-9/SinvObp3, was identified in the fire ant *S. Invicta* (Krieger and Ross, 2002). Then, a single antenna-specific CSP was identified in the Argentine ant *Linepithema humile* (Ishida *et al.*, 2002). The number of OBP and CSP genes identified in this study was lower than the number found in other ants, such as *S. invicta* (17 OBPs, 21 CSPs), *Camponotus floridanus* (13 OBPs, 13 CSPs) and *P. barbatus* (16 OBPs, 11 CSPs) (McKenzie *et al.*, 2014). The differences in the number of OBP and CSP genes may be due to the tissue used for sequencing. Some OBPs or CSPs can be expressed in other non-chemosensory tissues, such as the thorax, legs and wing, as well as in the antenna. In addition, the sequencing depth of different sequencing platforms influences the number of OBP genes identified (Gu *et al.*, 2015). The feature of conserved cysteines (Cys) is regarded as a signature for insect OBPs (Pelosi *et al.*, 2006). Based on the number of cysteines (Cys), all four CrogOBPs are classic OBPs with six Cys residues, consistent with the C_1-X_{15-39}-C_2-X_3-C_3-X_{21-44}-C_4-X_{7-12}-C_5-X_8-C_6 structural mode, which is considered to form interlocked disulfide bonds that stabilise the three-dimensional structure of the OBP (Zhou 2010; Vieira and Rozas 2011; Sun *et al.*, 2016). CSPs are more highly conserved than OBPs and are characterised by four cysteines that form two disulfide bridges (Pelosi *et al.*, 2006). Sequence analysis revealed that CrogCSPs have four conserved cysteine residues, in agreement with the strict structural characteristic model of C_1-X_6-C_2-X_{18}-C_3-X_2-C_4 of the CSP family. OBPs and CSPs evolved dynamically between insect orders, while they appear to be more conserved at lower taxonomic levels, such as in the wasp infraorder Aculeata, which contains ants (McKenzie *et al.*, 2014). Some studies have speculated that CSPs have replaced OBPs in ant olfaction, and recent studies have indicated that OBPs and CSPs primarily fulfil important conserved roles in ant olfaction rather than dynamically evolving to recognise species-specific odourants (McKenzie *et al.*, 2014). More experiments are required to explore their functions in this process.

Insect SNMPs, a type of membrane proteins, are homologous genes to human CD36 receptors (Rogers *et al.*, 2001). This olfactory-specific membrane proteins are expressed at different sites in insect antennal sensilla (Rogers *et al.*, 1997, 2001; Forstner *et al.*, 2008; Zhang *et al.*, 2015). SNMP1 is usually expressed in neuron cells that respond to pheromones, whereas SNMP2 is usually expressed in supporting cells (Forstner *et al.*, 2008). CrogSNMP1, CrogSNMP2, and CrogSNMP3 identified in this study clearly belong to different phylogenetic groups, which shows that the function of CrogSNMP1 is similar to that of *S. invicta* and *P. barbatus*. CrogSNMP2 and CrogSNMP3 have similar functions to proteins in *C. pipiens* and *Drosophila*.

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Table 3. Sequence information of ORs and IRs genes in *C. rogenhoferi*.

Unigene	Gene name	ORF	TMD	Full length	Name	Species	Acc. no.	*E*-value	Identity
c23886.graph_c0	CrogOR1	384	6	Yes	PREDICTED: odourant receptor 13a-like	*Vollenhovia emeryi*	XP_011880387.1	0	86
c21111.graph_c0	CrogOR2	338	4	No	PREDICTED: odourant receptor 22c-like	*Vollenhovia emeryi*	XP_011882711.1	0	84
c12215.graph_c0	CrogOR3	218	4	Yes	PREDICTED: odourant receptor 13a-like	*Vollenhovia emeryi*	XP_011878584.1	1.00×10^{-134}	89
c21670.graph_c0	CrogOR4	310	5	No	PREDICTED: odourant receptor 13a-like	*Vollenhovia emeryi*	XP_011880206.1	0	88
c16862.graph_c0	CrogOR5	396	3	No	PREDICTED: odourant receptor 13a-like	*Vollenhovia emeryi*	XP_011875306.1	0	81
c22200.graph_c0	CrogOR6	336	6	No	PREDICTED: odourant receptor 43a-like	*Vollenhovia emeryi*	XP_011861770.1	0	78
c24712.graph_c0	CrogOR7	419	6	No	PREDICTED: odourant receptor 43a-like isoform X1	*Vollenhovia emeryi*	XP_011861748.1	0	78
c16674.graph_c0	CrogOR8	382	6	No	PREDICTED: odourant receptor 46a,isoform A-like	*Atta cephalotes*	XP_012059522.1	0	83
c26353.graph_c0	CrogOR9	359	6	Yes	PREDICTED: odourant receptor Or2-like	*Atta cephalotes*	XP_012059523.1	1.00×10^{-167}	76
c12160.graph_c0	CrogOR10	404	6	Yes	PREDICTED: odourant receptor 49b-like	*Vollenhovia emeryi*	XP_011880220.1	0	93
c18157.graph_c0	CrogOR11	403	3	Yes	Odourant receptor 49b-like	*Temnothorax curvispinosus*	XP_024880487.1	0	86
c11184.graph_c0	CrogOR12	386	7	Yes	PREDICTED: odourant receptor 49b-like isoform X2	*Vollenhovia emeryi*	XP_011860058.1	0	72
c16269.graph_c0	CrogOR13	301	4	Yes	PREDICTED: odourant receptor 49b-like isoform X2	*Vollenhovia emeryi*	XP_011861183.1	2.00×10^{-125}	73
c22996.graph_c0	CrogOR14	303	6	Yes	PREDICTED: odourant receptor 4-like	*Trachymyrmex cornetzi*	XP_018374987.1	0	83
c23612.graph_c0	CrogOR15	342	6	Yes	PREDICTED: odourant receptor 4-like	*Vollenhovia emeryi*	XP_011867088.1	0	85
c24782.graph_c0	CrogOR16	439	8	Yes	PREDICTED: odourant receptor 4-like isoform X1	*Vollenhovia emeryi*	XP_011874600.1	0	76
c19888.graph_c0	CrogOR17	397	5	Yes	PREDICTED: odourant receptor 4-like isoform X3	*Vollenhovia emeryi*	XP_011878580.1	0	85
c27026.graph_c0	CrogOR18	403	6	Yes	Odourant receptor 10a-like	*Temnothorax curvispinosus*	XP_024886548.1	0	77
c22135.graph_c0	CrogOR19	292	5	Yes	PREDICTED: odourant receptor 67a-like isoform X2	*Vollenhovia emeryi*	XP_011878586.1	1.00×10^{-164}	72
c26848.graph_c0	CrogOR20	377	6	Yes	PREDICTED: odourant receptor 67c-like	*Vollenhovia emeryi*	XP_011862412.1	0	90
c25939.graph_c1	CrogOR21	312	4	No	Odourant receptor 82a-like	*Temnothorax curvispinosus*	XP_024869588.1	3.00×10^{-178}	84
c25088.graph_c0	CrogOR22	338	4	Yes	Odourant receptor 82a-like	*Temnothorax curvispinosus*	XP_024873896.1	0	85
c22845.graph_c0	CrogOR23	259	3	Yes	Odourant receptor 83a-like	*Temnothorax curvispinosus*	XP_024869573.1	3.00×10^{-167}	90
c22195.graph_c0	CrogOR24	354	5	Yes	PREDICTED: odourant receptor 85f-like isoform X1	*Vollenhovia emeryi*	XP_011876331.1	0	90
c21913.graph_c0	CrogOR25	405	6	No	PREDICTED: odourant receptor 94a-like	*Vollenhovia emeryi*	XP_011878857.1	0	88
c22515.graph_c0	CrogOR26	401	8	Yes	PREDICTED: odourant receptor 98a-like	*Vollenhovia emeryi*	XP_011878861.1	0	84
c22994.graph_c0	CrogOR27	481	7	Yes	Odourant receptor coreceptor	*Pogonomyrmex barbatus*	XP_011632710.1	0	97
c24091.graph_c0	CrogOR28	370	5	Yes	Odourant receptor 67a-like	*Monomorium pharaonis*	XP_028050636.1	0	84
c25254.graph_c1	CrogOR29	336	7	No	PREDICTED: odourant receptor Or1-like	*Vollenhovia emeryi*	XP_011882829.1	5.00×10^{-173}	80
c23044.graph_c0	CrogOR30	425	6	No	Odourant receptor Or2-like	*Temnothorax curvispinosus*	XP_024875354.1	0	86
c15540.graph_c0	CrogOR31	402	7	No	PREDICTED: odourant receptor Or2-like	*Vollenhovia emeryi*	XP_011861190.1	0	83
c21178.graph_c0	CrogOR32	402	6	No	Odourant receptor 13a-like	*Temnothorax curvispinosus*	XP_024876941.1	0	88
c24651.graph_c0	CrogOR33	202	3	No	Odourant receptor 13a-like	*Temnothorax curvispinosus*	XP_024883416.1	8.00×10^{-111}	72
c23530.graph_c0	CrogOR34	409	6	Yes	Odourant receptor 82a-like	*Temnothorax curvispinosus*	XP_024876932.1	0	83
c24896.graph_c0	CrogOR35	454	7	No	Odourant receptor 82a-like	*Temnothorax curvispinosus*	XP_024882437.1	0	73
c21714.graph_c0	CrogOR36	446	7	No	Odourant receptor Or1-like	*Temnothorax curvispinosus*	XP_024869344.1	0	85
c26160.graph_c0	CrogOR37	288	5	Yes	Odourant receptor 85b-like isoform X1	*Temnothorax curvispinosus*	XP_024871330.1	8.00×10^{-145}	74
c24161.graph_c0	CrogIR1	897	4	Yes	Ionotropic receptor 25a	*Temnothorax curvispinosus*	XP_024872632.1	0	93
c18801.graph_c0	CrogIR2	935	3	Yes	Ionotropic receptor 25a isoform X1	*Temnothorax curvispinosus*	XP_024885188.1	0	90

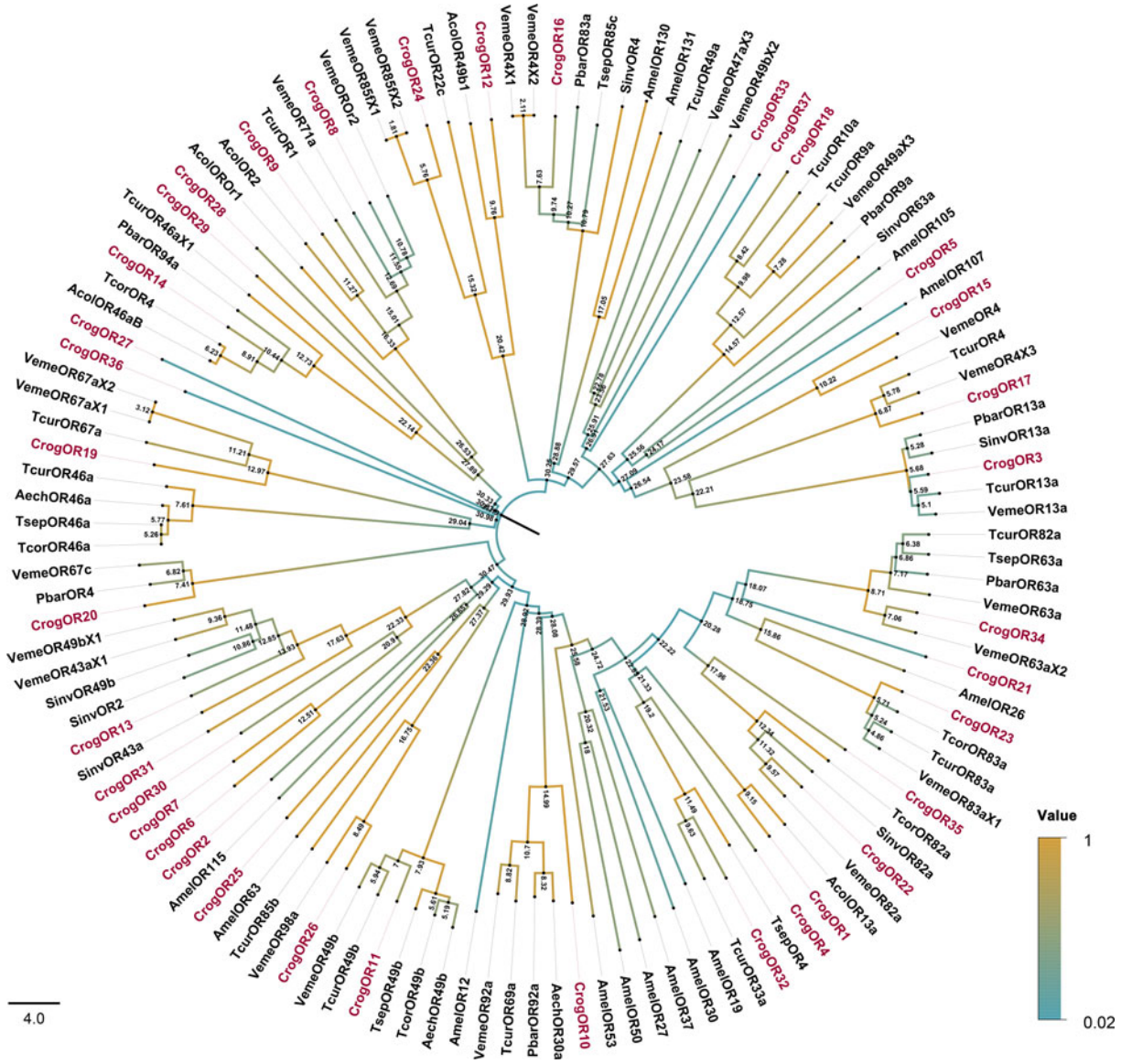


Figure 7. Phylogenetic relationships of candidate ORs. *A. colombica* (Acol), *A. echinatior* (Aech), *A. mellifera* (Amel), *P. barbatus* (Pbar), *S. invicta* (Sinv), *Trachymyrmex cornetzi* (Tcor), *T. septentrionalis* (Tsep), *Vollenhovia emeryi* (Veme). The tree was constructed in MEGA5.2 using the neighbour-joining method.

ORs are crucial for insects to detect general odours and sex pheromones (Leal, 2013). A large number of ORs have been reported in other ants based on genome studies, such as the red imported fire ant *S. invicta* (259 ORs), Florida carpenter ant *C. floridanus* (352 ORs), red harvester ant *P. barbatus* (344 ORs) and Argentine ant *L. humile* (337 ORs). The abundance of ORs may play a key role in olfactory codes to allow ants to detect and discriminate complex combinatorial odourant stimuli (Engsontia *et al.*, 2015). In our study, a total of 37 ORs were identified based on the transcriptome of *C. rogenhoferi*, which was much less than that of other ants. ORs expressed in other tissues or at low expression levels might be difficult to identify (Poivet *et al.*, 2013). CrogOR27 is hypothesised to function as a coreceptor, and homology analysis showed that CrogOR27 is highly conserved with Orcos of other ant species. They may form functional heteromeric complexes with other ORs, such as ORco in OR/ORco complexes (Abuin *et al.*, 2011). In addition, we obtained 2 *CrogIRs* from the transcriptome data. Usually, IRs are expressed in sensory neurons in a combinatorial fashion that responds to many distinct odours (Benton *et al.*, 2009). Homology analysis revealed that CrogIR1 and CrogIR2 cluster with IRs from Myrmicinae into a subfamily, indicating that these two genes are probably involved in the chemical sensing system of *C. rogenhoferi*.

In summary, we obtained comprehensive information on the *C. rogenhoferi* transcriptome by using RNA-seq analysis. Major genes related to chemoreception were identified, including four OBPs, three CSPs, 37 ORs, two IRs and three SNMPs. The findings provide a basis for elucidating the molecular mechanisms of the olfactory-related behaviours of *C. rogenhoferi*, which would facilitate a better application of *C. rogenhoferi* as a biological control agent.

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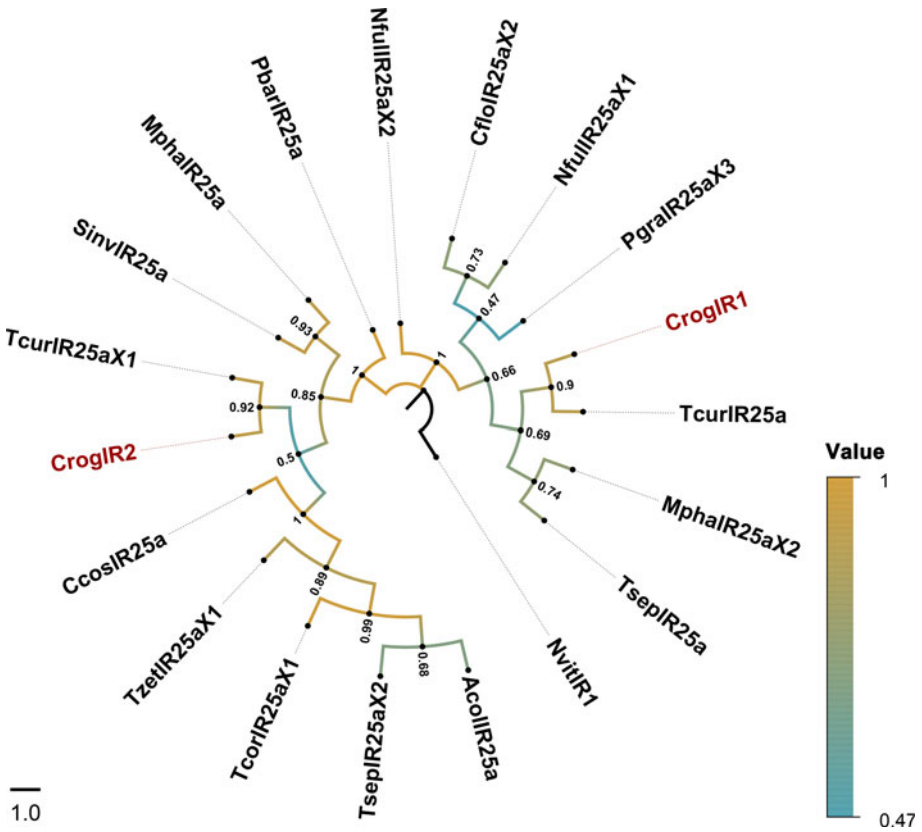


Figure 8. Phylogenetic relationships of candidate IRs. *T. curvispinosus* (Tcur), *M. pharaonis* (Mpha), *T. septentrionalis* (Tsep), *Nasonia vitripennis* (Nvit), *A. colombica* (Acol), *T. cornetzi* (Tcor), *Trachymyrmex zeteki* (Tzet), *Cyphomyrmex costatus* (Ccos), *S. invicta* (Sinv), *P. barbatus* (Pbar), *Nylanderia fulva (Nful), Camponotus_floridanus (Cflo), Pseudomyrmex gracilis(Pgra).* The tree was constructed in MEGA5.2 using the neighbour-joining method.

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Conflict of interest. The authors have no conflicts of interest to declare.

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