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Identification and Expression Analysis of an Olfactory Receptor Gene Family in the *Yemma signatus* Hsiao (Hemiptera: Berytidae)

Yue Qin Song, Hui Zhong Sun* and Jun Feng Dong

Forestry College, Henan University of Science and Technology, Luoyang 471000, China

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SYQ and SHZ designed and performed the research, analyzed the data, and wrote the paper. All authors were involved in writing and revisions of the manuscript.

Key words
***Yemma signatus*, Antennal transcriptome, Olfactory receptor family, Expression patterns.**

ABSTRACT

Olfactory receptors (ORs) in the dendritic membrane of olfactory cells are the key elements in the molecular recognition and discrimination of odorants. On the basis of female and male antennal transcriptomes of *Yemma signatus* adults, a total of 66 candidate *Y. signatus* olfactory receptor genes (YsigORs), including one olfactory co-receptor (Orco), were identified in this study. All the sequences were further validated by cloning and sequencing. Tissue expression profiles of all YsigOR genes in the antennae of females and males were analyzed using real-time quantitative PCR (RT-qPCR). The result showed that some YsigOR genes displayed significant differences in the expression levels between sexes. YsigOrco had the highest expression level in all YsigOR genes; however, the expression level in males was twice as that of females. Our study provides valuable biological information for studying the olfactory communication system of *Y. signatus*.

INTRODUCTION

The olfactory system plays a crucial role in most insects in the detection and discrimination between small, volatile compounds in the environment. The ability to sensitively and specifically recognize odors is crucial for their survival as these chemical signals are important for avoiding predators and can provide essential information about the sources of food, mating, and oviposition (Field *et al*., 2000; Zhang *et al*., 2015). Receptor proteins in the dendritic membrane of olfactory cells are the key elements in molecular recognition of odorants. These receptor proteins include three large, distinct families: olfactory receptors (ORs), gustatory receptors (GRs), and ionotropic receptors (IRs) (Clyne *et al*., 1999, 2000; Benton *et al*., 2009).

Insect OR genes were the first chemoreceptor family to be found in the *Drosophila melanogaster* genome (Gao *et al*., 1999). Unlike vertebrate ORs, which are G-protein coupled receptors (GPCRs), these ORs are seven transmembrane domain (TMD) receptors with an inverted membrane topology containing an intracellular N-terminus and an extracellular C-terminus (Benton *et al*., 2006; Lundin *et al*., 2007; Smart *et al*., 2008). These proteins are specifically expressed in the olfactory sensory neurons (OSNs) of the antennae and maxillary palps of the insect, where they are concentrated in the sensory dendrites of the cells. Subsequently, the OR genes of other insects have also been sequenced and identified using bioinformatics methods. A specific ligand-binding OR type that forms a heteromer with a second common co-receptor from the OR gene family has been reported (Benton *et al*., 2006). Formerly, it was named Or83b in *Drosophila* and OR2 or OR7 in other insects. According to the most recent nomenclature, this protein is referred to as Orco. The number of OR genes in different insect species varies greatly, from 10 in *Pediculus humanus* (Kirkness *et al*., 2010) to 400 in *Pogonomyrmex barbatus* (Smith *et al*., 2011). It is speculated that these variations in the number of OR genes among species reflects evolutionary adaption to certain ecological and physiological demands in the search for food or the major importance of odorants in social communication between insects living in colonies. In recent years, many Lepidopteran OR genes were explored using the Xenopus oocyte expression system (Mitsuno *et al*., 2008). However, to date, the exact functions of insect OR genes are largely unknown.

Yemma signatus (Hsiao) (Hemiptera: Berytidae) is an identified omnivorous insect that feeds on plants and small insects. It was recorded in China as a pest that sucks juices out of *Paulownia* tree leaves (Yang, 1982). Interestingly, *Y. signatus* is also considered beneficial as it feeds on small insects, such as Cicadellidae insects, that damage fruit trees (Liang *et al*., 1992). Currently, little molecular information is available on *Y. signatus*. In this study, we sought to identify and annotate olfactory receptor genes

* Corresponding author: huizhong66@163.com
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in *Y. signatus* antennae using *de novo* transcriptome sequencing and assembly. Next, tissue expression profiles of all YsigOR genes in the antennae of females and males were analyzed by real-time quantitative PCR (RT-qPCR). The present study provides bases for the functional study of ORs of *Y. signatus*.

MATERIALS AND METHODS

Insects and sample collection

The laboratory strain of *Y. signatus* was collected from cotton in Luoyang, Henan, China (112-26′E, 34-43′N) in 2014 and reared on cotton plants in a greenhouse at 27 ± 3°C with a 14 h:10 h light/dark cycle and 60%–80% relative humidity. Approximately 500 female and 500 male adult antennae were respectively dissected from *Y. signatus* (3–4 days old), immediately frozen in liquid nitrogen, and stored at −80°C until RNA isolation.

cDNA library construction and sequencing

Total RNA from the antennal tissue was extracted using RNAiso Plus kit (TaKaRa, Dalian, China) and treated with DNase I (TaKaRa, Dalian, China) following the manufacturer's instructions. The concentration, quality, quantity, and integrity of the RNA sample were detected using agarose gel electrophoresis, Nanodrop (Thermo Scientific, USA), Qubit 2.0 (Life Technologies, USA), and Agilent 2100 (Agilent, USA). The antennal RNAs from female and male adults were mixed in a 1:1 ratio to conduct transcriptome sequencing.

Following the TruSeq RNA Sample Preparation Guide v2 (Illumina), mRNA was enriched using magnetic beads crosslinked with oligo (dT) and was fragmented into small pieces using the fragmentation buffer. First-strand cDNA was synthesized using small mRNA fragments, random primers, and reverse transcriptase, and second-strand cDNA synthesis was conducted by adding dNTPs, DNA polymerase I, and RNase H. Next, the double-stranded cDNA was purified with AMPure XP beads (Beckman Coulter, USA) and then treated for end-repairing, poly-A tailing, and sequencing adapter linking processes. The size of the fragment was chosen using AMPure XP beads, and the cDNA library was constructed by PCR amplification (Veriti® 96-Well Thermal Cycle, Applied Biosystems, USA). The concentration and insert size of cDNA library were detected using Qubit 2.0 and Agilent 2100, respectively, and the DNA was quantified with q-PCR (CFX-96, Bio-Rad, USA).

Finally, sequencing was performed in Illumina HiSeq™ 2500 platform to generate 125-bp pair-end reads. Sequencing analysis was performed by the Genomics Services Lab of the Beijing Novogene Technologies Co., Ltd. (Beijing, China). Raw data processing and base calling were performed using the Illumina instrument software.

De novo *contig assembly and unigene annotation*

Clean reads were obtained by removing short or low quality and adaptor sequences. The transcriptome was assembled using Trinity (version: trinityrnaseq_r20131110) using default settings, except for setting min_kmer_cov to 2 (Grabherr *et al*., 2011). Unigene function was annotated based on searches against seven databases: NCBI non-redundant protein sequences (Nr, NCBI blast 2.2.28+, e-value = 1e-5), NCBI nucleotide sequences (Nt, NCBI blast 2.2.28+, e-value = 1e-5), protein family (Pfam, HMMER 3.0 package, hmmscan, e-value = 0.01), euKaryotic Ortholog Groups (KOG, NCBI blast 2.2.28+, e-value = 1e-3), Swiss-Prot (NCBI blast 2.2.28+, e-value = 1e-5), Kyoto Encyclopedia of Genes and Genomes (KEGG, KAAS, KEGG Automatic Annotation Server, e-value = 1e-10), and Gene Ontology (GO, Blast2GO v2.5, e-value = 1e-6). Coding sequences (CDS) were predicted by aligning transcriptome sequences to the Nr and Swiss-Prot databases or using ESTScan 3.0.3 (Iseli *et al.*, 1999). The read count of each gene was obtained by mapping clean reads back onto the assembled transcriptome using RSEM software (bowtie2 parameters: mismatch 0). Lastly, the read count was calculated as fragments per kilobase of transcript per million fragments mapped (FPKM).

Bioinformatics analyses

Similarity searches were performed with the NCBI Blast network server (http://blast.ncbi.nlm.nih.gov/). The transmembrane domains (TMDs) of ORs were predicted using TMHMM v. 2.0 (http://www.cbs.dtu.dk/services/TMHMM-2.0/). The amino acid sequence alignments of the candidate ORs were aligned using MAFFT (http://mafft.cbrc.jp/alignment/server/clustering.html), and phylogenetic trees were constructed using PhyML in Seaview v.4 based on the Jones–Taylor–Thomton (JTT) model with 1000-fold bootstrap replication in neighbor-joining method.

Tissue-specific expression of ORs

All total RNA samples were extracted using the RNAiso Plus kit (TaKaRa, Dalian, China), and the isolated RNA was transcribed to first-strand cDNA by PrimeScript™ RT reagent Kit with gDNA Eraser (TaKaRa, Dalian, China) following the manufacturer's instructions. The nucleotide sequences of all 66 YsigORs were confirmed by cloning and sequencing. Real-time quantitative PCRs (RT-qPCRs) were performed with SYBR® Premix Ex Taq™ II (TaKaRa, Dalian, China). The *Y. signatus* β-actin gene was used as endogenous

control correct for sample-to-sample variation. A 200-ng/l concentration cDNA sample was used for different tissues. Primers for RT-qPCR were designed using Primer Premier 5.0 software and are listed in Supplementary Table I. The RT-qPCR reactions were conducted in 20-μL reaction mixtures containing 10-μL SYBR Premix Ex Taq II, 20-ng cDNA templates, 0.2-μM of each primer, and nuclease-free water. The cycling conditions were as follows: one cycle of 95°C for 5 min, followed by 40 cycles of 95°C for 5 s and 55°C for 30 s. Melting curve conditions were 95°C for 10 s and 65°C for 30 s. A no-template control (NTC) was also included to detect for possible contamination. Three biological replicates were analyzed and relative expression levels of OR genes across the samples were measured by the $2^{-\Delta\Delta CT}$ method. Expression levels were calculated relative to the expression level in male antennae of YsigOR56, which was arbitrarily set at 1 (Xu *et al.*, 2019). The differences in the expression of YsigOR genes between tissues of females and males were compared by a one-way nested analysis of variance (ANOVA), followed by Tukey's honestly significance difference (HSD) test using SPSS software (SPSS Institute 17.0, IBM, Chicago, IL, USA).

RESULTS

Analysis of Y. signatus *antennae transcriptome*

To identify candidate OR genes from *Y. signatus*, the transcriptomes of the antennae of males and females were generated using the HiSeq 2500 platform. A total of 62530320 raw reads were produced from the female and male antennae mixture sample, and after filtering, 61080938 clean reads were assembled into 115491 (mean length, 578 bp) unigenes. All sequences from *Y. signatus* antennal transcriptome were registered in the NCBI database (GenBank: SRR3348966). The assembly of all clean reads together led to the generation of 148736 transcripts with a mean length of 687 bp (Supplementary Table II). BLASTx and BLASTn homology searches of all 115491 unigenes with an E-value < 1.0E-5 showed that 32842 unigenes (28.43%) had BLASTx hits in the Nr databases and 14986 (12.97%) had BLASTn hits in the Nt databases (Supplementary Table III).

GO assignments were used to functionally classify the predicted proteins. Of all the unigenes, 32216 (27.89%) could be classified into three functional categories: molecular function, biological process, and cellular component (Fig. 1). In molecular function category, the genes expressed in the antennae were mostly linked to binding (16903/52.47% unigenes) and catalytic activity (14742/45.76% unigenes). In terms of the biological process, the most represented biological processes were cellular processes (17,197/21.23% unigenes), metabolic processes (17756/22.98% unigenes), and the single-organism process (13552/17.54% unigenes). Among the cellular component terms, cell (9238/19.77% unigenes) and cell part (9237/19.76% unigenes) constituted the most abundant categories.

Identification of Y. signatus *ORs*

A total of 66 different sequences that encode candidate OR genes were identified, and six of the 66 analyzed candidate genes were partial sequences of genes. They were named YsigOR1 to YsigOR65, and one of the genes was named YsigOrco. The 58 full-length ORs had ORFs measuring about 1200 bp with 4–8 predicted transmembrane domains. We searched for homology of the OR sequences using BLASTx and found that the

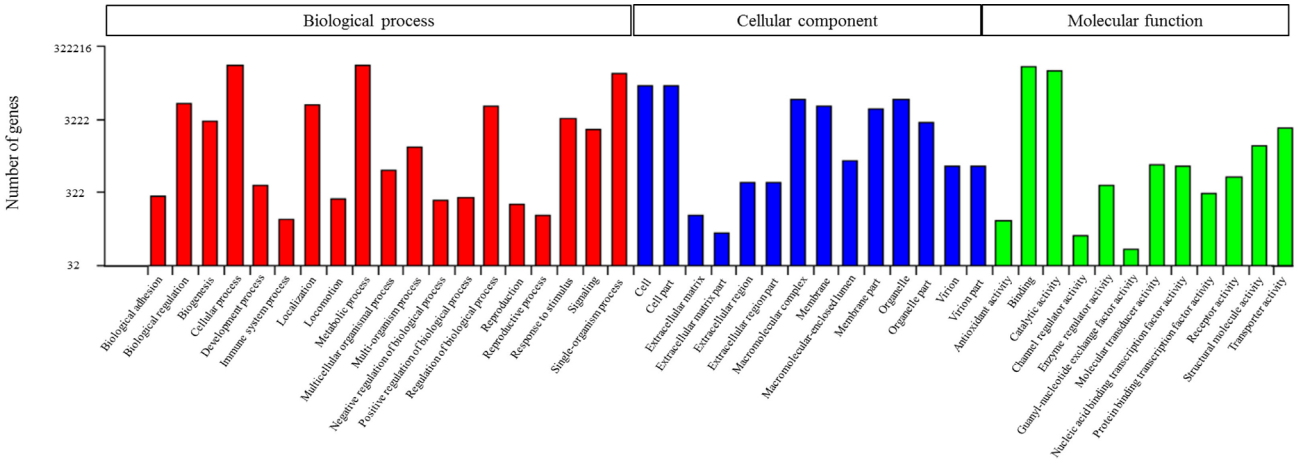

Fig. 1. Gene ontology (GO) classification of *Yemma signatus* antennal unigenes according to their involvement in biological processes, cellular components, and molecular functions.

amino acid sequences of candidate ORs had high sequence conservation with ORs from *Halyomorpha halys*, followed by *Cimex lectularius*. The Orco sequence of *Y. signatus* had very high nucleotide identity (92%) with *H. halys* Orco, which is often the only one conserved olfactory co-receptor in most insect species (Table I).

Phylogenetic analysis of YsigOR sequences

To better understand the relationship between the different OR genes that were identified in the transcriptome, we conducted a phylogenetic analysis of the 66 candidate YsigOR genes along with OR sequences from Hemipteran insects, *Apolygus lucorum*, *H. halys*, *Acyrthosiphon pisum*, *Nilaparvata lugens*, and *C. lectularius*. In the phylogenetic tree, OR genes were extremely divergent and formed various clades, indicating their distinct function in responding to different odors. By contrast, YsigOrco clustered with Orco sequences of different insect species and formed a clear orthologous lineage due to high sequence similarity (Fig. 2).

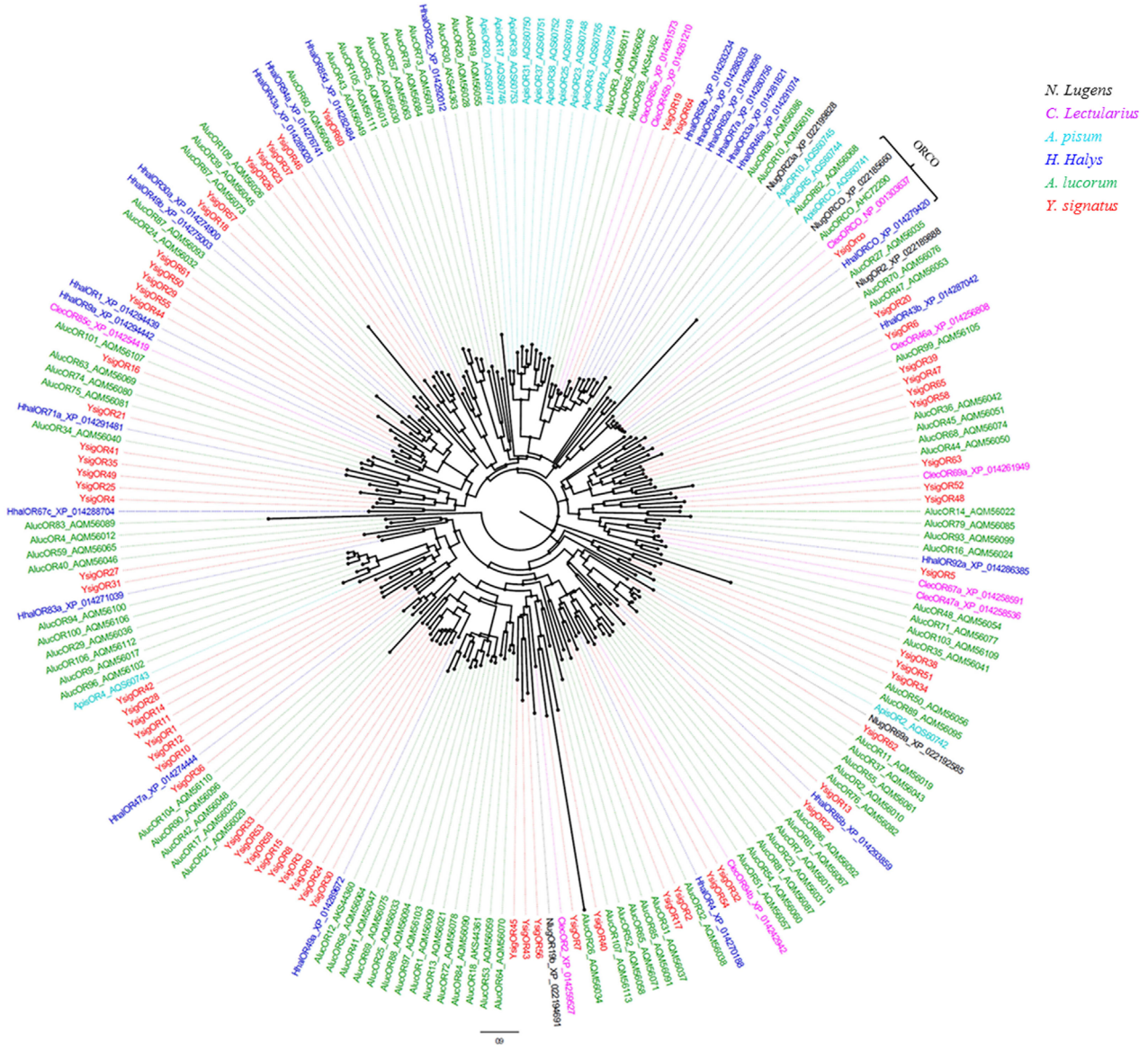

Fig. 2. A phylogenetic tree based on protein sequences of candidate ORs from *Yemma signatus* (Ysig). Included are ORs from *Apolygus lucorum* (Aluc), *Halyomorpha halys* (Hhal), *Acyrthosiphon pisum* (Apis), *Nilaparvata lugens* (Nlug), and *Cimex lectularius* (Clec). The branch containing Orco is marked with a brace. This tree was constructed using PhyML based on the alignment results of MAFFT.

Table I.- Sequences information of ORs in *Yemma signatus.*

Gene name	Accesion No.	ORF (aa)	BLASTx best hit (Reference/Name/Species)	E-value identity	Identity	Full length	TM (No.)
Olfactory co-receptor							
YsigOrco	MG2046701	474	ref|XP_014279419.1| odorant receptor coreceptor isoformX1 [*Halyomorpha halys*]	0.0	92%	Yes	7
Other olfactory receptors							
YsigOR1	MG204636	413	ref|XP_014271039.1| odorant receptor 83a-like [*Halyomorpha halys*]	8e-30	25%	Yes	6
YsigOR2	MG204637	410	ref|XP_014287492.1| odorant receptor 4-like [*Halyomorpha halys*]	8e-63	33%	Yes	6
YsigOR3	MG204638	434	ref|XP_014289672.1| odorant receptor 49a-like [*Halyomorpha halys*]	7e-68	35%	Yes	6
YsigOR4	MG204639	380	ref|XP_014288704.1| odorant receptor 67c-like isoform X1 [*Halyomorpha halys*]	8e-32	30%	Yes	6
YsigOR5	MG204640	376	ref|XP_014286385.1| putative odorant receptor 92a [*Halyomorpha halys*]	1e-55	33%	Yes	4
YsigOR6	MG204641	381	gb|XP_014257038| odorant receptor Or2-like isoform X2 [*Cimex lectularius*]	2e-47	30%	Yes	6
YsigOR7	MG204642	354	gb|KPJ01705.1| Putative odorant receptor 30a [*Papilio xuthus*]	2e-08	26%	Yes	4
YsigOR8	MG204643	437	ref|XP_014289672.1| odorant receptor 49a-like [*Halyomorpha halys*]	2e-60	35%	Yes	6
YsigOR9	MG204644	434	ref|XP_014289672.1| odorant receptor 49a-like [*Halyomorpha halys*]	1e-58	34%	Yes	6
YsigOR10	MG204645	415	ref|XP_014271039.1| odorant receptor 83a-like [*Halyomorpha halys*]	1e-64	32%	Yes	6
YsigOR11	MG204646	417	ref|XP_014274444.1| odorant receptor 47a-like [*Halyomorpha halys*]	2e-19	25%	Yes	7
YsigOR12	MG204647	429	ref|XP_014271039.1| odorant receptor 83a-like [*Halyomorpha halys*]	3e-53	31%	Yes	7
YsigOR13	MG204648	392	ref|XP_014273330.1| odorant receptor 85b-like [*Halyomorpha halys*]	4e-118	47%	Yes	6
YsigOR14	MG204649	418	ref|XP_014271039.1| odorant receptor 83a-like [*Halyomorpha halys*]	6e-38	26%	Yes	5
YsigOR15	MG204650	230	ref|XP_014289672.1| odorant receptor49a-like [*Halyomorpha halys*]	1e-46	38%	No (5′ lose)	3
YsigOR16	MG204651	379	ref|XP_014249919.1|putative odorant receptor 69a, isoform A [*Cimex lectularius*]	2e-17	26%	Yes	4
YsigOR17	MG204652	416	ref|XP_014287492.1| odorant receptor 4-like [*Halyomorpha halys*]	2e-73	35%	Yes	6
YsigOR18	MG204653	385	ref|XP_014274900.1| odorant receptor 30a-like [*Halyomorpha halys*]	5e-39	27%	Yes	6
YsigOR19	MG204654	364	ref|XP_014261210.1| odorant receptor 45b-like [*Cimex lectularius*]	1e-85	41%	Yes	3
YsigOR20	MG204655	391	ref|XP_014287040.1| odorant receptor 4-like [*Halyomorpha halys*]	7e-81	36%	Yes	6
YsigOR21	MG204656	404	ref|XP_014242040.1| odorant receptor 85b-like [*Cimex lectularius*]	2e-67	35%	No (3′ lose)	4
YsigOR22	MG204657	398	ref|XP_014273330.1| odorant receptor 85b-like [*Halyomorpha halys*]	3e-32	27%	Yes	5
YsigOR23	MG204658	407	ref|XP_014275988.1| odorant receptor 22c-like [*Halyomorpha halys*]	1e-37	26%	Yes	5
YsigOR24	MG204659	447	ref|XP_014289672.1| odorant receptor 49a-like [*Halyomorpha halys*]	2e-59	32%	Yes	6
YsigOR25	MG204660	379	ref|XP_014282544.1| odorant receptor 4-like [*Halyomorpha halys*]	4e-72	36%	Yes	6
YsigOR26	MG204661	231	ref|XP_014276741.1|odorantreceptor94a-like [*Halyomorpha halys*]	5e-31	36%	No (5′ lose)	2
YsigOR27	MG204662	225	gb|AIG51873.1| odorant receptor [Helicoverpa armigera]	4e-10	27%	No (5′ lose)	2
YsigOR28	MG204663	426	ref|XP_014271039.1| odorant receptor 83a-like [*Halyomorpha halys*]	1e-30	27%	Yes	6
YsigOR29	MG204664	383	ref|XP_014294439.1| odorant receptor Or1-like isoform X1 [*Halyomorpha halys*]	1e-52	30%	Yes	4
YsigOR30	MG204665	373	ref|XP_014289672.1| odorant receptor 49a-like [*Halyomorpha halys*]	4e-59	33%	Yes	5
YsigOR31	MG204666	422	ref|XP_014294765.1| odorant receptor 4-like [*Halyomorpha halys*]	9e-47	28%	Yes	5
YsigOR32	MG204667	431	ref|XP_014242937.1| odorant receptor Or2-like [*Cimex lectularius*]	3e-41	27%	Yes	7
YsigOR33	MG204668	437	ref|XP_014276985.1| odorant receptor 22c-like [*Halyomorpha halys*]	5e-59	32%	Yes	7
YsigOR34	MG204669	414	ref|XP_014281005.1| odorant receptor 4-like [*Halyomorpha halys*]	5e-59	33%	Yes	6
YsigOR35	MG204670	397	ref|XP_014292083.1| odorant receptor 85b-like [*Halyomorpha halys*]	3e-66	32%	Yes	6

Gene name	Accesion No.	ORF (aa)	BLASTx best hit (Reference/Name/Species)	E-value identity	Identity	Full length	TM (No.)
YsigOR36	MG204671	427	ref|XP_014274444.1| odorant receptor 47a-like [*Halyomorpha halys*]	5e-121	43%	Yes	5
YsigOR37	MG204672	402	ref|XP_014276741.1| odorant receptor 94a-like [*Halyomorpha halys*]	2e-42	29%	Yes	5
YsigOR38	MG204673	401	ref|XP_014281005.1| odorant receptor 4-like [*Halyomorpha halys*]	3e-24	26%	Yes	6
YsigOR39	MG204674	227	ref|XP_014257038.1| odorant receptor Or2-like isoform X2 [*Cimex lectularius*]	1e-19	27%	No (5′ lose)	3
YsigOR40	MG204675	282	ref|XP_014289672.1| odorantreceptor49a-like [*Halyomorpha halys*]	6e-09	26%	Yes	4
YsigOR41	MG204676	404	ref|XP_014292083.1| odorant receptor 85b-like [*Halyomorpha halys*]	9e-150	53%	Yes	5
YsigOR42	MG204677	427	ref|XP_014271039.1| odorant receptor 83a-like [*Halyomorpha halys*]	6e-34	27%	Yes	5
YsigOR43	MG204678	224	ref|XP_014282386.1| odorant receptor 49a-like isoform X1 [*Halyomorpha halys*]	1e-15	30%	No (5′ lose)	3
YsigOR44	MG204679	388	ref|XP_014294439.1| odorant receptor Or1-like isoform X1 [*Halyomorpha halys*]	2e-87	40%	Yes	6
YsigOR45	MG204680	444	ref|XP_014282386.1| odorant receptor 49a-like isoform X1 [*Halyomorpha halys*]	7e-08	27%	Yes	6
YsigOR46	MG204681	402	ref|XP_014275988.1| odorant receptor 22c-like [*Halyomorpha halys*]	3e-68	34%	Yes	8
YsigOR47	MG204682	392	ref|XP_014257038.1| odorant receptor Or2-likeisoformX2 [*Cimex lectularius*]	6e-24	24%	Yes	6
YsigOR48	MG204683	380	ref|XP_014287367.1| odorant receptor 85b-like [*Halyomorpha halys*]	1e-53	33%	Yes	4
YsigOR49	MG204684	375	ref|XP_014282544.1| odorant receptor 4-like [*Halyomorpha halys*]	3e-99	46%	Yes	6
YsigOR50	MG204685	396	ref|XP_014274900.1| odorant receptor 30a-like [*Halyomorpha halys*]	4e-84	37%	Yes	6
YsigOR51	MG204686	401	ref|XP_014281005.1| odorant receptor 4-like [*Halyomorpha halys*]	5e-39	28%	Yes	5
YsigOR52	MG204687	363	ref|XP_014287367.1| odorant receptor 85b-like [*Halyomorpha halys*]	4e-51	33%	Yes	4
YsigOR53	MG204688	437	ref|XP_014289672.1| odorant receptor 49a-like [*Halyomorpha halys*]	6e-61	32%	Yes	4
YsigOR54	MG204689	435	ref|XP_014242937.1| odorant receptor Or2-like [*Cimex lectularius*]	4e-46	28%	Yes	6
YsigOR55	MG204690	373	ref|XP_014294439.1| odorant receptor Or1-like isoform X1 [*Halyomorpha halys*]	6e-67	33%	Yes	4
YsigOR56	MG204691	157	ref|XP_014282386.1| odorant receptor 49a-like isoform X1 [*Halyomorpha halys*]	2e-14	29%	No (5′ lose)	2
YsigOR57	MG204692	381	ref|XP_014274900.1| odorant receptor 30a-like [*Halyomorpha halys*]	2e-32	25%	Yes	8
YsigOR58	MG204693	392	ref|XP_014257038.1| odorant receptor Or2-like isoform X2 [*Cimex lectularius*]	4e-28	25%	Yes	6
YsigOR59	MG204694	435	ref|XP_014289672.1| odorant receptor 49a-like [*Halyomorpha halys*]	3e-67	33%	Yes	6
YsigOR60	MG204695	415	ref|XP_014249551.1| putative odorant receptor 92a [*Cimex lectularius*]	2e-100	37%	Yes	5
YsigOR61	MG204696	394	ref|XP_014274900.1| odorant receptor 30a-like [*Halyomorpha halys*]	2e-129	47%	Yes	7
YsigOR62	MG204697	387	ref|XP_014273330.1| odorant receptor 85b-like [*Halyomorpha halys*]	1e-53	31%	Yes	6
YsigOR63	MG204698	372	ref|XP_014287367.1| odorant receptor 85b-like [*Halyomorpha halys*]	1e-12	25%	Yes	6
YsigOR64	MG204699	425	ref|XP_014261210.1| odorant receptor 45b-like [*Cimex lectularius*]	1e-112	43%	Yes	5
YsigOR65	MG204700	396	ref|XP_014257038.1| odorant receptor Or2-like isoform X2 [*Cimex lectularius*]	3e-33	29%	Yes	6

Transcript expressions of YsigOR genes

The expression profiles of 66 YsigORs in the antennae of females and males were evaluated using RT-qPCR. The result showed that YsigOrco had the highest expression level among all YsigOR genes; however, the expression level of males was twice as that of females. The expression levels of 11 YsigOR genes (*YsigOR2*, *YsigOR8*, *YsigOR21*, *YsigOR31*, *YsigOR35*, *YsigOR42*, *YsigOR48*, *YsigOR49*, *YsigOR50*, *YsigOR59*, and *YsigOR62*) were significantly higher in the antennae of males than in those of females, while the expression levels of YsigOR19 was significantly higher in the latter than in the former. Additionally, other YsigOR genes showed comparable expression levels in the antennae of both sexes (Fig. 3).

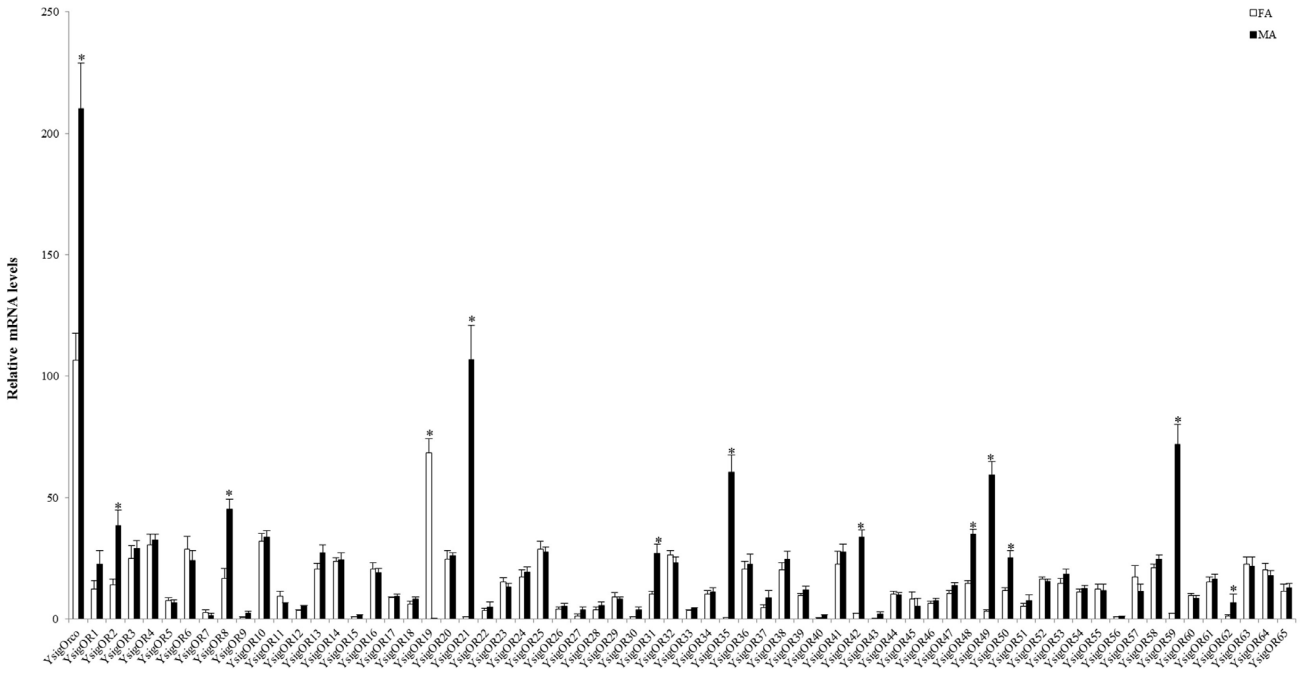

Fig. 3. Transcript abundances of *Yemma signatus* OR genes within the antennae of females and males determined by RT-qPCR. The standard errors of the means for three biological replicates are represented by error bars. Asterisk (*) indicates significant differences ($p < 0.05$).

DISCUSSION

In the present study, we generated a transcriptome of the *Y. signatus* antennae and identified candidate chemosensory genes encoding 66 ORs. To our knowledge, this is the first comprehensive study of olfactory genes in the Berytidae family. Subsequently, all the sequences were further validated by cloning and sequencing.

Previously, it was reported that the number of identified OR genes ranged from 10 in *P. humanus* (Kirkness *et al.*, 2010) to 400 in *P. barbatus* (Smith *et al*., 2011). In this study, 66 ORs were identified in the antennae of *Y. signatus*. This number is much lower than that for other Hemipteran insects, such as 83 ORs in *Nysius ericae* (Zhang *et al*., 2016), 110 ORs in *A. lucorum* (An *et al*., 2016), and 88 ORs in *Adelphocoris lineolatus* (Xiao *et al*., 2017); however, it is higher than 63 ORs in *Sogatella furcifera* (He *et al*., 2015) and 45 ORs in *Aphis gossypii* (Cao *et al*., 2014). This may be caused by the adaptation of distinct species to their hosts during evolution. In different insect species, OR genes are extremely divergent and formed different clades in our OR phylogenetic tree. This may be a result of adaptation of distinct species to their hosts during evolution (Sanchez-Gracia *et al*., 2009). We also found a co-receptor YsigOrco gene, which had characteristics common with those of other insect species' Orco genes, such as seven transmembrane domains, a high degree of similarity with Orco genes of other insects, and high expression levels (Smart *et al*., 2008; Dong *et al*., 2016). Unlike divergent ORs, Orcos from different insect species could be easily assigned and formed a clear orthologous lineage.

For a better understanding of the function of these *YsigORs*, the expressions of the antennae of females and males were evaluated using RT-qPCR methods. The results showed that 11 YsigOR genes (*YsigOR2*, *YsigOR8*, *YsigOR21*, *YsigOR31*, *YsigOR35*, *YsigOR42*, *YsigOR48*, *YsigOR49*, *YsigOR50*, *YsigOR59*, and *YsigOR62*) were expressed more in antennae of males than of females, whereas YsigOR19 was expressed more in the latter, indicating sex-specific functions of these genes. The male-dominant expression of ORs may be involved in the recognition of the sex pheromone or in other male-specific behaviors, while female-dominant expression of ORs may be related to oviposition site selection or male-produced courtship pheromone detection (Anderson *et al*., 2009; Zhang *et al*., 2014, 2015b). The sex-specific functions of these ORs need further investigation.

In conclusion, we successfully constructed the first antennal transcriptome of *Y. signatus*, and identified 66 candidate YsigOR genes. Simultaneously, the phylogenetic relationships between YsigORs and other Hemipteran

ORs were also analyzed. For a better understanding of their functions, the expression patterns of these YsigOR genes in the antennae of females and males were executed using RT-qPCR. We successfully identified 11 male antennae-specific genes and one female antenna-specific YsigOR gene. Our results will aid better understanding of the mechanisms of hemipteran chemosensory system.

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Supplementary material

There is supplementary material associated with this article. Access the material online at: https://dx.doi.org/10.17582/journal.pjz/20180616040644

Statement of conflict of interest

The authors declare no conflict of interest.

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