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**RESEARCH ARTICLE** 

## Identification of Candidate Odorant Receptors in Asian Corn Borer *Ostrinia furnacalis*

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## Abstract

In lepidopteran insects, odorant receptors are involved in the perception of sex pheromones and general odorants. In the Asian corn borer, Ostrinia furnacalis, although several pheromone receptors have been identified, no general odorant receptor has been reported. In this study, an RNA sequencing analysis was carried out to identify the whole repertoire of the odorant receptors expressed in the antennae of O. furnacalis. Among 12 million reads obtained from the antennae of male and female moths, 52 candidate odorant receptors were identified, including 45 novel ones. Expression levels of candidate odorant receptors were estimated by read mapping and quantitative reverse transcription PCR. These analyses confirmed that the expression of the previously identified pheromone receptors was highly male biased. In contrast, none of the newly identified odorant receptors showed male-biased expression. Three of the newly identified odorant receptors showed female-biased expression. Two of them were the most highly expressed odorant receptors in the female antennae, suggesting that they may be involved in the detection of odorants important for the induction of female-specific behaviors such as oviposition site selection. In addition, candidate genes of 21 ionotropic receptors, 5 gustatory receptors, 2 sensory neuron membrane proteins, and 26 odorant degrading enzymes were identified. Our results provide a basis for further analysis of the chemosensory system in the Ostrinia species.

### Introduction

Odorant receptors of lepidopteran insects are classified into two major groups, pheromone receptors and general odorant receptors, primarily based on their functions [1, 2]. Pheromone receptors are specialized for the perception of sex pheromones that mediate sexual communication between males and females [3, 4]. Most pheromone receptors are narrowly tuned to the respective components of sex pheromones, and their sensitivity is usually high [5]. On the other hand, general odorant receptors are considered to function in the perception of environmental odorants such as host-plant volatiles, the detection of which is crucial for the selection of oviposition

sites [2, 6, 7]. General odorant receptors are as important as pheromone receptors for understanding of the molecular basis of ecological characteristics of each lepidopteran species, but their identification and functional analysis has not been conducted to the same extent compared with those for pheromone receptors [8].

The genus *Ostrinia* (Lepidoptera: Crambidae) comprises 21 species including the Asian corn borer, *Ostrinia furnacalis*, an important agricultural pest [9]. Among the *Ostrinia* species worldwide, the sex pheromones of nine species have been characterized to date [10, 11]. Six pheromone components (Z9–14:OAc, E11–14:OAc, Z11–14:OAc, E12–14:OAc, Z12–14:OAc and E11–14:OH) were identified from these species, and the respective species use different combinations of these components in different proportions for species-specific signaling [12–18]. Nine pheromone receptors (including an odorant receptor coreceptor, *Orco*) have been identified in *O. nubilalis, O scapulalis*, and *O. furnacalis* [12, 19–21]. Electrophysiological analyses by ectopic expression in *Xenopus* oocytes have proven that these receptors in fact respond to the pheromone components [12, 19, 20]. The difference in ligand specificity between orthologous receptors was considered to be involved in the evolution of pheromone communication system in these species [21].

In spite of the intensive analyses of pheromone receptors in *Ostrinia* species, several important issues remain to be addressed. For example, it was not confirmed whether the nine previously identified receptors represent all of the pheromone receptors in the analyzed species. Because these receptors were primarily identified by degenerate PCR based on the conserved sequences at the 5' and 3' terminals of ORF sequences [12, 19], it is possible that receptors with divergent structures may have been overlooked. In fact, although the male sex pheromones were shown to be involved in mating acceptance by females in *O. nubilalis* [22], most of the previously identified pheromone receptors were reported to be expressed exclusively in the male antennae [12, 19, 20]. Odorant receptors responsible for perception of the male sex pheromones remain to be identified. Besides pheromone receptors, general odorant receptors in *Ostrinia* species are important for understanding of the molecular mechanisms underlying their ecological adaptation, such as host-plant specialization. Considering the fact that many *Ostrinia* species are important agricultural pests, general odorant receptors have the potential to be a target for novel pest control methods. However, no general odorant receptor has been identified in these species.

Besides odorant receptors, many other genes were involved in the odorant perception, such as ionotropic receptors (IRs), gustatory receptors (GRs), sensory neuron membrane proteins (SNMPs), and odorant degrading enzymes (ODEs) [23]. IRs evolved from ionotropic glutamate receptors (iGluRs), and function in detection of acids, amines, and aldehydes [24]. GRs are transmembrane domain receptors mostly expressed in gustatory receptor neurons. However, recent studies suggest that some GRs are expressed in the antennae and involved in the detection of  $CO_2$  [25]. SNMPs function in the pheromone-detecting ORNs [26]. ODEs are thought to inactivate odorant molecules by enzymatic degradation in the sensillar lymph [23, 27].

In this study, the RNA sequencing (RNA-seq) analysis of *O. furnacalis* was conducted to identify the entire repertoire of odorant receptors expressed in the antennae of males and females. We found 45 novel odorant receptor candidates, among which three showed female-biased expression. In addition, candidate genes of 21 IRs, 5 GRs, 2 SNMPs, and 26 ODEs were identified. Our results provide a basis for further study of the molecular mechanisms of chemical perception in the *Ostrinia* species.

## Materials and Methods

### Insect rearing

*O. furnacalis* were collected on the Eai river bank ( $38^{\circ}35'40''$ N,  $140^{\circ}57'20''$ E), Furukawa, Japan, in June 2010. This species is not endangered or protected. Collection of unprotected insects in this area does not require any permission. The collected insects were maintained in the laboratory on the artificial diet for silkworm (Silkmate 2M, Nosan Corporation Life-Tech Department, Yokohama, Japan) at 23°C, under a 16:8 light/dark cycle. The larvae were reared in the insect breeding jar (100 mm diameter × 80 mm height, 310122; SPL Lifesciences Co. Ltd., Seoul, Korea) at a density of 60–80 individuals per bottle until they became pupae. The pupae were collected and divided by sex. Eclosed adults were fed with water for 2 days, then allowed to mate in a net cage containing a plastic cup as the substrate for egg laying.

### RNA sequencing and assembly

Male and female antennae were dissected from 2-day-old adults, and frozen in liquid nitrogen. RNA was immediately isolated from the frozen antennae using the QuickGene RNA tissue Kit SII (RT-s2; KURABO, Neyagawa, Japan). The antennae from more than 20 individuals were pooled for a single RNA isolation experiment. Three biological repeats for each sex were made for the analysis of expression levels. Sequencing libraries were prepared using the TruSeq RNA Sample Preparation Kit v2 according to the LS protocol of the manufacturer's instructions (Illumina, Inc., San Diego, CA, USA) using 1 µg of total RNA from each sample, except for the following modifications to select the library with long inserts. Incubation time of purified mRNA fragmentation was changed from 8 min to 30 sec at 94°C, and 0.7×volume of the AMPure XP beads was used in the all purification steps. Prepared libraries were mixed at a concentration identical to each other in a 1.5 ml tube and applied for cluster generation on the MiSeq system using the MiSeq Reagent Kit v3 (Illumina, Inc., San Diego, CA, USA). A total of 6 libraries were indexed and applied for a single multiplex run in the 300 bp single-end mode. The raw data were deposited in the DDBJ Sequence Read Archive under accession number DRA002255. The reads were preprocessed with cutadapt v1.2.1 [28] for quality trimming at QV30 with a minimum length of 50 bp. The pass-through reads were pooled and assembled using Trinity r2013\_08\_14 (http://trinityrnaseq.sourceforge.net/) [29]. Open reading frames were extracted from the Trinity contigs with TransDecoder (http://transdecoder.sourceforge. net/) [30, 31, 32, 33] using the script that came with the Trinity distribution without modification.

### Screening of odorant receptors and read mapping

The extracted ORF sequences (referred to as the Trinity transcripts hereafter) were first screened by similarity to *Bombyx. mori* odorant receptors (*BmorORs*) using two different methods to maximize the possibility of identifying candidate odorant receptors (Fig. 1). A total of 68 protein sequences of *B. mori* were obtained from the database [34, 35], and each was used as a query in BLASTp searches against the Trinity transcripts. In parallel, PSI-BLAST searches were performed using alignments of *BmorORs* in various groupings as a query (Table 1). In both searches, the E-value cutoff was set to 0.0001. Overlapping variants were removed at this step by selecting the longest one as a representative transcript of a variant group. The results of two screenings were merged and duplications were removed. The remaining Trinity transcripts were screened for the presence of transmembrane domains using SOSUI (http://harrier.nagahama-i-bio.ac.jp/sosui/) and TMHMM (http://www.cbs.dtu.dk/services/TMHMM/) [36, 37]. The transcripts that contained transmembrane domains were finally screened using





Fig 1. Schematic diagram of sequence data analysis for odorant receptors. The numbers of reads or contigs at each step are indicated. See text for detailed explanation.

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Groups	genes	from	other	species	
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Groups	genes from other species
ORs	B. mori
1	OR26, OR45, OR46, OR47, OR48, OR50, OR51, OR57, OR58, OR59, OR63
2	Group1, OR10, OR11, OR12, OR13, OR15, OR16, OR24, OR25, OR33, OR34, OR41, OR61, OR62, OR65, OR66, OR67
3	OR1, OR5, OR7, OR18, OR35, OR37, OR38, OR39, OR43, OR68
4	OR5, OR7
5	OR27, OR29, OR49, OR53, OR54, OR55, OR56
6	Group2, Group3, Group5
7	Group6, OR3, OR23, OR28, OR42, OR64
8	Group7, OR40, OR44
9	Group8, OR2, OR8, OR19, OR20, OR21, OR22, OR30, OR36
10	Group9, OR4, OR6, OR9, OR14, OR17, OR32, OR52, OR60
lRs	B. mori
1	IR8a, IR25a, IR40a, IR76b, IR93a
2	IR7d2, IR7d3, IR87a, IR143
3	IR64a, IR75d, IR75p, IR75q2
4	Group2, IR21a, IR41a, IR68a
5	Group1, Group4
6	Group3, Group5
GRs	B. mori
1	GR39, GR41, GR42, GR43, GR44, GR45, GR46, GR, GR48, GR58, GR59, GR60, GR61, GR62
2	GR12, GR13, GR24, GR25, GR26, GR27, GR28, GR29, GR30, GR31, GR32, GR33, GR34, GR35, GR36, GR37, GR38, GR40, GR47, GR64, GR65, GR68
3	GR14, GR15, GR16, GR17, GR18, GR19, GR20, GR21, GR22, GR23, GR49, GR50, GR51, GR52, GR54, GR69
4	GR1, GR2, GR3, GR4, GR5, GR6, GR7, GR8, GR9, GR10, GR11, GR53, GR55, GR56, GR57, GR63, GR66, GR67
5	Group2, Group3
6	Group1, Group5
7	Group4, Group6
SNMPs	B. mori, O. furnacalis
1	BmorSNMP1,BmorSNMP2
2	OfurSNMP1, OfurSNMP2
3	BmorSNMP1, OfurSNMP1
4	BmorSNMP2, OfurSNMP2
5	BmorSNMP1,BmorSNMP2, OfurSNMP1, OfurSNMP2
ODEs	S. inferens
1	CXE2, CXE6, CXE12, CXE14, CXE28
2	CXE1, CXE18, CXE20
3	Group1, Group2
4	CXE5, CXE9, CXE11, CXE13, CXE16, CXE30
5	CXE3, CXE10, CXE26
6	Group3, Group4, Group5,CXE19
7	AD1, AD6
8	AD2, AD3, AD4, AD5
9	AOX1 AOX2 AOX3

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BLASTp against the NCBI non-redundant protein database (16.12.2013) (Fig. 1), and those that had an insect odorant receptor as a top-hit homolog were considered as candidate odorant receptors. The expression level of each receptor was estimated by mapping the raw reads to the ORF sequences of the candidate odorant receptors using bowtie2 v2.0.6 in local mode with -a option, followed by processing with eXpress v1.5.1 [38]. Expression levels were calculated as reads per kilobase of the ORF length per million total reads for each library (RPKM) [39].

## Quantitative reverse transcription PCR

The relative expression levels of the candidate odorant receptors in the antennae and the thorax were validated by quantitative reverse transcription PCR (qRT-PCR). Thorax (mixture from males and females) was used as a control to examine the tissue specificity of the expression pattern. Primers were designed to amplify an approximately 200 bp-long fragment at the 3' end of the ORF of each candidate (Table 2). Tissues were dissected from 2-days-old adults independently from those used in the RNA-seq analysis. Total RNA was isolated using the QuickGene RNA tissue Kit SII (RT-s2, KURABO, Neyagawa, Japan) and cDNAs were transcribed using the SuperScript III First-Strand Synthesis System (Invitrogen, Carlsbad, CA, USA). Three biological repeats (independent RNA extraction and cDNA synthesis) were made. Quantitative RT-PCR was done using the LightCycler Nano system (Roche, Mannheim, Germany) with the FastStart Essential DNA Green Master Kit (Roche). Three genes, *RpS3, actin,* and *NADH dehydrogenase*, were included in the analysis, and the average quantification cycle (Cq) value of these three genes was used as an internal control. Relative expression levels of candidates to the internal control.

## Phylogenetic analysis

Phylogenetic relationships of *O. furnacalis* odorant receptors (*OfurORs*) were analyzed against *BmorORs* and *Cydia pomonella* odorant receptors (*CpomORs*) [40, 41]. A total of 164 amino acid sequences were aligned using MAFFT v7.130 with the option E-INS-i [42]. Phylogenetic relationship was deduced by the maximum likelihood method using RAxML v8.0.17 [43, 44] with the GAMMA model for rate heterogeneity and the WAG model for substitution matrix. In addition, the rapid hill-climbing search algorithm (–f d) was used. Model optimization precision in log likelihood units for final optimization of tree topology (–e) was set at 0.0001. The tree image was created using FigTree v1.4.1 (http://tree.bio.ed.ac.uk/software/figtree) [45].

## Identification of other genes involved in the odorant perception

IRs, GRs, SNMPs, and ODEs were identified by the same method used in the identification of odorant receptors, except for the screening by presence of transmembrane domains which was not applied for SNMPs and ODEs. Protein sequences of IRs, GRs, SNMPs in *B. mori* were obtained from the database [46, 47, 48, 49]. SNMPs in *O. furnacalis* were also used as queries [50]. Because ODEs were not systematically studied in the *B. mori*, ODEs in *Sesamia inferens* were used [51].

## Results

### RNA sequencing and screening of candidate odorant receptors

From multiplexed sequencing with Illumina MiSeq for six cDNA libraries, more than 12 million reads were obtained, which consisted of 5,852,653 reads from female antennae and 6,167,215 reads from male antennae (Fig. 1). The average length of reads was approximately

### Table 2. Primers used in qRT-PCR.

Name	Primers
RpS3	CAGCTCCCATAGCAATCATGG/CCACGGAAGCATGATCTTTACC
Actin	CCGTCCTCCTGACCGAGGCTC/GGTGTGGGAGACACCATCTCCG
NADH	GCTGAAGGTGAGAGAGAATTAG/CGAGGTAATGTTCCTCGAACTC
OfurOR1	GTGCTGTTCCTGCTCTACAAC/GCTGAACGTTCGCAAGAACATG
OfurOR2	GCTCATCAGTGATGGAAGCAG/GCACCAAGTACAGAAGCGAAC
OfurOR3	TTGGTACTCAGAGCGAGACCC/GGTGAATGTTCGCAGTAGCATG
OfurOR4	GATGTTAGGTGCTGAGACGGAG/TTAATCATTCATTGTTTGTAGG
OfurOR5a	GGATTTACAGATGAAGTTTCGGT/GACCGTATATGAGTACAGTCATA
OfurOR5b	GGATTTACGGATGAACTTTCGGC/GACCGTATATGAGTAAAGTCAGT
OfurOR6	TGCAGTACTACGTTACGGACC/CAGTCCTAATGCCTTGAGACTG
OfurOR7	CCTTAGTCTTCGAACTGCTAGG/TAGCAATCATGGTCCTCGAGC
OfurOR8	GAGATGTTGGGTTCAGAGACTG/TCTTCAATATCCCGGTCATGG
OfurOR9	CAGAGGATGATGGATGCGTGC/TTACGCCATCATTGACCGCAG
OfurOR10	CGTACAGTGCCGATTGGATAC/CAGAAGCGTGAAGAACGAGTAC
OfurOR11	GGCTTCAATTTATGCCGGTGG/CACTGGTATGATATCAGCAGCC
OfurOR12	TTCTGTTGGCACAGCAACGAC/CACTTTGCTGATTCGCAGCTG
OfurOR13	ATTGCTGGCACAGCAACGACG/GCCACAGTGAGCTTGGTGAAC
OfurOR14	GAGTAGGTGAAGCAGTGTACTG/GAGACGTAGCAAGAGCGTCAATG
OfurOR15	GGACTTGTTGAAGAGGAGTCAG/CTCGTGGTTGACATGAACGTG
OfurOR16	GAGTGATGGATGCAAGCAAGGC/GCTGAAGCTCAACGTGGTGAC
OfurOR17	TAGCTATGGACTGCTGGACTG/CATGAGGCATTCGAAACTCAGC
OfurOR18	TTATTATACAGGCGGACCGCG/CACGACAAAAGTGTGGAGATCC
OfurOR19	CTCATCGTTTGCTACTGCAGTG/ACCATCGTAAATGTGGCTTGC
OfurOR20	GAAAGTACCCTAGTGAGCTACGG/CTGCAGCTTAATCGCAGGATC
OfurOR21	ACAGTAGAGAGCGACCGCATG/CAAAGGTGTCAAGTGAGAGCG
OfurOR22	GGCACAGTAACGAAGCTTTAG/GAGTGTAGTAGCTGTACGACC
OfurOR23	GTGGCCATGCTGCAGATTTAC/GACCACGACGTGCTAATAATC
OfurOR24	CATTAGAAGCAGCTCGCATCG/TACGCTGCCTTCATTATCGCG
OfurOR25	CTCTTCATGAGCTTGCTGCAAG/GCTCTCAAGTTGACATCTGCG
OfurOR26	ATCGCTGCTATGCTACTTCGG/GTAGTGAAGGCCGTCAAGTTC
OfurOR27	CGCTAGCAACTATGGAACAGAC/GGTTCCAGCAAGACAATGGTG
OfurOR28	CTGAAGTGCGTTTGTGAGAAC/ATCGTGTACATGGAATAAGCC
OfurOR29	ACTGCAGTTTATGTGCGCGAC/CACCGAAATGAATGGGCCTGC
OfurOR30	GAGTTAACTGCTACTAGCGAAG/ACGAACGTCTGCCTAGACATG
OfurOR31	TCGACTGTGAGCAGTCAAGTG/TCAATCTTCTCTTTGGAGCAC
OfurOR32	TGGAGCTTAGCTCTATTGAAC/TTACTCTCTCTTGTGCGTTGC
OfurOR33	ACAAGTCGATAATGAGTGCGC/ATCCTCCAGAACGGACATGAC
OfurOR34	GAGTGGCAGATGCTTTGTATA/CTATGGGTTATAAGTATTGAG
OfurOR35	GAACTGATTTGGAAGAGCACTGC/GACTGCGAATGCTTTGTAAGACC
OfurOR36	TTGACGTTCGTCGCGAGTATG/GAAGGCCTTCATGACAATCGG
OfurOR37	TATGATAGCCGGTTCAGCGTAC/CCTTCCACTTGCTGCAGCAATG
OfurOR38	CATCACTATCGAGGCAGCAAG/AACGGAGTATGCTGATTTCACG
OfurOR39	AGCGAGAGCGATCAGGTGTGC/GCGAATGTAGTAAGCGAGATGG
OfurOR40	CGTATCAGGCTTCACTGTTAC/GAGAATAAGTTCCCTTCAAGAC
OfurOR41	AGTTTCACATCTGTCGTCCAC/GAACAGGTTGAAAGCGGTGATG
OfurOR42	CATATCGCTAGCAGCATACGAG/TTAATTGACGACGTCTCGGAG
OfurOR43	AAATTGCTGACACGATGGCGC/AATGAAGAGCGTAGTTGACGC

(Continued)

### Table 2. (Continued)

Name	Primers
OfurOR44	ACGATGGTGGCACAGCTGTAC/ACAAAGGTAGGCCTCGACAGG
OfurOR45	GATGCGGCATACAATAGTAAATG/CTATTCTGGAGGCTTATAAACCG
OfurOR46	GCGTGTTGGGAGATTAGGTTC/GCCTGTCTCAGCATGTTGAAC
OfurOR47	CGCTAATACAGCGATGGATGTC/GTCAGCCGAGTTCACGATTTC
OfurOR48	GACGGCTTCACCAATATTAAC/TCATTCATCATCGTCATC
OfurOR49	CACCTTCGACATCCTGTTCATG/TGATGGAGATTGGATGCTGCG
OfurOR50	CGATAATCTGCGAATTGCAGCG/GAGTGAAGAATGAGTAGGCCG
OfurOR51	TCGGGCTTAGTGTATCATCAGC/GCACTGCATCAGAATCACCATC
OfurOR52	GCCTAACCGATGCCATATACTC/GCAGTGCGAAGCAGATTGAAG
OfurOR53	GGAGCTATTACCTACGTGAAGC/TTAAGCGCAGGCTGCGTTCATG

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160 bp (Table 3). All reads in a total number of 2 billion bases were pooled together to be assembled into 60,399 contigs, from which 24,629 open reading frame sequences (ORFs) were extracted (Table 4). In the first screening, 244 and 243 sequences were obtained from the homology searches against *B. mori* odorant receptors using BLASTp and PSI-BLAST, respectively. These sequences included groups of variants that were identical in their middle section but different from each other in the length of the two termini. Such variants were probably generated by sequencing errors that truncated the deduced ORF. For further analysis, the longest one was selected as a representative sequence of each group. The robustness of this method was confirmed by comparisons with the sequences of previously identified pheromone receptors (see below). After removing duplications between the two screening results (BLASTp and PSI-BLAST), 134 candidates remained. From the second screening using SOSUI and TMHMM, 117 sequences were found to contain transmembrane domains. These sequences were finally screened against the NCBI non-redundant protein database, of which 52 had an insect odorant receptor as the top-hit homolog (Table 5). Seven of the nine previously identified pheromone receptors were found in the candidates, with the exception of OfurOR1. Although two sequences were reported for OfurOR5 (OfurOR5a and OfurOR5b), only one sequence was found in our candidates, which was slightly different from either. In the other cases, the previously identified receptors and the corresponding candidates were completely identical at the amino acid level but with some differences at the nucleotide level. The 45 newly identified receptors were named from OfurOR9 to OfurOR53.

### Table 3. Summary of sequencing results.

Reads	Male			Female			
	Repeat1	Repeat2	Repeat3	Repeat1	Repeat2	Repeat3	
Total number	1837797	1896765	2118091	2409242	1503179	2254794	
Total bases (bp)	300350405	309419147	347769463	390381207	250725980	372529795	
Median length (bp)	156	162	160	157	157	158	
Q30 percentage	96.80%	96.61%	96.57%	96.67%	96.41%	96.72%	

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### Table 4. Summary of assembly results.

	Contigs	ORFs
Total number	60399	24629
Total length (bp)	65431963	29858679
Mean length (bp)	1083	1212
Median length (bp)	617	921
N50 length (bp)	1865	1578

doi:10.1371/journal.pone.0121261.t004

## Expression levels of the candidate odorant receptors estimated by read mapping

To estimate the expression level of the candidate odorant receptors in males and females, the reads were mapped onto the ORF sequences of the candidate receptors. Because OfurOR1 was not found in our candidates, its sequence was obtained from the database. Sequences for Ofur-OR5a and OfurOR5b were also obtained from the database and treated as independent receptors in the mapping. As expected, OfurOR2 (Orco) was expressed at the highest level in both male and female antennae (Table 5, Fig. 2A). Most of the previously identified pheromone receptors (OfurOR3, 4, 5a, 5b, 6, 7, and 8) showed male-specific expression, which was consistent with previous studies [12, 19, 20]. Among these, OfurOR4, the receptor for the major pheromone component in O. furnacalis, was expressed at the highest level. OfurOR7 was expressed not only in males but also in females at an intermediate level. Surprisingly, but consistently with the results of the candidate screening, the number of reads mapped onto OfurOR1 was very low, suggesting that it was not expressed in our samples. None of the 45 novel candidate receptors showed strongly male-biased expression as observed with the previously identified pheromone receptors. Because the read counts were normalized by the total read number, and a large part of the reads were mapped onto the pheromone receptors in males, the RPKM values for the other receptors tended to be higher in females. Nevertheless, OfurOR15, 39, 52, and 53 should be recognized as female-biased receptors. In particular, OfurOR15 and OfurOR39 were expressed at the next highest levels after OfurOR2 (Orco) in female antennae.

## Expression levels of the candidate odorant receptors confirmed by qRT-PCR

To confirm the expression levels of the candidate odorant receptors, qRT-PCR was carried out using the independently prepared cDNA libraries. The primers for qRT-PCR were designed to specifically recognize the sequence at the 3' end of each candidate (Table 2). Thorax cDNA libraries were used as the negative control, and no expression was detected for any of the odorant receptors (Table 5). In the antennae, the results were generally consistent with those of the read mapping but with some exceptions (Fig. 2B). The inconsistency with the results of read mapping was probably caused by high sequence similarity between two receptors. Because we used -a option in the bowtie2 mapping, single reads derived from the high-homology regions were mapped to both of the receptors with a 0.5 count each, resulting in a similar RPKM value in both receptors. Such cases were likely in *OfurOR21, 25*, and *36* that were expressed at lower levels than estimated by the read mapping. Female-biased expression was confirmed for *OfurOR15, 39*, and *53*. In particular, *OfurOR53* was highly female-specific, suggesting its dedicated role in females. Expression of *OfurOR7* in females was also confirmed. Because the male-female expression ratio was more accurately estimated by qRT-PCR than read mapping, the



### Table 5. List of candidate odorant receptors in O. furnacalis.

Itale         Fende         Itale         Fende         Itale         Fende         Theres           Ohr/ORP1         AB467327*         425         0.29         0.00         0.000         0.0000         <	Name	Accession Number	aa length	RPKM		RT-PCR**		
OkurOR1         AB48/3227*         425         0.29         0.00         0.000         0.000         0.0000           OhurOR2         L.G002667         474         678.43         448.04         0.366         0.301         0.0000           OhurOR3         L.G002669         429         376.26         0.57         0.383         0.000         0.0000           OhurOR5         AB503303*         408         61.85         1.21         0.199         0.000         0.0000           OhurOR5         AB503303*         408         45.79         1.10         0.24         0.001         0.0000           OhurOR6         L.G002701         448         21.43         4.27         0.111         0.014         0.0000           OhurOR7         L.G002701         448         21.43         4.27         0.011         0.126         0.0011         0.0000           OhurOR1         L.C002704         404         17.35         31.60         0.046         0.66         0.0000           OhurOR1         L.C002705         398         15.75         18.22         0.027         0.038         0.0000           OhurOR14         L.C002706         423         12.32         23.11         0.039				Male	Female	Male	Female	Thorax
OkurOR2         LC002897         474         678.43         449.04         0.365         0.301         0.0000           OkurOR4         LC002898         426         61.07         0.15         0.150         0.000         0.0000           OhurOR4         LC002899         423         376.28         0.57         0.383         0.000         0.0000           OhurOR5s         AB503302*         408         64.87         1.10         0.334         0.001         0.0000           OhurOR5s         LC002701         448         21.43         4.27         0.111         0.168         0.002         0.0000           OhurOR8         LC002701         422         37.69         33.48         0.104         0.126         0.001         0.0000           OhurOR1         LC002705         386         15.75         16.22         0.027         0.021         0.0000           OhurOR13         LC002706         423         12.17         18.09         0.046         0.022         0.001         0.0000           OhurOR13         LC002708         424         12.32         23.11         0.022         0.033         0.0000           OhurOR14         LC0002716         423         12.75	OfurOR1	AB467327*	425	0.29	0.00	0.000	0.000	0.0000
Ohm Orga         LC002698         426         61.07         0.15         0.150         0.000         0.0000           Ohm Ork         LC002699         423         376.26         0.57         0.383         0.000         0.0000           Ohm Ork         AB503302*         408         61.85         1.21         0.199         0.000         0.0000           Ohm Ork         LC002701         448         41.73         1.10         0.234         0.011         0.010         0.0000           Ohm ORK         LC002701         448         21.43         4.27         0.111         0.014         0.0000           Ohm ORK         LC002703         324         27.58         33.48         0.104         0.126         0.0001           Ohm ORK         LC002703         324         27.55         33.48         0.104         0.022         0.0021         0.0000           Ohm ORK         LC002706         407         13.95         16.74         0.036         0.0421         0.0000           Ohm ORK         LC002706         423         12.17         18.00         0.022         0.012         0.0000           Ohm ORK         LC002711         423         12.17         9.023	OfurOR2	LC002697	474	678.43	449.04	0.366	0.301	0.0000
OhmOH4         LO02699         423         376.26         0.57         0.139         0.000         0.0000           OhmOR5x         AB508502*         408         61.85         1.21         0.199         0.001         0.0000           OhmOR5x         AB508503*         408         45.78         1.10         0.234         0.001         0.0000           OhmOR7         LO02700         422         37.69         1.01         0.162         0.001         0.0000           OhmOR7         LC002701         448         44.57         0.01         0.162         0.001         0.0000           OhmOR8         LC002703         324         27.69         33.48         0.014         0.162         0.0001           OhmOR1         LC002705         398         15.75         16.22         0.027         0.027         0.020           OhmOR14         LC002707         425         12.75         19.00         0.022         0.019         0.0000           OhmOR14         LC002708         424         12.32         23.11         0.002         0.0000           OhmOR14         LC002710         422         10.16         22.44         0.018         0.0002           OhmOR16 </td <td>OfurOR3</td> <td>LC002698</td> <td>426</td> <td>61.07</td> <td>0.15</td> <td>0.150</td> <td>0.000</td> <td>0.0000</td>	OfurOR3	LC002698	426	61.07	0.15	0.150	0.000	0.0000
OrkurOR5a         AB508302*         408         61.85         1.21         0.199         0.000         0.0000           OrkurOR5b         AB508303*         408         45.79         1.10         0.234         0.001         0.0000           OrkurOR5         LC002700         422         37.69         1.01         0.168         0.002         0.0000           OrkurOR7         LC002701         448         21.43         4.47         0.111         0.014         0.0000           OrkurOR8         LC002703         324         27.69         33.48         0.104         0.126         0.0001           OrkurOR10         LC002705         398         15.75         16.22         0.027         0.0000           OrkurOR13         LC002705         423         12.11         88.09         0.034         0.22         0.0000           OrkurOR15         LC002705         423         12.11         88.09         0.034         0.203         0.00000           OrkurOR16         LC002710         422         10.16         2.44         0.012         0.0000           OrkurOR18         LC002711         239         10.68         6.46         0.033         0.0000           OrkurOR16	OfurOR4	LC002699	423	376.26	0.57	0.383	0.000	0.0000
OhmoRbb         ABS0803*         408         45.79         1.10         0.234         0.001         0.0000           OhmoRF         LC002700         422         37.69         1.01         0.134         0.001         0.0000           OhmoR7         LC002701         448         21.43         4.457         0.011         0.111         0.014         0.0000           OhmoR8         LC002702         438         44.57         0.01         0.046         0.0061         0.0000           OhmoR91         LC002705         388         15.75         16.22         0.027         0.021         0.0000           OhmOR11         LC002705         425         12.75         19.00         0.022         0.019         0.0000           OhmOR13         LC002707         425         12.11         88.09         0.034         0.210         0.0000           OhmOR16         LC002710         422         10.16         22.84         0.012         0.012         0.0000           OhmOR17         LC002713         422         833         15.95         0.039         0.047         0.0000           OhmOR17         LC002713         422         833         5.95         0.033         0.0003	OfurOR5a	AB508302*	408	61.85	1.21	0.199	0.000	0.0000
OhmORE         LC002701         442         37.69         1.01         0.168         0.002         0.0000           OhmOR7         LC002701         448         21.43         4.27         0.111         0.014         0.0000           OhmOR9         LC002703         324         27.69         33.48         0.104         0.126         0.0001           OhmOR10         LC002705         398         15.75         16.22         0.027         0.021         0.0000           OhmOR11         LC002706         407         13.95         16.74         0.036         0.027         0.0000           OhmOR13         LC002706         423         12.11         88.09         0.034         0.210         0.0000           OhmOR15         LC002710         422         10.16         2.84         0.012         0.0001           OhmOR15         LC002711         239         10.08         6.46         0.034         0.043         0.0000           OhmOR18         LC002712         471         9.72         14.54         0.012         0.0001         0.0003         0.0002         0.0003         0.0000         0.0071         0.0003         0.0000         0.0071         0.0003         0.0001	OfurOR5b	AB508303*	408	45.79	1.10	0.234	0.001	0.0000
OhroR7         LC002701         448         21.43         4.27         0.111         0.014         0.0001           OhroR8         LC002702         438         44.57         0.01         0.162         0.0001         0.0000           OhroR9         LC002703         324         27.69         33.48         0.104         0.126         0.0001           OhroR10         LC002705         398         15.75         16.22         0.027         0.021         0.0000           OhroR11         LC002706         407         13.95         16.74         0.030         0.022         0.019         0.0000           OhroR13         LC002709         423         12.175         19.00         0.022         0.019         0.0000           OhroR16         LC002710         423         10.16         22.84         0.019         0.023         0.0000           OhroR17         LC002711         239         10.08         6.46         0.012         0.0001         0.0002           OhroR17         LC002713         422         8.93         15.96         0.039         0.047         0.0000           OhroR27         LC002713         422         8.93         15.96         0.039         0	OfurOR6	LC002700	422	37.69	1.01	0.168	0.002	0.0000
OhroRB         LC002702         438         44.57         0.01         0.162         0.001         0.0000           OhroRB         LC002703         524         27.69         33.48         0.104         0.126         0.0001           OhroR10         LC002705         398         15.75         16.22         0.027         0.021         0.0000           OhroP11         LC002707         425         12.75         19.00         0.022         0.019         0.0000           OhroP13         LC002707         425         12.75         19.00         0.022         0.038         0.0000           OhroP14         LC002708         424         12.32         23.11         0.027         0.038         0.0000           OhroP17         LC002710         422         10.16         2.484         0.012         0.0010           OhroP17         LC002711         239         10.08         6.46         0.033         0.047         0.0000           OhroP17         LC002713         422         8.93         15.96         0.039         0.047         0.0000           OhroP28         LC002714         411         8.79         12.19         0.018         0.018         0.0000	OfurOR7	LC002701	448	21.43	4.27	0.111	0.014	0.0000
OhroRP9         LC002703         324         27.69         33.48         0.104         0.126         0.0001           OhroR110         LC002704         404         17.35         31.60         0.046         0.066         0.0000           OhroR111         LC002705         398         15.75         16.22         0.027         0.019         0.0000           OhroR13         LC002707         425         12.75         19.00         0.022         0.019         0.0000           OhroR14         LC002708         423         12.11         88.09         0.034         0.210         0.0000           OhroR16         LC002710         422         10.16         22.84         0.012         0.0012         0.0001           OhroR17         LC002711         239         10.08         6.46         0.034         0.043         0.0000           OhroR17         LC002713         422         8.93         15.96         0.039         0.047         0.000           OhroR2         LC002715         363         8.37         8.91         0.003         0.003         0.0000           OhroR2         LC002717         404         7.71         14.27         0.023         0.039         0	OfurOR8	LC002702	438	44.57	0.01	0.162	0.001	0.0000
OhmOR10         LC002704         404         17.35         31.60         0.046         0.066         0.0000           OhmOR11         LC002705         398         15.75         16.22         0.027         0.021         0.0000           OhmOR13         LC002707         425         12.75         19.00         0.022         0.019         0.0000           OhmOR15         LC002708         424         12.32         23.11         0.034         0.210         0.0000           OhmOR15         LC002710         422         10.16         22.84         0.019         0.023         0.0000           OhmOR17         LC002711         239         10.08         6.45         0.034         0.043         0.0000           OhmOR18         LC002713         422         8.93         15.96         0.039         0.047         0.0000           OhmOR20         LC002714         411         8.79         12.19         0.018         0.0018         0.0000           OhmOR21         LC002715         363         8.37         8.91         0.002         0.0000           OhmOR23         LC002716         409         8.27         14.28         0.022         0.022         0.0000	OfurOR9	LC002703	324	27.69	33.48	0.104	0.126	0.0001
OhroPH11         LC002705         398         15.75         16.22         0.027         0.021         0.0000           OhroPH12         LC002706         407         13.95         16.74         0.036         0.027         0.0000           OhroPH13         LC002707         425         12.75         19.00         0.022         0.019         0.0000           OhroPH15         LC002709         423         12.11         86.09         0.034         0.210         0.0000           OhroPH15         LC002710         422         10.16         22.84         0.012         0.012         0.0000           OhroPH18         LC002711         239         10.08         6.46         0.034         0.043         0.0000           OhroPH18         LC002714         411         8.79         12.19         0.018         0.018         0.0000           OhroPR21         LC002716         409         8.27         14.28         0.022         0.028         0.0000           OhroPR22         LC002718         448         6.44         8.52         0.014         0.017         0.000           OhroPR24         LC002719         418         6.14         8.19         0.002         0.0001     <	OfurOR10	LC002704	404	17.35	31.60	0.046	0.066	0.0000
OhurOR12         LC002706         407         13.95         16.74         0.036         0.027         0.0000           OhurOR13         LC002707         425         12.75         19.00         0.022         0.019         0.0000           OhurOR14         LC002708         424         12.32         23.11         0.027         0.038         0.0000           OhurOR16         LC002710         422         10.16         22.84         0.019         0.033         0.0000           OhurOR17         LC002711         239         10.08         6.46         0.034         0.043         0.0000           OhurOR19         LC002713         422         8.93         15.96         0.039         0.047         0.0000           OhurOR19         LC002714         411         8.79         12.19         0.018         0.018         0.0000           OhurOR22         LC002716         409         8.27         14.28         0.022         0.028         0.0000           OhurOR23         LC002719         418         6.14         8.19         0.004         0.002         0.0000           OhurOR24         LC002718         448         6.44         8.52         0.014         0.0000	OfurOR11	LC002705	398	15.75	16.22	0.027	0.021	0.0000
OhroR13         LC002707         425         12.75         19.00         0.022         0.019         0.0000           OhroR14         LC002708         424         12.32         23.11         0.027         0.038         0.0000           OhroR15         LC002709         423         12.11         88.09         0.034         0.210         0.0000           OhroR16         LC002710         422         10.16         22.84         0.012         0.012         0.0000           OhroR18         LC002712         471         9.72         14.54         0.012         0.012         0.0000           OhroR18         LC002713         422         8.93         15.96         0.039         0.047         0.0000           OhroR21         LC002715         363         8.37         8.91         0.003         0.039         0.0000           OhroR22         LC002717         404         7.71         14.27         0.023         0.039         0.0000           OhroR24         LC002719         418         6.44         8.52         0.014         0.017         0.0001           OhroR25         LC002719         418         6.14         8.19         0.010         0.0000         0.00	OfurOR12	LC002706	407	13.95	16.74	0.036	0.027	0.0000
OhurOR14         LC002708         424         12.32         23.11         0.027         0.038         0.0000           OhurOR15         LC002709         423         12.11         88.09         0.034         0.210         0.0000           OhurOR16         LC002710         422         10.16         22.84         0.019         0.023         0.0000           OhurOR18         LC002711         229         10.08         6.46         0.034         0.047         0.0000           OhurOR19         LC002713         422         8.93         15.96         0.039         0.047         0.0000           OhurOR21         LC002716         499         8.27         14.28         0.022         0.028         0.0000           OhurOR22         LC002717         404         7.71         14.27         0.023         0.039         0.0000           OhurOR25         LC002719         418         6.14         8.19         0.002         0.0000           OhurOR26         LC002721         402         5.94         6.89         0.012         0.0001           OhurOR26         LC002722         433         6.01         7.07         0.011         0.015         0.0000 <td< td=""><td>OfurOR13</td><td>LC002707</td><td>425</td><td>12.75</td><td>19.00</td><td>0.022</td><td>0.019</td><td>0.0000</td></td<>	OfurOR13	LC002707	425	12.75	19.00	0.022	0.019	0.0000
OhurOR15         LC002709         423         12.11         88.09         0.034         0.210         0.0000           OhurOR16         LC002710         422         10.16         22.84         0.019         0.023         0.0000           OhurOR17         LC002711         239         10.08         6.46         0.034         0.043         0.0000           OhurOR19         LC002712         471         9.72         14.54         0.012         0.001         0.0000           OhurOR20         LC002714         411         8.79         12.19         0.018         0.018         0.0000           OhurOR21         LC002715         363         8.37         8.91         0.003         0.0003         0.0000           OhurOR23         LC002717         404         7.71         14.27         0.023         0.039         0.0000           OhurOR24         LC002718         448         6.14         8.19         0.004         0.002         0.0000           OhurOR25         LC002721         402         5.94         6.89         0.012         0.202         0.0001           OhurOR26         LC002722         420         5.93         11.78         0.022         0.0021	OfurOR14	LC002708	424	12.32	23.11	0.027	0.038	0.0000
OhurOR16         LC002710         422         10.16         22.84         0.019         0.023         0.0000           OhurOR17         LC002711         239         10.08         6.46         0.034         0.043         0.0000           OhurOR18         LC002712         471         9.72         14.54         0.012         0.001         0.0000           OhurOR18         LC002713         422         8.93         15.96         0.039         0.047         0.0000           OhurOR20         LC002714         411         8.79         12.19         0.018         0.018         0.0000           OhurOR21         LC002716         409         8.27         14.28         0.022         0.028         0.0000           OhurOR23         LC002717         404         7.71         14.27         0.023         0.039         0.0000           OhurOR26         LC002719         418         6.14         8.19         0.004         0.002         0.0000           OhurOR27         LC002721         402         5.94         6.89         0.011         0.014         0.0000           OhurOR30         LC002724         402         5.98         10.89         0.014         0.0000 <td>OfurOR15</td> <td>LC002709</td> <td>423</td> <td>12.11</td> <td>88.09</td> <td>0.034</td> <td>0.210</td> <td>0.0000</td>	OfurOR15	LC002709	423	12.11	88.09	0.034	0.210	0.0000
OhroR17         LC002711         239         10.08         6.46         0.034         0.043         0.000           OhroR18         LC002712         471         9.72         14.54         0.012         0.012         0.0001           OhroR19         LC002713         422         8.93         15.96         0.039         0.047         0.0000           OhroR20         LC002715         363         8.37         8.91         0.003         0.003         0.0000           OhroR22         LC002716         409         8.27         14.28         0.022         0.028         0.0000           OhroR23         LC002717         404         7.71         14.27         0.023         0.039         0.0000           OhroR24         LC002718         448         6.14         8.19         0.004         0.002         0.0000           OhroR25         LC002721         402         5.93         11.78         0.022         0.0001           OhroR26         LC002721         402         5.93         11.78         0.022         0.0001           OhroR31         LC002725         396         5.43         6.52         0.008         0.0090         0.0001           OhroR32	OfurOR16	LC002710	422	10.16	22.84	0.019	0.023	0.0000
OhurOR18         LC002712         471         9.72         14.54         0.012         0.012         0.0001           OhurOR19         LC002713         422         8.93         15.96         0.039         0.047         0.0000           OhurOR20         LC002714         411         8.79         12.19         0.018         0.018         0.0000           OhurOR21         LC002716         409         8.27         14.28         0.022         0.028         0.0000           OhurOR23         LC002717         404         7.71         14.27         0.023         0.039         0.0000           OhurOR24         LC002719         418         6.14         8.19         0.004         0.002         0.0000           OhurOR25         LC002720         433         6.01         7.07         0.011         0.015         0.0000           OhurOR26         LC002721         402         5.93         11.78         0.022         0.022         0.0001           OhurOR28         LC002724         402         5.93         11.78         0.022         0.002         0.0001           OhurOR31         LC002726         396         5.43         6.52         0.008         0.009 <td< td=""><td>OfurOR17</td><td>LC002711</td><td>239</td><td>10.08</td><td>6.46</td><td>0.034</td><td>0.043</td><td>0.0000</td></td<>	OfurOR17	LC002711	239	10.08	6.46	0.034	0.043	0.0000
OhinOR19         LC002713         422         8.93         15.96         0.039         0.047         0.000           OhinOR20         LC002714         411         8.79         12.19         0.018         0.003         0.000           OhinOR21         LC002715         363         8.37         8.91         0.003         0.003         0.0000           OhinOR22         LC002716         409         8.27         14.28         0.022         0.028         0.0000           OhinOR24         LC002717         404         7.71         14.27         0.023         0.039         0.0000           OhinOR25         LC002719         418         6.14         8.19         0.004         0.002         0.0000           OhinOR26         LC002721         402         5.94         6.89         0.012         0.000         0.001           OhinOR27         LC002723         441         5.71         12.74         0.008         0.014         0.000           OhinOR30         LC002724         402         5.58         10.89         0.010         0.014         0.000           OhinOR31         LC002726         412         5.11         14.27         0.008         0.009         0.00	OfurOR18	LC002712	471	9.72	14.54	0.012	0.012	0.0001
OhurOR20         LC002714         411         8.79         12.19         0.018         0.018         0.000           OhurOR21         LC002715         363         8.37         8.91         0.003         0.003         0.0000           OhurOR22         LC002716         409         8.27         14.28         0.022         0.028         0.0000           OhurOR23         LC002717         404         7.71         14.27         0.023         0.039         0.0000           OhurOR24         LC002718         448         6.44         8.52         0.014         0.017         0.0000           OhurOR26         LC002719         418         6.14         8.19         0.004         0.002         0.0000           OhurOR27         LC002721         402         5.94         6.89         0.012         0.022         0.0001           OhurOR28         LC002723         441         5.71         12.74         0.008         0.014         0.0000           OhurOR30         LC002725         396         5.43         6.52         0.008         0.009         0.0010           OhurOR31         LC002725         396         5.43         6.52         0.008         0.0000         0.	OfurOR19	LC002713	422	8.93	15.96	0.039	0.047	0.0000
OhimOR21         LC002715         363         8.37         8.91         0.003         0.003         0.0000           OhimOR22         LC002716         409         8.27         14.28         0.022         0.028         0.0000           OhimOR23         LC002717         404         7.71         14.27         0.023         0.039         0.0000           OhimOR24         LC002718         448         6.44         8.52         0.014         0.017         0.0005           OhimOR25         LC002719         418         6.14         8.19         0.004         0.002         0.0000           OhimOR26         LC002721         402         5.94         6.89         0.012         0.020         0.0001           OhimOR27         LC002723         441         5.71         12.74         0.008         0.014         0.0000           OhimOR30         LC002725         396         5.43         6.52         0.008         0.009         0.0001           OhimOR31         LC002726         412         5.11         14.27         0.004         0.006         0.0000           OhimOR32         LC002728         439         5.01         8.82         0.008         0.008         0.	OfurOR20	LC002714	411	8.79	12.19	0.018	0.018	0.0000
OhurOR22         LC002716         409         8.27         14.28         0.022         0.028         0.0000           OhurOR23         LC002717         404         7.71         14.27         0.023         0.039         0.0000           OhurOR24         LC002718         448         6.44         8.52         0.014         0.017         0.0005           OhurOR25         LC002719         418         6.14         8.19         0.004         0.002         0.0000           OhurOR26         LC002720         433         6.01         7.07         0.011         0.015         0.0000           OhurOR27         LC002721         402         5.94         6.89         0.012         0.022         0.0010           OhurOR30         LC002723         441         5.71         12.74         0.008         0.014         0.0000           OhurOR31         LC002725         396         5.43         6.52         0.008         0.0000         0.0001           OhurOR33         LC002728         439         5.01         8.82         0.008         0.0000           OhurOR34         LC002731         390         4.76         7.34         0.009         0.011         0.0000	OfurOR21	LC002715	363	8.37	8.91	0.003	0.003	0.0000
OhroR23         LC002717         404         7.71         14.27         0.023         0.039         0.000           OhroR24         LC002718         448         6.44         8.52         0.014         0.017         0.005           OhroR25         LC002719         418         6.14         8.19         0.004         0.002         0.0000           OhroR26         LC002720         433         6.01         7.07         0.011         0.015         0.0000           OhroR27         LC002721         402         5.94         6.89         0.012         0.022         0.001           OhroR28         LC002723         441         5.71         12.74         0.008         0.014         0.0000           OhroR30         LC002724         402         5.58         10.89         0.010         0.014         0.0000           OhroR31         LC002726         412         5.11         14.27         0.004         0.006         0.0000           OhroR33         LC002728         439         5.01         8.82         0.008         0.008         0.0000           OhroR34         LC002732         430         4.72         5.60         0.001         0.0000         0.0073	OfurOR22	LC002716	409	8.27	14.28	0.022	0.028	0.0000
OhurOR24         LC002718         448         6.44         8.52         0.014         0.017         0.0005           OhurOR25         LC002719         418         6.14         8.19         0.004         0.002         0.0000           OhurOR26         LC002720         433         6.01         7.07         0.011         0.015         0.0000           OhurOR27         LC002721         402         5.94         6.89         0.012         0.020         0.0011           OhurOR28         LC002723         441         5.71         12.74         0.008         0.014         0.0000           OhurOR30         LC002724         402         5.58         10.89         0.010         0.014         0.0000           OhurOR31         LC002725         396         5.43         6.52         0.008         0.009         0.0010           OhurOR32         LC002726         412         5.11         14.27         0.004         0.006         0.0000           OhurOR33         LC002728         439         5.01         8.82         0.008         0.0000           OhurOR35         LC002730         398         4.72         5.60         0.001         0.001         0.0000	OfurOR23	LC002717	404	7.71	14.27	0.023	0.039	0.0000
And Chrones         And Chroes	OfurOR24	LC002718	448	6.44	8.52	0.014	0.017	0.0005
OlivOR26         LC002720         433         6.01         7.07         0.011         0.015         0.0000           OlivOR27         LC002721         402         5.94         6.89         0.012         0.022         0.0001           OlivOR28         LC002722         420         5.93         11.78         0.022         0.022         0.0001           OlivOR29         LC002723         441         5.71         12.74         0.008         0.014         0.0000           OfurOR30         LC002724         402         5.58         10.89         0.010         0.014         0.0000           OfurOR31         LC002726         412         5.11         14.27         0.004         0.006         0.0000           OfurOR33         LC002728         439         5.01         8.82         0.008         0.008         0.0000           OfurOR35         LC002730         398         4.72         5.60         0.001         0.001         0.0000           OfurOR36         LC002731         390         4.69         5.36         0.008         0.008         0.0000           OfurOR37         LC002731         390         4.69         5.36         0.010         0.004         0.	OfurOR25	LC002719	418	6.14	8.19	0.004	0.002	0.0000
Ohron R27         LC002721         402         5.94         6.89         0.012         0.020         0.0001           Ohron R27         LC002722         420         5.93         11.78         0.022         0.022         0.0001           Ohron R29         LC002723         441         5.71         12.74         0.008         0.014         0.0000           Ohron R30         LC002724         402         5.58         10.89         0.010         0.014         0.0000           Ohron R31         LC002725         396         5.43         6.52         0.008         0.009         0.0001           Ohron R32         LC002726         412         5.11         14.27         0.004         0.006         0.0000           Ohron R33         LC002727         410         5.06         7.58         0.023         0.028         0.0001           Ohron R34         LC002730         398         4.72         5.60         0.001         0.001         0.0000           Ohron R35         LC002731         390         4.69         5.36         0.008         0.008         0.0000           Ohron R37         LC002731         390         4.69         5.36         0.008         0.0000	OfurOR26	LC002720	433	6.01	7.07	0.011	0.015	0.0000
Number         No.         No.<	OfurOR27	LC002721	402	5.94	6.89	0.012	0.020	0.0001
Altorne         Lood 112         Herio         Lood 112         Herio         Herio </td <td>OfurOR28</td> <td>L C002722</td> <td>420</td> <td>5.93</td> <td>11.78</td> <td>0.022</td> <td>0.022</td> <td>0.0001</td>	OfurOR28	L C002722	420	5.93	11.78	0.022	0.022	0.0001
OfurOR30         LC002724         402         5.58         10.89         0.010         0.014         0.000           OfurOR31         LC002725         396         5.43         6.52         0.008         0.009         0.001           OfurOR32         LC002726         412         5.11         14.27         0.004         0.006         0.0000           OfurOR33         LC002727         410         5.06         7.58         0.023         0.028         0.001           OfurOR34         LC002728         439         5.01         8.82         0.008         0.008         0.0000           OfurOR35         LC002729         430         4.76         7.34         0.009         0.011         0.0003           OfurOR36         LC002730         398         4.72         5.60         0.001         0.001         0.0000           OfurOR37         LC002731         390         4.69         5.36         0.008         0.008         0.0000           OfurOR38         LC002733         386         4.46         44.89         0.010         0.064         0.0000           OfurOR39         LC002734         432         4.44         10.99         0.005         0.006         0.000	OfurOR29	LC002723	441	5.71	12.74	0.008	0.014	0.0000
OfurOR31         LC002725         396         5.43         6.52         0.008         0.009         0.0001           OfurOR32         LC002726         412         5.11         14.27         0.004         0.006         0.0000           OfurOR33         LC002727         410         5.06         7.58         0.023         0.028         0.0011           OfurOR34         LC002728         439         5.01         8.82         0.008         0.008         0.0000           OfurOR35         LC002729         430         4.76         7.34         0.009         0.011         0.0000         0.0000           OfurOR36         LC002730         398         4.72         5.60         0.001         0.011         0.0000         0.0000         0.0001         0.0001         0.0001         0.0000         0.0000         0.00170R37         LC002731         390         4.69         5.36         0.008         0.008         0.0000         0.0001         0.0001         0.0001         0.0000         0.0001         0.0001         0.0001         0.0000         0.0001         0.0001         0.0000         0.0001         0.0001         0.0000         0.0001         0.0000         0.0001         0.0000         0.0001	OfurOR30	LC002724	402	5.58	10.89	0.010	0.014	0.0000
OfurOR32         LC002726         412         5.11         14.27         0.004         0.006         0.0000           OfurOR33         LC002727         410         5.06         7.58         0.023         0.028         0.001           OfurOR34         LC002728         439         5.01         8.82         0.008         0.0000         0.011         0.003           OfurOR35         LC002729         430         4.76         7.34         0.009         0.011         0.0000           OfurOR36         LC002730         398         4.72         5.60         0.001         0.001         0.0000           OfurOR37         LC002731         390         4.69         5.36         0.008         0.008         0.0000           OfurOR38         LC002732         415         4.59         7.55         0.010         0.009         0.0010           OfurOR39         LC002734         432         4.44         10.99         0.005         0.006         0.0000           OfurOR40         LC002735         421         4.24         13.06         0.008         0.025         0.0004           OfurOR42         LC002736         346         4.16         7.13         0.010         0.01	OfurOB31	1 C002725	396	5.43	6.52	0.008	0.009	0.0001
OfurOR33         LC002727         410         5.06         7.58         0.023         0.028         0.0001           OfurOR34         LC002728         439         5.01         8.82         0.008         0.008         0.0000           OfurOR35         LC002729         430         4.76         7.34         0.009         0.011         0.0003           OfurOR36         LC002730         398         4.72         5.60         0.001         0.001         0.0000           OfurOR37         LC002731         390         4.69         5.36         0.008         0.008         0.0000           OfurOR38         LC002731         390         4.69         5.36         0.010         0.009         0.0011           OfurOR37         LC002731         390         4.69         5.36         0.008         0.008         0.0000           OfurOR38         LC002732         415         4.59         7.55         0.010         0.009         0.0011           OfurOR40         LC002734         432         4.44         10.99         0.005         0.006         0.0000           OfurOR41         LC002736         346         4.16         7.13         0.010         0.015         0.000	OfurOR32	LC002726	412	5.11	14.27	0.004	0.006	0.0000
OfurOR34         LC002728         439         5.01         8.82         0.008         0.008         0.0000           OfurOR35         LC002729         430         4.76         7.34         0.009         0.011         0.0003           OfurOR36         LC002730         398         4.72         5.60         0.001         0.001         0.0000           OfurOR37         LC002731         390         4.69         5.36         0.008         0.008         0.0000           OfurOR38         LC002732         415         4.59         7.55         0.010         0.009         0.0011           OfurOR39         LC002734         432         4.44         10.99         0.005         0.006         0.0000           OfurOR41         LC002736         346         4.16         7.13         0.010         0.015         0.0000           OfurOR42         LC002736         346         4.16         7.13         0.010         0.015         0.0000           OfurOR43         LC002737         198         4.00         8.34         0.008         0.010         0.0003           OfurOR44         LC002738         436         3.54         4.33         0.003         0.006         0.000	OfurOR33	LC002727	410	5.06	7.58	0.023	0.028	0.0001
OfurOR35         LC002729         430         4.76         7.34         0.009         0.011         0.0003           OfurOR36         LC002730         398         4.72         5.60         0.001         0.001         0.0000           OfurOR37         LC002731         390         4.69         5.36         0.008         0.008         0.0000           OfurOR38         LC002732         415         4.59         7.55         0.010         0.009         0.0001           OfurOR39         LC002733         386         4.46         44.89         0.010         0.064         0.0000           OfurOR40         LC002735         421         4.24         13.06         0.008         0.025         0.0004           OfurOR42         LC002736         346         4.16         7.13         0.010         0.015         0.0000           OfurOR43         LC002737         198         4.00         8.34         0.008         0.010         0.0033           OfurOR44         LC002738         436         3.54         4.33         0.003         0.006         0.0000	OfurOR34	L C002728	439	5.01	8.82	0.008	0.008	0.0000
OfurOR36         LC002730         398         4.72         5.60         0.001         0.001         0.0000           OfurOR37         LC002731         390         4.69         5.36         0.008         0.008         0.0000           OfurOR38         LC002732         415         4.59         7.55         0.010         0.009         0.0001           OfurOR39         LC002733         386         4.46         44.89         0.010         0.064         0.0000           OfurOR40         LC002734         432         4.44         10.99         0.005         0.006         0.0000           OfurOR41         LC002736         346         4.16         7.13         0.010         0.015         0.0000           OfurOR42         LC002737         198         4.00         8.34         0.008         0.010         0.0033           OfurOR44         LC002738         436         3.54         4.33         0.003         0.006         0.0000	OfurOR35	LC002729	430	4.76	7.34	0.009	0.011	0.0003
OfurOR37         LC002731         390         4.69         5.36         0.008         0.008         0.0000           OfurOR38         LC002732         415         4.59         7.55         0.010         0.009         0.0001           OfurOR39         LC002733         386         4.46         44.89         0.010         0.064         0.0000           OfurOR40         LC002734         432         4.44         10.99         0.005         0.006         0.0000           OfurOR41         LC002736         421         4.24         13.06         0.008         0.025         0.0004           OfurOR42         LC002737         198         4.00         8.34         0.008         0.010         0.0003           OfurOR44         LC002738         436         3.54         4.33         0.003         0.006         0.0000	OfurOR36	LC002730	398	4.72	5.60	0.001	0.001	0.0000
OfurOR38         LC002732         415         4.59         7.55         0.010         0.009         0.0001           OfurOR39         LC002733         386         4.46         44.89         0.010         0.064         0.0000           OfurOR40         LC002734         432         4.44         10.99         0.005         0.006         0.0000           OfurOR41         LC002735         421         4.24         13.06         0.008         0.025         0.0004           OfurOR42         LC002736         346         4.16         7.13         0.010         0.015         0.0000           OfurOR43         LC002738         436         3.54         4.33         0.003         0.006         0.0000	OfurOB37	L C002731	390	4.69	5.36	0.008	0.008	0.0000
OfurOR39         LC002733         386         4.46         44.89         0.010         0.064         0.0000           OfurOR40         LC002734         432         4.44         10.99         0.005         0.006         0.0000           OfurOR41         LC002735         421         4.24         13.06         0.008         0.025         0.0004           OfurOR42         LC002736         346         4.16         7.13         0.010         0.015         0.0000           OfurOR43         LC002737         198         4.00         8.34         0.008         0.010         0.0003           OfurOR44         LC002738         436         3.54         4.33         0.003         0.006         0.0000	OfurOR38	LC002732	415	4.59	7.55	0.010	0.009	0.0001
OfurOR40         LC002734         432         4.44         10.99         0.005         0.006         0.0000           OfurOR41         LC002735         421         4.24         13.06         0.008         0.025         0.0004           OfurOR42         LC002736         346         4.16         7.13         0.010         0.015         0.0000           OfurOR43         LC002737         198         4.00         8.34         0.008         0.010         0.0003           OfurOR44         LC002738         436         3.54         4.33         0.003         0.006         0.0000	OfurOR39	LC002733	386	4.46	44.89	0.010	0.064	0.0000
OfurOR41         LC002735         421         4.24         13.06         0.008         0.025         0.0004           OfurOR42         LC002736         346         4.16         7.13         0.010         0.015         0.0000           OfurOR43         LC002737         198         4.00         8.34         0.008         0.010         0.0003           OfurOR44         LC002738         436         3.54         4.33         0.003         0.006         0.0000	OfurOR40	L C002734	432	4.44	10.99	0.005	0.006	0.0000
OfurOR42         LC002736         346         4.16         7.13         0.010         0.015         0.0000           OfurOR43         LC002737         198         4.00         8.34         0.008         0.010         0.0003           OfurOR44         LC002738         436         3.54         4.33         0.003         0.006         0.0000	OfurOR41	LC002735	421	4.24	13.06	0.008	0.025	0.0004
OfurOR43         LC002737         198         4.00         8.34         0.008         0.010         0.0003           OfurOR44         LC002738         436         3.54         4.33         0.003         0.006         0.0000	OfurOR42	LC002736	346	4.16	7.13	0.010	0.015	0.0000
OfurOR44         LC002738         436         3.54         4.33         0.003         0.006         0.0000	OfurOR43	LC002737	198	4.00	8.34	0.008	0.010	0.0003
	OfurOR44	LC002738	436	3.54	4.33	0.003	0.006	0.0000
OfurOR45 LC002739 407 3.51 6.69 0.013 0.013 0.000	OfurOR45	LC002739	407	3.51	6.69	0.013	0.013	0.0000

(Continued)



### Table 5. (Continued)

Name	Accession Number	aa length	RPKM		RT-PCR**		
			Male	Female	Male	Female	Thorax
OfurOR46	LC002740	431	3.05	8.02	0.015	0.016	0.0006
OfurOR47	LC002741	103	2.86	1.63	0.002	0.003	0.0000
OfurOR48	LC002742	265	2.65	7.15	0.037	0.015	0.0001
OfurOR49	LC002743	361	2.49	5.72	0.006	0.010	0.0002
OfurOR50	LC002744	355	1.88	4.52	0.003	0.006	0.0000
OfurOR51	LC002745	380	1.56	2.86	0.006	0.008	0.0000
OfurOR52	LC002746	409	0.79	6.31	0.002	0.003	0.0000
OfurOR53	LC002747	407	0.00	7.55	0.000	0.018	0.0000

\*: Reported in the previous paper [12].

\*\*: Relative expression level to the internal control.

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difference between males and females was smaller for most receptors than that estimated by read mapping.

### Phylogenetic analysis

Phylogenetic relationships between *OfurORs* and *BmorORs*, as well as with odorant receptors in *C. pomonella* are shown in Fig. 3. As expected, *Orco* was highly conserved among the three





represent standard error calculated from the results of three biological replicates.

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0.4

Fig 3. Phylogenetic relationship of *O. furnacalis* odorant receptors (*OfurORs*) with those of B. mori (*BmorORs*) and C. pomonella (*CpomORs*). The tree was constructed by the maximum likelihood method using RAxML and visualized using FigTree. *OfurORs* are indicated in red. Green, pink, and yellow shading indicates the clades of *Orco*, pheromone receptors, and *OfurOR53*, respectively.

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species. All the previously identified pheromone receptors of *O. furnacalis* formed a single clade with other pheromone receptors from *B. mori* and *C. pomonella*. Within this clade, however, receptors from the same species tended to form subclusters, suggesting that pheromone receptors have undergone species-specific duplication events. The female-specific receptor *OfurOR53* formed a clade with *BmorOR30* and *CpomOR30*, among which *BmorOR30* was reported to exhibit female-specific expression [34, 40], whereas *CpomOR30* was not [41]. The other two female-biased receptors, *OfurOR15* and *OfurOR39*, belonged to independent clades. *OfurOR15* formed a clade with *OfurOR28*, *OfurOR41*, *BmorOR14*, *CpomOR14*, and *Cpo-mOR20*. Among these, *OfurOR41* showed slightly female-biased expression (Fig. 2B), but the others were expressed both in males and females [34, 40, 41]. *OfurOR39* formed a clade with *OfurOR51*, *BmorOR50*, *BmorOR51*, and *CpomOR43*. None of these were reported to be female biased [34, 40, 41].

### Identification of other genes involved in the odorant perception

We also identified candidate genes of 21 IRs, 5 GRs, 2 SNMPs and 26 ODEs (<u>Table 6</u>). All the genes were novel in *O. furnacalis* except for SNMPs [50]. The phylogenetic relationships between *OfurIRs, BmorIRs*, and *CpomIRs* are shown in Fig. 4. ODEs were divided into three families, including eight aldehyde oxidases (*OfurAOX1* to *OfurAOX8*), fifteen carboxylesterase (*OfurCXE1* to *OfurCXE15*) and three alcohol dehydrogenase (*OfurAD1* to *OfurAD3*) (<u>Table 6</u>). Most of the identified genes were full length. However, all of the GR genes were partial, probably due to their low expression levels in the antennae.

## Discussion

### Pheromone receptors

In the previous study, pheromone receptors in *O. furnacalis* were cloned by degenerate PCR [12]. For this reason, the 5' and 3' terminal sequences of the ORFs were not known. In the present study, we identified complete ORF sequences for seven of the nine previously identified pheromone receptors. On the other hand, *OfurOR1* was not found in our RNA-seq analysis. It was also not detected in the independent qRT-PCR analysis, indicating that *OfurOR1* was not expressed in our sample. This might be due to intraspecies polymorphism because our samples and those used in the previous studies were derived from different localities in Japan [12]. None of the 45 novel receptors found in this study showed male-biased expression as observed in the previously identified pheromone receptors. The previously identified pheromone receptors were structurally distinct from the other receptors; they formed a single clade in the phylogenetic analysis. Thus, it is likely that there are no additional pheromone receptors in *O. furnacalis* other than the already identified ones. However, the presence of some other receptors that incidentally respond to pheromone components was not excluded. Identification of novel odorant receptors in *O. furnacalis* provides the opportunity to experimentally examine this possibility.

# Phylogenetic relationship of odorant receptors with sexually biased expression

Genome wide analysis of the expression pattern of odorant receptors has been carried out in several lepidopteran species including *B. mori* [34, 40], *Manduca sexta* [52], *C. pomonella* [41], *Helicoverpa armigera* [53], and *Spodoptera littoralis* [54]. In each species, receptors with sexspecific expression have been identified. Some of these receptors are phylogenetically close to each other. The most significant example is the pheromone receptor group, which contains



### Table 6. List of other candidate genes involved in olfactory perception in O. furnacalis.

Number         Number         Number         Number           Ökurlökurft         LC017781         900         5.83         5.83           Ökurlökurft         LC017781         900         5.83         5.83           Ökurlökurft         LC017781         902         37.14         908           Ökurläka         LC017783         849         2.68         3.66           Ökurläka         LC017785         709         3.28         5.45           Ökurläka         LC017785         506         5.79         6.65           Ökurläka         LC017785         606         5.79         6.65           Ökurläka         LC017789         665         13.28         6.66           Ökurläka         LC017789         663         3.32         6.60           Ökurläka         LC017789         663         3.83         6.64           Ökurläka         LC017789         639         5.42         1.04           Ökurläka         LC01778         639         6.44         7.05           Ökurläka         LC01778         547         27.01         4.67           Ökurläka         LC01778         547         2.01         4.67	Name	Accession Number	aa length	RPKM		
Durkliker         LOD17780         923         5.67         7.7           OkunGluR2         LC017781         900         5.93         6.3           OkunGluR2         LC017782         902         37.14         5.08           OkunR25a         LC017785         709         3.26         5.54           OkunR40a         LC017785         709         3.26         5.54           OkunR45a         LC017786         596         12.15         17.0           OkunR45a         LC017787         606         5.79         8.4           OkunR75a         LC017789         626         13.28         16.0           OkunR75a         LC017790         274         2.29         3.1           OkunR75a         LC017792         609         5.42         10.4           OkunR75a2         LC017792         609         5.42         10.4           OkunR75a3         LC017793         639         3.30         0.0           OkunR75a2         LC017794         890         6.64         7.0           OkunR75a2         LC017797         890         6.64         7.0           OkunR75a2         LC017797         890         6.64         7.0				Male	Female	
OkurGlu/2         LC017781         900         5.93         6.3           OkurRa         LC017782         902         37.14         508           OkurRa         LC017783         849         26.88         36.6           OkurRa         LC017784         942         71.03         668.0           OkurRa         LC017786         596         12.15         17.0           OkurRa         LC017787         606         5.79         6.8           OkurRa         LC017788         300         4.34         6.66           OkurRa         LC017789         628         13.28         16.0           OkurRa         LC017792         609         5.42         10.4           OkurRa         LC017793         633         3.0         0.0           OkurRa         LC017794         637         6.99         11.5           OkurRa         LC017796         644         5.25         7.4           OkurRa         LC01779         890         6.64         7.0           OkurRa         LC01779         890         6.64         7.0           OkurRa         LC01779         890         6.64         7.0           OkurRa	OfuriGluR1	LC017780	923	5.67	7.99	
OkufR8a         LC017782         902         37.14         608           OkufR2ia         LC017783         849         26.88         36.60           OkufR2ia         LC017785         709         3.26         6.45           OkufR4ia         LC017786         506         1.215         1.70           OkufR4ia         LC017787         606         5.79         8.1           OkufR5a         LC017789         626         13.28         16.0           OkufR75d         LC017790         274         2.29         3.3           OkufR75d         LC017791         630         4.40         10.9           OkufR75g2         LC017792         609         5.42         10.4           OkufR75g3         LC017793         6.37         6.99         11.5           OkufR75g2         LC017795         547         27.01         46.7           OkufR75g3         LC017796         358         2.02         4.1           OkufR75g         LC01779         357         9.06         18.2           OkufR7         169         1.11         1.6         1.6         1.2           OkufR7         100         0.30         2.6         0.00	OfuriGluR2	LC017781	900	5.93	6.37	
OkurR25a         LC017783         849         26.88         36.66           OkurR25a         LC017784         942         71.03         66.8.0           OkurR41a         LC017785         709         3.26         5.4.5           OkurR43a         LC017786         596         12.15         17.0           OkurR45a         LC017788         340         4.34         6.6           OkurR55         LC017789         625         13.28         16.0           OkurR753         LC017790         27.4         2.29         3.1           OkurR7532         LC017791         630         4.40         10.3           OkurR7532         LC017793         637         6.99         11.5           OkurR754         LC017793         647         2.25         7.1           OkurR755         LC017796         644         5.25         7.1           OkurR753         LC017796         647         5.25         7.1           OkurR75         140         1.62         3.2         1.6           OkurR3         LC01779         357         9.06         18.2           OkurR4         LC01779         357         9.05         14.2	OfurlR8a	LC017782	902	37.14	50.82	
OkurR25a         LC017784         942         71.03         6630           OkurR440         LC017785         709         3.28         5.4           OkurR44a         LC017786         596         12.15         17.0           OkurR64a         LC017789         626         13.28         16.6           OkurR753         LC017789         625         13.28         16.0           OkurR7531         LC017791         609         5.42         10.3           OkurR7532         LC017792         609         5.42         10.4           OkurR7532         LC017793         639         3.30         0.0           OkurR7532         LC017795         547         27.01         46.7           OkurR7532         LC017795         547         27.01         46.7           OkurR753         LC017795         547         27.01         46.7           OkurR754         LC017795         357         9.06         18.2           OkurR75         140         1.62         3.2         14.2           OkurR76         LC01777         11         1.05         1.4           OkurR76         LC017775         140         1.62         3.2 <t< td=""><td>OfurlR21a</td><td>LC017783</td><td>849</td><td>26.88</td><td>36.66</td></t<>	OfurlR21a	LC017783	849	26.88	36.66	
OhurRADQ         LC017785         709         3.28         5.4           OhurRAH         LC017786         596         12.15         17.0           OhurRAB         LC017787         606         5.79         6.81           OhurRBBa         LC017789         626         13.28         16.0           OhurR750         LC017790         274         2.29         6.11           OhurR7501         LC017791         630         4.40         10.0           OhurR7502         LC017793         639         3.30         0.00           OhurR7503         LC017793         637         6.99         11.5           OhurR7504         LC017795         547         27.01         44.7           OhurR7502         LC017796         654         5.25         7.1           OhurR33         LC017797         890         6.64         7.0           OhurR3         LC01779         357         9.06         14.2           OhurR3         LC017776         130         2.36         0.3           OhurR4         LC017776         140         1.62         0.3           OhurR3         LC017776         121         1.05         1.4	OfurlR25a	LC017784	942	71.03	68.03	
Ohurikata         LC017786         596         12.15         17.0           Ohurikada         LC017787         606         5.79         8.1           Ohurikada         LC017788         340         4.34         6.6           Ohurikata         LC017789         626         13.28         16.0           Ohurik75         LC017791         630         4.40         10.3           Ohurik75p2         LC017792         609         5.42         10.4           Ohurik75p3         LC017791         633         3.0         0.0           Ohurik75p2         LC017794         637         6.99         11.5           Ohurik75p3         LC017795         547         27.01         467           Ohurik73a         LC01779         890         6.64         7.0           Ohurik7a         LC01779         890         6.64         7.0           Ohurik7a         LC017780         357         9.06         182           Ohurik7         LC017776         140         1.62         32           Ohurik7         LC017777         121         1.05         1.4           Ohurik7         LC01778         100         0.00         2.3 <t< td=""><td>OfurlR40a</td><td>LC017785</td><td>709</td><td>3.26</td><td>5.41</td></t<>	OfurlR40a	LC017785	709	3.26	5.41	
OkurRR44         LC017787         606         5.79         6.1           OkurR75         LC017789         626         13.28         10.00           OkurR75d         LC017790         274         2.29         3.1           OkurR75d         LC017791         630         4.40         10.3           OkurR75p1         LC017792         609         5.42         10.40           OkurR75p2         LC017793         639         3.30         0.00           OkurR75p2         LC017795         547         27.01         4.67           OkurR75p2         LC017796         654         5.25         7.1           OkurR75p3         LC017797         890         6.64         7.0           OkurR83         LC017797         890         6.64         7.0           OkurR75         LC017797         357         9.06         18.2           OkurR83         LC017775         100         0.0         2.5           OkurB73         LC017776         130         2.36         0.0           OkurB75         LC017776         130         2.36         0.0           OkurG81         LC017775         121         1.05         1.4	OfurlR41a	LC017786	596	12.15	17.01	
OHURREBa         LC017788         340         4.34         6.6           OHUR75         LC017780         626         13.28         16.0           OHUR75         LC017790         274         2.29         3.1           OHUR7502         LC017791         630         4.40         10.3           OHUR7502         LC017792         609         5.42         10.4           OHUR7502         LC017794         637         6.99         11.5           OHUR7502         LC017795         547         27.01         467           OHUR7502         LC017796         654         5.25         7.1           OHUR7503         LC017797         890         6.64         7.0           OHUR7504         LC017798         358         2.02         4.1           OHUR7505         LC01779         890         6.64         7.0           OHUR78         LC01779         357         9.06         13.2         3.2           OHUR78         LC01779         140         1.62         3.2         3.2           OHUR78         LC01777         121         1.05         4.4         4.0           OHUR78         LC01777         124         3.0 <td>OfurlR64a</td> <td>LC017787</td> <td>606</td> <td>5.79</td> <td>8.17</td>	OfurlR64a	LC017787	606	5.79	8.17	
OhurR75         LC017789         626         13.28         10.0           OhurR75d         LC017790         274         2.29         3.1           OhurR75p1         LC017791         630         4.40         10.3           OhurR75p2         LC017792         609         5.42         10.4           OhurR75p3         LC017793         639         3.30         0.0           OhurR75p2         LC017796         547         27.01         46.7           OhurR75p2         LC017796         547         27.01         46.7           OhurR75p2         LC017796         654         5.25         7.1           OhurR75p2         LC017797         890         6.64         7.0           OhurR75p2         LC017798         358         2.02         4.1           OhurR75p2         LC01779         357         9.06         13.2           OhurR75p2         LC01776         140         1.62         3.3           OhurR75         140         1.62         3.3         0.0           OhurR75         140         1.62         3.3         0.0         0.2         5.0           OhurR75         140         1.62         3.3 <t< td=""><td>OfurlR68a</td><td>LC017788</td><td>340</td><td>4.34</td><td>6.67</td></t<>	OfurlR68a	LC017788	340	4.34	6.67	
OhunR75d         LC017790         274         2.29         3.1           OhunR75p1         LC017791         630         4.40         10.3           OhunR75p2         LC017792         609         5.42         10.40           OhunR75p3         LC017793         639         3.30         0.00           OhunR75p2         LC017796         5.47         27.01         46.7           OhunR75p3         LC017796         654         5.25         7.1           OhunR75p4         LC017796         654         5.25         7.1           OhunR3a         LC017796         654         5.25         7.1           OhunR3a         LC017796         357         9.06         11.8           OhunR3         LC017779         357         9.06         11.8           OhunR3         LC017775         140         1.62         3.2           OhunR4         LC017777         121         1.05         1.4           OhunGR5         LC017777         121         1.05         1.4           OhunGR4         LC017778         100         0.00         2.5           OhunGR5         LC01775         124         3.5         3.36	OfurIR75	LC017789	626	13.28	16.00	
OlufR75p1         LC017791         630         4.40         10.3           OlufR75p2         LC017792         609         5.42         10.4           OlufR75p3         LC017793         639         3.30         0.00           OlufR75p2         LC017795         547         27.01         46.7           OlufR75p2         LC017796         654         5.25         7.1           OlufR73q2         LC017797         890         6.64         7.00           OlufR73a         LC017798         357         9.06         18.2           OlufR3         LC017790         357         9.06         18.2           OlufR3         LC017775         140         1.62         3.2           OlufR4         LC017775         140         1.62         3.3           OlufR3         LC017775         140         1.62         3.3           OlufR4         LC017775         140         1.62         3.3           OlufR3         LC01777         121         1.05         1.4           OlufGR4         LC01777         121         3.6         3.3           OlufSNMP2         LC01775         128         135.03         1317.2	OfurlR75d	LC017790	274	2.29	3.13	
OfurlR75p2         LC017792         609         5.42         10.4           OfurlR75p3         LC017793         639         3.30         0.00           OfurlR75p3         LC017794         637         6.99         11.5           OfurlR76b         LC017795         547         27.01         48.7           OfurlR76b         LC017797         890         6.64         7.0           OfurlR783         LC017799         357         9.06         18.2           OfurlR2         LC017799         357         9.06         18.2           OfurlR3         LC017790         140         1.62         3.2           OfurlR4         LC017775         140         1.62         3.2           OfurlGR3         LC017776         130         2.36         0.0           OfurlGR4         LC017777         121         1.05         1.4           OfurlSNMP1         LC017752         1275         134.36         3.3           OfurAX1         LC017752         1275         134.36         161.1           OfurAX2         LC017752         1275         134.36         161.1           OfurAX3         LC017755         766         6.35         10.4	OfurlR75p1	LC017791	630	4.40	10.33	
OtufR75p3         LC017793         639         3.30         0.0           OtufR75q2         LC017794         637         6.99         11.5           OtufR76b         LC017795         547         27.01         46.7           OtufR87a         LC017796         654         5.25         7.1           OtufR93a         LC017797         890         6.64         7.0           OtufR1         LC017798         357         9.06         18.2           OtufR3         LC017800         178         1.11         16.2           OtufGR1         LC017775         140         1.62         3.2           OtufGR2         LC017776         130         2.36         0.0           OtufGR3         LC017777         121         1.05         1.4           OtufGR5         LC017779         194         2.36         3.3           OtufGR5         LC017751         122         135.03         135.03         135.03           OtufGR5         LC017753         1275         134.36         161.1         1.5           OtufGR5         LC017754         1280         19.36         15.9           OtufAOX1         LC017755         766         6.35 </td <td>OfurlR75p2</td> <td>LC017792</td> <td>609</td> <td>5.42</td> <td>10.47</td>	OfurlR75p2	LC017792	609	5.42	10.47	
OturlR75g2         LC017794         637         6.99         11.5           OfurlR75b         LC017795         547         27.01         46.7           OfurlR87a         LC017796         654         5.25         7.1           OfurlR97a         LC017797         690         6.64         7.0           OfurlR1         LC017798         357         9.06         18.2           OfurlR3         LC01775         140         1.62         3.2           OfurlGR2         LC017776         130         2.36         0.0           OfurGR3         LC017776         121         1.05         1.4           OfurGR4         LC017777         194         2.36         3.3           OfurGR5         LC017778         194         2.36         3.3           OfurGR4         LC017775         194         2.36         3.3           OfurADX1         LC017752         1275         134.3         161.1           OfurADX1         LC017753         1279         189.54         297.3           OfurAOX3         LC017755         766         6.35         10.4           OfurAOX4         LC017755         766         6.35         10.4 <t< td=""><td>OfurlR75p3</td><td>LC017793</td><td>639</td><td>3.30</td><td>0.00</td></t<>	OfurlR75p3	LC017793	639	3.30	0.00	
OturlR76b         LC017795         547         27.01         46.7           OturlR87a         LC017796         664         5.25         7.1           OturlB93a         LC017797         890         6.64         7.0           OturlR1         LC017798         357         9.06         18.2           OturlR2         LC017799         357         9.06         18.2           OturlR3         LC017797         140         1.62         3.2           OturdR4         LC017775         140         1.62         3.2           OturGR3         LC017777         121         1.05         1.4           OturGR4         LC017778         194         2.36         3.3           OturGR5         LC017777         121         1.05         1.4           OturGR5         LC017778         194         2.36         3.3           OturA0X1         LC017801         528         191.31         389.5           OturA0X2         LC017752         127.5         134.36         161.1           OturA0X3         LC017754         1280         19.36         3.3           OturA0X4         LC017755         766         6.35         10.4 <tr< td=""><td>OfurlR75q2</td><td>LC017794</td><td>637</td><td>6.99</td><td>11.58</td></tr<>	OfurlR75q2	LC017794	637	6.99	11.58	
OturlR87a         LC017796         664         5.25         7.1           OfurlR93a         LC017797         890         6.64         7.0           OfurlR1         LC017798         358         2.02         4.1           OfurlR3         LC017799         357         9.06         182           OfurlR3         LC017800         178         1.11         1.6           OfurGR2         LC017776         121         1.05         3.2           OfurGR3         LC017777         121         1.05         1.4           OfurGR4         LC017778         100         0.00         2.5           OfurGR5         LC017779         194         2.36         3.3           OfurSNMP1         LC017801         528         919.31         389.5           OfurAX1         LC017752         1275         134.36         161.1           OfurAX3         LC017754         1280         19.36         159.9           OfurAX4         LC017755         766         6.35         10.4           OfurAX5         LC017755         766         6.35         10.4           OfurAX4         LC017755         766         6.35         10.4	OfurlR76b	LC017795	547	27.01	46.73	
Ofur/R93a         LC017797         890         6.64         7.0           Ofur/R1         LC017798         358         2.02         4.1           Ofur/R2         LC017799         357         9.06         18.2           Ofur/R3         LC017790         357         9.06         18.2           Ofur/R1         LC017775         140         1.62         3.2           OfurGR1         LC017776         130         2.36         0.0           OfurGR3         LC017777         121         1.05         1.4           OfurGR4         LC017778         100         0.00         2.5           OfurGR5         LC017779         194         2.36         3.3           OfurSNMP1         LC017801         528         919.31         389.5           OfurAX1         LC017752         1275         134.36         161.1           OfurAX3         LC017753         1279         189.54         27.3           OfurAX4         LC017754         1280         19.36         59.3           OfurAX5         LC017755         766         6.35         10.4           OfurAX6         LC017757         1268         13.28         13.9	OfurlR87a	LC017796	654	5.25	7.12	
OturIR1         LC017798         358         2.02         4.1           OfurIR2         LC017799         357         9.06         18.2           OfurIR3         LC017800         178         1.11         1.6           OfurGR1         LC017775         140         1.62         3.2           OfurGR2         LC017776         130         2.36         0.0           OfurGR3         LC017777         121         1.05         1.4           OfurGR4         LC017778         100         0.00         2.5           OfurGR5         LC017779         194         2.36         3.3           OfurSNMP1         LC017801         528         919.31         386.5           OfurAX1         LC017752         1275         134.36         161.1           OfurAX2         LC017753         1279         189.54         297.3           OfurAX3         LC017755         766         6.35         10.4           OfurAX4         LC017757         1268         13.28         13.9           OfurAX5         LC017758         778         7.68         5.1           OfurAX6         LC017759         378         2.44         4.0	OfurlR93a	LC017797	890	6.64	7.06	
OturiR2         LC017799         357         9.06         18.2           OfuriR3         LC017800         178         1.11         1.6           OfurGR1         LC017775         140         1.62         3.2           OfurGR2         LC017776         130         2.36         0.0           OfurGR3         LC017777         121         1.05         1.4           OfurGR4         LC017778         100         0.00         2.56           OfurGR5         LC017779         124         2.36         3.3           OfurSNMP1         LC017801         528         919.31         389.5           OfurAX1         LC017752         1275         134.36         161.1           OfurAX2         LC017753         1279         189.54         297.3           OfurAX3         LC017755         766         6.35         10.4           OfurAX4         LC017755         766         6.35         10.4           OfurAX4         LC017759         378         2.44         4.0           OfurAX5         LC017759         378         2.44         4.0           OfurAX6         LC017761         541         135.42         11.63	OfurIR1	LC017798	358	2.02	4.12	
Ofur/R3         LC017800         178         1.11         1.6           OfurGR1         LC017775         140         1.62         3.2           OfurGR2         LC017776         130         2.36         0.0           OfurGR3         LC017777         121         1.05         1.4           OfurGR4         LC017778         100         0.00         2.5           OfurGR5         LC017779         194         2.36         3.3           OfurGR4         LC01778         100         0.00         2.5           OfurSNMP1         LC017801         528         919.31         389.5           OfurAX1         LC017752         1275         134.36         161.1           OfurAOX3         LC017754         1280         19.36         15.9           OfurAOX3         LC017755         766         6.35         10.9           OfurAOX4         LC017756         593         3.36         6.8           OfurAOX5         LC017759         378         2.44         4.0           OfurAOX6         LC017761         541         135.42         116.3           OfurAOX8         LC017761         541         135.42         116.3 <t< td=""><td>OfurIR2</td><td>LC017799</td><td>357</td><td>9.06</td><td>18.22</td></t<>	OfurIR2	LC017799	357	9.06	18.22	
OfurGR1         LC017775         140         1.62         3.2           OfurGR2         LC017776         130         2.36         0.0           OfurGR3         LC017777         121         1.05         1.4           OfurGR4         LC017778         100         0.00         2.5           OfurGR5         LC017779         194         2.36         3.3           OfurSNMP1         LC017801         528         919.31         389.5           OfurACX1         LC017752         1275         134.36         161.1           OfurAOX2         LC017753         1279         189.54         297.3           OfurAOX3         LC017754         1280         19.36         15.9           OfurAOX4         LC017755         766         6.35         10.4           OfurAOX5         LC017758         778         7.68         5.1           OfurAOX6         LC017760         560         5.93         5.2           OfurAOX8         LC017761         5411         135.42         116.3           OfurACX5         LC017763         559         66.51         34.9           OfurAOX6         LC017763         559         66.51         34.9 </td <td>OfurIR3</td> <td>LC017800</td> <td>178</td> <td>1.11</td> <td>1.65</td>	OfurIR3	LC017800	178	1.11	1.65	
OfurGR2         LC017776         130         2.36         0.0           OfurGR3         LC017777         121         1.05         1.4           OfurGR4         LC017778         100         0.00         2.5           OfurGR5         LC017779         194         2.36         3.3           OfurSNMP1         LC017801         528         919.31         389.5           OfurSNMP2         LC017802         523         1352.03         1317.2           OfurAOX1         LC017752         1275         134.36         161.1           OfurAOX2         LC017753         1279         189.54         297.3           OfurAOX3         LC017754         1280         19.36         15.9           OfurAOX4         LC017755         766         6.35         10.4           OfurAOX5         LC017756         593         3.36         6.8           OfurAOX6         LC017757         1268         13.28         13.9           OfurAOX7         LC017759         378         2.44         4.0           OfurAOX8         LC017761         541         135.42         116.3           OfurCXE1         LC017761         541         135.42         116.3 </td <td>OfurGR1</td> <td>LC017775</td> <td>140</td> <td>1.62</td> <td>3.25</td>	OfurGR1	LC017775	140	1.62	3.25	
OfurGR3         LC017777         121         1.05         1.4           OfurGR4         LC017778         100         0.00         2.5           OfurGR5         LC017779         194         2.36         3.3           OfurSNMP1         LC017801         528         919.31         389.5           OfurANMP2         LC017802         523         1352.03         1317.2           OfurAX1         LC017752         1275         134.36         161.1           OfurAX3         LC017753         1279         189.54         297.3           OfurAX4         LC017755         766         6.35         10.4           OfurAX4         LC017756         593         3.36         68.8           OfurAX5         LC017757         1268         13.28         13.9           OfurAX4         LC017758         778         7.68         5.1           OfurAX5         LC017759         378         2.44         4.0           OfurAX5         LC017761         541         135.42         116.3           OfurAX24         LC017763         559         66.51         34.9           OfurAX5         LC017761         541         135.42         116.3	OfurGR2	LC017776	130	2.36	0.00	
OfurGR4         LC017778         100         0.00         2.5           OfurGR5         LC017779         194         2.36         3.3           OfurSNMP1         LC017801         528         919.31         389.5           OfurANMP2         LC017802         523         1352.03         1317.2           OfurANMP2         LC017752         1275         134.36         161.1           OfurAOX1         LC017753         1279         189.54         297.3           OfurAOX3         LC017754         1280         19.36         15.9           OfurAOX4         LC017755         766         6.35         10.4           OfurAOX5         LC017756         593         3.36         66.8           OfurAOX6         LC017757         1268         13.28         13.9           OfurAOX7         LC017758         778         7.68         5.1           OfurAOX8         LC017761         560         5.93         5.2           OfurACXE1         LC017761         541         135.42         116.3           OfurACXE3         LC017761         541         135.42         116.3           OfurCXE3         LC017763         559         66.51 <t< td=""><td>OfurGR3</td><td>LC017777</td><td>121</td><td>1.05</td><td>1.47</td></t<>	OfurGR3	LC017777	121	1.05	1.47	
OfurGR5         LC017779         194         2.36         3.3           OfurGNNMP1         LC017801         528         919.31         389.5           OfurGNNMP2         LC017802         523         1352.03         1317.2           OfurGNX1         LC017752         1275         134.36         161.1           OfurAOX1         LC017753         1279         189.54         297.3           OfurAOX3         LC017754         1280         19.36         15.9           OfurAOX4         LC017755         766         6.35         10.4           OfurAOX5         LC017757         1268         13.28         13.9           OfurAOX6         LC017759         378         2.44         4.0           OfurAOX8         LC017760         560         5.93         5.2           OfurACX2         LC017761         541         135.42         116.3           OfurCXE1         LC017761         541         135.42         116.3           OfurCXE3         LC017763         559         66.51         34.9           OfurCXE4         LC017763         559         66.51         34.9           OfurCXE5         LC017764         566         15.20	OfurGR4	LC017778	100	0.00	2.50	
OfurSNMP1         LC017801         528         919.31         389.5           OfurSNMP2         LC017802         523         1352.03         1317.2           OfurAOX1         LC017752         1275         134.36         161.1           OfurAOX2         LC017753         1279         189.54         297.3           OfurAOX3         LC017754         1280         19.36         15.9           OfurAOX4         LC017755         766         6.35         10.4           OfurAOX5         LC017756         593         3.36         6.8           OfurAOX6         LC017757         1268         13.28         13.9           OfurAOX7         LC017758         778         7.68         5.1           OfurAOX8         LC017760         560         5.93         5.2           OfurCXE1         LC017761         541         135.42         116.3           OfurCXE2         LC017761         541         135.42         116.3           OfurCXE3         LC017763         559         66.51         34.9           OfurCXE4         LC017763         559         66.51         34.9           OfurCXE5         LC017764         566         15.20	OfurGR5	LC017779	194	2.36	3.38	
OfurSNMP2         LC017802         523         1352.03         1317.2           OfurAOX1         LC017752         1275         134.36         161.1           OfurAOX2         LC017753         1279         189.54         297.3           OfurAOX3         LC017754         1280         19.36         15.9           OfurAOX4         LC017755         766         6.35         10.4           OfurAOX5         LC017756         593         3.36         6.8           OfurAOX6         LC017757         1268         13.28         13.9           OfurAOX7         LC017758         778         7.68         5.1           OfurAOX8         LC017760         560         5.93         5.2           OfurAOX7         LC017760         560         5.93         5.2           OfurCXE1         LC017761         541         135.42         116.3           OfurCXE2         LC017763         559         66.51         34.9           OfurCXE3         LC017763         559         66.51         34.9           OfurCXE5         LC017764         566         15.20         19.4           OfurCXE6         LC017765         511         13.59         15.	OfurSNMP1	LC017801	528	919.31	389.54	
OfurAOX1         LC017752         1275         134.36         161.1           OfurAOX2         LC017753         1279         189.54         297.3           OfurAOX3         LC017754         1280         19.36         15.9           OfurAOX4         LC017755         766         6.35         10.4           OfurAOX5         LC017756         593         3.36         6.8           OfurAOX6         LC017757         1268         13.28         13.9           OfurAOX7         LC017758         778         7.68         5.1           OfurAOX8         LC017759         378         2.44         4.0           OfurCXE1         LC017760         560         5.93         5.2           OfurCXE2         LC017761         541         135.42         116.3           OfurCXE3         LC017763         559         66.51         34.9           OfurCXE4         LC017763         559         66.51         34.9           OfurCXE5         LC017764         566         15.20         19.4           OfurCXE6         LC017765         511         13.59         15.4	OfurSNMP2	LC017802	523	1352.03	1317.27	
OfurAOX2         LC017753         1279         189.54         297.3           OfurAOX3         LC017754         1280         19.36         15.9           OfurAOX4         LC017755         766         6.35         10.4           OfurAOX5         LC017756         593         3.36         6.8           OfurAOX6         LC017757         1268         13.28         13.9           OfurAOX7         LC017758         778         7.68         5.1           OfurAOX8         LC017759         378         2.44         4.0           OfurCXE1         LC017761         541         135.42         116.3           OfurCXE2         LC017763         559         66.51         34.9           OfurCXE4         LC017763         559         66.51         34.9           OfurCXE5         LC017763         559         66.51         34.9           OfurCXE5         LC017764         566         15.20         19.4           OfurCXE6         LC017765         511         13.59         15.4	OfurAOX1	LC017752	1275	134.36	161.16	
OfurAOX3         LC017754         1280         19.36         15.90           OfurAOX4         LC017755         766         6.35         10.4           OfurAOX5         LC017756         593         3.36         6.8           OfurAOX6         LC017757         1268         13.28         13.9           OfurAOX7         LC017758         778         7.68         5.1           OfurAOX8         LC017760         560         5.93         5.2           OfurCXE1         LC017761         541         135.42         116.3           OfurCXE2         LC017763         559         66.51         34.9           OfurCXE4         LC017763         559         66.51         34.9           OfurCXE5         LC017764         566         15.20         19.4           OfurCXE6         LC017765         511         13.59         15.4	OfurAOX2	LC017753	1279	189.54	297.30	
OfurAOX4         LC017755         766         6.35         10.4           OfurAOX5         LC017756         593         3.36         6.8           OfurAOX6         LC017757         1268         13.28         13.9           OfurAOX7         LC017758         778         7.68         5.1           OfurAOX8         LC017759         378         2.44         4.0           OfurAOX8         LC017760         560         5.93         5.2           OfurCXE1         LC017761         541         135.42         116.3           OfurCXE3         LC017762         532         11.35         14.7           OfurCXE4         LC017763         559         66.51         34.9           OfurCXE5         LC017764         566         15.20         19.4           OfurCXE6         LC017765         511         13.59         15.4	OfurAOX3	LC017754	1280	19.36	15.92	
OfurAOX5         LC017756         593         3.36         6.8           OfurAOX6         LC017757         1268         13.28         13.9           OfurAOX7         LC017758         778         7.68         5.1           OfurAOX8         LC017759         378         2.44         4.0           OfurAOX8         LC017760         560         5.93         5.2           OfurCXE1         LC017761         541         135.42         116.3           OfurCXE3         LC017762         532         11.35         14.7           OfurCXE4         LC017763         559         66.51         34.9           OfurCXE5         LC017764         566         15.20         19.4           OfurCXE6         LC017765         511         13.59         15.4	OfurAOX4	LC017755	766	6.35	10.49	
OfurAOX6         LC017757         1268         13.28         13.9           OfurAOX7         LC017758         778         7.68         5.1           OfurAOX8         LC017759         378         2.44         4.0           OfurCXE1         LC017760         560         5.93         5.2           OfurCXE2         LC017761         541         135.42         116.3           OfurCXE3         LC017762         532         11.35         14.7           OfurCXE4         LC017763         559         66.51         34.9           OfurCXE5         LC017764         566         15.20         19.4           OfurCXE6         LC017765         511         13.59         15.4	OfurAOX5	LC017756	593	3.36	6.88	
OfurAOX7         LC017758         778         7.68         5.1           OfurAOX8         LC017759         378         2.44         4.0           OfurCXE1         LC017760         560         5.93         5.2           OfurCXE2         LC017761         541         135.42         116.3           OfurCXE3         LC017762         532         11.35         14.7           OfurCXE4         LC017763         559         66.51         34.9           OfurCXE5         LC017764         566         15.20         19.4           OfurCXE6         LC017765         511         13.59         15.4	OfurAOX6	LC017757	1268	13.28	13.98	
OfurAOX8         LC017759         378         2.44         4.0           OfurCXE1         LC017760         560         5.93         5.2           OfurCXE2         LC017761         541         135.42         116.3           OfurCXE3         LC017762         532         11.35         14.7           OfurCXE4         LC017763         559         66.51         34.9           OfurCXE5         LC017764         566         15.20         19.4           OfurCXE6         LC017765         511         13.59         15.4	OfurAOX7	LC017758	778	7.68	5.12	
OfurCXE1         LC017760         560         5.93         5.2           OfurCXE2         LC017761         541         135.42         116.3           OfurCXE3         LC017762         532         11.35         14.7           OfurCXE4         LC017763         559         66.51         34.9           OfurCXE5         LC017764         566         15.20         19.4           OfurCXE6         LC017765         511         13.59         15.4	OfurAOX8	LC017759	378	2.44	4.03	
OfurCXE2         LC017761         541         135.42         116.3           OfurCXE3         LC017762         532         11.35         14.7           OfurCXE4         LC017763         559         66.51         34.9           OfurCXE5         LC017764         566         15.20         19.4           OfurCXE6         LC017765         511         13.59         15.4	OfurCXE1	LC017760	560	5.93	5.25	
OfurCXE3         LC017762         532         11.35         14.7           OfurCXE4         LC017763         559         66.51         34.9           OfurCXE5         LC017764         566         15.20         19.4           OfurCXE6         LC017765         511         13.59         15.4	OfurCXE2	LC017761	541	135.42	116.35	
OfurCXE4         LC017763         559         66.51         34.9           OfurCXE5         LC017764         566         15.20         19.4           OfurCXE6         LC017765         511         13.59         15.4	OfurCXE3	LC017762	532	11.35	14.77	
OfurCXE5         LC017764         566         15.20         19.4           OfurCXE6         LC017765         511         13.59         15.4	OfurCXE4	LC017763	559	66.51	34.96	
OfurCXE6 LC017765 511 13.59 15.4	OfurCXE5	LC017764	566	15.20	19.43	
	OfurCXE6	LC017765	511	13.59	15.40	
OfurCXE7 LC017766 317 82.91 68.7	OfurCXE7	LC017766	317	82.91	68.76	
OfurCXE8 LC017767 566 354.93 237.8	OfurCXE8	LC017767	566	354.93	237.89	
OfurCXE9 LC017768 544 23.12 30.3	OfurCXE9	LC017768	544	23.12	30.35	
OfurCXE10 LC017769 542 23.89 28.6	OfurCXE10	LC017769	542	23.89	28.67	

(Continued)



Name	Accession Number	aa length	RPKM	
			Male	Female
OfurCXE11	LC017770	527	22.83	32.79
OfurCXE12	LC017771	519	17.16	30.97
OfurCXE13	LC017772	511	44.64	61.26
OfurCXE14	LC017773	562	175.11	229.03
OfurCXE15	LC017774	515	2.32	5.19
OfurAD1	LC017749	325	32.41	43.19
OfurAD2	LC017750	356	5.34	8.79
OfurAD3	LC017751	365	46.62	53.06

### Table 6. (Continued)

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nine receptors from three species (BmorOR1, 3, 4, 5, 6; HarmOR14, 15; SlitOR6, 13) that were male specific [34, 53, 54]. The previously identified pheromone receptors in O. furnacalis belonged to this group, and most of them were male specific [12]. However, not all of the members were male biased. Seven receptors from three species (CpomOR3, 5; HarmOR1, 2, 11; SlitOR11, 16) were equally expressed in males and females [41, 53, 54]. Furthermore, CpomOR15 was shown to be female specific [41]. Another example is a group of receptors including OfurOR53 and BmOR30. Although these two receptors were specifically expressed in female antennae ([34, 40], this study), orthologous receptors in other species (*CpomOR30* and *SlitOR30*) were also expressed in the male antennae [41, 54]. These examples indicated that sexually biased expression is under the influence of phylogenetic constraint to some extent, but it also evolves dynamically from sex-specific expression to sex-independent expression and vice versa. Nevertheless, it should be noted that in most of the previous studies, the expression levels were determined by non-quantitative methods, leaving the possibility that the difference between sexes was over- or under-estimated [34, 41, 52-54]. Quantitative analysis of the expression level is necessary to gain insight into the evolutionary pattern of sexually biased expression of odorant receptors.

### Biological function of female-biased receptors in O. furnacalis

In this study, the expression levels of the all receptors were estimated quantitatively by two independent methods, which demonstrated that *OfurOR53*, *15*, and *39* had female-biased expression. Importantly, the latter two were the receptors with the highest expression level in female antennae next to *OfurOR2* (*Orco*). One possible function of these receptors is the perception of male sex pheromone, which was reported to be required for acceptance of mating by females in *O. nubilalis* [21]. The *OfurOR7* is also a candidate for the male pheromone receptor. It belongs to the pheromone receptor group, and it was also expressed in the female antennae. Another possible function of the female-biased receptors is to recognize host-plant volatiles. Finding an appropriate host plant is crucial for reproduction in the herbivorous lepidopteran insects. Odorant receptors involved in host-plant detection would serve as a potential target for novel pest control techniques. In this regard, *OfurOR15* and *OfurOR39*, the receptors with the highest expression levels in female antennae, should be considered as the primary candidates for further characterization of their molecular function.

Openmines         Openmines <t< th=""><th>1 <b>)</b>.</th></t<>	1 <b>)</b> .
ofuniR15d OfuniR15d OfuniR15d CpomIR3 CpomIR1 LalungO Callurogo Callurogo	
0.4	

Fig 4. Phylogenetic relationship of *O. furnacalis* ionotropic receptors (*OfurIRs*) with those of B. mori (*BmorIRs*) and C. pomonella (*CpomIRs*). The tree was constructed by the maximum likelihood method using RAxML and visualized using FigTree. *OfurORs* are indicated in red.

doi:10.1371/journal.pone.0121261.g004

### Repertoire of odorant receptors in O. furnacalis

Although an intensive analysis of the antennal transcriptome was conducted in this study, other tissues were not investigated. Therefore, odorant receptors not expressed in the antennae were not included in our analysis. Furthermore, receptors with extremely low expression levels

may not have been identified. In fact, the ORF sequences appeared to be incomplete for a few receptors with low expression levels (<u>Table 5</u>). Two receptors with a similar sequence, such as recently duplicated pairs, were indistinguishable in our analysis, as seen in the case of *Ofur-OR5a* and *OfurOR5b*. Finally, extremely divergent receptors that were not similar to any of the other insect odorant receptors may not be identified in our analysis, although the candidates excluded at the third screening (homology search against the NCBI nr database) were significantly similar to non-odorant-receptor proteins. These limitations mean that our method is conservative, and whole genome sequence analysis may identify additional odorant receptors in *O. furnacalis*. Nevertheless, our results provide a list of odorant receptors with significant expression in the antennae, thus they are considered to be biologically functional. Our present results will serve as a basis for studies to understand the evolution of the pheromone communication system, as well as for the development of novel control methods of agriculturally important pests.

## **Author Contributions**

Conceived and designed the experiments: TM. Performed the experiments: BY KO. Analyzed the data: BY TM. Contributed reagents/materials/analysis tools: YI KO TM. Wrote the paper: BY KO YI TM.

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