# Identification and expression profiles of candidate chemosensory receptors in *Histia rhodope* (Lepidoptera: Zygaenidae)

Haibo Yang [Corresp., 1], Junfeng Dong [Corresp., 1], Ya-Lan Sun [1], Zhenjie Hu [1], Qihui Lv [1], Dingxu Li [1]

[1] College of Forestry, Henan University of Science and Technology, Luoyang, Henan, China

Corresponding Authors: Haibo Yang, Junfeng Dong
Email address: hbyang@haust.edu.cn, junfengdong@haust.edu.cn

Insect olfaction and vision play important roles in survival and reproduction. Diurnal butterflies mainly rely on visual cues whereas nocturnal moths rely on olfactory signals to locate external resources. *Histia rhodope* Cramer (Lepidoptera: Zygaenidae) is an important pest of the landscape tree *Bischofia polycarpa* in China and other Southeast Asian regions. As a diurnal moth, *H. rhodope* represents a suitable model for studying the evolutionary shift from olfactory to visual communication. However, only a few chemosensory soluble proteins have been characterized and information on *H. rhodope* chemoreceptor genes is currently lacking. In this study, we identified 45 odorant receptors (ORs), 9 ionotropic receptor s (IRs), 8 gustatory receptors (GRs) and 2 sensory neuron membrane proteins (SNMPs) from our previously acquired *H. rhodope* antennal transcriptomic data. The number of chemoreceptors of *H. rhodope* was less compared with that found in many nocturnal moths. Some specific chemoreceptors such as OR co-receptor (ORco), ionotropic receptor co-receptor, $CO_2$ receptor, sugar receptors and bitter receptors were predicted by phylogenetic analysis. Notably, two candidate pheromone receptors (PRs) were identified within a novel PR lineage. qRT-PCR results showed that almost all tested genes (22/24) were predominantly expressed in antennae, indicating that they may be important in olfactory function. Among these antennae-enriched genes, 6 ORs, 5 IRs and 2 GRs displayed female-biased expression, while 2 ORs displayed male-biased expression. Additionally, HrhoIR75q.2 and HrhoGR67 were more highly expressed in heads and legs. This study enriches the olfactory gene inventory of *H. rhodope* and provides the foundation for further research of the chemoreception mechanism in diurnal moths.

## Identification and expression profiles of candidate chemosensory receptors in *Histia rhodope* (Lepidoptera: Zygaenidae)

Hai-Bo Yang, Jun-Feng Dong, Ya-Lan Sun, Zhen-Jie Hu, Qi-Hui Lv, Ding-Xu Li

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

Corresponding Author:
Hai-Bo Yang
Email address: hbyang@haust.edu.cn
Jun-Feng Dong
Email address: junfengdong@haust.edu.cn

16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41

## Abstract

Insect olfaction and vision play important roles in survival and reproduction. Diurnal butterflies mainly rely on visual cues whereas nocturnal moths rely on olfactory signals to locate external resources. *Histia rhodope* Cramer (Lepidoptera: Zygaenidae) is an important pest of the landscape tree *Bischofia polycarpa* in China and other Southeast Asian regions. As a diurnal moth, *H. rhodope* represents a suitable model for studying the evolutionary shift from olfactory to visual communication. However, only a few chemosensory soluble proteins have been characterized and information on *H. rhodope* chemoreceptor genes is currently lacking. In this study, we identified 45 odorant receptors (ORs), 9 ionotropic receptors (IRs), 8 gustatory receptors (GRs) and 2 sensory neuron membrane proteins (SNMPs) from our previously acquired *H. rhodope* antennal transcriptomic data. The number of chemoreceptors of *H. rhodope* was less compared with that found in many nocturnal moths. Some specific chemoreceptors such as OR co-receptor (ORco), ionotropic receptor co-receptor, $CO_2$ receptor, sugar receptors and bitter receptors were predicted by phylogenetic analysis. Notably, two candidate pheromone receptors (PRs) were identified within a novel PR lineage. qRT-PCR results showed that almost all tested genes (22/24) were predominantly expressed in antennae, indicating that they may be important in olfactory function. Among these antennae-enriched genes, 6 ORs, 5 IRs and 2 GRs displayed female-biased expression, while 2 ORs displayed male-biased expression. Additionally, HrhoIR75q.2 and HrhoGR67 were more highly expressed in heads and legs. This study enriches the olfactory gene inventory of *H. rhodope* and provides the foundation for further research of the chemoreception mechanism in diurnal moths.

## Introduction

*Histia rhodope* Cramer (Lepidoptera: Zygaenidae), a diurnal moth, is a destructive forest pest widely distributed in China and other Southeast Asian regions (Huang, 1980). Although *H. rhodope* is an oligophagous pest, its larvae mainly feed on the leaves of *Bischofia polycarpa* (Chinese bishopwood), resulting in severe defoliation. Mature larvae always fall from the tree by spinning silk, which not only affects the appearance but also disturbs human activities (Huang, 1980; Yang et al., 2019). Upon eclosion, males court females through nuptial flight in the afternoon. In addition, females release sex pheromones for attracting males. After mating, females then search for suitable oviposition sites and lay eggs (Yang et al., 2019). Therefore, the detection of sex pheromones is crucially important for reproduction.

Olfaction plays an important role in many behaviors in insects. Insects depend on their sensitive olfactory system to detect semiochemicals in complex environments (Gadenne, Barrozo & Anton, 2016; Haverkamp, Hansson & Knaden, 2018). A myriad of proteins is implicated in the process of olfactory perception. Chemoreceptors are key proteins that interact directly with odorants and play an important role in the specificity and sensitivity of insect peripheral chemoreception (Leal, 2013). Chemosensory receptors include odorant receptors (ORs) (Clyne et al., 1999), gustatory receptors (GRs) (Agnihotri, Roy & Joshi, 2016) and ionotropic receptors (IRs) (Benton et al., 2009). Additionally, sensory neuron membrane proteins (SNMPs), located

82    in different dendrites of the olfactory receptor neurons, function in odor detection (Rogers,
83    Krieger & Vogt, 2001).
84        Insect ORs were first identified in the *Drosophila melanogaster* genome. They are
85    characterized by seven transmembrane domains (TMDs) with an intracellular N-terminus and an
86    extracellular C-terminus (Clyen et al., 1999). During chemosensory signal transduction, ORs
87    serve as signal-transducers by converting chemical signals into electric signals in the insect
88    olfactory process (Hallem & Carlson, 2006). Functional insect ORs consist of a ligand-gated
89    nonselective cation channel, composed of one divergent OR subunit that confers odor specificity,
90    and one conserved OR co-receptor (ORco) subunit (Sato et al., 2008; Buttrerwick et al., 2018).
91    The OR repertoire size is considerably variable among insect species, and the genes are rapidly
92    evolving (Andersson, Löfstedt & Newcomb, 2015; Robertson, 2019).
93        IRs belong to the ionotropic glutamate receptor family (iGluRs). Their molecular structures
94    consist of an extracellular ligand-binding domain with two lobes and an ion channel (Benton et
95    al. 2009). IRs can be divided into two subfamilies: conserved 'antennal IRs', involved in
96    olfaction, thermo- and hygrosensation, and species-specific 'divergent IRs', found in peripheral
97    and internal gustatory organs and involved in gustation (Croset et al., 2010; Koh et al., 2014).
98    Similar to ORco, IRs have highly conserved IR co-receptors in different insect species, including
99    IR8a, IR25a and IR76b, which are widely expressed and play different roles in the process of
100   odorant and taste sensation (Abuin et al., 2011). Besides the perception of acids, amines and
101   amino acids (Hussain et al., 2016), IRs are also involved in sensing temperature and humidity
102   stimuli, auditory function and regulating the circadian clock (Senthilan et al., 2012; Chen et al.,
103   2015; Ni et al., 2016; Frank et al., 2017). To date, IRs function reported in lepidopteran species
104   is quite limited. However, recent research showed that IR8a was essential for acid-mediated
105   feces avoidance in ovipositioning hawkmoth, *Manduca sexta* (Zhang et al., 2019).
106       GRs and ORs have a similar membrane topology. GRs are mainly expressed in the gustatory
107   organs (Dunipace et al., 2001; Touhara & Vosshall, 2009; Guo et al., 2017), although some
108   genes are also expressed in insect antennae (Croset et al., 2010). Generally, GRs are not highly
109   conserved among insect species, and mainly detect some nonvolatile compounds and contact
110   stimuli (Clyne, Warr & Carlson, 2000; Scott et al., 2001). However, candidate carbon dioxide
111   ($CO_2$) receptors are highly conserved in lepidopteran species, such as BmorGR1-3 receptor from
112   *Bombyx mori* (Wanner & Robertson, 2008), HmelGR1-3 from *Heliconius melpomene* (Briscoe et
113   al. 2013) and HarmGR1-3 from *Helicoverpa armigera* (Ning et al., 2016).
114       SNMPs belong to the cluster of differentiation 36 (CD36) receptor family with two TMDs and
115   are mainly expressed in pheromone sensitive neurons in *Drosophila* and moths (Rogers, Krieger
116   & Vogt, 2001; Benton, Vannice & Vosshall, 2007; Vogt et al., 2009). SNMPs are divided into
117   SNMP1 and SNMP2 subgroups (Forstner et al., 2008). Notably, SNMPs are known to participate
118   in pheromone detection (Jin, Ha & Smith, 2008; Pregitzer et al., 2014; Sun et al., 2019), although
119   the molecular mechanisms of their involvement in chemoreception are unknown.
120       Although various olfactory related genes have recently been identified in numerous moth
121   species, most of them are from nocturnal moths, such as Noctuidae and Pyralidae (Yuvaraj et al.,

122   2018). However, some moth species are diurnal, such as Castniidae, Phaudidae, Sphingidae and
123   Zygaenidae (Subchev, 2014; Monteys et al., 2016; Zheng et al., 2019; Chen et al., 2020). Their
124   activities are only observed during the daytime. Studies investigating the chemosensory genes in
125   diurnal moths are scarce. These genes have only been studied in one non-ditrysian moth,
126   *Eriocrania semipurpurella* (Eriocraniidae), belonging to the oldest lineages of Lepidoptera
127   (Yuvaraj et al., 2017). Visual and/or olfactory cues mediate the orientation of most lepidopteran
128   adults during mating. The contrasting lifestyles between the diurnal and nocturnal insects result
129   in a substantial distinction in their sensory biology: diurnal butterflies primarily rely on visual
130   cues while nocturnal moths largely rely on olfactory signals (Martin et al., 2011; Arikawa, 2017).
131   However, this does not imply that butterflies are anosmic or moths are blind. Both butterflies and
132   moths employ olfactory signals for sex communication. Besides, even at night, male moths
133   strongly rely on visual cues from the landscape to track a pheromone trail to find a receptive
134   female (Preiss & Kramer, 1986). While diurnal moths search for mating partners using olfactory
135   cues over relatively long distances, they employ visual and auditory cues within short distances
136   or even a combination of visual, olfactory and auditory signals (Chen et al., 2020).
137      Nevertheless, there has been a notable chemosensory/visual shift between moths and
138   butterflies that at least affect long-range/short-range sex-attraction/courtship, as well as certain
139   aspects of host plant recognition (Costanzo & Monteiro, 2007). A previous study of gene gain
140   and loss within the general odorant binding proteins (GOBP)/pheromone binding proteins (PBP)
141   of two moths and butterflies revealed that diurnal butterflies might have lost a PBP gene
142   compared with nocturnal moths (Vogt, Grosse-Wilde & Zhou, 2015). Moreover, in our previous
143   study, we constructed transcriptomes from both male and female adult antennae of *H. rhodope*
144   using *de novo* transcriptome sequencing and assembly. We identified some chemosensory
145   soluble proteins, such as odorant binding proteins (OBPs), chemosensory proteins (CSPs) and
146   Niemann-Pick type C2 proteins (NPC2s) in this diurnal moth. We found that *H. rhodope* might
147   also have lost a PBP gene (Yang et al., 2020). Therefore, we hypothesize that *H. rhodope* could
148   also have lost chemoreceptor genes associated with moth pheromone detection. However, the
149   numbers, expression profiles and functions of chemoreceptor genes in *H. rhodope* are unknown.
150      In the present study, we identified candidate chemoreceptor genes based on our previous
151   antennal transcriptomic data of *H. rhodope* (Yang et al., 2020). The phylogenetic relationships
152   between the ORs, IRs, GRs and SNMPs of *H. rhodope* and other insect species were further
153   investigated. Finally, we examined their tissue expression using quantitative real-time PCR
154   (qRT-PCR). This work provides the basis for future functional characterization of olfactory
155   genes in *H. rhodope*.
156
## Materials & Methods
### Insect rearing and tissue collection
159   *H. rhodope* was collected from *B. polycarpa* forest in Sui and Tang Dynasties City Ruins
160   Botanical Garden (112.45°E, 34.64°N) in Luoyang city, Henan province, China. We received
161   verbal permission to collect insects in Sui and Tang Dynasties City Ruins Botanical Garden from

162   the management of the Garden. The larvae were reared on fresh *B. polycarpa* leaves under
163   constant conditions of 25 ± 1ºC and 70 ± 5% relative humidity with 16 h light: 8 h dark
164   photoperiod. Adult moths were grouped based on their sex and fed with 10% honey solution
165   after emergence. For RNA-seq samples, 500 pairs of 1 day old adult antennae from each gender
166   were dissected. For qRT-PCR analysis, we collected 50 male antennae (MA), 50 female
167   antennae (FA), 50 heads (♀:♂ = 1:1; H) whose antennae were cut off and 50 legs (♀:♂ = 1:1; L)
168   in three replications from 1 day old virgin adults. After collection, the samples were preserved in
169   liquid nitrogen for further use.
170
171   **Identification of putative chemosensory receptor proteins**
172   The antenna transcriptomes of unmated *H. rhodope* were reported in our previous study. Six
173   female and male antennae samples (three biological replicates each gender) were separately
174   sequenced using the Illumina Hiseq4000 platform. The female and male antennae yielded at least
175   21.62 and 21.39 million clean reads per sample, respectively. All clean reads from male and
176   female samples were combined into an assembly that generated 50,218 unigenes with a mean
177   length of 1,031.08 bp and an N50 length of 2,247 bp (Yang et al., 2020). A tBLASTn analysis
178   was performed using available ORs, IRs, GRs and SNMPs protein sequences from lepidopteran
179   species as 'queries' to identify candidate unigenes in *H. rhodope.* All putative ORs, IRs, GRs
180   and SNMPs were manually checked using the BLASTx program in the National Center for
181   Biotechnology Information (NCBI) with a cut-off E-value of $10^{-5}$. The OR genes were numbered
182   arbitrarily, while the IR, GR and SNMP genes were named based on the highest scoring Blastx
183   match from the NR database. Open reading frames (ORFs) were predicted using the ORF Finder
184   (https://www.ncbi.nlm.nih.gov/orffinder/). The TMDs of the putative chemoreceptor genes were
185   predicted on the TOPCONS website (http://topcons.net/).
186
187   **Comparison of transcript abundances of chemosensory receptor genes**
188   Using the assembled transcriptome as reference sequences, the clean data from various samples
189   of *H. rhodope* were mapped back onto the reference sequences using Bowtie2 v2.1.0 software
190   (Langmead et al., 2009). The unigene expression levels among various samples were estimated
191   with RSEM (Li & Dewey, 2011) according to the read count values of the unigenes for each
192   sample, which were obtained from the mapping results. The transcript abundance of candidate
193   chemoreceptor genes in male and female antennal transcriptomes was calculated using fragments
194   per kb per million fragments (FPKM) values (Li & Dewey, 2011). Heatmap plots of the
195   chemoreceptor gene expression were generated in Microsoft Excel, using the conditional
196   formatting option. For each plot, blue color was used for the minimum value, yellow for the
197   midpoint and red for the maximum value. For all gene families, the range was specified for each
198   tissue type independently, such that the color gradient was set based upon the highest FPKM
199   values within each tissue, not across all tissues. The criteria for estimating significant
200   differentially expressed genes were set as the absolute value of log2 Fold Change >1.
201   **Phylogenetic analysis**

202 Phylogenetic trees of candidate chemoreceptors were constructed using MEGA7.0 software
203 (Kumar, Stecher &Tamura, 2016). Amino acid sequences were first aligned using the program
204 ClustalX (http://www.clustal.org/clustal2/). Maximum likelihood (ML) statistical method was
205 used to infer evolutionary relationship and unrooted trees were built with the Jones-Taylor-
206 Thornton (JTT) model used to obtain the initial trees for the heuristic search. The tree calculation
207 was carried out using 1000 bootstrap replicates. Generated trees were edited using Figtree
208 software (http://tree.bio.ed.ac.uk/software/figtree/). The protein sequences of chemoreceptors
209 used for building phylogenetic trees are listed in Supplementary file 1.
210

211 **Expression level analysis**
212 We selected 10 ORs, all IRs, 3 GRs and 2 SNMPs to verify their expression profiles. 10 ORs and
213 3 GRs were selected for qRT-PCR analysis because they were significant DEGs between male
214 and female antennae according to the criteria as described above. The relative expression levels
215 of these genes in different tissues were determined using qRT-PCR. Different tissues were
216 collected from both male and female adults as described above. Total RNA was extracted using
217 TRIzol reagent. First-strand cDNA was synthesized from 2 μg of total RNA using the
218 PrimeScript RT reagent kit with gDNA Eraser (Takara, Dalian, China) according to the
219 manufacturer's instructions. qRT-PCR primers (Supplementary Table S1) were designed with
220 Primer Premier 5 (Premier Biosoft International, CA, USA) and synthesized by Sangon Biotech
221 Co., Ltd (Shanghai, China). The glyceraldehyde-phosphate dehydrogenase (GAPDH) gene was
222 identified from the *H. rhodope* antennal transcriptome and used as the internal reference (Yang
223 et al., 2020). The qRT-PCR was conducted on a StepOne Plus Real-time PCR System (Applied
224 Biosystems, Foster City, CA, USA) using SYBR Premix ExTaq II (Tli RNaseH Plus) (Takara,
225 Dalian, China). Each reaction (20 μL volume) contained 2 μL cDNA, 10 μL SYBR® Premix Ex
226 Taq, 0.4 μL forward and reverse primers (10 μM), and 7.2 μL RNase-free double distilled water.
227 qRT-PCR was performed as follows: initial denaturation at 95°C for 3 min, 40 cycles at 95°C for
228 10 s and 60°C for 30 s. Melting curve analysis was performed from 55 °C to 95 °C to determine
229 the specificity of qPCR primers. To determine the efficiency of the qPCR primers, a standard
230 curve (cDNA concentration vs. Ct) was produced with a 5-fold dilution series of legs cDNA
231 corresponding to one microgram total RNA. qRT-PCR efficiencies were then calculated
232 according to the equation: $E = (10^{[-1/slope]} - 1)*100$ (Pfaffl, 2001; Radonić et al., 2004). The
233 $2^{-\Delta\Delta Ct}$ method was used to analyze gene expression profiles (Pfaffl, 2001). All data were
234 normalized to endogenous GAPDH rRNA levels from the identical tissue sample, and the
235 relative fold change in the different tissues was calculated with the transcript level of the legs as
236 the calibrator. Each reaction was performed in triplicate (from three biological replicates).
237    Relative expressions of each test gene from the different tissues were compared using a one-
238 way analysis of variance (ANOVA) in SPSS 20.0 software (IBM, Chicago, IL, USA) followed
239 by the least significant difference test (LSD) (critical values corresponding to $P = 0.05$).
240

241 **Results**

**Identification and phylogenetic analyses of ORs in *H. rhodope***

A total of 45 candidate ORs, including 44 conventional ORs and one ORco, were identified in *H. rhodope* antennal transcriptome (Table 1). Among these genes, 25 candidate OR genes had 7 TMDs and full ORFs encoding more than 370 amino acids. HrhoORco shared 85.44% sequence identity with *Galleria mellonella* ORco (NCBI ID: QEI46859) and 84.81% sequence identity with *Ostrinia furnacalis* ORco (NCBI ID: XP_028178675). Gene expression levels of all 45 ORs were assessed using the FPKM values, where 23 *ORs* were relatively highly expressed in the FA and two *ORs* were relatively highly expressed in the MA. The remaining 20 ORs showed no differences in expression levels between sexes (Fig. 1A).

A phylogenetic tree was constructed with 195 ORs from *H. rhodope*, *B. mori*, *H. armigera*, *O. furnacalis*, *Cnaphalocrocis medinalis*, *Heliothis virescens*, *Dendrolimus punctatus*, *Spodoptera littoralis* and *M. sexta*. Most of the HrhoORs have orthologous relationships with the other lepidopteran species (Fig. 2). As anticipated, HrhoORco clustered in the ORco family. In addition, four HrhoORs (HrhoOR4, 16, 39 and 40) were segregated into one unique clade whereas HrhoOR15/23, HrhoOR2/32 and HrhoOR21/28 clustered together. Interestingly, *H. rhodope* ORs lacked in the classical moth pheromone receptor (PR) clade, while HrhhoOR14 and HrhoOR30 clustered within a novel PR clade containing SlitOR5 in *S. littoralis* and several candidate PRs from *D. punctatus*. These ORs were recently identified as a novel lineage of PRs detecting type Ⅰ pheromones in Lepidoptera (Bastin-Héline et al., 2019; Shen et al., 2020).

**Identification and phylogenetic analyses of IRs in *H. rhodope***

Nine putative IRs were identified from the antennal transcriptome of *H. rhodope* and named as HrhoIR8a, 21a, 76b, 41a, 60a, 75q2, 68a, 75p and 40a based on homologous sequences from other insects (Table 2). All candidate IRs were partial sequences and the TMDs of IRs ranged from 1 to 4.Three IRs (*HrhoIR8a*, *60a* and *75q.2*) were significantly higher expressed in FA than in MA. The highest FPKM value of IRs (*HrhoIR68a*, FPKM = 202.80) was found in the FA (Fig. 1B).

A phylogenetic tree was constructed with 99 IRs from *H. rhodope*, *Cydia pomonella*, *Epiphyas postvittana* and *D. melanogaster* (Fig. 3). We found that HhroIR8a, HrhoIR76b, HrhoIR75p, HrhoIR75q.2, HrhoIR21a, HrhoIR68a, HrhoIR40a, HhroIR60 and HrhoIR41a were clustered into IR8a, IR76b, IR75, IR21a, IR68a, IR40a, IR60a and IR41a clades with high bootstrap values, respectively. According to their positions in the phylogenetic tree and based on the strong bootstrap support, the candidate HrhoIRs names were consistent with the number and suffix of known IRs. However, no orthologs for IR25a, IR75d, IR93a and IR87a were identified from *H. rhodope*.

**Identification and phylogenetic analyses of GRs in *H. rhodope***

We identified eight candidate GR genes in the antennal transcriptomes of *H. rhodope* (Table 3). All GR genes had incomplete ORFs ranging from 390 to 1218 bp in length with 1-5 TMDs. The FPKM values of all *HrhoGRs* were less than those of OR, IR and SNMP genes. Three GR genes

282  (*HrhoGR3*, *HrhoIR29* and *HrhoIR64a*) were significantly higher expressed in the FA than in the
283  MA (Fig. 1C).
284      Phylogenetic analysis of 81 GRs from five lepidopteran species showed that several candidate
285  GRs were closely related to other known insect GRs that function as sugar receptors, bitter
286  receptors and $CO_2$ receptors. HrhoGR7 and HhroGR64a clustered into sugar receptor clade,
287  HrhoGR67 clustered into bitter receptor subfamily and HrhoGR2 clustered into the $CO_2$ receptor
288  clade. Notably, the other four GRs (HrhoGR3/4/29/68.1) had orthologous relationships with *C.*
289  *pomonella* (Fig. 4).
290
291  **Identification and phylogenetic analyses of SNMPs in *H. rhodope***
292  We identified two candidate SNMPs (HrhoSNMP1 and HrhoSNMP2) from antennal
293  transcriptomes of *H. rhodope* (Table 4). Two SNMP genes had intact ORFs (length: 1422-1581
294  bp) with 1-2 TMDs. According to the FPKM values of SNMPs, *HrhoSNMP1* and *HrhoSNMP2*
295  were equally expressed in both the FA and the MA groups (Fig. 1D). SNMPs phylogenetic tree
296  revealed that HrhoSNMP1 and HrhoSNMP2 clustered into SNMP1 and SNMP2 clades,
297  respectively (Fig. 5).
298
299  **Tissue-specific expression analysis by qRT-PCR**
300  To assess the difference in expression of chemoreceptor genes between male and female
301  antennae, heads (without antennae) and legs and test the RNA-Seq results, 10 ORs, all IRs, 3
302  GRs and 2 SNMPs were selected for qRT-PCR. The results showed that the expression levels of
303  the tested genes in the male and female antennae were consistent with the RNA-Seq results. The
304  qRT-PCR results revealed that all 10 *HrhoORs* were significantly higher expressed in the
305  antennae than in the heads and legs. Furthermore, 6 OR genes (*HrhoOR9, 24, 26, 33, 37* and *40*)
306  were significantly higher expressed in the FA while *HrhoOR4* and *HrhoOR13* were significantly
307  higher expressed in the MA. Interestingly, *HrhoORco* and *HrhoOR11* showed no differences in
308  expression between sexes (Fig. 6A-J). Notably, *HrhoORco*, *HrhoOR4* and *HrhoOR37* showed
309  higher expression levels in the antennae compared with the expression levels of other *HrhoORs*.
310      The qRT-PCR results revealed that the expression of 5 IRs (*HrhoIR8a, 21a, 40a, 60a* and *76b*)
311  was significantly higher in the FA while that of 3 genes (*HrhoIR41a, 68a* and *75p*) was equally
312  expressed in the antennae of both sexes. Additionally, we found that *HrhoIR75q.2* and *HrhoIR40*
313  had higher expression in the heads compared with other genes (Fig. 6K-S).
314      *HrhoGR2* and *HrhoGR64a* displayed significant FA-specific expressions, while *HrhoGR67*
315  had significantly higher expression in the heads than in other tissues (Fig. 6T-V). *HrhoSNMP1*
316  and *HrhoSNMP2* were significantly expressed at high levels in the antennae in both sexes;
317  *HrhoSNMP2* was also highly expressed in heads (Fig. 6W-X).
318
319  **Discussion**
320  To date, a growing number of chemosensory genes have been identified in moths. However,
321  most of the genes are from nocturnal moths (Ditrysia) (Yuvaraj et al., 2018). Currently, the

322 molecular basis of chemoreception in the Zygaenidae family of diurnal moths is poorly
323 understood. The identification and characterization of the chemoreceptor genes of *H. rhodope*,
324 an important forest pest and diurnal moth, will improve our understanding of olfaction
325 mechanisms in Zygaenidae and provide the basis for further exploration of sensory disparities
326 between the diurnal and nocturnal moths.
327     In the present study, we identified 45 ORs, 9 IRs, 8 GRs and 2 SNMPs from our previous *H.*
328 *rhodope* transcriptomic data (Yang et al., 2020). The number of chemoreceptor genes in *H.*
329 *rhodope* was less compared to previously reported numbers in other nocturnal lepidopteran
330 species, such as 62 ORs, 20 IRs and 16 GRs in *Mythimna separata* (Du et al., 2018), 64 ORs, 22
331 IRs and 30 GRs in *Spodoptera exigua* (Zhang et al., 2018), 60 ORs, 21 IRs and 197 GRs in *H.*
332 *armigera* (Liu et al., 2014; Xu et al., 2016), 60 ORs, 17 IRs and 17 GRs in *S. littoralis* (Walker
333 III et al., 2019) and 58 ORs, 21 IRs and 22 GRs in *C. pomonella* (Walker III et al., 2016).
334 Previous studies reported that different chemosensory behavior (Zhang et al., 2017), life stage
335 (He et al., 2017), ecological niche breadth (Gouin et al., 2017), phenotype (Purandare & Brisson,
336 2020), mating status (Jin et al., 2017), circadian rhythm (Gadenne, Barrozo & Anton, 2016) and
337 feeding trait (Taparia, Ignell & Hill, 2017) influence the variation in chemosensory gene number.
338 The smaller number of genes identified in the current study may be due to the following reasons.
339 Firstly, we identified olfactory related genes from antenna transcriptome data only, whereas
340 previous studies used transcriptome data from different developmental stages and organs,
341 especially for GRs. Secondly, the number of chemosensory genes may be related to its ability for
342 diverse host-odor detection to feed on the range size of host plants (Gouin et al., 2017). *H.*
343 *rhodope* is an oligophagous pest requiring no additional olfactory proteins to perceive single host
344 chemicals (Gouin et al., 2017; Yang et al., 2020). Finally, *H. rhodope* is a diurnal insect,
345 although it is a moth and not a butterfly. Diurnal moths mainly depend on visual cues while
346 nocturnal moths mainly depend on their odorant signals (Vogt, Grosse-Wilde & Zhou, 2015).
347 Chemosensory gene loss in diurnal moths compared to nocturnal moths could result from the
348 shift from olfactory to visual communication.
349     Odorant receptors act as signal-transducers that convert chemical molecules to electrical
350 signals in insects (Leal, 2013). ORs are desirable targets for alternative strategies to control
351 insect populations due to their critical roles in the initial steps of the olfactory response process
352 (Venthur & Zhou, 2018). In this study, 44 specific ORs and 1 ORco were identified. ORco genes
353 are widely expressed and highly conserved among lepidopteran species and play a key role in
354 insect olfaction (Touhara & Vosshall, 2009). HrhoORco shared a high degree of similarity with
355 the ORco genes of other insects and clustered into the ORco subfamily. Previous studies showed
356 that many ORco mutants were anosmic. For instance, mutagenesis of BmolORco and MsexORco
357 severely disrupted the olfactory system (Liu et al., 2017a; Fandino et al., 2019), suggesting that
358 the ORco genes might be the primary potential target genes for pest management.
359     Generally, phylogenetic analysis of lepidopteran ORs showed two specific branches
360 comprising the ORco orthologs and the traditional PR clade. Interestingly, no PRs were related
361 to the conserved lepidopteran PR clade. However, two ORs (HrhoOR14 and 30) clustered into a

362   novel lineage distantly related to the conserved lepidopteran PR clade. Notably, several
363   DpunPRs (Shen et al., 2020) and SlitOR5 (Bastin-Héline et al., 2019) that grouped with
364   HrhoOR14 and 30 were recently characterized as a new evolution origin of PRs in Lepidoptera.
365   We speculate that these two ORs may be involved in the detection of *H. rhodope* sex
366   pheromones, although further functional analysis is needed to confirm this hypothesis. Besides
367   phylogenetic analysis, the expression pattern is another main criterion used to select candidate
368   PRs for functional studies. SlitOR5 and DpunPRs were all highly or specifically expressed in the
369   MA. Unfortunately, we did not conduct the expression levels of HrhoOR14 and 30 by qRT-PCR.
370   However, according to the FPKM values, HrhoOR14 exhibited male-biased expression while
371   HrhoOR30 exhibited female-biased expression. This expression pattern was similar to the novel
372   lineage of PRs, whereby approximately one-half of the ORs were male-biased and the other half
373   were female-biased.
374        The expression patterns of candidate genes may reveal important clues into their functions.
375   Overall, sexually differential expressions of ORs in antennae suggest a possible involvement of
376   PRs in sexual behaviors. Female-biased ORs are believed to play a role in detecting oviposition-
377   related odorant (Pelletier et al., 2010) or pheromones (signals) released by males (Anderson et al.,
378   2009), whereas male-biased ORs potentially function in the detection of sex pheromones
379   released by females (Zhang & Löfstedt, 2013). ORs expressed evenly between female and male
380   antennae are predicted to take part in general odorant perception (Yan et al., 2015). Therefore,
381   we speculate that male-biased expressed ORs (*HrhoOR4* and *13*) are likely to participate in
382   female pheromone perception. On the other hand, female-biased expressed HrhoORs, including
383   *HrhoOR9*, *24*, *26*, *33*, *37* and *40*, may function in the detection of oviposition-related plant odors
384   or male-produced courtship pheromones. The other HrhoORco and HrhoOR11 with
385   approximately equal expression levels between female and male antennae are likely to function
386   in the detection of food source odors. In addition, HrhoOR37 was orthologous to CmedOR32
387   according to phylogenetic analysis, and the expression levels of these two OR genes were higher
388   in the FA than in the MA (Liu et al., 2017b). Therefore, we speculate that these two orthologous
389   OR genes may play similar roles in odor detection. Detailed functional studies of these sex-
390   specific ORs should be performed in further studies.
391        IRs are a new subfamily of chemosensory receptors. IRs are relatively conserved in both the
392   sequence and the expression patterns and are widely distributed throughout the body parts,
393   including the labellum, pharynx, leg and wing compared with ORs (Koh et al., 2014; Rimal &
394   Lee 2018). In this study, 9 IRs were identified. IR8a, IR25a and IR76b were regarded as co-
395   receptors just like ORco as they were co-expressed along with other stimulus-specific IRs
396   (Abuin et al., 2011). HrhoIR8a and HrhoIR76b clustered in the IR8a/IR25a/IR76b subfamily,
397   indicating that these two genes may perform a function similar to that of the co-receptors in *D.
398   melanogaster*. Interestingly, IR25a, one of the conserved IR co-receptors, was not identified in
399   this study. The failure to identify IR25a could be due to the low expression levels in the antennae
400   in this species. Other IRs found in antennae belong to the conserved "antennal IRs" (Croset et al.,
401   2010) and have their respective orthologs. This revealed that IRs are highly conserved across

402    insect orders and HrhoIRs may have conserved functions, retaining their roles as IRs in other
403    Lepidoptera species. However, the function of these IRs has been studied in *D. melanogaster*.
404    For example, DmelIR21a and 25a are essential for cool sensing (Ni et al., 2016), IR40 and IR68a
405    are involved in the detection of temperature and humidity (Enjin et al., 2016; Frank et al., 2017),
406    IR75 functions in acid sensing (Prieto-Godino et al., 2017) and IR76b is involved in the
407    sensation of taste (Ganguly et al., 2017). Therefore, HrhoIR21a, HrhoIR40a, HrhoIR68a,
408    HrhoIR75 and HrhoIR76b are predicted to perform functions similar to those in *D. melanogaster*,
409    which may involve activation by acids, temperatures, humidity and other factors. The function of
410    IRs in lepidopteran species is still unclear, warranting further functional studies. In the
411    expression patterns of the 9 *IRs* identified, 8 *HrhoIRs* were highly expressed in the antennae,
412    indicating that these IRs may participate in odor, thermo- and hygrosensation. Three *IRs*,
413    *HrhoIR40a*, *HrhoIR75p* and *HrhoIR75q.2*, were also expressed in the heads and legs except for
414    the antennae. Notably, *HrhoIR75q.2* had the highest expression level in the heads compared with
415    the other four tissues studied. The expression of these three *IRs* in different tissues suggests that
416    they may have multiple functions. In addition to olfaction, IRs are associated with gustation,
417    hygrosensation and thermosensation (Rimal & Lee, 2018). In *D. melanogaster*, *IR21a*, *IR40a*,
418    *IR68a* and *IR93a* were expressed in the antennae and played critical roles in thermosensation and
419    hygrosensation (Enjin et al., 2016; Knecht et al., 2016; Ni et al., 2016; Frank et al., 2017; Rimal
420    & Lee, 2018). In our study, HrhoIR21a and HrhoIR40a showed a close evolutionary relationship
421    to DmelIR21a and DmelIR40a. Additionally, *HrhoIR21a* and *HrhoIR40a* showed high
422    expression levels in the antennae (Fig. 6L-M). However, further analysis should be carried out to
423    establish whether these two IRs function in the mediation of thermotransduction in *H. rhodope*.
424         GRs function in detecting $CO_2$ and nonvolatile bitter, sugar, amino acid and plant secondary
425    metabolite compounds via contact chemosensation (Agnihotri, Roy & Joshi, 2016). Phylogenetic
426    tree analysis showed that HhroGR2 clustered with BmorGR2 in the $CO_2$ receptor subfamily (Fig.
427    4). Moreover, just like *BmorGR2*, *HrhoGR2* exhibits higher expression levels in the antennae
428    compared with other tissues (Guo et al., 2017). Therefore, HrhoGR2 may contribute to $CO_2$
429    detection. Furthermore, HrhoGR67 was clustered into a bitter receptor family and highly
430    expressed in the heads (Fig. 4 and 6V). Kasubuchi et al. (2018) reported three bitter receptors
431    (*BmorGR16/18/53*) expressed at different levels in the labrum, maxillary palp and maxillary
432    galea, which perceived various feeding deterrents such as coumarin and caffeine. Further studies
433    should explore whether HrhoGR67 has a similar function to the three receptors.
434         Generally, there are two SNMPs in insects, which were also identified in the present study.
435    *HrhoSNMP1* and *HrhoSNMP2* were highly expressed in *H. rhodope* antennae, suggesting that
436    they might be involved in olfactory functions. However, *HrhoSNMP2* was also expressed in the
437    heads. Similar broad expression patterns were also observed in other moth species, such as
438    *Sesamia inferens*, *C. medinalis*, *Spodoptera litura* and *H. armigera* (Zhang et al., 2013; Zhang et
439    al., 2015; Zhang et al., 2020). The ubiquitous expression pattern of *SNMP2* means that in
440    addition to odorant detection, they may have various functions specific to different organs (Vogt
441    et al., 2009). On the other hand, SNMP1 subfamilies are implicated in mediating responses to

442 lipid pheromones (Li et al., 2014). *HrhoSNMP1* showed higher expression levels in the FA,

443 indicating that *HrhoSNMP1* may be associated with the detection of sex pheromones. However,

444 SNMP1 identified from *M. destructor* was reported not to be crucial in pheromone perception

445 (Andersson et al., 2016). This finding calls for further investigation to determine whether

446 HrhoSNMP1 functions in pheromone detection in *H. rhodope*.

447

## Conclusion

449 In conclusion, 45 ORs, 9 IRs, 8 GRs and 2 SNMPs were identified in antennae transcriptomes of

450 *H. rhodope*. The putative functions of some genes were predicted by comparative phylogenetic

451 analyses and tissue expression assays. Our results enrich the olfactory gene inventory of *H.*

452 *rhodope* and provide the foundation for further research on the molecular mechanism and

453 evolution of the olfactory system in diurnal moths.

454

## Acknowledgements

456 We thank students Deng-Gao Ji, Jian-Wei Ding and An-Ran Tan (Henan University of Science

457 and Technology) for their help in collecting insects. We also thank Joseph Gillespie, Arthur de

458 Fouchier, Nicolas Montagné and the other anonymous referee for their helpful comments that

459 improved the manuscript.

460

## References

462 Abuin L, Bargeton B, Ulbrich MH, Isacoff EY, Kellenberger S, Benton R. 2011. Functional architecture of
463    olfactory ionotropic glutamate receptors. *Neuron* 69:44–60 DOI: 10.1016/j.neuron.2010.11.042.

464 Agnihotri AR, Roy AA, Joshi RS. 2016. Gustatory receptors in Lepidoptera: chemosensation and beyond.
465    *Insect Molecular Biology* 25:519–529 DOI: 10.1111/imb.12246.

466 Anderson AR, Wanner KW, Trowell SC, Warr CG, Jaquin-Joly E, Zagatti P, Robertson H, Newcom RD.
467    2009. Molecular basis of female-specific odorant responses in *Bombyx mori. Insect Biochemistry and*
468    *Molecular Biology* 39:189–197 DOI: 10.1016/j.ibmb.2008.11.002.

469 Andersson MN, Corcoran JA, Zhang DD, Hillbur Y, Newcomb RD, Löfstedt C. 2016. A sex pheromone
470    receptor in the hessian fly *Mayetiola destructor* (Diptera, Cecidomyiidae). *Frontiers in Cellular*
471    *Neuroscience* 10:212 DOI: 10.3389/fncel.2016.00212.

472 Andersson MN, Löfstedt C, Newcomb RD. 2015. Insect olfaction and the evolution of receptor tuning.
473    *Frontiers in Ecology and Evolution* 3:53 DOI: 10.3389/fevo.2015.00053

474 Arikawa K. 2017. The eyes and vision of butterflies. *Journal of Physiology-London* 595:5457–5464 DOI:
475    10.1113/JP273917.

476 Bastin-Héline L, de Fouchier A, Cao S, Koutroumpa F, Caballero-Vidal G, Robakiewicz S, Monsempes C,
477    Francois MC, Ribeyre T, Maria A, Chertemps T, de Cian A, Walker III WB, Wang GR, Jacquin-Joly E,
478    Montagné N. 2019. A novel lineage of candidate pheromone receptors for sex communication in moths.
479    *eLife* 8:e49826. DOI: 10.7554/eLife.49826.

480 Benton R, Vannice KS, Gomez-Diaz C, Vosshall LB. 2009. Variant ionotropic glutamate receptors as
481    chemosensory receptors in *Drosophila. Cell* 136:149–162 DOI: 10.1016/j.cell.2008.12.001.

482 Benton R, Vannice KS, Vosshall LB, 2007. An essential role for a CD36-related receptor in pheromone
483    detection in *Drosophila. Nature* 450:289–293 DOI: 10.1038/nature06328.

484 Briscoe AD, Macias-Muñoz A, Kozak KM, Walters JR, Yuan F, Jamie GA, Martin SH, Dasmahapatra KK,
485    Ferguson LC, Mallet J, Jacquin-Joly E, Jiggins CD. 2013. Female behaviour drives expression and
486    evolution of gustatory receptors in butterflies. *PLoS Genetics* 9:e1003620 DOI:
487    10.1371/journal.pgen.1003620.

488  Butterwick JA, del Mármol J, Kim KH, Kahison MA, Rogow JA, Walz T, Ruta V. 2018. Cryo-EM structure of
489      the insect olfactory receptor Orco. *Nature* 560:447-452 DOI: 10.1038/s41586-018-0420-8.
490  Chen CH, Buhl E, Xu M, Croset V, Rees JS, Lilley KS, Benton R, Hodge JJL, Stanewsky R. 2015. *Drosophila*
491      ionotropic receptor 25a mediates circadian clock resetting by temperature. *Nature* 527:516–520 DOI:
492      10.1038/nature16148.
493  Chen L, Wang XY, Lu W, Zheng XL. 2020. Sexual communication in diurnal moths: behaviors and
494      mechanisms. *International Journal of Tropical Insect Science* Published: 01 June DOI:  10.1007/s42690-
495      020-00174-z.
496  Clyne PJ, Warr CG, Carlson JR. 2000. Candidate taste receptors in *Drosophila*. *Science* 287:1830–1834 DOI:
497      10.1126/science.287.5459.1830.
498  Clyne PJ, Warr CG, Freeman MR, Lessing D, Kim J, Carlson JR. 1999. A novel family of divergent seven-
499      transmembrane proteins: candidate odorant receptors in *Drosophila*. *Neuron* 22:327–338 DOI:
500      10.1016/S0896-6273(00)81093-4.
501  Costanzo K, Monteiro A. 2007. The use of chemical and visual cues in female choice in the butterfly *Bicyclus*
502      *anynana*. *Proceedings of the Royal Society B-Biological Science B* 274:845e851 DOI:
503      10.1098/rspb.2006.3729.
504  Croset V, Rytz R, Cummins SF, Budd A, Brawand D, Kaessmann H, Gibson TJ, Benton R. 2010. Ancient
505      protostome origin of chemosensory ionotropic glutamate receptors and the evolution of insect taste and
506      olfaction. *PLoS Genetocs* 6:e1001064 DOI: 10.1371/journal.pgen.1001064.
507  Du LX, Zhao XC, Liang XZ, Gao XW, Liu Y, Wang GR. 2018. Identification of candidate chemosensory
508      genes in *Mythimna separata* by transcriptomic analysis. *BMC Genomics* 19:518 DOI: 10.1186/s12864-
509      018-4898-0.
510  Dunipace L, Meister S, McNealy C, Amrein H. 2001. Spatially restricted expression of candidate taste
511      receptors in the *Drosophila* gustatory system. *Current Biology* 11:822–835 DOI: 10.1016/s0960-
512      9822(01)00258-5.
513  Enjin A, Zaharieva EE, Frank DD, Mansourian S, Suh GSB, Gallio M, Stensmyr MC. 2016. Humidity sensing
514      in *Drosophila. Current Biology* 26:1352-1358 DOI: 10.1016/j.cub.2016.03.049.
515  Fandino RA, Haverkamp A, Bisch-Knaden S, Zhang J, Bucks S, Nguyen TAT, Schröder K, Werckenthin A,
516      Rybak J, Stengl M, Knaden M, Hansson BS, Große-Wilde E. 2019. Mutagenesis of odorant coreceptor
517      Orco fully disrupts foraging but not oviposition behaviors in the hawkmoth *Manduca sexta. Proceedings*
518      *of the National Academy of Science*s *of the United States of America* 116:15677-15685 DOI:
519      10.1073/pnas.1902089116.
520  Forstner M, Gohl T, Gondesen I, Raming K, Breer H, Krieger J. 2008. Differential expression of SNMP-1 and
521      SNMP-2 proteins in pheromone-sensitive hairs of moths. *Chemical Senses* 33:291–299 DOI:
522      10.1093/chemse/bjm087.
523  Frank DD, Enjin A, Jouandet GC, Zaharieva EE, Para A, Stenamyr MC, Gallio M. 2017. Early integration of
524      temperature and humidity stimuli in the *Drosophila* brain. *Current Biology* 27:2381-2388.e4 DOI:
525      10.1016/j.cub.2017.06.077.
526  Gadenne CR, Barrozo B, Anton S. 2016. Plasticity in insect olfaction to smell or not to smell? *Annual Review*
527      *of Entomology* 61:317–333 DOI: 10.1146/annurev-ento-010715-023523.
528  Ganguly A, Pang L, Duong VK, Lee A, Schoniger H, Varady E. Dahanukar A. 2017. A molecular and cellular
529      context-dependent role for Ir76b in detection of amino acid taste. *Cell Reports* 18:737–750 DOI:
530      10.1016/j.celrep.2016.12.071.
531  Gouin A, Bretaudeau A, Nam K, Gimenez S, Aury JM, Duvic B, Hilliou F, Durand N, Montagne N, Darboux
532      I, Kuwar S, Chertemps T, Siaussat D, Bretscheider A, Mone Y, Ahn SJ, Hanniger S, Gosselin Grenet AS,
533      Neunemann D, Maumus F, Luyten I, Labadie K, Xu W, Koutroumpa F, Escoubas JM, Llopis A,
534      Maibeche-Coisne M, Salasc F, Tomar A, Anderson AR, Khan SA, Dumas P, Orsucci M, Guy J, Belser C,
535      Alberti A, Noel B, Couloux A, Mercier J, Nidelet S, Dubois E, Liu NY, Boulogne I, Mirabeau O, Goff
536      GL, Gordon K, Oakeshott J, Consoli FL, Volkoff AN, Fescemyer HW, Marden JH, Luthe DS, Herrero S,
537      Heckel DG, Wincker P, Kergoat GJ, Amselem J, Quesneville H, Groot AT, Jacquin-Joly E, Negre N,
538      Lemaitre C, Legeai F, Alencon E, Fournier P. 2017. Two genomes of highly polyphagous lepidopteran
539      pests (*Spodoptera frugiperda* Noctuidae) with different host-plant ranges. *Scientific Reports* 7:11816 DOI:
540      10.1038/s41598-017-10461-4.

541 Guo H, Cheng T, Chen Z, Jiang L, Guo Y, Liu J, Li S, Taniai K, Asaoka K, Kadono-Okuda K, Arunkumar KP,
542     Wu J, Kishino H, Zhang H, Seth RK, Gopinathan KP, Montagné N, Jacquin-Joly E, Goldsmith MR, Xia
543     Q, Mita K. 2017. Expression map of a complete set of gustatory receptor genes in chemosensory organs of
544     *Bombyx mori. Insect Biochemistry and Molecular Biology* 82:74-82 DOI: 10.1016/j.ibmb.2017.02.001
545 Hallem EA, Carlson JR. 2006. Coding of odors by a receptor repertoire. *Cell* 125:143–160 DOI:
546     10.1016/j.cell.2006.01.050.
547 Haverkamp AB, Hansson S, Knaden M. 2018. Combinatorial codes and labeled lines: how insects use
548     olfactory cues to find and judge food mates and oviposition sites in complex environments. *Frontiers in*
549     *Physiology* 9:49 DOI: 10.3389/fphys.2018.00049.
550 He YQ, Feng B, Guo QS, Du YJ. 2017. Age influences the olfactory profiles of the migratory oriental
551     armyworm *mythimna separate* at the molecular level. *BMC Genomics* 18:32 DOI: 10.1186/s12864-016-
552     3427-2.
553 Huang BK. 1980. A study on the bionomic characteristics and control of the *Bischofia* burnet *Histia rhodope*
554     Cramer. *Jouranl of Fujian Agricultural College* 1:61-79.
555 Hussain A, Zhang M, Üçpunar HK, Svensson T, Quillery E, Gompel N, Ignell R, Grunwald Kadow IC. 2016.
556     Ionotropic chemosensory receptors mediate the taste and smell of polyamines. *PLoS Biology* 14:e1002454
557     DOI: 10.1371/journal.pbio.1002454.
558 Jin S, Zhou X, Gu F, Zhong G, Yi X. 2017. Olfactory plasticity: variation in the expression of chemosensory
559     receptors in *Bactrocera dorsalis* in different physiological states. *Frontiers in Physiology* 8:672 DOI:
560     10.3389/fphys.2017.00672.
561 Jin X, Ha TS, Smith DP. 2008. SNMP is a signaling component required for pheromone sensitivity in
562     *Drosophila*. *Proceedings of the National Academy of Sciences of the United States of America* 105:10996–
563     11001 DOI: 10.1073/pnas.0803309105.
564 Kasubuchi M, Shii F, Tsuneto K, Yamagishi T, Adegawa S, Endo H, Sato R. 2018. Insect taste receptors
565     relevant to host identification by recognition of secondary metabolite patterns of non-host plants.
566     *Biochemical and Biophysical Research Communications* 499:901–906 DOI: 10.1016/j.bbrc.2018.04.014.
567 Knecht ZA, Silbering AF, Ni L, Klein M, Budelli G, Bell R, Abuin L, Ferrer AJ, Samuel ADT, Benton R,
568     Garrity PA. 2016. Distinct combinations of variant ionotropic glutamate receptors mediate
569     thermosensation and hygrosensation in *Drosophila. eLife* 5:e17879 DOI: 10.7554/eLife.17879.
570 Koh TW, He Z, Gorur-Shandilya S, Menuz K, Larter NK, Stewart S, Carlson JR. 2014. The *Drosophila* IR20a
571     clade of ionotropic receptors are candidate taste and pheromone receptors. *Neuron* 83:850–865 DOI:
572     10.1016/j.neuron.2014.07.012.
573 Kumar S, Stecher G, Tamura K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for
574     bigger datasets. *Molecular Biology and Evolution* 33:1870–1874 DOI: 10.1093/molbev/msw054.
575 Langmead B, Trapnell C, Pop M, Salzberg SL. 2009. Ultrafast and memory-efficient alignment of short DNA
576     sequences to the human genome. *Genome Biology* 10:R25 DOI: 10.1186/gb-2009-10-3-r25.
577 Leal WS. 2013. Odorant reception in insects: roles of receptors binding proteins and degrading enzymes.
578     *Annual Review Entomology* 58:373–391 DOI: 10.1146/annurev-ento-120811-153635.
579 Li B, Dewey CN. 2011. RSEM: accurate transcript quantification from RNA-Seq data with or without a
580     reference genome. *BMC Bioinformatics* 12:323 DOI: 10.1186/1471-2105-12-323.
581 Li ZZ, Ni JD, Huang J, Montell C. 2014. Requirement for *Drosophila* SNMP1 for rapid activation and
582     termination of pheromone-induced activity. *PLoS Genetics* 10:e1004600 DOI:
583     10.1371/journal.pgen.1004600.
584 Liu NY, Xu W, Papanicolaou A, Dong SL, Anderson A. 2014. Identification and characterization of three
585     chemosensory receptor families in the cotton bollworm *Helicoverpa armigera*. *BMC Genomics* 15:597
586     DOI: 10.1186/1471-2164-15-597.
587 Liu Q, Liu W, Zeng BS, Wang GR, Hao DJ, Huang YP. 2017a. Deletion of the *Bombyx mori* odorant receptor
588     co-receptor (*BmOrco*) impairs olfactory sensitivity in silkworms. *Insect Biochemistry and Molecular*
589     *Biology* 86:58-67 DOI: 10.1016/j.ibmb.2017.05.007.
590 Liu S, Wang WL, Zhang YX, Zhang BX, Rao XJ, Liu XM, Wang DM, Li SG. 2017b. Transcriptome
591     sequencing reveals abundant olfactory genes in the antennae of the rice leaffolder *Cnaphalocrocis*
592     *medinalis* (Lepidoptera:Pyralidae). *Entomological Science* 20:177-188 DOI: 10.1111/ens.12253.
593 Martin JP, Beyerlein A, Dacks AM, Reisenman CE, Riffell JA, Lei H, Hildebrand JG. 2011. The neurobiology

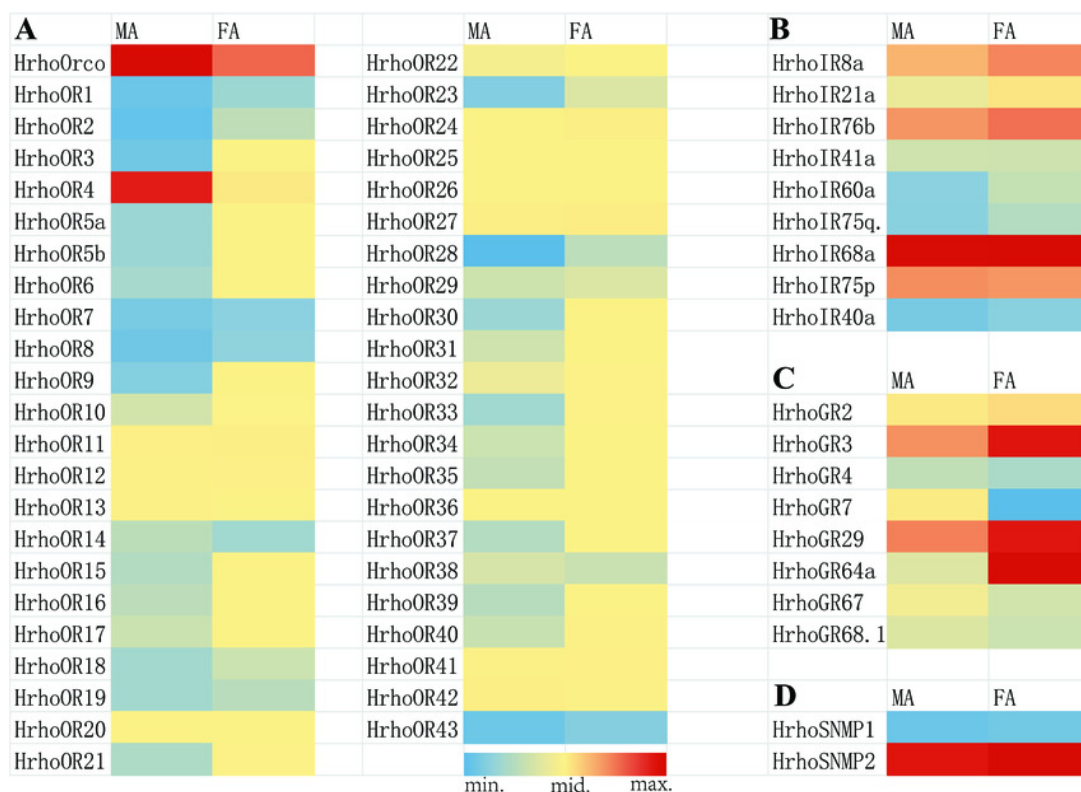
594    of insect olfaction: Sensory processing in a comparative context. *Progress in Neurobiology* 95:427–447
595        DOI: 10.1016/j.pneurobio.2011.09.007.
596    Monteys VSI, Quero C, Santa–Cruz MC, Rosell G, Guerrero A. 2016. Sexual communication in day–flying
597        Lepidoptera with special reference to castniids or 'butterfly–moths'. *Bulletin of Entomological Research*
598        106:421–431 DOI: 10.1017/S0007485316000158.
599    Ni L, Klein M, Svec KV, Budelli G, Chang EC, Ferrer AJ, Benton R, Damuel AD, Garrity PA. 2016. The
600        ionotropic receptors IR21a and IR25a mediate cool sensing in *Drosophila. eLife* 5:e13254 DOI:
601        10.7554/eLife.13254.
602    Ning C, Yang K, Xu M, Huang LQ, Wang CZ. 2016. Functional validation of the carbon dioxide receptor in
603        labial palps of *Helicoverpa armigera* moths. *Insect Biochemistry and Molecular Biology* 73:12-19 DOI:
604        10.1016/j.ibmb.2016.04.002.
605    Pelletier J, Hughes DT, Luetje CW, Leal WS. 2010. An odorant receptor from the southern house mosquito
606        *Culex pipiens* quinquefasciatus sensitive to oviposition attractants. *PLoS ONE* 5:e10090 DOI:
607        10.1371/journal.pone.0010090.
608    Pfaffl MW. 2001. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids*
609        *Research* 29:e45 DOI: 10.1093/nar/29.9.e45.
610    Pregitzer P, Greschista M, Breer H, Krieger J. 2014. The sensory neurone membrane protein SNMP1
611        contributes to the sensitivity of a pheromone detection system. *Insect Molecular Biology* 23:733–742 DOI:
612        10.1111/imb.1211.
613    Preiss R, Kramer E. 1986. Mechanism of pheromone orientation in flying moths. *Naturwissenschaften*
614        73:555e557.
615    Prieto-Godino LL, Rytz R, Cruchet S, Bargeton B, Abuin L, Silbering AF. Ruta V, Peraro MD, Benton R.
616        2017. Evolution of acid-sensing olfactory circuits in Drosophilids. *Neuron* 93:661–676.e6 DOI:
617        10.1016/j.neuron.2016.12.024
618    Purandare SR, Brisson JA. 2020. Divergent chemosensory gene expression accompanies ecological
619        specialisation of pea aphid morphs. *Ecological Entomology* 45:364-368 DOI: 10.1111/een.12803.
620    Radonić A, Thulke S, Mackay IM, Landt O, Siegert W, Nitsche A. 2004. Guideline to reference gene selection
621        for quantitative real-time PCR. *Biochemical and Biophysical Research Communications* 313:856–62 DOI:
622        10.1016/j.bbrc.2003.11.177.
623    Rimal S, Lee Y. 2018. The multidimensional ionotropic receptors of *Drosophila melanogaster. Insect*
624        *Molecular Biology* 27:1–7 DOI: 10.1111/imb.12347.
625    Robertson HM. 2019. Molecular evolution of the major arthropod chemoreceptor gene families. *Annual*
626        *Review Entomology* 64:227–242 DOI: 10.1146/annurev-ento-020117-043322.
627    Rogers ME, Krieger J, Vogt RG. 2001. Antennal SNMPs (sensory neuron membrane proteins) of lepidoptera
628        define a unique family of invertebrate CD36-like proteins. *Journal of Neurobiology* 49:47–61 DOI:
629        10.1002/neu.1065.
630    Sato K, Pellegrino M, Nakagawa T, Vosshall LB, Touhara K. 2008. Insect olfactory receptors are heteromeric
631        ligand-gated ion channels. *Nature* 452:1002–1006 DOI: 10.1038/nature06850.
632    Scott K, Brady RJr, Cravchik A, Morozov P, Rzhetsky A, Zuker C, Axel R. 2001. A chemosensory gene
633        family encoding candidate gustatory and olfactory receptors in *Drosophila. Cell* 104:661–673. DOI:
634        10.1016/s0092-8674(01)00263-x.
635    Senthilan PR, Piepenbrock D, Ovezmyradov G, Nadrowski B, Bechstedt S, Pauls S, Winkler M, Möbius W,
636        Howard J, Göpfert MC. 2012. *Drosophila* auditory organ genes and genetic hearing defects. *Cell*
637        150:1042-1054 DOI: 10.1016/j.cell.2012.06.043.
638    Shen SF, Cao S, Zhang Z, Kong XB, Liu F, Wang GR, Zhang SF. 2020. Evolution of sex pheromone receptors
639        in *Dendrolimus punctatus* Walker (lepidoptera: Lasiocampidae) is divergent from other moth species.
640        *Insect biochemistry and molecular biology* 122:10335 DOI:10.1016/j.ibmb.2020.103375.
641    Subchev M. 2014. Sex pheromone communication in the family Zygaenidae (Insecta: Lepidoptera): A review.
642        *Acta Zoologica Bulgarica* 66:147-157.
643    Sun L, Wang Q, Zhang YX, Yan YT, Guo HW, Xiao Q, Zhang YJ. 2019. Expression patterns and
644        colocalization of two sensory neurone membrane proteins in *Ectropis obliqua* Prout a geometrid moth pest
645        that uses Type-II sex pheromones. *Insect Molecular Biology* 28:342-354 DOI: 10.1111/imb.12555.
646    Taparia T, Ignell R, Hill SR. 2017. Blood meal induced regulation of the chemosensory gene repertoire in the

647      southern house mosquito. *BMC Genomics* 18:393 DOI: 10.1186/s12864-017-3779-2.
648    Touhara K, Vosshall LB. 2009. Sensing odorants and pheromones with chemosensory receptors. *Annual*
649      *Review Physiology* 71:307–332 DOI: 10.1146/annurev.physiol.010908.163209.
650    Venthur H, Zhou JJ. 2018. Odorant receptors and odorant-binding proteins as insect pest control targets: a
651      comparative analysis. *Frontiers in Physiology* 9:1163 DOI: 10.3389/fphys.2018.01163.
652    Vogt RG, Grosse-Wilde E, Zhou JJ. 2015. The lepidoptera odorant binding protein gene family:gene gain and
653      loss within the GOBP/PBP complex of moths and butterflies. *Insect Biochemistry and Molecular Biology*
654      62:142–153 DOI: 10.1016/j.ibmb.2015.03.003.
655    Vogt RG, Miller NE, Litvack R, Fandino RA, Sparks J, Staples J, Friedman R, Dickens JC. 2009. The insect
656      SNMP gene family. *Insect Biochemistry and Molecular Biology* 39:448–456 DOI:
657      10.1016/j.ibmb.2009.03.007.
658    Walker III WB, Gonzalez F, Garczynski SF, Witzgall P. 2016. The chemosensory receptors of codling moth
659      *Cydia pomonella*–expression in larvae and adults. *Scientific Reports* 6:23518 DOI: 10.1038/srep23518.
660    Walker III WB, Roy A, Anderson P, Schlyter F, Hansson BS, Larsson MC. 2019. Transcriptome analysis of
661      gene families involved in chemosensory function in *Spodoptera littoralis* (Lepidoptera: Noctuidae). *BMC*
662      *Genomics* 20:428 DOI: 10.1186/s12864-019-5815-x.
663    Wanner KW, Robertson HM. 2008. The gustatory receptor family in the silkworm moth *Bombyx mori* is
664      characterized by a large expansion of a single lineage of putative bitter receptors. *Insect Molecular*
665      *Biology* 17:621-629 DOI: 10.1111/j.1365-2583.2008.00836.x.
666    Xu W, Papanicolaou A, Zhang HJ, Anderson A. 2016. Expansion of a bitter taste receptor family in a
667      polyphagous insect herbivore. *Scientific Reports* 6:23666. DOI: 10.1038/srep23666.
668    Yan SW, Zhang J, Liu Y, Li GQ, Wang GR. 2015. An olfactory receptor from *Apolygus lucorum* (Meyer-Dur)
669      mainly tuned to volatiles from flowering host plants. *Journal of Insect Physiology* 79:36–41 DOI:
670      10.1016/j.jinsphys.2015.06.002.
671    Yang HB, Dong JF, Sun YL, Hu ZJ, Lv QH, Li DX. 2020. Antennal transcriptome analysis and expression
672      profiles of putative chemosensory soluble proteins in *Histia rhodope* Cramer (Lepidoptera:Zygaenidae).
673      *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics* 33:100654 DOI:
674      101016/jcbd2020100654.
675    Yang HB, Hu ZJ, Dong JF, Zhu PH, Li DX. 2019. Changes in the cold hardiness of overwintering larvae of
676      *Histia rhodope* (Lepidoptera: Zygaenidae). *Acta Entomology Sinica* 62:979-986.
677    Yuvaraj JK, Andersson MN, Zhang DD, Löfstedt C. 2018. Antennal transcriptome analysis of the
678      chemosensory gene families from trichoptera and basal Lepidoptera. *Frontiers in Physiology* 9:1365 DOI:
679      10.3389/fphys.2018.01365.
680    Yuvaraj JK, Corcoran JA, Andersson MN, Newcomb RD, Anderbrant O, Löfstedt C. 2017. Characterization of
681      odorant receptors from a non-ditrysian moth, *Eriocrania semipurpurella* sheds light on the origin of sex
682      pheromone receptors in Lepidoptera. *Molecular Biology and Evolution* 34:2733–2746 DOI:
683      10.1093/molbev/msx215.
684    Zhang DD, Löfstedt C. 2013. Functional evolution of a multigene family: orthologous and paralogous
685      pheromone receptor genes in the turnip moth *Agrotis segetum*. *PLoS ONE* 8:e77345 DOI:
686      10.1371/journal.pone.0077345.
687    Zhang HJ, Xu W, Chen QM, Sun LN, Anderson A, Xia QY, Papanicolaou A. 2020. A phylogenomics
688      approach to characterizing sensory neuron membrane proteins (SNMPs) in Lepidoptera. *Insect*
689      *Biochemistry and Molecular Biology* 118:103313 DOI: 10.1016/j.ibmb.2020.103313.
690    Zhang J, Bisch-Knaden S, Fandino RA, Yan SW, Obiero GF, Frosse-Wilde E, Han BS, Knaden M. 2019. The
691      olfactory coreceptor IR8a governs larval feces mediated competition avoidance in a hawkmoth.
692      *Proceedings of the National Academy of Sciences* of *the United States of America* 116:21828-21833 DOI:
693      10.1073/pnas.1913485116.
694    Zhang J, Liu Y, Walker WB, Dong SL, Wang GR. 2015. Identification and localization of two sensory neuron
695      membrane proteins from *Spodoptera litura* (Lepidoptera:Noctuidae). *Insect Science* 22:399-408 DOI:
696      10.1111/1744-7917.12131.
697    Zhang YN, Jin JY, Jin R, Xia YH, Zhou JJ, Deng JY, Dong SL, 2013. Differential expression patterns in
698      chemosensory and non-chemosensory tissues of putative chemosensory genes identified by transcriptome
699      analysis of insect pest the purple stem borer *Sesamia inferens* (Walker). *PLoS ONE* 8:e69715 DOI:

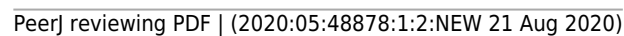
700   10.1371/journal.pone.0069715.
701 Zhang YN, Qian JL, Xu JW, Zhu XY, Li MY, Xu XX, Liu CX, Xue T, Sun L. 2018. Identification of
702   chemosensory genes based on the transcriptomic analysis of six different chemosensory organs in
703   *Spodoptera exigua. Frontiers in Physiology* 9:432 DOI: 10.3389/fphys.2018.00432.
704 Zhang YN, Zhu XY, Ma JF, Dong ZP, Xu JW, Kang K, Zhang LW. 2017. Molecular identification and
705   expression patterns of odorant binding protein and chemosensory protein genes in *Athetis lepigone*
706   (Lepidoptera:Noctuidae). *PeerJ* 5:e3157 DOI: 10.7717/peerj.3157.
707 Zheng XL, Liu JY, Zhang ZL, Wang P, Lu W. 2019. Diel rhythms of sexual behavior and pheromone
708   responses in *Phauda flammans* Walker (Lepidoptera: Zygaenidae). *Pest Management Science* 75:3070–
709   3075 DOI: 10.1002/ps.5423.

# Figure 1

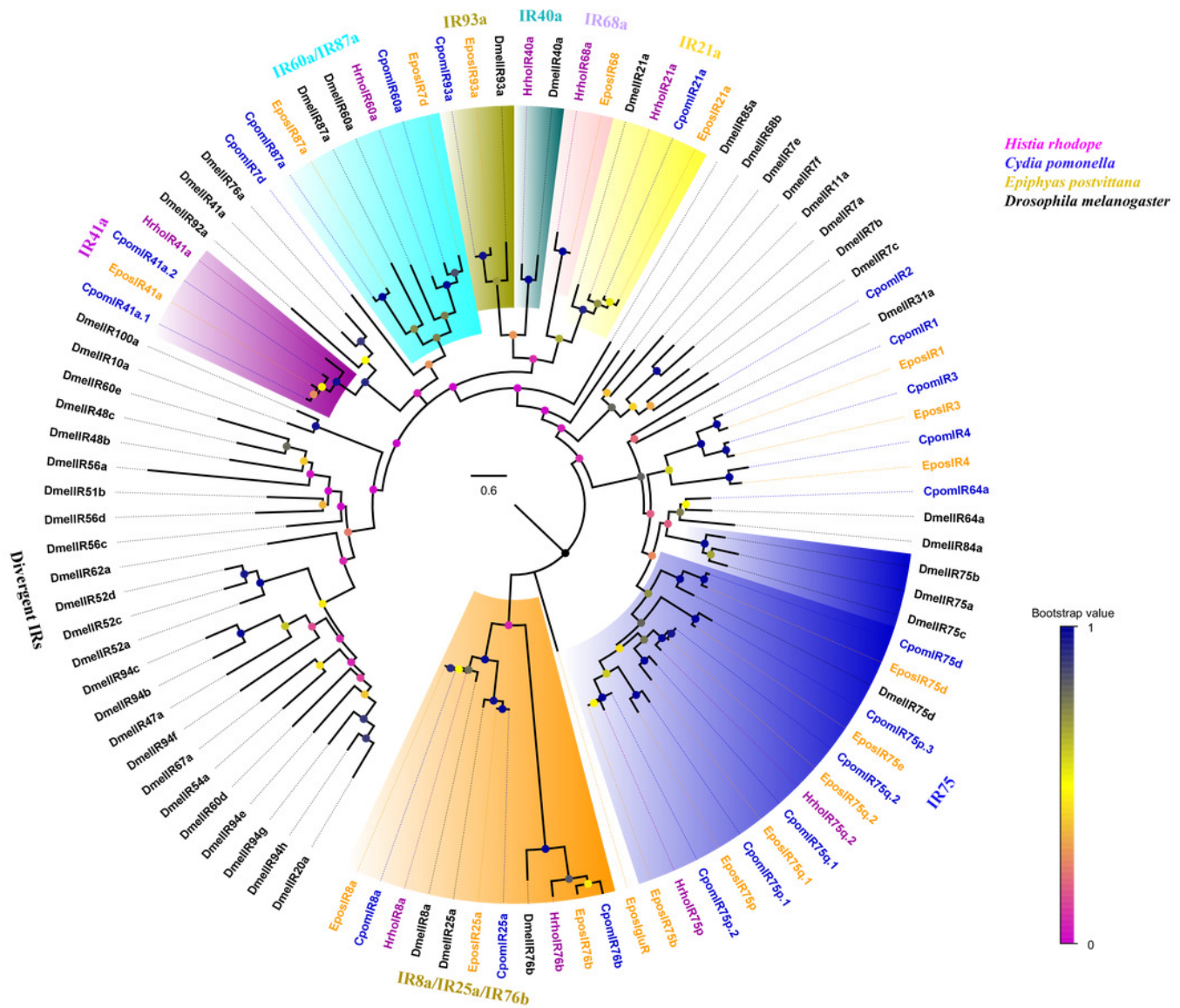
Heat map of FPKM values for ORs, IRs, GRs and SNMPs in male antennae (MA) and female antennae (FA).

Blue color indicates low expression, yellow color indicates moderate expression and red color indicates high expression. FA: female antennae, MA: male antennae. (A): ORs, odorant receptors, (B): IRs, ionotropic receptors, (C): GRs, gustatory receptors, (D): SNMPs, sensory neuron membrane proteins.

# Figure 2

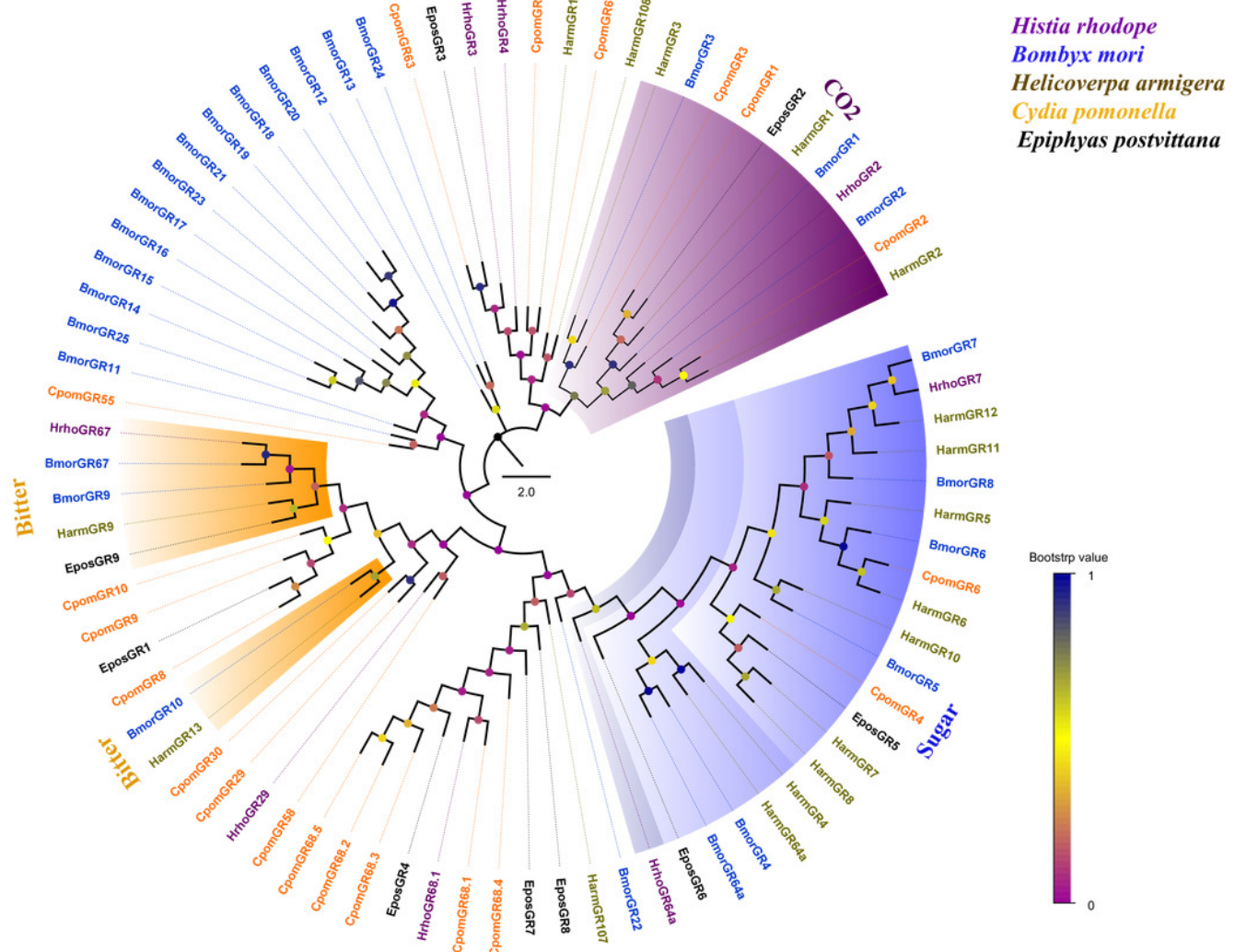
Phylogenetic tree of putative odorant receptors (ORs).

Branch support (circles at the branch nodes) was estimated using an approximate likelihood ratio test based on the scale indicated at the bottom right. The clade of traditional PR clade is marked in yellow, the novel lineage of candidate PR clade is marked in blue and ORco in purole.

# Figure 3

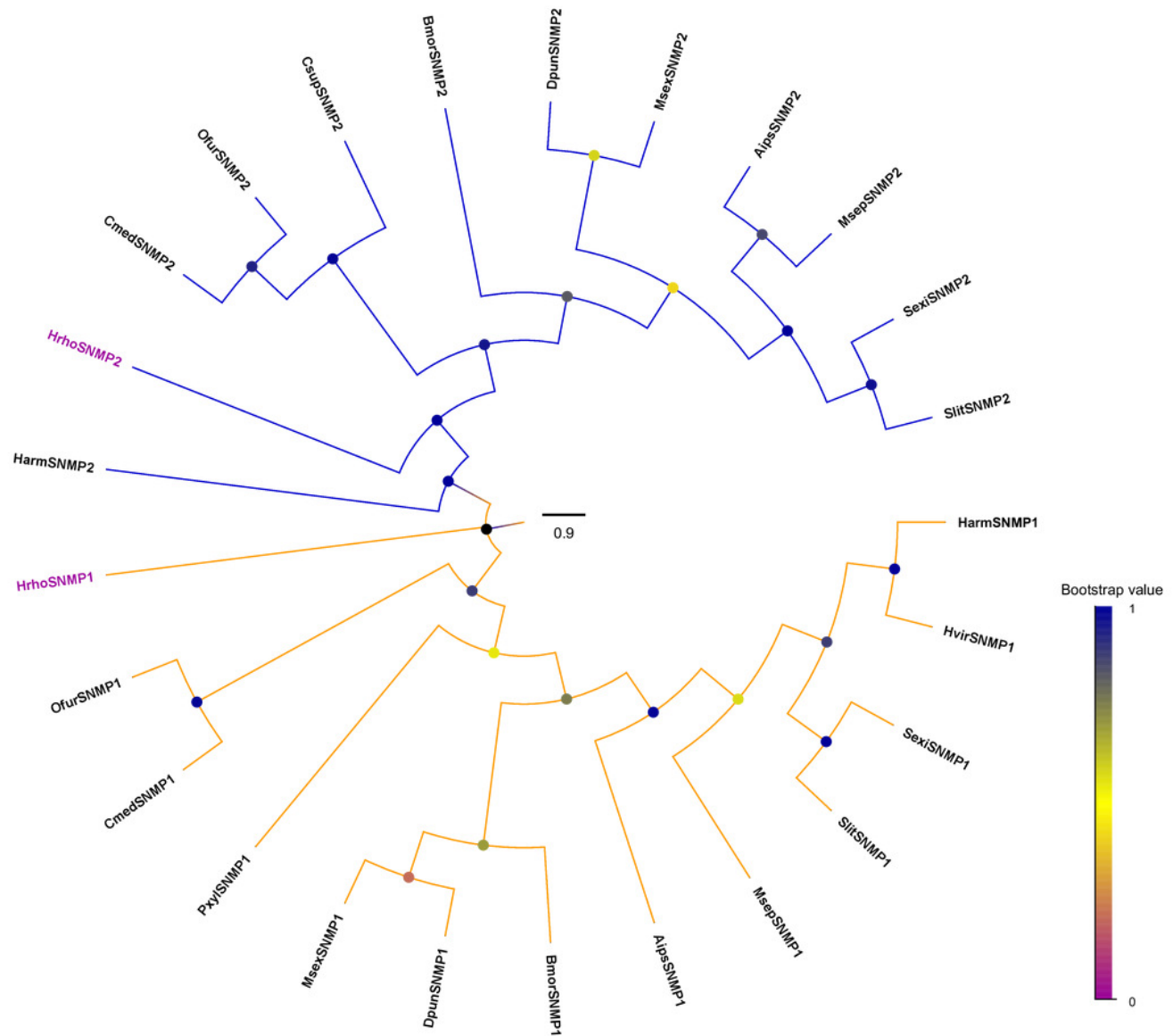
Phylogenetic tree of putative ionotropic receptors (IRs).

Branch support (circles at the branch nodes) was estimated using an approximate likelihood ratio test based on the scale indicated at the bottom right. Branches containing putative IR co-receptors and conversed antennal IRs are colored.

# Figure 4

Phylogenetic tree of putative gustatory receptors (GRs).

Branch support (circles at the branch nodes) was estimated using an approximate likelihood ratio test based on the scale indicated at the bottom right. The sugar receptor clade, bitter receptor clade and CO2 receptor clade are colored.

# Figure 5

Phylogenetic tree of putative sensory neuron membrane proteins (SNMPs).

Branch support (circles at the branch nodes) was estimated using an approximate likelihood ratio test based on the scale indicated at the bottom right. The SNMP1 clade and SNMP2 clade are colored.

# Figure 6

Relative expression levels of the chemoreceptor genes in the male antennae, female antennae, heads and legs from *H. rhodope*.

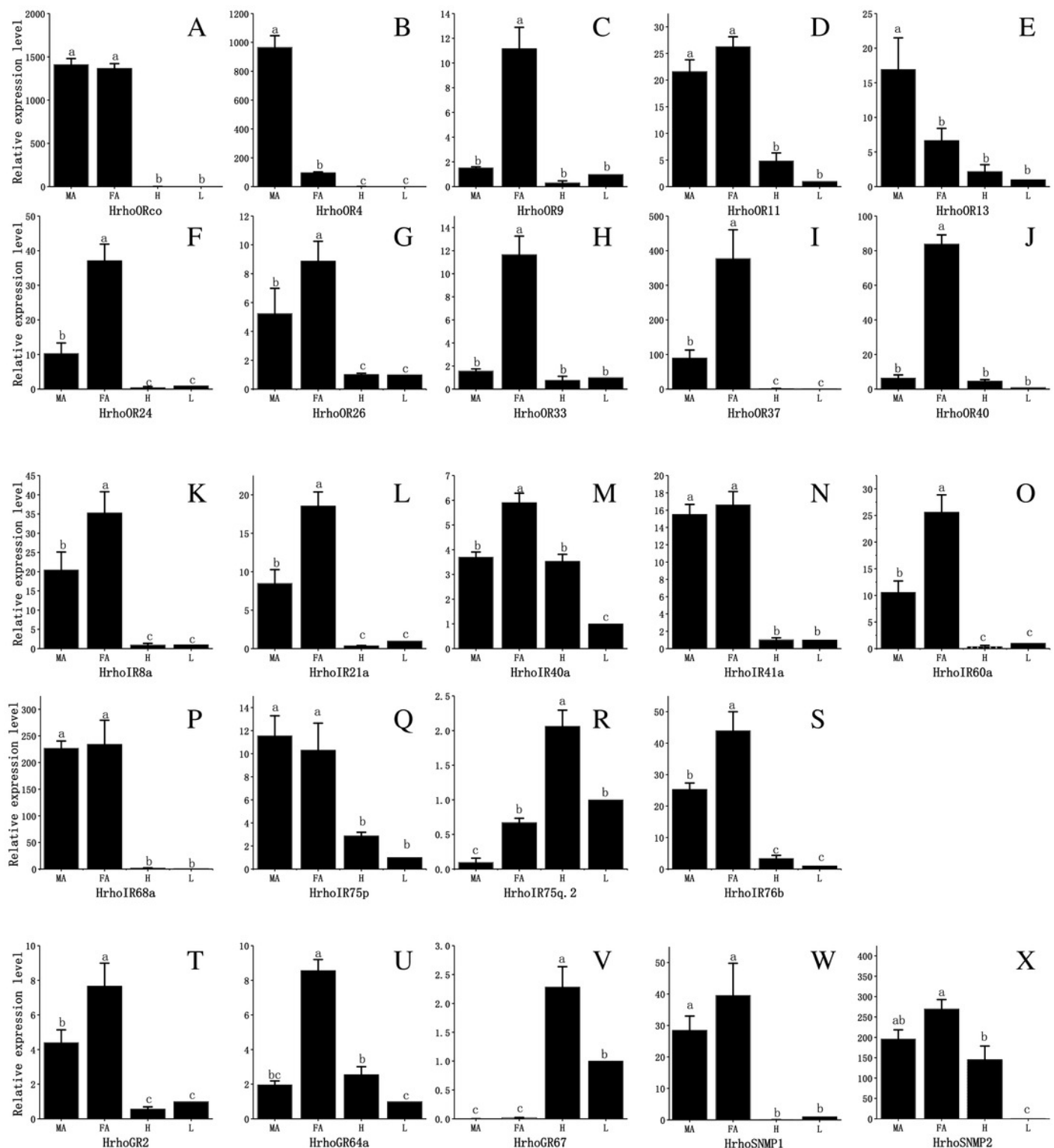
(A-J): ORs. (K-S): IRs. (T-V): GRs. (W-X): SNMPs. MA: male antennae, FA: female antennae, H: heads (without antennae), L: legs. The GAPDH was used as the reference gene, Gene expressions in various tissues are normalized relative to that in legs. Different small letters mean significant difference between tissues (p < 0.05, ANOVA, LSD).

The best Blast match of candidate odorant receptors (ORs) in *H. rhodope*

**Table 1 The best Blast match of candidate odorant receptors (ORs) in *H. rhodope***

| Accession number | Gene name | ORF (bp) | Complete ORF | TMD (No.) | Blastx annotation (Description/Species) | Accession number | E-value | Identity (%) |
|---|---|---|---|---|---|---|---|---|
| MN515166 | HrhoOrco | 1422 | Y | 7 | Orco [*Galleria mellonella*] | QEI46859.1 | 0 | 85.44 |
| MN515167 | HrhoOR1 | 687 | N | 4 | olfactory receptor 11 [*Ctenopseustis obliquana*] | AIT71985.1 | 5.00E-40 | 36.65 |
| MN515168 | HrhoOR2 | 978 | N | 6 | putative odorant receptor OR57 [*Cydia nigricana*] | AST36406.1 | 2.00E-57 | 34.97 |
| MN515169 | HrhoOR3 | 1215 | Y | 7 | odorant receptor [*Eogystia hippophaecolus*] | AOG12952.1 | 1.00E-149 | 51.36 |
| MN515170 | HrhoOR4 | 1203 | Y | 7 | olfactory receptor 66 [*Ctenopseustis herana*] | AIT69908.1 | 3.00E-79 | 35.09 |
| MN515171 | HrhoOR5a | 669 | N | 4 | odorant receptor 94a-like isoform X2 [*Manduca sexta*] | XP_030035689.1 | 8.00E-47 | 47.32 |
| MN515172 | HrhoOR5b | 603 | N | 2 | putative olfactory receptor 21 [*Ostrinia furnacalis*] | BAR43463.1 | 2.00E-86 | 64.14 |
| MN515173 | HrhoOR6 | 1212 | Y | 7 | putative odorant receptor OR27 [*Hedya nubiferana*] | AST36262.1 | 0 | 67.33 |
| MN515174 | HrhoOR7 | 1224 | Y | 7 | odorant receptor [*Eogystia hippophaecolus*] | AOG12916.1 | 1.00E-135 | 49.63 |
| MN515175 | HrhoOR8 | 714 | N | 3 | odorant receptor [*Eogystia hippophaecolus*] | AOG12915.1 | 3.00E-102 | 63.14 |
| MN515176 | HrhoOR9 | 417 | N | 3 | putative odorant receptor 92a [*Bombyx mandarina*] | XP_028031179.1 | 1.00E-22 | 36.96 |
| MN515177 | HrhoOR10 | 1341 | Y | 7 | odorant receptor 1 [*Cnaphalocrocis medinalis*] | ALT31655.1 | 0 | 63.33 |
| MN515178 | HrhoOR11 | 1185 | Y | 7 | odorant receptor 85c-like [*Vanessa tameamea*] | XP_026493935.1 | 3.00E-88 | 38.66 |
| MN515179 | HrhoOR12 | 792 | N | 6 | odorant receptor [*Eogystia hippophaecolus*] | AOG12948.1 | 1.00E-143 | 74.62 |
| MN515180 | HrhoOR13 | 1152 | Y | 7 | odorant receptors OR25 [*Lobesia botrana*] | AXF48775.1 | 1.00E-158 | 56.49 |
| MN515181 | HrhoOR14 | 1206 | Y | 7 | putative odorant receptor OR30 [*Cydia pomonella*] | AFC91738.2 | 7.00E-122 | 44.56 |
| MN515182 | HrhoOR15 | 378 | N | 3 | putative odorant receptor 57 [*Conopomorpha sinensis*] | AXY83406.1 | 1.00E-30 | 47.54 |
| MN515183 | HrhoOR16 | 1188 | Y | 7 | olfactory receptor 66 [*Ctenopseustis herana*] | AIT69908.1 | 2.00E-97 | 40.54 |
| MN515184 | HrhoOR17 | 1263 | Y | 7 | putative odorant receptor OR13 [*Hedya nubiferana*] | AST36253.1 | 9.00E-156 | 55.81 |
| MN515185 | HrhoOR18 | 1176 | Y | 7 | odorant receptor [*Eogystia hippophaecolus*] | AOG12907.1 | 0 | 67.70 |
| MN515186 | HrhoOR19 | 1302 | Y | 7 | putative odorant receptor OR63 [*Hedya nubiferana*] | AST36285.1 | 1.00E-154 | 49.07 |
| MN515187 | HrhoOR20 | 951 | N | 4 | odorant receptor [*Eogystia hippophaecolus*] | AOG12933.1 | 2.00E-119 | 54.78 |
| MN515188 | HrhoOR21 | 1233 | Y | 7 | olfactory receptor 11 [*Ctenopseustis herana*] | AIT69876.1 | 6.00E-101 | 40.35 |
| MN515189 | HrhoOR22 | 1200 | Y | 7 | olfactory receptor 20 [*Helicoverpa armigera*] | ACC63240.1 | 6.00E-121 | 44.99 |
| MN515190 | HrhoOR23 | 1110 | Y | 7 | olfactory receptor OR54 [*Planotortrix octo*] | AJF23812.1 | 2.00E-145 | 54.30 |
| MN515191 | HrhoOR24 | 1212 | Y | 7 | olfactory receptor 27 [*Helicoverpa armigera*] | ARF06962.1 | 8.00E-115 | 45.18 |
| MN515192 | HrhoOR25 | 1197 | Y | 7 | odorant receptor [*Eogystia hippophaecolus*] | AOG12927.1 | 0 | 71.43 |
| MN515193 | HrhoOR26 | 1332 | Y | 7 | olfactory receptor 71 [*Ctenopseustis herana*] | AIT69911.1 | 0 | 65.08 |
| MN515194 | HrhoOR27 | 378 | N | 2 | olfactory receptor 37 [*Carposina sasakii*] | AYD42255.1 | 2.00E-42 | 51.22 |
| MN515195 | HrhoOR28 | 352 | N | 2 | putative olfactory receptor 25 [*Ostrinia furnacalis*] | BAR43467.1 | 4.00E-22 | 36.75 |
| MN515196 | HrhoOR29 | 1230 | Y | 7 | odorant receptor 24a-like [*Ostrinia furnacalis*] | XP_028158823.1 | 0 | 71.15 |
| MN515197 | HrhoOR30 | 1194 | N | 6 | odorant receptor [*Eogystia hippophaecolus*] | AOG12934.1 | 3.00E-91 | 36.57 |
| MN515198 | HrhoOR31 | 1284 | Y | 7 | odorant receptor [*Eogystia hippophaecolus*] | AOG12926.1 | 0 | 75.93 |
| MN515199 | HrhoOR32 | 927 | N | 3 | putative odorant receptor OR57 [*Cydia nigricana*] | AST36406.1 | 3.00E-47 | 33.00 |
| MN515200 | HrhoOR33 | 756 | N | 5 | olfactory receptor 14 [*Heortia vitessoides*] | AZB49428.1 | 7.00E-84 | 53.57 |
| MN515201 | HrhoOR34 | 1212 | Y | 7 | odorant receptors OR29 [*Lobesia botrana*] | AXF48779.1 | 0 | 65.58 |

| MN515202 | HrhoOR35 | 612 | N | 3 | Olfactory receptor 23 [*Manduca sexta*] | CUQ99405.1 | 5.00E-64 | 52.24 |
| MN515203 | HrhoOR36 | 1218 | Y | 7 | odorant receptor [*Eogystia hippophaecolus*] | AOG12941.1 | 2.00E-134 | 46.56 |
| MN515204 | HrhoOR37 | 1143 | Y | 7 | olfactory receptor 32 [*Cnaphalocrocis medinalis*] | ANZ03145.1 | 4.00E-159 | 56.74 |
| MN515205 | HrhoOR38 | 1215 | N | 0 | putative odorant receptor [*Peridroma saucia*] | AVF19641.1 | 0 | 67.48 |
| MN515206 | HrhoOR39 | 1005 | N | 6 | Olfactory receptor 65 [*Manduca sexta*] | CUQ99419.1 | 1.00E-69 | 38.74 |
| MN515207 | HrhoOR40 | 489 | N | 3 | putative odorant receptor OR23 [*Athetis lepigone*] | AOE48028.1 | 3.00E-41 | 43.90 |
| MN515208 | HrhoOR41 | 1335 | Y | 7 | gustatory receptor 6 [*Helicoverpa armigera*] | ASW18695.1 | 0 | 67.59 |
| MN515209 | HrhoOR42 | 720 | N | 1 | putative odorant response protein ODR-4 [*Danaus plexippus plexippus*] | OWR50364.1 | 2.00E-144 | 84.45 |
| MN515210 | HrhoOR43 | 526 | N | 3 | odorant receptor [*Eogystia hippophaecolus*] | AOG12949.1 | 2.00E-28 | 37.42 |

The best Blast match of candidate ionotrpic receptors (IRs) in *H. rhodope*

| Accession number | Gene name | ORF (bp) | Complete ORF | TMD (No.) | Blastx annotation (Description/Species) | Accession number | E-value | Identity (%) |
|---|---|---|---|---|---|---|---|---|
| MN515211 | HrhoIR8a | 2631 | N | 4 | ionotropic receptor 8a [*Ostrinia furnacalis*] | BAR64796.1 | 0 | 73.84 |
| MN515212 | HrhoIR21a | 1182 | N | 2 | ionotropic receptor 21a [*Eogystia hippophaecolus*] | AOG12851.1 | 0 | 68.77 |
| MN515213 | HrhoIR76b | 693 | N | 1 | putative ionotropic receptor IR76b [*Cydia nigricana*] | AQM73618.1 | 4E-99 | 63.22 |
| MN515214 | HrhoIR41a | 1128 | N | 1 | ionotropic receptor 41a [*Ostrinia furnacalis*] | BAR64800.1 | 2E-142 | 56.75 |
| MN515215 | HrhoIR60a | 1959 | N | 3 | ionotropic receptor IR60a [*Cnaphalocrocis medinalis*] | APY22698.1 | 0 | 49.92 |
| MN515216 | HrhoIR75q.2 | 1686 | N | 3 | putative ionotropic receptor IR75q.2 [*Cydia fagiglandana*] | AST36364.1 | 0 | 62.14 |
| MN515217 | HrhoIR68a | 1302 | N | 4 | ionotropic receptor 68a [*Eogystia hippophaecolus*] | AOG12853.1 | 0 | 74.88 |
| MN515218 | HrhoIR75p | 1707 | N | 2 | ionotropic receptor 75a-like [*Bombyx mandarina*] | XP_028025288.1 | 0 | 63.46 |
| MN515219 | HrhoIR40a | 1632 | N | 2 | ionotropic receptor 40a [*Manduca sexta*] | XP_030034643.1 | 0 | 79.96 |

2
3
4

The best Blast match of candidate gustatory receptors (GRs) in *H. rhodope.*

**Table 3 The best Blast match of candidate gustatory receptors (GRs) in *H. rhodope***

| Accession number | Gene name | ORF (bp) | Complete ORF | TMD (No.) | Blastx annotation (Description/Species) | Accession number | E-value | Identity (%) |
|---|---|---|---|---|---|---|---|---|
| MN515222 | HrhoGR2 | 612 | N | 4 | putative gustatory receptor GR2 [*Hedya nubiferana*] | AST36211.1 | 7E-116 | 88.24 |
| MN515223 | HrhoGR3 | 390 | N | 2 | antennal gustatory receptor 9 [*Dendrolimus punctatus*] | ARO70281.1 | 5E-36 | 50.38 |
| MN515224 | HrhoGR4 | 198 | N | 1 | antennal gustatory receptor 9 [*Dendrolimus punctatus*] | ARO70281.1 | 7E-10 | 55.32 |
| Mn515229 | HrhoGR7 | 237 | N | 1 | Gustatory receptor 7 [*Operophtera brumata*] | KOB71247.1 | 1E-11 | 47.06 |
| MN515225 | HrhoGR29 | 393 | N | 2 | putative gustatory receptor GR29 [*Cydia fagiglandana*] | AST36346.1 | 1E-29 | 45.45 |
| MN515226 | HrhoGR64a | 1218 | N | 5 | gustatory receptor for sugar taste 64a-like [*Bombyx mandarina*] | XP_028037655.1 | 7E-153 | 53.50 |
| MN515227 | HrhoGR67 | 693 | N | 4 | gustatory receptor 67 [*Bombyx mori*] | NP_001233216.1 | 4E-48 | 38.29 |
| MN515228 | HrhoGR68.1 | 879 | N | 4 | putative gustatory receptor GR68.1 [*Hedya nubiferana*] | AST36219.1 | 1E-43 | 34.56 |

The best Blast match of candidate sensory neuron membrane proteins (SNMPs) in *H. rhodope.*

**Table 4 The best Blast match of candidate sensory neuron membrane proteins (SNMPs) in *H. rhodope***

| Accession number | Gene name | ORF (bp) | Complete ORF | TMD (No.) | Blastx annotation (Description/Species) | Accession number | E-value | Identity (%) |
|---|---|---|---|---|---|---|---|---|
| MN515220 | HrhoSNMP1 | 1581 | Y | 2 | Sensory neuron membrane protein 1 [*Papilio xuthus*] | KPI90909.1 | 0 | 66.22 |
| MN515221 | HrhoSNMP2 | 1422 | Y | 1 | Sensory neuron membrane protein 2 [*Ostrinia nubilalis*] | E5EZW9.1 | 0 | 64.08 |

2
3