Temporal and Spatial Expression of Parasitism-Related Olfactory Genes in Larvae of the Ectoparasitoid *Dastarcus helophoroides* (Coleoptera: Bothrideridae)[1]

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Abstract *Dastarcus helophoroides* (Fairmaire) (Coleoptera: Bothrideridae) is an ectoparasitoid of the pine sawyer beetle, *Monochamus alternatus* Hope (Coleoptera: Cerambycidae). A sensitive and precise olfactory system is required for the accurate location of the coleopteran host by *D. helophoroides* neonates. Herein, we characterized the relative expression patterns of six representative olfactory-related genes at different stages of *M. alternatus* parasitism and in different body regions of *D. helophoroides* neonates. The genes encoding chemosensory protein 2 (*DhelCSP2*), odorant receptor 2 (*DhelOR2*), and ionotropic receptor 2 (*DhelIR2*) were significantly upregulated before parasitization was initiated, whereas the genes encoding odorant binding protein 8 (*DhelOBP8*), gustatory receptor 5 (*DhelGR5*), and sensory neuron membrane protein 1 (*DhelSNMP1*) were significantly upregulated 4–5 d after initiation of parasitism. In *D. helophoroides* neonates, four genes (*DhelOBP8*, *DhelCSP2*, *DhelOR2*, and *DhelIR2*) were significantly upregulated in the head compared with the thoracoabdominal region, and one gene (*DhelGR5*) was significantly upregulated in the thoracoabdominal area compared with the head. Double-stranded RNAs (dsRNAs) targeting the six olfactory-related genes were synthesized and delivered to *D. helophoroides* neonates via immersion. After dsRNA treatment, the transcript levels of four olfactory-related genes (*DhelOBP8*, *DhelCSP2*, *DhelOR2*, and *DhelSNMP1*) were significantly reduced compared with that of the controls. These results provide a basis for further functional explorations of *D. helophoroides* olfactory genes, which may lead to the development of improved biological pest control methods using *D. helophoroides* larvae.

Key Words olfactory genes, *Dastarcus helophoroides*, parasitism, RNA interference

Dastarcus helophoroides (Fairmaire) (Coleoptera: Bothrideridae) is an ectoparasitoid of the pine sawyer beetle, *Monochamus alternatus* Hope (Coleoptera: Cerambycidae), the primary insect vector of the pine wood nematode, *Bursaphelenchus xylophilus* (Steiner et Buhrer) Nickle (Aphelenchida: Parasitaphelenchidae) (Mamiya and Enda 1972, Taketsune 1982). In China, large-scale releases of laboratory-reared *D. helophoroides* adults and eggs have been used to reduce the

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population density of *M. alternatus* and inhibit the proliferation of *B. xylophilus* (Yang et al. 2014). In field experiments, *D. helophoroides* larvae effectively parasitized *M. alternatus* larvae from a distance of 7.5 m (Li et al. 2013). Therefore, we hypothesized that the keen olfactory system of the *D. helophoroides* larva plays an essential role in the parasitic process.

Insect behavioral processes, such as host search, foraging, pairing, and egg laying, require the olfactory system (Becher et al. 2012, Birkett et al. 2004, Carey et al. 2010, Leal 2013, Syed and Leal 2009, Todd et al. 1992). For example, the odorant binding protein (OBP) *DhelOBP21* was shown to play a vital role in the recognition of (+)-β-pinene in adult *D. helophoroides* (Li 2019). However, it is larval *D. helophoroides* that exhibit parasitic behavior; thus, the role of olfactory genes in the host search and parasitism of *D. helophoroides* neonates requires investigation.

To address this knowledge gap, we previously published the transcriptome of *D. helophoroides* neonates and characterized the expression patterns of 62 olfactory genes encoding six types of proteins: 20 encoding putative OBPs, 11 encoding putative chemosensory proteins (CSPs), 9 encoding putative odorant receptors (ORs), 11 encoding putative ionotropic receptors (IRs), 7 encoding putative gustatory receptors (GRs), and 4 encoding putative sensory neuron membrane proteins (SNMPs) (Li et al. 2020). To continue this investigation and to clarify the functions of the olfactory genes during that parasitism process, we aimed to determine the spatiotemporal specificity of parasitism-related olfactory genes by profiling olfactory gene expression levels in *D. helophoroides* larvae at different parasitic stages and in various body regions. Furthermore, we investigated the silencing efficiency of double-stranded RNA (dsRNA) with respect to the olfactory genes by targeting representative olfactory genes in *D. helophoroides* larvae *in vitro* by using RNA interference (RNAi). Our overall objective was to provide a theoretical basis for further exploration of the molecular mechanisms underlying the parasitism of *M. alternatus* by *D. helophoroides* larvae.

Materials and Methods

Insect and tissue collection. Adult *D. helophoroides* were reared in our laboratory, and females laid eggs on vellum tied to pine wood blocks. We incubated the vellum containing the eggs at 25°C and 50% relative humidity (RH), monitoring the eggs every 24 h and retrieving neonates with a small brush.

Larvae of the *M. alternatus* host were obtained from the xylem of *Pinus massoniana* Lambert trees in the Miaoshou Forest Farm (Jingde County, Anhui Province, China) and kept in separate centrifuge tubes with air-permeable holes at 8°C and 50% RH.

Using sterile forceps, we separated the heads and thoracoabdominal parts of 100 *D. helophoroides* neonates, placed the heads and thoracoabdominal parts in separate 1.5-ml centrifuge tubes, and stored the tubes at −80°C. We performed this procedure three times (a total of 300 larvae), creating three biological replicates.

Parasitism was initiated by placing five *D. helophoroides* neonates on the surface of each *M. alternatus* larva by using a small brush (day 0). We collected 20 *D. helophoroides* larvae from each *M. alternatus* larva on days 2–6 after parasitism initiation; the collected larvae were placed in 1.5-ml centrifuge tubes and stored at

Table 1. Primers used for reverse transcription-quantitative polymerase chain reaction (RT-qPCR).

Gene*	Primer	Product Size (bp)
OBP8	Forward: TACTCGCCCTCACCTTCGT	133
	Reverse: CCTTTGCGTGCTTTGGTGAT	
CSP2	Forward: TTCGCTTTGGTGACTCTGCT	84
	Reverse: GTTATCCACGTCCACATTGTCG	
OR2	Forward: ACAAAAGGAGATCATGCTGGTG	112
	Reverse: GGCACTTCGGACAATTTTGATGA	
IR2	Forward: TGTTGGAAGATGGGACGGTG	146
	Reverse: AGTACCATTGGCGGATTCCC	
GR5	Forward: TCATGAACGTGGTGGTGCAA	80
	Reverse: AATCGTGGGGAGAAGGAACC	
SNMP1	Forward: TCTTGGCCACCGTAAGTACT	135
	Reverse: CAAAACCTCCGGCTTCTCCT	
Actin	Forward: GGCCGGCTCTATACAGTTCC	89
	Reverse: AAGCCTCATCGAACCTGGTG	

* OBP8 = odorant binding protein 8; CSP2 = chemosensory protein 2; OR2 = odorant receptor 2; IR2 = ionotropic receptor 2; GR5 = gustatory receptor 5; SNMP1 = sensory neuron membrane protein 1; Actin = actin-like protein 1.

−80°C. We performed this procedure three times, collecting a total of 60 *D. helophoroides* larvae at each stage of parasitism.

cDNA library preparation. Total RNA was extracted from *D. helophoroides* neonates. The larvae were collected at different stages of parasitism, and the larval heads and thoracoabdominal parts were analyzed using *EasyPure* RNA kits (TransGen, Beijing, China) following the manufacturer's instructions. RNA integrity was determined using 1% agarose gel electrophoresis, and RNA concentration was measured using a Multiskan GO microplate spectrophotometer with a μDrop plate (Thermo Fisher Scientific, Waltham, MA). RNA was reverse transcribed to obtain complementary DNA (cDNA) by using an *EasyScript* One-Step gDNA Removal and cDNA Synthesis SuperMix kit (TransGen) following the manufacturer's instructions.

Target gene selection and primer synthesis. In a previous study, we identified 62 olfactory-related genes encoding six types of proteins in the transcriptomes of *D. helophoroides* neonates (Li et al. 2020). From the genes identified in this previous study as strongly upregulated in *D. helophoroides* neonates, we selected one gene in each category for further investigation: *DhelOBP8*, *DhelCSP2*, *DhelOR2*, *DhelIR2*, *DhelGR5*, and *DhelSNMP1*. Primers for reverse transcription-quantitative polymerase chain reaction (RT-qPCR) were designed based on the previously published gene sequences using Primer3web (Version 4.1.0) (Table 1).

dsRNA synthesis. Using the *TransStart* KD Plus PCR SuperMix kit (TransGen), we amplified the target olfactory genes (*DhelOBP8*, *DhelCSP2*, *DhelOR2*, *DhelIR2*, *DhelGR5*, and *DhelSNMP1*) from the *D. helophoroides* larval cDNA and the green fluorescent protein (*EGPF*) gene fragment (as the control) from the PEGFP-C1 plasmid (Miaolingbio, Wuhan, China). PCRs were performed using a PCR instrument (Langji, Hangzhou, China), and primers were designed using Primer3-web (Version 4.1.0) (Table 2). All primers were supplemented with T7 promoter sequences to produce double-stranded DNA. PCR amplicon integrity was confirmed on 1% agarose gels before purification with SanPrep Column DNA Gel Extraction kits (Sangon, Shanghai, China). The purified products were sequenced by Sangon by using a 3730XL DNA Analyzer (Applied Biosystems, Foster, CA) to guarantee sequence accuracy and then used as templates for the synthesis of dsRNA with the MEGAscript T7 Transcription kit (Thermo Fisher Scientific).

dsRNA delivery. Using a small brush, 100 *D. helophoroides* neonates were placed in the center of a clean slide, and 10-μl drops of synthesized dsRNA (500 ng/μl) were aspirated onto the gathered larvae with a pipette. After 10 min, the dsRNA solution was absorbed with filter paper and the larvae were washed twice with sterile water. The treated larvae were transferred to 1.5-ml of air-permeable centrifuge tubes by using a small brush and incubated at 25°C for 24 h. Three independent biological replicates of the dsRNA inhibition experiment were performed for each target olfactory gene and EGFP (the control). After total RNA was extracted from the treated larvae and reverse transcribed into cDNA as described previously, RNAi-mediated knockdown efficiency was determined using RT-qPCR.

RT-qPCR. The six olfactory genes and EGFP (control) were amplified from the cDNA templates by using *PerfectStart* Green qPCR SuperMix (TransGen) following the manufacturer's instructions. RT-qPCRs were performed on a LineGene 4800 Fluorescent Quantitative PCR Detection System (Bioer, Hangzhou, China). The thermal cycling conditions were 94°C for 30 s, followed by 40 cycles of 94°C for 5 s and 60°C for 30 s. Actin was used as the internal reference gene, and relative mRNA expression levels were calculated using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001).

Statistical analysis. Significant differences in the relative expression profiles of olfactory genes between *D. helophoroides* neonates and larvae at various stages of parasitism were identified using one-way analyses of variance and Tukey's tests. We also used Student's paired-samples *t* tests to compare transcript levels between larval heads and thoracoabdominal parts as well as between treatment and control groups in the RNAi experiments. All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 26.0 (IBM Corp., Armonk, NY).

Results

Relative expression of olfactory genes in *D. helophoroides* larvae at different stages of parasitism. RT-qPCR analysis showed that the relative expression patterns of the six target olfactory genes varied considerably among the parasitic stages of the *D. helophoroides* larvae (i.e., neonates and days 2–6 of parasitism; Fig. 1). Three of the six genes were significantly upregulated at the neonatal stage compared with the parasitic stages: *DhelCSP2* ($F = 549.400$; df = 5,

Table 2. Primers used for polymerase chain reactions to produce double-stranded DNA.

Gene*	Primer†	Product Size (bp)
OBP8	Forward: **TAATACGACTCACTATAGGGAGA** CTCACTGAAGAGCAAAAG	347
	Reverse: **TAATACGACTCACTATAGGGAGA** GCTAAGCTAATGTGTTGG	
CSP2	Forward: **TAATACGACTCACTATAGGGAGA** CCGAGGAAGGAAAATTTC	331
	Reverse: **TAATACGACTCACTATAGGGAGA** GGCCTTAAGAACTTGAAC	
OR2	Forward: **TAATACGACTCACTATAGGGAGA** ATTCTGATGAGATCCACC	401
	Reverse: **TAATACGACTCACTATAGGGAGA** GTAGCGAAGAGGTGATAG	
IR2	Forward: **TAATACGACTCACTATAGGGAGA** GGACCTCAGGAAAACTAT	436
	Reverse: **TAATACGACTCACTATAGGGAGA** CGAGGAATACGATCAAAG	
GR5	Forward: **TAATACGACTCACTATAGGGAGA** CTTCGCTTACTTATTCGG	424
	Reverse: **TAATACGACTCACTATAGGGAGA** GAATCTATCAACGATGCC	
SNMP1	Forward: **TAATACGACTCACTATAGGGAGA** GACTTTTGATGTCCTTGG	414
	Reverse: **TAATACGACTCACTATAGGGAGA** GGAATGGGTCGAATAAAC	
EGFP	Forward: **TAATACGACTCACTATAGGGAGA** GCTGACCCTGAAGTTCATCT	436
	Reverse: **TAATACGACTCACTATAGGGAGA** GGTGTTCTGCTGGTAGTGGT	

* OBP8 = odorant binding protein 8; CSP2 = chemosensory protein 2; OR2 = odorant receptor 2; IR2 = ionotropic receptor 2; GR5 = gustatory receptor 5; SNMP1 = sensory neuron membrane protein 1; EGFP = enhanced green fluorescent protein.

† These are the primers used to produce double-stranded DNA; the text in bold is the sequence of the T7 promoter.

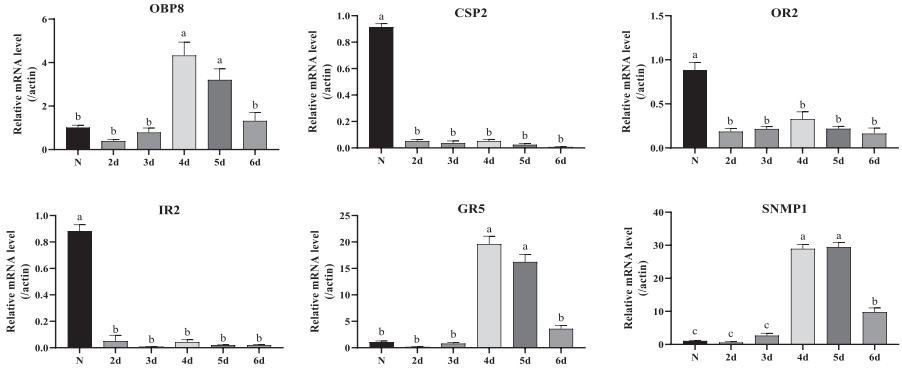

Fig. 1. Relative expression levels of olfactory genes in *Dastarcus helophoroides* larvae at different parasitic stages: neonates (N) and days 2–6 after the initiation of parasitization. Actin was used as the internal reference. Values graphed represent means ± SE of three independent replicates. Lowercase letters above the bars indicate significant differences in expression levels among parasitic stages ($P \leq 0.05$, analysis of variance).

12; $P < 0.001$), *DhelOR2* ($F = 21.536$; df = 5, 12; $P < 0.001$), and *DhelIR2* ($F = 136.728$; df = 5, 12; $P < 0.001$; Fig. 1). The remaining three genes were significantly upregulated on days 4 and 5 postparasitism compared with day 0: *DhelOBP8* ($F = 20.356$; df = 5, 12; $P < 0.001$), *DhelGR5* ($F = 114.373$; df = 5, 12; $P < 0.001$), and *DhelSNMP1* ($F = 228.312$; df = 5, 12; $P < 0.001$; Fig. 1).

Relative expression patterns of olfactory genes in the heads and thoracoabdominal parts of *D. helophoroides* larvae. We also identified significant differences in the relative expression levels of five of the six target olfactory genes between the heads and thoracoabdominal parts of *D. helophoroides* neonates by using RT-qPCR. Four genes were significantly upregulated in the head compared with the thoracoabdominal area: *DhelOBP8* ($t = 15.115$; df = 2; $P = 0.004$), CSP2 ($t = 15.068$; df = 2; $P = 0.004$), *DhelOR2* ($t = 5.404$; df = 2; $P = 0.033$), and *DhelIR2* ($t = 11.937$; df = 2; $P = 0.007$; Fig. 2). Conversely, *DhelGR5* was significantly downregulated in the head compared with the thoracoabdominal area ($t = -10.099$; df = 2; $P = 0.010$; Fig. 2). The sixth gene, *DhelSNMP1*, was upregulated in the head compared with the thoracoabdominal area, but this upregulation was not significant ($t = 3.580$; df = 2; $P = 0.070$; Fig. 2).

RNAi knockdown efficiency. Based on transcription levels before and after dsRNA treatment, RNAi efficiently knocked down four of the six target olfactory genes in *D. helophoroides* neonates. The genes significantly downregulated after dsRNA treatment were *DhelOBP8* ($t = 42.220$; df = 2; $P = 0.001$), *DhelCSP4* ($t = 11.912$; df = 2; $P = 0.007$), *DhelOR2* ($t = 10.856$; df = 2; $P = 0.008$), and *DhelSNMP1* ($t = 19.815$; df = 2; $P = 0.003$; Fig. 3). The remaining two target genes were also downregulated, but not significantly: *DhelIR2* ($t = 4.115$; df = 2; $P = 0.054$), and *DhelGR5* ($t = 2.903$; df = 2; $P = 0.101$; Fig. 3).

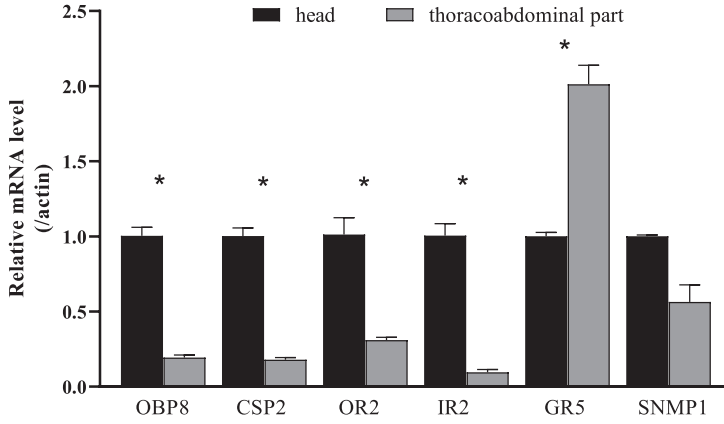

Fig. 2. Relative expression levels of olfactory genes in the heads and thoracoabdominal parts of *Dastarcus helophoroides* neonates. Actin was used as the internal reference. Values graphed represent means ± SE of three independent replicates. Asterisks above the bars indicate a significant difference in expression level between the head and the thoracoabdominal part ($P < 0.05$, pairwise *t* test).

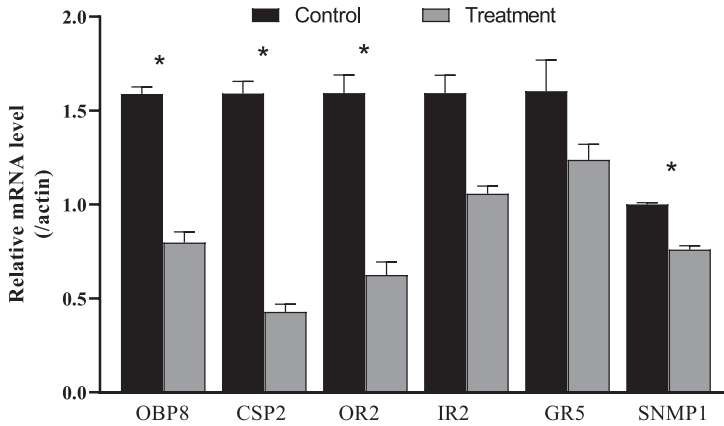

Fig. 3. Knockdown efficiency of double-stranded RNA (dsRNA) treatments in *Dastarcus helophoroides* neonates. Actin was used as the internal reference. Values graphed represent means ± SE of three independent replicates. Asterisks above the bars indicate a significant difference in expression level between the target olfactory gene (*DhelOBP8*, *DhelCSP2*, *DhelOR2*, *DhelIR2*, *DhelGR5*, or *DhelSNMP1*) and the control gene (enhanced green fluorescent protein [*EGFP*]) after dsRNA treatment ($P < 0.05$, pairwise *t* test).

Discussion

Insects rely on a keen sense of olfaction to perceive various chemical signals in the natural environment (Sachse and Krieger 2011). *Dastarcus helophoroides* mainly use host volatiles and host-associated plant volatiles to locate the host *M. alternatus* (Lu et al. 2012, Wei et al. 2009, Yu 2014). Examination of the parasitism-related expression patterns of the six representative olfactory genes included in this study showed that *DhelCSP2*, *DhelOR2*, and *DhelIR2* were significantly upregulated before the initiation of parasitism, suggesting that these three genes might play crucial roles in host recognition by *D. helophoroides* larvae. Conversely, *DhelOBP8*, *DhelGR5*, and *DhelSNMP1* were significantly and strongly upregulated 4–5 d after parasitism initiation, suggesting that these genes might be involved in parasitic functions in *D. helophoroides* larvae other than or as well as locating the host.

Four of the six representative olfactory genes included in this study were significantly upregulated in the heads of *D. helophoroides* neonates compared with the thoracoabdominal area, suggesting that the primary functions of *DhelCSP2*, *DhelOR2*, *DhelOBP8*, and *DhelIR2* were concentrated in the head. However, *DhelGR5* was significantly upregulated in the thoracoabdominal area compared with the head, suggesting that the primary functions of this gene may be nonolfactory. Indeed, all six olfactory genes were constitutively expressed in both the heads and the thoracoabdominal area of *D. helophoroides* larvae, suggesting that all six genes may play both olfactory and nonolfactory roles. Similar results have been reported in other insects. For example, OBPs and CSPs may be involved in development as well as the olfactory system in *Apis mellifera* L. (Hymenoptera: Apidae) (Forêt et al. 2007, Maleszka et al. 2007). In addition, the OBP of *Phormia regina* (Meigen) (Diptera: Calliphoridae) binds long-chain fatty acids and contributes to nutrient uptake (Ishida et al. 2013). However, further investigations of the nonolfactory functions of insect olfactory genes are required.

RNAi is a posttranscriptional gene-silencing mechanism in eukaryotic cells that is a potent tool for studying gene function (Wang et al. 2018). Herein, gene-specific dsRNAs were successfully delivered to *D. helophoroides* neonates by immersion, resulting in the significant knockdown of four of the six target olfactory genes. Our results demonstrate the effectiveness of RNAi in *D. helophoroides* larvae, providing a framework for further functional explorations of *D. helophoroides* olfactory genes, which may lead to the development of improved biological pest control methods using *D. helophoroides*.

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References Cited

Becher, P.G., G. Flick, E. Rozpędowska, A. Schmidt, A. Hagman, S. Lebreton, M.C. Larsson, B.S. Hansson, J. Piškur and P. Witzgall. 2012. Yeast, not fruit volatiles,

mediate *Drosophila melanogaster* attraction, oviposition and development. Funct. Ecol. 26: 822–828.

Birkett, M.A., N. Agelopoulos, K.M. Jensen, J.B. Jespersen, J.A. Pickett, H.J. Prijs, G. Thomas, J.J. Trapman, L.J. Wadhams and C.M. Woodcock. 2004. The role of volatile semiochemicals in mediating host location and selection by nuisance and disease-transmitting cattle flies. Med. Vet. Entomol. 18: 313–322.

Carey, A.F., G. Wang, C.Y. Su, L.J. Zwiebel and J.R. Carlson. 2010. Odorant reception in the malaria mosquito *Anopheles gambiae*. Nature 464: 66–71.

Forêt, S., K.W. Wanner and R. Maleszka. 2007. Chemosensory proteins in the honey bee: Insights from the annotated genome, comparative analyses and expressional profiling. Insect Biochem. Mol. Biol. 37: 19–28.

Ishida, Y., J. Ishibashi and W.S. Leal. 2013. Fatty acid solubilizer from the oral disk of the blowfly. PloS One 8: e51779.

Leal, W.S. 2013. Odorant reception in insects: roles of receptors, binding proteins, and degrading enzymes. Annu. Rev. Entomol. 58: 373–391.

Li, D. 2019. Analysis of olfactory function of DhelOBP21 in *Dastarcus helophoroides* and transcriptional regulatory mechanism of MaltOBP19 in *Monochamus alternatus*. PhD Dissertation, Huazhong Agricultural Univ., Wuhan, China.

Li, X.J., G.P. Dong, L. Yang and W.L. Guo. 2013. Parasitic distance of *Dastarcus helophoroides* on *Monochamus alternatus* and overwintering of the artificial breeding *D. helophoroides* adult in the field. For. Pest Dis. 32: 40–43.

Li, X., G. Dong, J. Fang, H. Liu, W. Guo and H. Yin. 2020. Identification of putative olfactory genes in newly hatched larvae of a coleopteran ectoparasitoid *Dastarcus helophoroides* (Fairmaire) (Coleoptera: Bothrideridae) by transcriptome analysis. Entomol. Res. 50: 329–342.

Livak, K.J. and T.D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2-$\Delta\Delta$CT method. Methods 25: 402–408.

Lu, X., L. Jiang and J.R. Wei. 2012. Searching behavior of adult *Dastarcus helophoroides* (Fairmaire) (Coleoptera: Zopheridae) for *Apriona swainsoni* (Hope) (Coleoptera: Cerambycidae). Chin. J. Biol. Control 28: 594–598.

Maleszka, J., S. Forêt, R. Saint and R. Maleszka. 2007. RNAi-induced phenotypes suggest a novel role for a chemosensory protein CSP5 in the development of embryonic integument in the honeybee (*Apis mellifera*). Dev. Genes Evol. 217: 189–196.

Mamiya, Y. and N. Enda. 1972. Transmission of *Bursaphelenchus lignicolus* (Nematoda: Aphelenchoididae) by *Monochamus alternatus* (Coleoptera: Cerambycidae). Nematology 18: 159–162.

Sachse, S. and J. Krieger. 2011. Olfaction in insects. e-Neuroforum 17: 49–60.

Syed, Z. and W.S. Leal. 2009. Acute olfactory response of *Culex* mosquitoes to a human-and bird-derived attractant. Proc. Natl. Acad. Sci. USA 106: 18803–18808.

Taketsune, A. 1982. *Dastarcus helophoroides*, a natural enemy against *Monochamus alternatus*. For. Pests 31: 228–230.

Todd, J.L., K.F. Haynes and T.C. Baker. 1992. Antennal neurones specific for redundant pheromone components in normal and mutant *Trichoplusia ni* males. Physiol. Entomol. 17: 183–192.

Wang, J., L. Gu and D.C. Knipple. 2018. Evaluation of some potential target genes and methods for RNAi-mediated pest control of the corn earworm *Helicoverpa zea*. Pestic. Biochem. Physiol. 149: 67–72.

Wei, J.R., Z.Q. Yang, T.M. Poland and J.W. Du. 2009. Parasitism and olfactory responses of *Dastarcus helophoroides* (Coleoptera: Bothrideridae) to different cerambycid hosts. Biol. Control 54: 733–742.

Yang, Z.Q., X.Y. Wang and Y.N. Zhang. 2014. Recent advances in biological control of important native and invasive forest pests in China. Biol. Control 68: 117–128.

Yu, L. 2014. Researches on the host selection behavior of *Dastarcus helophoroides* (Fairmaire), a parasitoid of *Monochamus alternatus* (Hope). MS Thesis, Shandong Agricultural Univ., Shandong, China.