Cloning and Expression Analysis of Sensory Neuron Membrane Protein *AoSNMP1* from *Anastatus orientalis* (Hymenoptera: Eupelmidae)[1]

Yan-Yan Bai[2], Xin-Yu Liu[2], Ying-Ying Zhao, Yu Qi, Bao-Zhi Liu, Jia-Ting Yang, Shu-Jie Lv, and Shou-An Xie[3]

Key Laboratory of National Forestry and Grassland Administration on Management of Forest Bio-Disaster, College of Forestry, Northwest A&F University, Yangling, Shaanxi 712100, China

J. Entomol. Sci. 61(2): 000–000 (Month 2025)
DOI: 10.18474/JES25-21

Abstract Sensory neuron membrane proteins (SNMPs) are widely distributed in parasitoid wasps and play a pivotal role in multiple essential activities including feeding, mating, egg laying, and host localizing. In this study, the *AoSNMP1* of *Anastatus orientalis* Yang and Chou (Hymenoptera: Eupelmidae) was cloned by rapid amplification of complementary DNA (cDNA) ends based on the transcriptome data of *A. orientalis*. The nucleotide and deduced amino acid sequences of *AoSNMP1* were analyzed by bioinformatics software. The bioinformatic analysis showed that the full-length cDNA of *AoSNMP1* was 1,928 bp (GenBank accession PV545919), with a noncoding region of 211 and 121 bp at the 5′and 3′ end, respectively. *AoSNMP1* contains a 1,596-bp open reading frame encoding 531 amino acids, with a molecular mass of 60.873 kDa and isoelectric point of 5.54. Phylogenetic analysis revealed that the *AoSNMP1* of *A. orientalis* was closely related to the evolution of the *SNMP1* in *Nasonia vitripennis* (Walker). Real-time quantitative polymerase chain reaction indicated that *AoSNMP1* is expressed in both male and female adults as well as in various tissues. Notably, expression levels are significantly higher in female adults than in male adults. In addition, the expression level in the abdomen is markedly greater than that in both the head and thorax. This study provides a theoretical basis for further research on the function of *AoSNMP1*, especially on the olfactory receptive mechanism in *A. orientalis.*

Key Words *Anastatus orientalis*, sensory neuron membrane protein, gene cloning, bioinformatic analysis, real-time quantitative polymerase chain reaction

Lycorma delicatula (White) (Hemiptera: Fulgoridae) is native to China, Japan, Vietnam, and India, but is considered a widely distributed pest in the United States, Japan, Korea, Vietnam, and other countries (Kim et al. 2011). *Lycorma delicatula* has been discovered in many provinces of China (e.g., Ningxia, Beijing, Hebei, Shandong, and Shaanxi), where it mainly harms forest trees, urban street trees, and other important economic trees, including *Zanthoxylum bungeanum* Maximowicz, *Vitis vinifera* L., *Cerasus pseudocerasus* (Lindl) G.Don, *Actinidia chinensis* Planchon, *Toona sinensis* A.Juss, and *Ailanthus altissima* Miller (Gould et al. 2024, Leach et al. 2019). The high reproduction rate, resistance to pesticides, and host plant transfer

[1]Received 30 April 2025; accepted for publication 6 June 2025.
[2]Co–first authors contributed equally to this work.
[3]Corresponding author (email: shouanxie@163.com).

behavior of *L. delicatula* are problematic for pest management strategists, making it crucial to enhance monitoring and green control of *L. delicatula* populations.

Anastatus orientalis Yang and Chou (Hymenoptera: Eupelmidae) is a dominant egg parasitoid wasp of *L. delicatula*. *Anastatus orientalis* was first discovered in China in 2013 and formally described and published in 2015 (Yang et al. 2015). In China, the distribution pattern of *A. orientalis* is the same as that of *L. delicatula* (Hou 2013). It is a key biological factor in controlling the population of the *L. delicatula*, capable of accurately locating and identifying its egg masses for parasitism (Bao 2022). Under natural conditions, the parasitism rate of the *A. orientalis* can reach up to 40.2% and the parasitism rate can reach up to 77.3% in laboratory artificial breeding (Yang et al. 2015). Moreover, *A. orientalis* is easy to rear in captivity and has strong host-seeking abilities. Bao et al. (2025) showed that the parasitism rate of *A. orientalis* decreased with the increase of host densities of *Antherea pernyi* Guérin and Méneville, and the daily maximum parasitism amount on *A. pernyi* was 16.2%, thereby demonstrating the high biocontrol potential of the *A. orientalis*.

Olfaction is a crucial foundation for insects to accomplish interaction and communication in their environment (Guo et al. 2025, Lehmann et al. 2024). Insects can identify insect pheromones and plant volatiles through olfaction to perform behaviors such as host location, host recognition, mating, and oviposition (Emmanuelle and Christine 2004, Li et al. 2006, Liu et al. 2025). In the process of insects recognizing external odor molecules, various proteins are involved that mainly include odor-binding proteins (OBPs), chemosensory proteins (CSPs), odor receptors, odor-degrading enzymes, and sensory neuron membrane proteins (SNMPs) (Li et al. 2006). As a crucial member of the CD36 gene family, SNMPs are important receptors involved in olfactory perception in holometabolous insects and other species (Hu et al. 2013a). SNMPs were first discovered in *Antheraea polyphemus* Cramer and their structural prediction indicates that they are transmembrane proteins on olfactory sensory neurons (OSNs) (Rogers et al. 1997). In recent years, the identification and analysis of SNMPs have been applied to various insects, such as *Plutella xylostella* (L.) (Li and Qin 2011), *Agrotis ipsilon* Hufnagel (Gu et al. 2013), *Apis cerana* F. (Hu et al. 2013b), *Sitophilus zeamais* Motschulsky (Xia et al. 2019), and *Microplitis mediator* (Haliday) (Shan et al. 2020).

There is a different relative expression level of various SNMP genes in tissues and sexes of insects, and different SNMP subfamilies may play distinct roles in insect physiological activities. For example, it has been confirmed in various insects that SNMP1 plays a crucial role in pheromone perception (Rogers et al. 2001, Sina et al. 2022). The presence of SNMP1 significantly increases the response of the *Heliothis virescens* (F.) antennal neurons and thus to the remarkable sensitivity of the pheromone detection system (Pregitzer et al. 2014). *BmSNMP1* is highly expressed in the antennae of *Bactrocera minax* Enderlein, indicating the functional role of SNMP1 in sensing external odorants by antennae (Zhang et al. 2018). The expression level of *OsSNMP1* is significantly higher in male antennae than in female antennae, implying that *OsSNMP1* may participate in recognizing the sex pheromone of female *Orthosia songi* Chen & Zhang (Huang et al. 2023). Expression levels of *CpSNMP2* are significantly higher in the antennae both male and female *Cydia pomonella* (L.) than in tissues from the thorax, abdomen, legs, and wings, providing evidence for olfactory roles of *CpSNMP2* (Huang et al. 2016). The expression of SNMP2 in supporting cells

(SCs) enables the uptake of fatty acids resulting from sex pheromone inactivation from the sensillum lymph, suggesting that SNMP2 plays an integral role of in the removal of lipophilic "waste products" (Sina and Jurgen 2023). When *Bombyx mori* L. are infected by viruses and bacteria, *BmSNMP3* is highly expressed in the midgut and can stimulate the gut to produce corresponding immune responses (Zhang et al. 2020).

In this study, *AoSNMP1* was identified from the transcriptome data of *A. orientalis*, cloned by polymerase chain reaction (PCR) and rapid amplification of complementary DNA (cDNA) ends (RACE). Its sequence and structural characteristics were analyzed. A phylogenetic tree was constructed by MEGA12.0 software using the neighbor-joining method. Real-time quantitative (q)PCR was used to examine the relative expression levels of the *AoSNMP1* in different tissues and sexes of *A. orientalis*. for Our results establish a foundation for further research on the function of SNMPs and olfactory mechanisms.

Materials and Methods

Insects. The *A. orientalis* specimens used in this study were collected from parasitized egg masses of the *L. delicatula* on 9 December 2024 in Xi Lijiagou Village (34°15′N, 109°45′E), Puhua Town, Lantian County, Xi'an City, Shaanxi Province, in China. After emergence of parasitoid wasps, adults are fed with 10% honey water solution. Male and female adults were separated according to morphological characteristics, and the head with antennae, thorax, and abdomen of the *A. orientalis* adults were dissected for RNA extraction. For each treatment, we had 3 biological replicates. All collected tissues were placed in 1.5-ml Eppendorf tubes, frozen immediately with liquid nitrogen, and stored at −80°C until use.

Total RNA extraction and cDNA synthesis. RNA was extracted from male and female adults, as well as 3 tissues of male and female adults, by using TRIzol reagent (Sangon Biotech, Shanghai, China) according to the manufacturer's instructions. Simultaneously, the concentration and purity of RNA were assayed by NanoPhotometer N60 Touch spectrophotometer (Implen GmbH, Munich, Germany) at absorbance ratios of *A*260/230 and *A*260/280. The integrity of the total RNA was verified via 1% agarose gel electrophoresis. In accordance with the manufacturer's instructions, total RNA was reverse transcribed using the PrimeScript™ II 1st Strand cDNA Synthesis kit (Takara Biomedical Technology Co., Ltd., Beijing, China): 1.0 μl of random 6 mers, 1.0 μl of dNTP mixture, and 8 μl of total RNA were mixed to reach 10 μl in the tube that was then incubated at 65°C for 5 min to improve reverse transcription efficiency. Next, 4.0 μl of 5×PrimeScript II buffer, 0.5 μl of RNase Inhibitor, and 1.0 μl of Primer Script II RTase and RNase-free water were added to reach 20 μl. Finally, the mixture was incubated at 45°C for 50 min and then incubated at 70°C for 15 min. The cDNA was stored at −20°C for subsequent experiments.

Molecular cloning of the *AoSNMP1*. Fragments of the putative *AoSNMP1* gene were procured from the transcriptome database for *A. orientalis* (Sequence Read Archive PRJNA1235513). The veracity of the sequences was established by polymerase chain reaction (PCR) using the primers listed in Table 1. Full-length cDNA was obtained by 5′ and 3′ RACE by using SMARTer® RACE5′/3′ kit (Takara Biomedical Technology Co., Ltd.), with the specific primers listed in Table 1. We subsequently recovered and purified the PCR product. The purified DNA was ligated

Table 1. The primers for amplification of full-length cDNA and real-time qPCR of *AoSNMP1*.[a]

Primer Name	Primer Sequence (5′ to 3′)	Primer Length (bp)	Use
AoSNMP1-F	CAAGGTCGGATGCTGAG	17	Amplification of midpart sequence
AoSNMP1-R	GAGCCTCTTGTATCGTACTCA	21	
AoSNMP1-R1	TCTCAGCATCCGACCTT	17	5′ RACE
AoSNMP1-R2	ATCCACCGATAATAACAAAT	20	
AoSNMP1-F1	TGTGCGTTGGATTAGGAA	18	3′ RACE
AoSNMP1-F2	ACGATACAAGAGGCTCAA	18	
β-Actin-F	GTGCGACGTGGACGTGAGAA	20	Real-time qPCR
β-Actin-R	AGACGGAGCAAGAGCGGTGA	20	
α-Tubulin-F	TTTCGACGGAGCTTTGAATGTAG	23	
α-Tubulin-R	TTGGTGATTTCAGCAACGGATAA	23	
QS1-F	GCTCGCCAATACACGCTCATACA	23	
QS1-R	TTGGTAGTACCGCCTCTGGAAAC	23	
M13-F	GTTGTAAAACGACGGCCAG	19	Universal primer
M13-R	CAGGAAACAGCTATGAC	17	
NUP	AAGCAGTGGTAACAACGCAGAGT	23	
UPM short	CTAATACGACTCACTATAGGGC	22	
UPM long	CTAATACGACTCACTATAGGGCA AGCAGTGGTATCAACGCAGAGT	45	

[a] qPCR, quantitative polymerase chain reaction; bp, base pair(s); F, forward, R, reverse; RACE, rapid amplification of complementary DNA ends.

onto the PGEM-Teasy Vector (Shanghai Promega Trading Co., Ltd., Shanghai, China), transformed into DH5a *Escherichia coli* cells (Takara Biomedical Technology Co., Ltd.) by thermal excitation, cultured with LB medium containing Amp resistance, and screened for positive colonies. After verification by bacterial PCR, the positive clone strains were used for sequencing (Sangon Biotech, Shanghai, China) by the dideoxy-nucleotide method.

Bioinformatic and phylogenetic analyses. The cDNA sequence of *AoSNMP1* was translated with the Translate tool (http://www.expasy.org/translate/). Amino acid sequences were deduced using ExPASy (http://web.expasy.org/translate). The open reading frame (ORF) of the *AoSNMP1* was predicted by the ORF Finder online software available on the National Center for Biotechnology Information (NCBI) website (https://www.ncbi.nlm.nih.gov/orffinder/). The molecular weight (Mw) and isoelectric point (pI) of the deduced amino acid sequences were predicted by Compute pI/Mw

(http://web.expasy.org/compute_pi/). Sequence comparisons were performed using DNAMAN. In addition, a phylogenetic tree was constructed using a total of 16 insect SNMP1 protein sequences obtained from NCBI via MEGA 12.0 software and Clustal X 1.83 by the maximum likelihood method. Bootstrap values were calculated based on 1,000 replicates.

Expression of *AoSNMP1* gene. The cDNA templates derived from both sexes and different tissues of *A. orientalis* were used for expression tests. Primers were designed for real-time qPCR by Prime 5.0 and are listed in Table 1. Expression of the target gene was measured by real-time qPCR and normalized with two stable reference genes, β-actin and α-tubulin. Each PCR reaction was mixed with 10 μl of TB green, 7.8 μl of double stilled water, 1.0 μl of cDNA, 0.4 μl of Rox dye, and 0.4 μl of each primer. The thermal cycling profile consisted of an initial denaturation at 95°C for 5 min and 40 cycles at 95°C for 10 s and 60°C for 20 s. The reactions were performed with the StepOnePlus™ Real-Time PCR instrument (Thermo Fisher Scientific, Singapore). The relative expression level was calculated using the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis. The data are summarized as the mean ± SE for all datasets. A one-way analysis of variance was performed on more than 3 groups of data by using IBM SPSS Statistics 26.0. Differences among means were tested using a Student–Newman–Keuls test for multiple comparisons. The independent sample *t* tests were used for data with <3 groups. All experiments were performed with 3 biological replicates. Each biological replicate was performed with 3 technical repetitions. Differences were considered statistically significant at the 5% level ($P < 0.05$).

Results

Cloning and sequence analysis of *AoSNMP1*. The full-length cDNA sequence of *AoSNMP1* was cloned and deposited in the GenBank database (GenBank accession PV545919). The product was analyzed by agarose gel electrophoresis, as shown in Fig. 1. Our results show that the full length of the *AoSNMP1* from *A. orientalis* was 1,928 bp and that the ORF of *AoSNMP1* is 1,596 bp, encoding 531 amino acids (Fig. 2). The start codon (ATG) was located at 212 bp, and the stop codon (TAG) was located at 1807 bp. The sequence included 5′ untranslated region of 211 bp and 3′ untranslated region of 121 bp. Physicochemical properties of protein coded by *AoSNMP1* were as follows: the molecular formula of its protein was derived as $C_{2757}H_{4269}N_{707}O_{797}S_{25}$; the molecular mass was 60.873 kDa; and the pI was 5.54. The grand average of hydropathicity was −0.258, which indicates the protein was a hydrophilic protein. The aliphatic index was 89.17, and the instability index was 36.50 (<40), suggesting that it was stable protein. The amino acids encoded by the *AoSNMP1* contained a conserved domain of the insect CD36 protein family from positions 11 to 484 (E-value of 3.24e−127). Hydrophobic regions in the N terminus and C terminus were found in *AoSNMP1* (Fig. 3). The two hydrophobic regions were inferred to be transmembrane helices, which are the structural components of the CD36 protein family. Furthermore, it was predicted that amino acid sequence contains two transmembrane regions (amino acids 9–28 and 463–485) (Fig. 4), exhibiting typical characteristics of the SNMP gene family.

Sequence alignment and phylogenetic analysis of *AoSNMP1*. Sequence alignment and phylogenetic analysis indicated that the sequence identity of *AoSNMP1* from *A. orientalis* with other selected hymenopteran SNMPs ranged

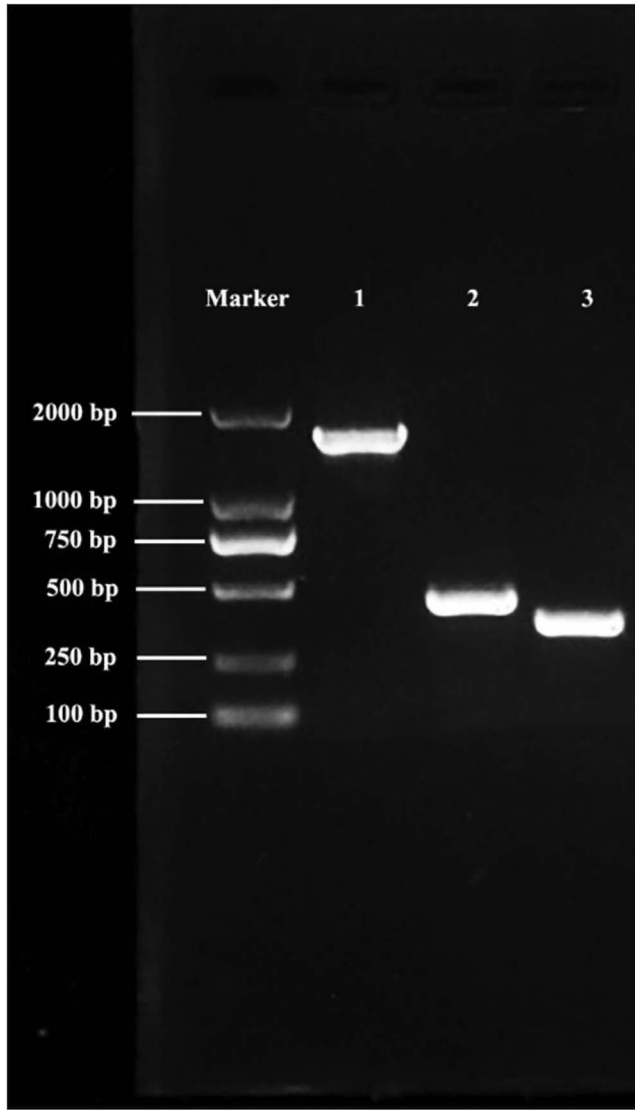

Fig. 1. Polymerase chain reaction products of *AoSNMP1*. M represents DL 2000 DNA Marker, 1 represents the middle segment of *AoSNMP1*, 2 represents the 5′ rapid amplification of complementary DNA ends (RACE) segment of *AoSNMP1*, and 3 represents the 3′ RACE segment of *AoSNMP1*.

from 58.44 to 61.82% (Fig. 5). The highest sequence homology is observed with *NvSNMP1* from *N. vitripennis* at 61.82%, whereas the amino acid sequence identity with SNMP1 of *Phymastichus coffea* LaSalle and *Trichogramma pretiosum* Riley is 60.52 and 58.44%, respectively. The phylogenetic tree based on SNMPs from

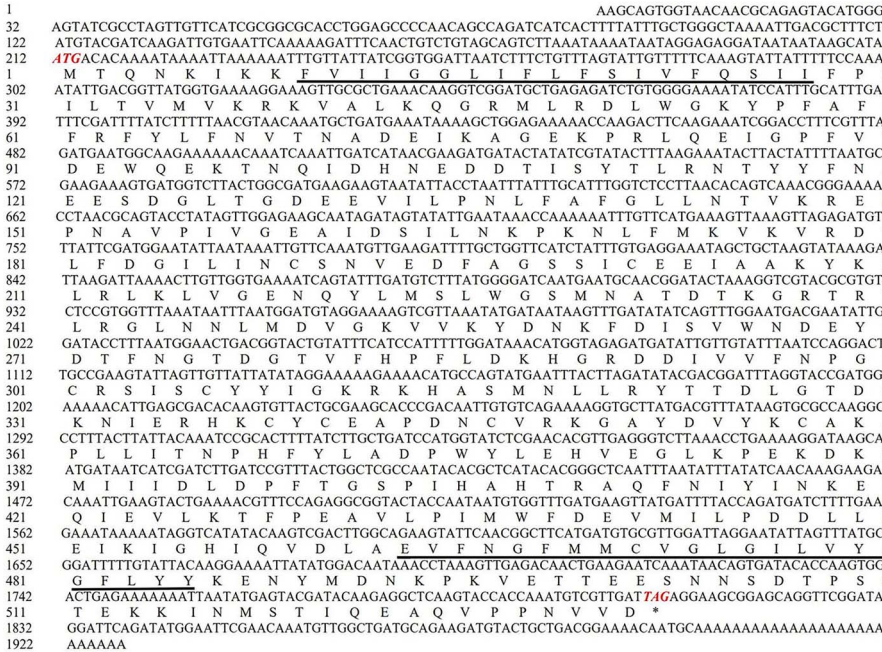

Fig. 2. Nucleotide and amino acid sequences of *AoSNMP1*. The start and stop codons are indicated in bold red italic font, and the asterisk (*) represents the stop codon. The two transmembrane domains are represented by black underlines.

A. orientalis and other species of Hymenoptera was constructed by the neighbor-joining method (Fig. 6). The *AoSNMP1* in *A. orientalis* was clustered closely with the *NvSNMP1* in *N. vitripennis* with 73% confidence, indicating that the genetic distance between *AoSNMP1* and *NvSNMP1* is the shortest. The SNMP1 of *P. coffea* and *T. pretiosum* shared a closer evolutionary relationship with *AoSNMP1*. This is consistent with the results of the amino acid sequence homology analysis.

Expression of *AoSNMP1* in male and female adults and various tissues. The expression pattern of *AoSNMP1* was investigated by real-time qPCR (Fig. 7). The result analysis revealed that the relative expression level of females was higher than that of males ($P < 0.05$). The relative expression level of *AoSNMP1* was detected in the head, thorax, and abdomen. The relative expression level of *AoSNMP1* in various tissues was from high to low in the order of abdomen, head, and thorax, with the relative expression level in abdomen significantly higher than in those in the head and thorax ($P < 0.01$). The expression levels of *AoSNMP1* in the head and thorax were not significantly different ($P > 0.05$).

Discussion

Over the process of long-term evolution, insects have developed highly sensitive olfactory systems that enable them to detect and filter out relevant odor substances

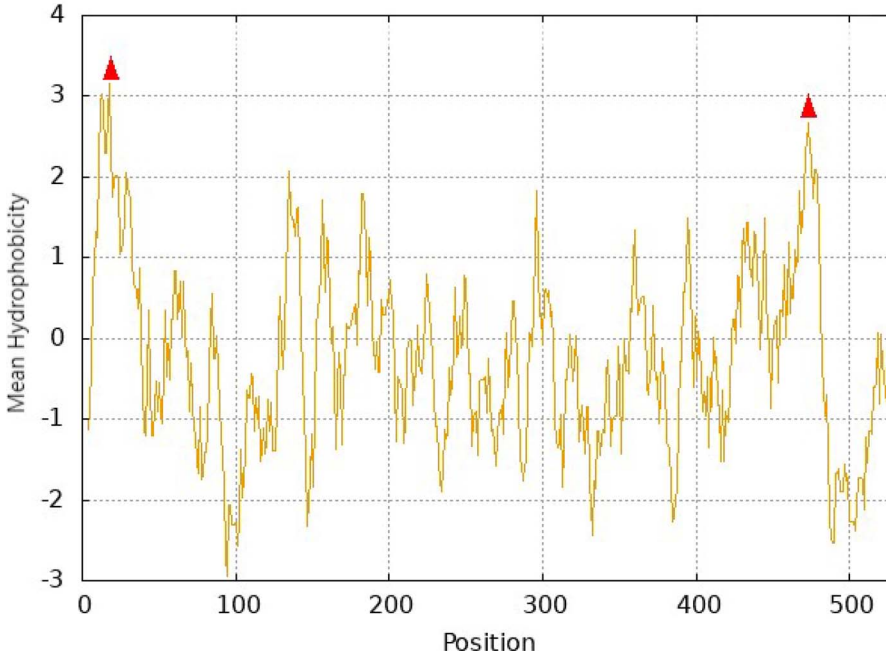

Fig. 3. Hydropathy plot of *AoSNMP1*. Two conserved hydrophobic regions are marked with red triangles.

within a complex environment, finally eliciting appropriate behavioral responses (Michel and Sylvia 2020). Odorant detection highly depends on the interaction of various OSNs and different proteins related to SCs in insects (Sina and Jurgen 2020). The ORF of *AoSNMP1* from *A. orientalis* successfully was cloned in this study: it was 1,596 bp and encoded 531 amino acids. The protein was encoded by *AoSNMP1* with a molecular

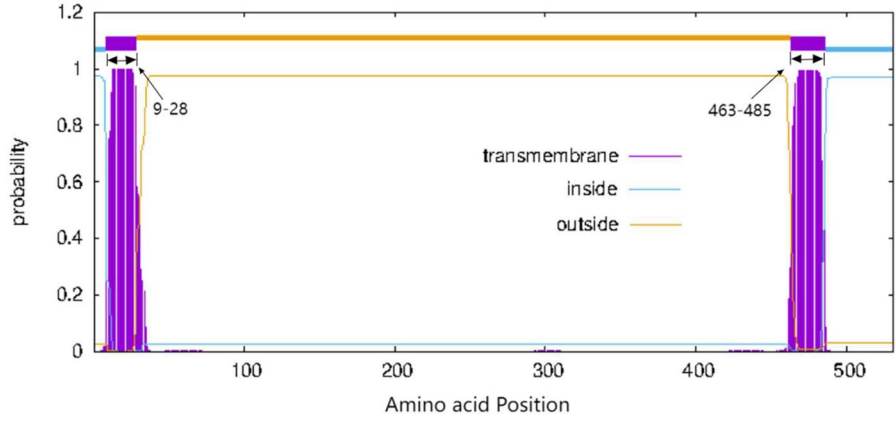

Fig. 4. Prediction of transmembrane regions in *AoSNMP1*.

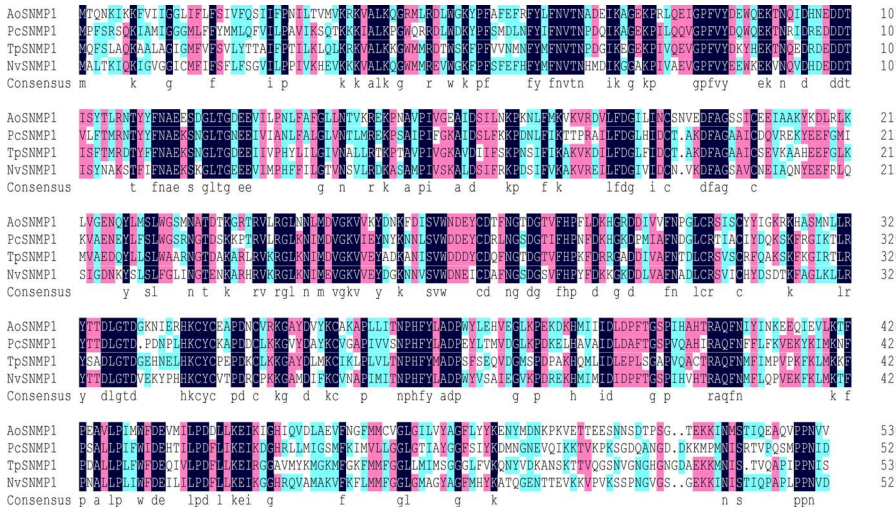

Fig. 5. Multiple sequence alignment among *AoSNMP1* with sensory neuron membrane proteins (SNMPs) from other parasitoid wasps. Black, pink, blue, and white represent 100% identity, identity above 75%, identity above 50% and identity below 30%, respectively. GenBank ID and its corresponding SNMP of other parasitoid wasps: PV545919 *Anastatus orientalis* (*AoSNMP1*), XP_058792824.1 *Phymastichus coffea* (*PcSNMP1*), XP_014231879.1 *Trichogramma pretiosum* (*TpSNMP1*), and XP_032451925.1 *Nasonia vitripennis* (*NvSNMP1*).

formula of $C_{2757}H_{4269}N_{707}O_{797}S_{25}$, a pI of 5.54, and a molecular mass of 60.873 kDa. Similar characteristics of *AoSNMP1* were shared with other SNMPs, such as having two transmembrane regions near the N terminus and C terminus of the amino acid sequence, demonstrating that *AoSNMP1* exhibits the typical characteristics of membrane proteins (Huang et al. 2016, Zhang et al. 2018).

The phylogenetic tree of *AoSNMP1* and homologous SNMPs from hymenopterans indicated that *AoSNMP1* and *NvSNMP1* were closely clustered into the same clade, with a bootstrap support of 73%. The results showed that *AoSNMP1* had the closest evolutionary relationship with *NvSNMP1*. However, there was evidently low homology with species from Braconidae in Hymenoptera. Although SNMP2 and SNMP3 subfamily genes had been identified in Lepidoptera, these genes were not found in the transcriptome data of the *A. orientalis*, suggesting that it may be related to the function of genes (Sina and Jurgen 2023, Zhang et al. 2020).

The expression characteristics of genes in insects are often associated with functions (Johny et al. 2024, Zhang et al. 2022). The expression results revealed that expression levels of females were higher than those of males, suggesting that the function of *AoSNMP1* is related to its role in females. In parasitoid wasps, females typically use their ovipositors as detectors to assess host acceptance (Vinson 1998). The ovipositors of systematic and persistent probing in response to exogenous volatiles will happen in female parasitoid wasps (Kaiser et al. 2010). Compared with Lepidoptera

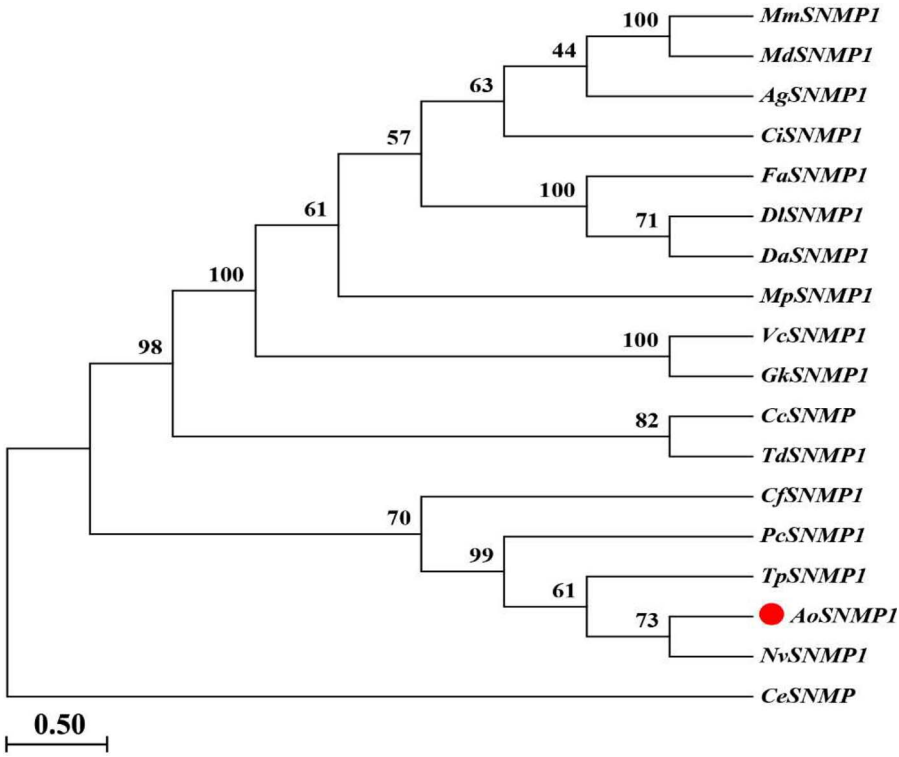

Fig. 6. Phylogenetic tree of *AoSNMP1* and its homologs from other hymenopterans based on amino acid sequences of sensory neuron membrane proteins (SNMPs) by using neighbor-joining method. GenBank ID and its corresponding SNMP: PV545919 *Anastatus orientalis* (*AoSNMP1*), XP_058792824.1 *Phymastichus coffea* (*PcSNMP1*), XP_014231879.1 *Trichogramma pretiosum* (*TpSNMP1*), XP_032451925.1 *Nasonia vitripennis* (*NvSNMP1*), XP_014206677.1 *Copidosoma floridanum* (*CfSNMP1*), XP_057329642.1 *Microplitis mediator* (*MmSNMP1*), XP_011306891.1 *Fopius arisanus* (*FaSNMP1*), AQN78521.1 *Meteorus pulchricornis* (*MpSNMP1*), XP_043284448.1 *Venturia canescens* (*VcSNMP1*), QGW50408.1 *Chouioia cunea* (*CcSNMP*), ANG08489.1 *Trichogramma dendrolimi* (*TdSNMP1*), XP_034946984.1 *Chelonus insularis* (*CiSNMP1*), XP_063976400.1 *Diachasmimorpha longicaudata* (*DlSNMP1*), XP_008543235.3 *Microplitis demolitor* (*MdSNMP1*), AWS20447.1 *Aphidius gifuensis* (*AgSNMP1*), XP_015114628.1 *Diachasma alloeum* (*DaSNMP1*), and UEN71286.1 *Gregopimpla kuwanae* (*GkSNMP1*), CAB11566.3 *Caenorhabditis elegans* (*CeSNMP*).

and other insect orders, the expression levels of the *AoSNMP1* gene in the abdomen was found with the highest levels, suggesting that the genes might be involved in parasitic functions in *A. orientalis* adults other than or as well as locating the host (Liu et al. 2013, Yin et al. 2023). Some olfactory-related genes were highly expressed in

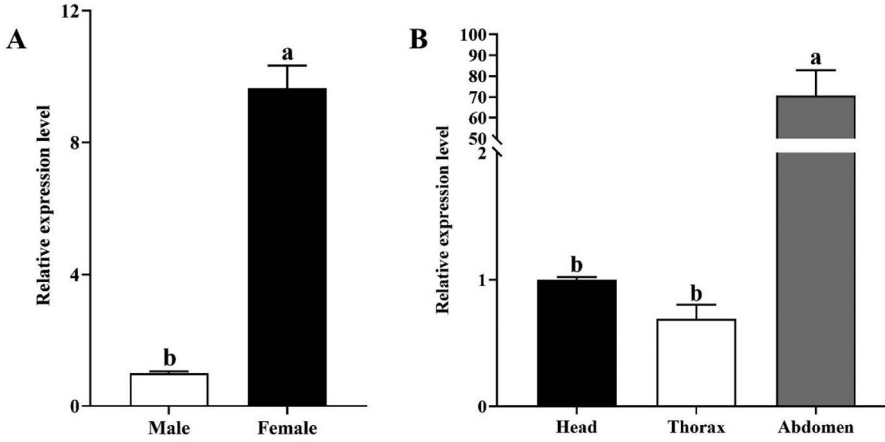

Fig. 7. Relative expression level of *AoSNMP1* in (A) male and female adults and (B) various tissues (head, thorax, and abdomen). Values are means ± SE. The relative expression of *AoSNMP1* in male and female adults was analyzed by independent sample *t* test, and the different lowercase letters above each bar show significant differences ($P < 0.05$). The relative expression of *AoSNMP1* in different tissues (head, thorax, and abdomen) was analyzed by one-way analysis of variance, and there was a significant difference between the relative expression of *AoSNMP1*, expressed by different lowercase letters ($P < 0.05$).

abdominal tissues of *Halyomorpha halys* Stål, such as OBPs and CSPs (Sun et al. 2020). It was supposed that the insect ovipositor played an important role as an olfactory organ to some extent (Pratibha and Renee 2017). The scarcity of current research on hymenopterans, particularly parasitoid wasps, is notable. Furthermore, the relative expression level of *AoSNMP1* in various tissues hinted at the existence of nonolfactory functions. The expression of olfactory genes in nonolfactory organs will provide useful insight toward the functions of olfactory genes and the behavioral process of parasitoid insects.

In this study, *AoSNMP1* of *A. orientalis* was cloned and the physicochemical properties of the protein were predicted. In addition, the relative expression of *AoSNMP1* was investigated in male and female adults and in the various tissues. The findings lay the foundation for further investigation of the chemical communication mechanism of *A. orientalis* and provide reference for research on gene function. In the future, the functions of *AoSNMP1* can be further investigated by fluorescence competition binding experiments, thereby facilitating the development of control strategies for pest insects via parasitoid wasps.

Acknowledgments

This study was supported by the "Green Pollution Control Technology Based on Chemical Pheromone in *Agrilus zanthoxylum*," The National Public Welfare Forestry projects in China (grant 201504324); "Shaanxi Province's Second Batch of Special Support Program for High-Level Talents Leading Talents Project"

(Shaanxi Group 2020-44); Xi'an Innovation Strong Foundation Plan-Agricultural technology research and development projects "Lantian County's Development and application technology of pheromone lure for *Agrilus zanthoxylum*" (2022JH-JSYF-0261), National Special Support Program for High-level Talents (2022).

References Cited

Bao, K.X. 2022. Studies on the field lethal factor of spotted lanternfly and biology of its natural enemy at egg stage-*Anastatus orientalis*. Agricultural University of Hebei. Baoding, Hebei, China.

Bao, K.X., Y.Q. Dang, Y.T. Zhuang, M.X. Fan and X.Y. Wang. 2025. Evaluation of parasitic capacity of *Anastatus orientalis* (Hymenoptera: Eupelmidae) on eggs of *Antherea pernyi* (Lepidoptera: Saturniidae) based on functional response model. Chin. J. Biol. Control 41(02): 260–268.

Emmanuelle, J.J. and M. Christine. 2004. Insect olfactory receptors: Contributions of molecular biology to chemical ecology. J. Chem. Ecol. 30(12): 2359–2397.

Gould, R.J., C. Losch, L. Sullivan, Y.K. Wu, X.Y. Wang, L.M. Cao and H.J. Broadley. 2024. Lifecycle of *Anastatus orientalis* (Hymenoptera: Eupelmidae) and synchrony with its host, the spotted lanternfly, *Lycorma delicatula* (Hemiptera: Fulgoridae). Environ. Entomol. 53(06): 954–965.

Gu, S.H., R.N. Yang, M.B. Guo, G.R. Wang, K.M. Wu, Y.Y. Guo, J.J. Zhou and Y.J. Zhang. 2013. Molecular identification and differential expression of sensory neuron membrane proteins in the antennae of the black cutworm moth *Agrotis ipsilon*. J. Insect. Physiol. 59(4): 430–443.

Guo, X.L., D.H. Yang, X.W. Sun, W. Song and J.B. Wen. 2025. Research progress of the functions of sensory neuron membrane proteins (SNMPs) in insects. Acta Entomol. Sin. 68(5): 679–696. (http://kns.cnki.net/kcms/detail/11.1832.q.20250318.1131.002.html).

Hou, Z.R. 2013. Study on the *Lycorma delicatula* and egg parasitoids. Chinese Academy of Forestry, Beijing, China.

Hu, Y.Y., S.F. Xu, A.J. Wubie, W. Li, J.X. Huang, Z.B. Guo and T. Zhou. 2013a. Research advance of olfactory proteins and olfactory mechanism in insects. Genomics Appl. Biol. 32(5): 667–676.

Hu, Y.Y., S.F. Xu, A.J. Wubie, W. Li, J.X. Huang, Z.B. Guo and T. Zhou. 2013b. Molecular characterization and tissue localization of sensory neuron membrane protein from Chinese honey bee, *Apis cerana* (Hymenoptera: Apidae). Appl. Entomol. Zool. 48(4): 533–545.

Huang, X.L., L. Liu, Y.Q. Fang and J.N. Feng. 2016. Expression of a sensory neuron membrane protein SNMP2 in olfactory sensilla of codling moth *Cydia pomonella* (Lepidoptera: Tortricidae). J. Econ. Entomol. 109(4): 1907–1913.

Huang, X.R., J. Yang, X. Wen, C.H. Hu, Y.X. Zhang and X.L. Huang. 2023. Research on the cloning and tissue distribution of *Orthosia songi* CD36 family homologous genes. For. Res. 36(5): 72–82.

Johny, J., M. Nihad, H.A. Alharbi, M.A. AlSaleh and B. Antony. 2024. Silencing sensory neuron membrane protein *RferSNMPu1* impairs pheromone detection in the invasive Asian palm weevil. Sci. Rep. 14(1): 16541.

Kaiser, L., C. Bartheye, V. Kerguelen and M.H. Delegue. 2010. Odour conditioning of ovipositor probing in a parasitic wasp. Ethol. Ecol. Evol. 7(3): 245–255.

Kim, G.J., E. Lee, Y. Seo and N. Kim. 2011. Cyclic behavior of *Lycorma delicatula* (Insecta: Hemiptera: Fulgoridae) on host plants. J. Insect. Behav. 24(6): 423–435.

Leach, H.J., D. Biddinger, G. Krawczyk, E. Smyers and J.M. Urban. 2019. Evaluation of insecticides for control of the spotted lanternfly, *Lycorma delicatula* (Hemiptera: Fulgoridae), a new pest of fruit in the northeastern U.S. Crop. Prot. 124: 104833.

Lehmann, J., Y. Günzel, M. Khosravian, S. Cassau, S. Kraus, J.S. Libnow, H. Chang, B.S. Hansson, H. Breer, E.C. Fuchs, J. Fleischer and J. Krieger. 2024. SNMP1 is critical for sensitive detection of the desert locust aromatic courtship inhibition pheromone phenylacetonitrile. BMC Biol. 22(1): 150.

Li, P.Y. and Y.C. Qin. 2011. Molecular cloning and characterization of sensory neuron membrane protein and expression pattern analysis in the diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae). Appl. Entomol. Zool. 46(4): 497–504.

Li, W.H., H.T. Tu, X.X. Miao and X.R. Guo. 2006. Research progresses of proteins related with insect olfactory. J. Appl. Entomol. 6: 757–762.

Liu, S., Y.R. Zhang, W.W. Zhou, Q.M. Liang, X. Yuan, J.A. Cheng, Z.R. Zhu and Z.J. Gong. 2013. Identification and characterization of two sensory neuron membrane proteins from *Cnaphalocrocis medinalis* (Lepidoptera: Pyralidae). Arch. Insect Biochem. 82: 29–42.

Liu, X.Y., Y.Y. Bai, Y.Y. Zhao, Y. Qi, N. Jiang, B.Z. Liu, S.J. Lv and S.A. Xie. 2025. Morphology and ultrastructure of the antennal sensilla of adult *Anastatus orientalis* (Hymenoptera: Eupelmidae), a parasitoid of *Lycorma delicatula* (Hemiptera: Fulgoridae). Acta Entomol. Sin. 64(1): 123–132.

Michel, R. and A. Sylvia. 2020. Insect olfactory communication in a complex and changing world. Curr. Opin. Insect Sci. 42: 1–7.

Pratibha, Y. and M.B. Renee. 2017. The insect ovipositor as a volatile sensor within a closed microcosm. J. Exp. Biol. 220(9): 1554–1557.

Pregitzer, P., M. Greschista, H. Breer and J. Krieger. 2014. The sensory neurone membrane protein SNMP1 contributes to the sensitivity of a pheromone detection system. Insect Mol. Biol. 23(6): 733–742.

Rogers, M.E., J. Krieger and R.G. Vogt. 2001. Antennal SNMPs (sensory neuron membrane proteins) of Lepidoptera define a unique family of invertebrate CD36-like proteins. J. Neurobiol. 49(1): 47–61.

Rogers, M.E., M. Sun, M.R. Lerner and R.G. Vogt. 1997. SNMP-1, a novel membrane protein of olfactory neurons of the silk moth *Antheraea polyphemus* with homology to the CD36 family of membrane proteins. J. Biol. Chem. 272(23): 14792–14799.

Shan, S., S.N. Wang, X. Song, A. Khashaveh, Z.Y. Lu, H.D. Khalid, R.J. Li, X.W. Gao and Y.J. Zhang. 2020. Molecular characterization and expression of sensory neuron membrane proteins in the parasitoid *Microplitis mediator* (Hymenoptera: Braconidae). Insect Sci. 27(3): 425–439.

Sina, C., S. Doreen, K. Thomas, L. Michael, H. Gerd, B. Heinz and K. Jurgen. 2022. The sensilla-specific expression and subcellular localization of SNMP1 and SNMP2 reveal novel insights into their roles in the antenna of the desert locust *Schistocerca gregaria*. Insects 13(7): 579–579.

Sina, C. and K. Jurgen. 2020. The role of SNMPs in insect olfaction. Cell Tissue Res. 383: 21–33.

Sina, C. and K. Jurgen. 2023. Evidence for a role of SNMP2 and antennal support cells in sensillum lymph clearance processes of moth pheromone-responsive sensilla. Insect Biochem. Mol. 164: 104046.

Sun, D.D., Y. Huang, Z.J. Qin, H.X. Zhan, J.P. Zhang, Y. Liu and S.Y. Yang. 2020. Identification of candidate olfactory genes in the antennal transcriptome of the stink bug *Halyomorpha halys*. Front. Physiol. 11: 876.

Vinson, S.B. 1998. The general host selection behavior of parasitoid Hymenoptera and a comparison of initial strategies utilized by larvaphagous and oophagous species. Biol. Control 11(2): 79–96.

Xia, D.S., C. Shen, Y. Zhang, Q.F. Tang and J. Wang. 2019. Identification and expression patterns of sensory neuron membrane protein genes from *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). Coleopt. Bull. 73(3): 735–745.

Yang, Z.Q., W.Y. Choi, L.M. Cao, X.Y. Wang and Z.R. Hou. 2015. A new species of *Anastatus orientalis* (Hymenoptera: Eulpelmidae) from China, parasitizing eggs of *Lycorma delicatula* (Homoptera: Fulgoridae). Zool. Syst. 40(3): 290–302.

Yin, H.Y., X.J. Li, G.P. Dong, W.L. Guo, J.M. Fang and H.J. Liu. Temporal and spatial expression of parasitism-related olfactory genes in larvae of the ectoparasitoid *Dastarcus helophoroides* (Coleoptera: Bothrideridae). J. Entomol. Sci. 58(3): 335–343.

Zhang, G.H., J. Chen, F.L. Wang, L.Y. Gui and J.M. Zhang. 2018. Characterization and levels of expression of sensory neuron membrane proteins in the adult citrus fruit fly (Diptera: Tephritidae). J. Insect Sci. 18(6): 1–8.

Zhang, H.J., W. Xu, Q.M. Chen, L.N. Sun, A. Anderson, Q.Y. Xia and A. Papanicolaou. 2020. A phylogenomics approach to characterizing sensory neuron membrane proteins (SNMPs) in Lepidoptera. Insect Biochem. Mol. 118: 103313.

Zhang, Y., K. Feng, R.L. Mei, W. Li and F. Tang. 2022. Analysis of the antennal transcriptome and identification of tissue-specific expression of olfactory-related genes in *Micromelalopha troglodyta* (Lepidoptera: Notodontidae). J. Insect. Sci. 22(5): 1–14.