Studies on olfactory systems of two parasitoid wasps: *Diadegma semiclausum* (Hellén) (Hymenoptera: Ichneumonidae) and *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) of the Diamondback moth *Plutella xylostella* (L.) (Lepidoptera: Plutellidae)

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A thesis submitted to
College of Science, Health, Engineering and Education
Murdoch University
Perth, Western Australia
Australia

in partial fulfilment of the requirements of the degree of
Doctor of Philosophy

September 2020

Declaration

I hereby declare that the work presented in this thesis is my own account of my research work, and its content has not been previously submitted elsewhere for the award of a degree at any tertiary education institute.

To the best of my knowledge, all work performed by others, published or unpublished, has been acknowledged.

Basman Haseb Al-Jalely

September 2020

In the name of Allah, Most Gracious, Most Merciful

Who taught by the pen (4) Taught man that which he knew not (5)

Sura 96: AL-ALAQ

الحمد والشكر لله من قبل ومن بعد

Acknowledgement

Humbly, I would like to thank my home country, **Iraq**, for sponsoring my scholarship and my home university, **Baghdad University**, for nominating me for it.

I also would like to thank my supervisory panel: Drs. **Wei Xu**, **Manjree Agarwal**, **Yonglin Ren** and **Bernard Dell** for their time in producing this thesis.

I would like to thank **Andras Szito**, **Ashleigh Roberts**, **Dale Banks**, **Gavin D'Mello**, **Emad Aziz**, **Greg Baker**, **Hatem Taha**, **Ian McKernan**, **Jacqueline Dyer**, **James Altmann**, **Janet Box**, **Jeremy Shaw**, **Jodie-Lee McLeod**, **Jose Minetto**, **Julia Hobson**, **Julie Blake**, **Karen Richardson**, **Karen Sutherland**, **Kellie O'Toole**, **Kimberly Baker**, **Kirsty Townsend**, **Louise Pallant**, **Lyn Kirilak**, **Michael Taylor**, **Mark Tan**, **Rob Emery**, **Stephen Johnson**, **Bob Du, Yvette Toyne** and everyone who helped me or supported me by any mean at all the stages of this long journey. I hope I can one day return the favour.

George Graeme, **Gordon Thomson** and **James Newman**, interacting with you and your support were always pleasant to me and a key to solve many technical issues. Thank you.

My mentors, Drs: **Husam Al-Din Abdullah**, **Mohammad Sadiq Hasan** and **Hamza Al-Zubaidi**, I greatly appreciate the opportunities and the confidence you provided me with. Thank you.

Again, I would like to thank Dr **Wei Xu**, the person. Thank you for being there every time I needed your help, above and beyond supervisory affiliations.

My great friends, **Nazih Wais** and **Ihab Al-Najim** thank you for your invaluable support at all the stages of my study.

I deeply appreciate the contributions of **Penghao Wang** and **Yalin Liao** in parts of the proposed journal manuscripts.

I would like to thank: **Murdoch University** for the "**Graduate Research Conference Travel Award**" to attend the "**International Society of Chemical Ecology 34th. Annual Meeting-2018-Budapest**", the **Grain Industry Association of Western Australia** for the "**Student Award**" to attend "**Grains Research Update 2017**" and **Western Australian State Agricultural Biotechnology Centre** (SABC) for using their laboratories in some of my experiments.

I also acknowledge and respect the **Aboriginal** and **Torres Strait Islander people** as the traditional custodians of the land and pay respect to the elders, past, present and future, alongside the **Aussie community** and its amazing **cultural harmony**.

My always loving **Mum (Yom)** *mercy upon her soul*, **sisters** and **in-laws** who supported me with their prayers the whole time. I sincerely appreciate it for you

Finally, my kind wife, **Khulood**, and lovely daughters, **Riyam** and **Yaqeen**; For your support and bearing with me all the difficulties and hard times; thank you with my love.

This acknowledgement represents a fraction of my gratitude; If I unintentionally missed someone, I would sincerely apologize and say: Rest assure, I am grateful to you in my heart.

With all the modesty and gratitude, I would like to thank you all, but I feel it's not enough.

PS: (Titles are honoured and preserved)
(Names are alphabetically ordered)

Basman Haseb Al-Jalely
Perth, Western Australia
2020

Dedication

To my dad's soul.

To my life's beacon, mom.

To every single person laid a layer in my learning life milestones

With humble and gratitude

Abstract

The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is one of the most destructive insect pests of brassicaceous crops, which has shown problematic resistance to almost every common insecticide. In certain parts of the world, the economic production of crucifers has become nearly impossible due to the failure in controlling DBM. Consequently, increased efforts worldwide have been undertaken to implement biological control programs that are principally employing its natural enemies such as *Diadegma semiclausum* and *Trichogramma pretiosum*. *D. semiclausum* is a specialist DBM larvae parasitoid and one of the most common parasitoids in Australia. *T. pretiosum* is a generalist egg parasitoid, and it inserts its eggs into the host eggs, including DBM.

However, limited studies have been conducted on these two parasitoid wasps regarding how they detect DBM, how their olfactory systems guide them to localize the DBM and what olfactory genes are involved in these DBM-seeking and oviposition behaviours. Without this knowledge, it is difficult to utilize them at maximum efficiency and effectiveness in DBM management programs.

In this study, analytical chemistry, electrophysiology, scanning electron microscopy, genomics, transcriptomics, bioinformatics and molecular biology approaches were applied to investigate the olfactory systems of *D. semiclausum* and *T. pretiosum*. Eight candidate attractants were identified from DBM-infested canola *Brassica napus*, while some of them were found to be able to initiate significant antennal responses from the parasitoid wasp *D. semiclausum*. Male and female *D. semiclausum* exhibited different antennal responses to various tested volatile compounds.

Candidate olfactory genes, including 17 odorant-binding proteins (OBPs) and 67 odorant receptors (ORs), were identified and characterized from *D. semiclausum*. Similarly, a total of 22 OBPs and 121 ORs were identified and characterized from *T. pretiosum*. These genes may play pivotal roles in the host-seeking and oviposition behaviours.

This study improves our understanding of the olfactory systems of these two wasps and their host-seeking behaviours, which will assist in developing more efficient and environmentally friendly biological control strategies to manage DBM.

Key words

Diamondback moth, *Plutella xylostella*, *Diadegma semiclausum*, *Trichogramma pretiosum*, Biological control, Odorant binding proteins, Odorant receptors, Semiochemicals, Electroantennography (EAG), Olfactory sensilla, Coeloconica, Polymerase chain reaction (PCR), Canola Volatile organic compounds (VOCs), Scanning electron Microscope (SEM), Parasitoid, Phylogenetics.

Australian and New Zealand Standard Research Classifications (ANZSRC)

ANZSRC code: 060808, Invertebrate Biology, 50%

ANZSRC code: 060806, Animal Physiological Ecology, 30%

ANZSRC code: 060201, Behavioural Ecology, 20%

Fields of Research (FoR) Classification

FoR code: 0602, Ecology, 35%

FoR code: 0608, Zoology, 65%

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Chapter 1

1.1 Literature review

1.1.1 The Diamondback Moth (DBM)

The diamondback moth, *Plutella xylostella* (L.), is a serious insect pest of crucifers throughout the world (Figure 1). Cultivated brassicas are considered of European origin, so it was suggested that DBM had also originated in the same area and spread with the cultivated brassicas around the world (Kfir 1998). However, a recent study (You et al. 2020) showed that its origin is South America. DBM is a member of the Plutellidae family that falls under the Lepidoptera order (Charleston et al. 2006).

Scientific name: *Plutella xylostella* (Linnaeus)
Kingdom: Animalia
Phylum: Arthropoda
Class: Insecta
Order: Lepidoptera
Family: Plutellidae
Genus: *Plutella*
Species: *xylostella*

1.1.1.1 Life cycle

Females deposit their eggs singly or in patches of two to eight, which are oval, flattened, yellow or pale green and around 0.44 mm (length) × 0.26 mm (width) (Figure 1). The average number of eggs deposited by one female adult is 150, but it may reach 250-300. It takes about 5.6 days on average for an egg to hatch to the first instar larva. The first, second, third and fourth instars last around 4.5, 4, 4 and 5 days, respectively. The average DBM pupation period is around 8.5 days. Although the first instar is colourless, the other instars are green (Capinera 2000a). The 7-9 mm yellowish-green pupae form a loose silk cocoon on the lower or outer host leaves, which may occur in the florets of cauliflower and broccoli (Figure 1). Adults are small, slender and greyish-brown moths with pronounced 6-mm long antennae. Wings have a broad cream or light brown band along the back, forming the shape of one or more diamonds (Figure 1). A side view shows wings' tips turned upward. Male adults have yoked abdominal ends, whilst female adults are bigger in body size and have round-shaped abdominal ends (Chen et al. 2011). The average development time from egg to the pupal stage is 25-30 days, but the range can be 17-51 days. Therefore, the number of generations varies from 4-12 generations per year depending on various factors, including temperature, humidity and sunlight. Male adults live about 12 days. Female adults live about 16 days, but they can lay eggs for only ten days (CABI 2020)

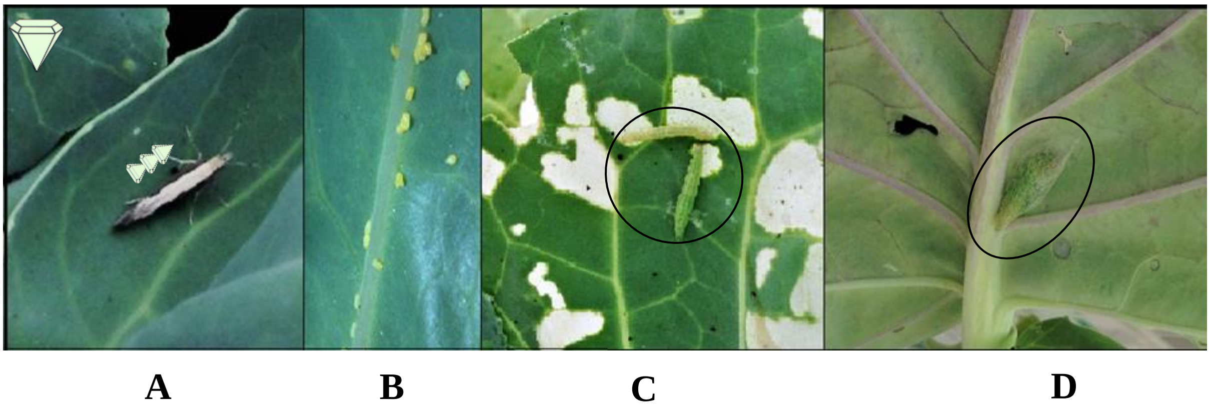

Figure 1. Diamondback moth *Plutella xylostella* (L.) A, an adult; B, eggs; C, larvae; and D, a pupa.

1.1.1.2 Dispersal

DBM is highly dispersive and often found in areas where it cannot successfully overwinter, including most parts of Canada (Capinera 2000b). DBM is also found throughout America, Europe, Asia and Australia (Capinera 2000a) and was reported as a pest in South Africa in the early 1900s (Charleston and Kfir 2000). It occurs wherever crucifer crops are grown and is believed to be the most universally distributed species of all Lepidoptera (Grzywacz et al. 2010).

1.1.1.3 Damage caused by DBM

The feeding habit for the first instar DBM is leaf mining, but the mines are too small to be noticed. At the conclusion of the first instar, the second instar larvae emerge from the mines and start chewing the lower part of the leaf, leaving the upper epidermis intact (Capinera 2000a). On canola, larvae may feed on all above-ground plant structures but are particularly damaging when leaves senesce late in the season because they feed on pericarp of canola pods, preventing ripening, and reducing yields (Dosdall, Soroka, and Olfert 2011).

DBM is one of the most destructive insects of cruciferous plants throughout the world (Sarfraz, Dosdall, and Keddie 2006, Grzywacz et al. 2010), causing 50 to 80% loss in marketable yields (Singh, Satyanarayana, and Sunitha 2015) or even up to 90% crop loss (Talekar and Shelton 1993). The economic cost of the DBM can reach between 1.3 and 2.3 billion US$ depending on various reasons, not to mention the chemical insecticides in pest management that would increase the cost estimate by 2.7 billion US$ in a conservative manner (Zalucki et al. 2012).

1.1.1.4 Host plants

DBM natural host range is limited to cultivated and wild Brassicaceae that are characterized by having glucosinolates and sulphur-containing secondary plant compounds. DBM adults utilize an integrated suite of chemicals and morphological cues for host plant localization and recognition (Sarfraz, Dosdall, and Keddie 2006). Although DBM attacks all the cruciferous plants, there is variation in oviposition preference. Collard is more preferred than cabbage, and cruciferous weeds are the hosts when the cultivated crops are unavailable (Capinera 2000a). A total of 175 wild plant species in the Brassicaceae have been recorded in South Africa (Kfir 1998), which provides plenty of shelter and feeding resources for DBM till the next cultivation season.

1.1.1.5 Control

1.1.1.5.1 Chemical control

The management of DBM costs up to US$ 1 billion per year globally, and the control measures constitute about 38% of the cost of production of main brassica crops in India (Singh, Satyanarayana, and Sunitha 2015). The farmers often increase the doses of insecticides, so insecticides alone account for 30 – 50% of the total cost of production (Weinberger and Srinivasan 2009). Some insecticides that are used to control DBM are neem-based insecticides such as Agroneem, Ecozin and Neemix, which have been proved to be effective as antifeedants but failed as oviposition deterrents (Liang, Chen, and Liu 2003). Pheromone traps and the synthetic insecticide phosalone are also used in attempts to control the DBM (Reddy and Guerrero 2000).

However, DBM has displayed an ability to develop resistance to most insecticides rapidly due to a range of biochemical and behavioural factors (Abro et al. 2013, Singh, Satyanarayana, and Sunitha 2015). Moreover, DBM's genetic elasticity has enabled it to develop resistance to almost every insecticide applied in the field (Sarfraz, Dosdall, and Keddie 2006). DBM was the first crop pest that was reported to resist DDT (Sarfraz, Keddie, and Dosdall 2005). The resistance of this species to insecticides is a significant obstacle to its effective management (Guo et al. 2015). Resistance to synthetic pyrethroids and organophosphates is widespread in DBM populations throughout all Australian canola production areas, rendering these chemicals partially or totally ineffective for DBM control. The insecticide products currently available and effective for DBM control can be in short supply during outbreaks. DBM showed resistance not only to chemical pesticides but also to biological pesticides, for example, *Bacillus thuringiensis* var. Kurustaki (Tabashnik et al. 1990).

1.1.1.5.2 Biological control

Insect control using predators and parasitoids has been successfully applied in protecting cropping systems, orchards and forestry (Bradburne and Mithen 2000). Several biological control programs using parasitoids in various regions have limited impacts because of the continuous spraying of broad-spectrum insecticides by farmers, which kill parasitoids and thus exacerbate DBM outbreaks (Grzywacz et al. 2010). Parasitoids are known to be attracted to damaged host-plant volatiles to localize the host insects (Turlings and Fritzsche 1999). Therefore, manipulating the host-plant chemistry may provide an avenue of enhancing the attraction of parasitoids to their prey efficiently (Bradburne and Mithen 2000). For example, variation in the emission of volatile compounds after jasmonic acid treatment was reflected in the behaviour of the parasitoid *Diadegma semiclausum* when it was offered the headspace volatiles of several combinations of accessions in two-choice experiments (Snoeren, Kappers, et al. 2010).

1.1.2 DBM parasitoids

Unlike parasites, a parasitoid is an insect that kills (parasitizes) its host, which is usually another insect, in order to complete its lifecycle. Over 130 parasitoid species are known as biological enemies of DBM on various stages of its life cycle. DBM biological control is achieved by a number of hymenopteran species belonging to the ichneumonid genera *Diadegma* and *Diadromus*, the braconid genera *Microplitis* and *Cotesia*, and the eulophid genus *Oomyzus* (Sarfraz, Keddie, and Dosdall 2005). Twenty-two species of parasitoids and hyperparasitoids have been reared from larvae and pupae of DBM in South Africa (Kfir 1998, Ooi 1992b). *Cotesia plutellae* (Kurdjumov), *Diadegma semiclausum* (Hellén) and *Diadromus collaris* (Gravenhorst) are three major primary parasitoids of the DBM. *C. plutellae* was discovered in the early 1970s in the Cameron Highlands, Malaysia (Ooi 1992a), but it was introduced to several Caribbean countries in 1970 from India (Sarfraz, Keddie, and Dosdall 2005). *D. semiclausum* and *D. collaris* were introduced in the mid-1970s from New Zealand to Australia. *D. collaris*, which has been widely used in biological control projects against DBM, is an abundant pupal parasitoid of DBM in South Africa (Kfir 1998). In brassica vegetable crops in the suburbs of Hangzhou, eight species of primary parasitoids were recorded, including: *Trichogramma chilonis* Ishii, *Cotesia plutellae* Kurdjumov, *Microplitis sp.*, *Oomyzus sokolowskii* Kurdjumov, *D. collaris*, *Itoplectis naranyae* (Ashmead), *Exochus sp.* and *Brachymeria excarinata* Gahan (Liu et al. 2000).

1.1.2.1 *Diadegma semiclausum* Hellen (Hymenoptera: Ichneumonidae)

D. semiclausum (Figure 2) is a specialist solitary parasitoid wasp that lays its eggs into the developing larvae of DBM (Ohara, Takafuji, and Takabayashi 2003). It has been recorded in many parts of the world as an important parasitoid (Khatri 2011). Its introduction in some parts of the world led to high levels of success in DBM control. For example, it was introduced in Kenya in 2002 and resulted in a pesticide-free Brassica production for three years of its release in the highland (Löhr et al. 2007). This encouraged its introduction into Ethiopia and resulted in a decline in DBM density, predicting a pesticide-free Brassica production there too (Ayalew and Hopkins 2013). *D. semiclausum* was amongst eight parasitoid species that emerged from the collected DBM at the east of Africa (Mt. Kilimanjaro and Taita hills), but the abundance and diversity significance was found to be related to the altitude (Ngowi et al. 2019).

In southern Queensland, Australia, it has been demonstrated that the active and dominant roles of *D. semiclausum* in controlling DBM are in winter, suggesting that an IPM strategy that utilizes *D. semiclausum* alongside *B. thuringiensis* is effective in managing *P. xylostella* in seasons or regions with a mild temperature (Wang et al. 2004). Once female *D. semiclausum* finds DBM larvae, it inserts its ovipositor into the larvae and lays an egg into it. The parasitoid eggs hatch, and the wasp larvae start feeding on the internal tissues of the host, mummifying the caterpillar by making its cocoon in the dead caterpillar skin in which it will pupate. However, (Furlong and Zalucki 2017) raised the alarm that climate change may compromise the use of the parasitoid as temperature increases will have a greater negative impact on the distribution of the parasitoid than on its host.

Scientific name: *Diadegma semiclausum* (Helen)

Kingdom: Animalia

Phylum: Arthropoda

Class: Insecta

Order: Hymenoptera

Superfamily: Ichneumonidea

Family: Ichneumonidae

Genus: *Diadegma*

Species: *semiclausum*.

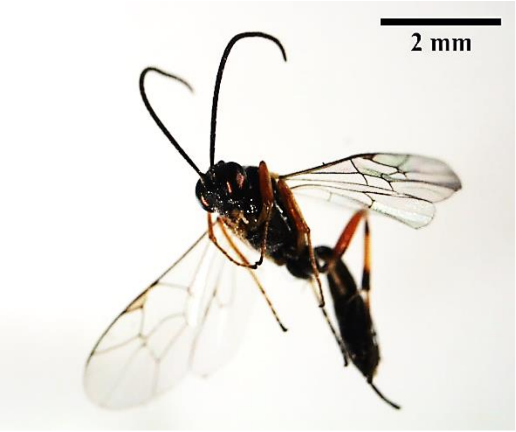

Figure 2. *Diadegma Semiclausum* Hellen.

1.1.2.2 *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae)

Trichogramma spp. are very tiny wasps of the family Trichogrammatidae. The commercial development of this natural enemy and the fact that it attacks various important caterpillar pests have earned it a place in the popular vocabulary of numerous pest management advisors and producers (Knutson 1998).

T. pretiosum Riley is an obligate endoparasitic wasp of lepidopteran eggs. It is a natural enemy of 45 different insect species (CABI 2020) (Figure 3), including DBM, *Ectomyelois ceratoniae* and *Helicoverpa armigera* (Pereira et al. 2019). It showed excellent-searching capacity and percentage of parasitism when compared to the other 11 Trichogramatidae species (Klemm et al. 1992). In 1998, more than a thousand scientific papers were published on *Trichogramma spp.* and its use as a biological control agent, making it one of the most researched natural enemies in the world (Knutson 1998).

Figure 3. *Trichogramma pretiosum* Riley (SimFRUIT 2016)

A *T. pretiosum* adult is approximately 1 mm or less. It often has wing hairs (setae) arranged in rows. Its body is relatively compact, and the antennae are short. Due to their minute size, *Trichogramma* species are hard to differentiate (UC 2017)

T. pretiosum is the most widely distributed *Trichogramma* species in North America and the most important member of its genus (Pinto, Oatman, and Platner 1986). It was imported from the USA to Australia in 1974 (Grimm and Lawrence 1975). All members of this family are parasitoids of insect eggs. Seven Australian *Trichogramma* species have been identified and described (Glenn, Hercus, and Hoffmann 1997).

Scientific name: *Trichogramma pretiosum* Riley

Kingdom: Animalia

Phylum: Arthropoda

Class: Insecta

Order: Hymenoptera

Family: Trichogrammatidae

Genus: *Trichogramma*

Species: *pretiosum*

Female *T. pretiosum* adults use chemical cues (Keromones) and visual clues (such as egg shape and colour) to locate the host eggs. Once a female adult finds a host egg, it drills a hole in the shell and inserts its egg(s) into the host egg. The hole excretes a small droplet of yolk out of the oviposition hole, offering a good feeding source for increasing the longevity of the female wasp. Large females parasitize more eggs than smaller females (Knutson 1998). The life span of female adults varies from 1 to 11 days based on diet, temperature, and humidity. Eggs hatch in about 24 hours, and the larvae develop quickly through three instars and transform to the pupal stage. After about 4.5 days, an adult wasp emerges from the pupa and escape the egg by chewing a hole in the shell. The whole life cycle is about nine days but varies from eight days (when temperatures are high) to as many as 17 days (Knutson 1998), but it usually ranges from 7 to 10 days (UC 2017). *T. pretiosum* life cycle is relatively short compared to their hosts, which enables them to build up more generations in the same period, enhancing their efficiency in the IPM programs.

1.1.3 Chemical communication between plants, herbivorous insects and parasitoids

Phytophagous insects use blends of volatiles released from plants to select hosts for feeding and oviposition (Binyameen et al. 2014). Locating a host plant is crucial for a herbivorous insect to fulfil its nutritional requirements and to find suitable oviposition sites. Plant volatiles play an essential role in this host-location process (Bruce, Wadhams, and Woodcock 2005). For herbivorous insects, it is crucial to find host plants for feeding and reproduction, and these insects must be able to differentiate suitable from unsuitable plants. Therefore, volatiles are important cues for insect herbivores to assess host plant quality (Zakir et al. 2013).

Herbivore feeding activates plant defences at the site of damage as well as systematically induced defences which can be induced internally by signals transported via phloem or xylem or externally by volatiles emitted by the damaged tissues (Rodriguez-Saona, Rodriguez-Saona, and Frost 2009). For example, the volatiles from the yellow rocket *Barbarea vulgaris* (R. Br.) infested with DBM minimize the number of surviving DBM larvae as compared to *Brassica oleracea* L., suggesting that it is a plant with a high potential to be used as a trap crop for DBM (Badenes-Perez, Nault, and Shelton 2006). Moreover, upon herbivore attack, plants activate an indirect define, that is, the release of a complex mixture of volatiles that attracts natural enemies of the herbivore (Zhang et al. 2013). Plants emit specific blends of volatiles that attract natural enemies of the herbivore insects. The natural enemies of herbivore insects commonly make use of plant-produced volatiles in order to locate their victims (Sobhy et al. 2014). However, (Snoeren, Mumm, et al. 2010) believe that some plants may emit volatiles, such as methyl salicylate, that has negative effects on parasitoid host-finding behaviour. Moreover, plants response and induced changes in plant quality may impact the higher trophic levels, such as the development of parasitoids (Bukovinszky et al. 2012)

1.1.3.1 Herbivore induced plant volatiles (HIPVs)

Herbivore-induced plant volatiles (HIPVs) can affect the diversity and composition of plant-associated arthropod communities (Fatouros et al. 2012). HIPVs are potent attractants for entomophagous arthropods (Kaplan 2012) (Figure 4). Caterpillar feeding induces direct and indirect defences in brassicaceous plants. Thus, feeding by the biting–chewing *P. xylostella* resulted in significantly increased endogenous levels of jasmonic acid (JA), a central component in the octadecanoid signalling pathway that mediates induced plant defence (Bruinsma et al. 2009). Insects, at various stages of their development, use a range of environmental cues to locate their mates, food sources and oviposition sites and avoid dangerous situations or unsuitable habitats and hosts (Field, Pickett, and Wadhams 2000).

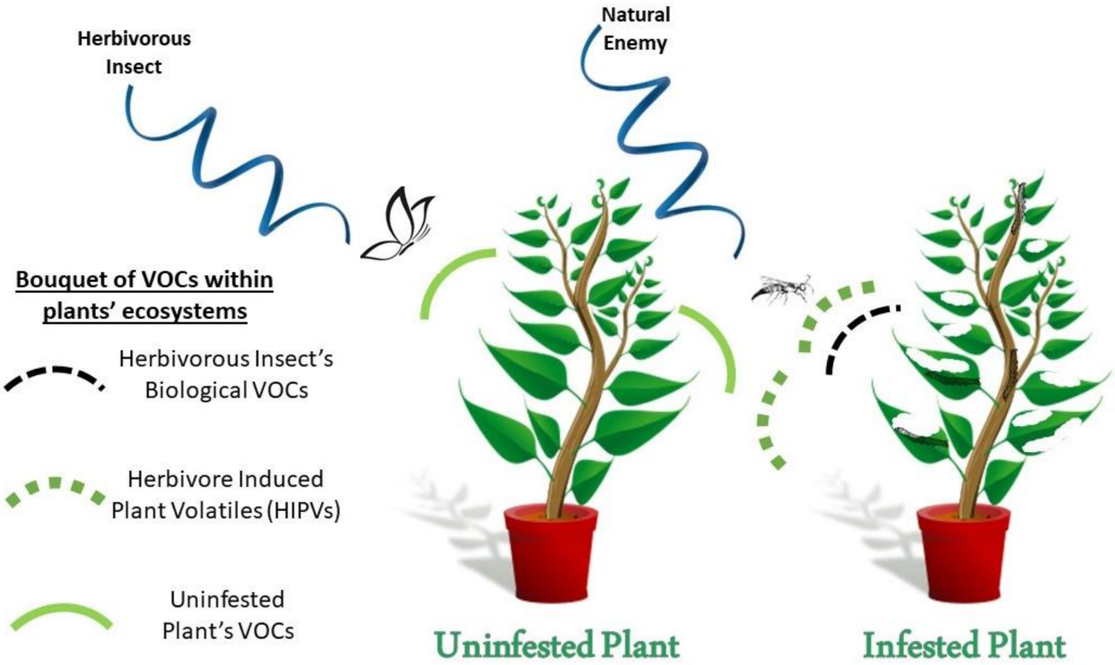

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Figure 4. Herbivore induced plant volatiles (HIPVs) help natural enemies to locate their host insects.

1.1.3.2 Attraction of insect parasitoids to HIPVs

Insects belonging to different feeding guilds are known to induce different responses in the host plant (Li et al. 2014). The attraction of parasitoids has been shown to be determined in many systems by a wide variety of chemical cues acting at different spatial and temporal scales (Bradburne and Mithen 2000). Glucosinolates are sulphur-containing secondary metabolites characteristic of Brassicaceous plants. Glucosinolate breakdown products, which include isothiocyanates, are released following tissue damage when hydrolytic enzymes act on them (Bruce 2014). The volatile and toxic isothiocyanates originated from the hydrolysis of secondary metabolite glucosinolate and are present in the Brassica tissues are the primary cause for the biofumigant effect of the Brassiceae (Dutta, Khan, and Phani 2019). On the other hand, green leaf volatiles are widespread in plants, but many groups of plants also produce specific volatiles to their taxon. Insect herbivores which are specialized in feeding on these groups of plants have been shown to utilize these specific chemicals as attractants for feeding and oviposition (Visser 1986). It is very likely that the parasitoids of these insects will also have evolved to use these same specific chemicals as cues to find their hosts (Bradburne and Mithen 2000). To be more specific, it has been noticed that *Tersilochus heterocerus*, a parasitoid on the ichneumonid pollen beetle of the oilseed rape, *Brassica napus*, was attracted to odours of flowering rape unlike other parasitoids, *Phradis interstitialis* Thomson and *P. morionellus* (Holmgr), which were only attracted to the bud stage odours (Jönsson, Lindkvist, and Anderson 2005).

The mustard oils (Isothiocyanates), which are formed after tissue damage by glucosinolates, are examples of taxon-specific volatile chemicals found in Brassica and related genera. They have been shown to attract specialist herbivores to Brassica (Chew 1988). However, isothiocyanates have toxic effects on generalist herbivores when they attempt to feed on oilseed rape, *B. napus*, and also function as repellents (Bruce 2014). It has been reported that Brassica specialists are attracted to isothiocyanates (Bruce 2014).

D. semiclausum females are more attracted to host-infested plants than uninfested ones, suggesting that the attraction is not related to the host itself or its products. They initiated specific antennal contact with a host-damaged site on a leaf to search for hosts (Ohara, Takafuji, and Takabayashi 2003). The quality, along with the quantity of the volatile blend, is vital in the host-seeking behaviour of the parasitic wasps such as *Cotesia glomerata*, *C. rubecula,* and *D. semiclausum*, whereas they preferred volatiles from herbivore-induced plants over volatiles from JA-induced plants (Bruinsma et al. 2009) as parasitoids can distinguish between the volatile of compound (VOC) profiles of currently and formerly infested plants (Kugimiya et al. 2010). In contrast, benzyl cyanide and dimethyl trisulfide attracted *Cotesia vestalis* in a dose-dependent manner (Kugimiya et al. 2010).

1.1.4. Insect olfaction

Insect behaviours are regulated mainly by olfaction through major olfactory organs such as antennae (Wang et al. 2015). To locate and evaluate food, mates, shelter and breeding substrates in addition to avoiding predators and other dangers, or simply just to move around, insects rely on a wide range of sensory systems, in which olfaction plays a pivotal role, supplying the nervous system with information subsequently used to generate a simplified internal representation of the complex external world. Then this process, in turn, allows the insect to decide and execute the appropriate behavioural response that fits the situation (Hansson and Stensmyr 2011).

1.1.4.1 Antennae

Apart from members of the subclass Protura, which have neither antennae nor eyes, all insects possess a pair of antennae (Figure 5). The importance of olfaction is evident from the elaborate antennal structures, the functional equivalents of the human nose, found in many insects. Apart from antennae, insects also detect odours using their maxillary palps and/or labial palps. The antennae (and palps) come in a multitude of shapes but nevertheless conform to the same basic principles (Schneider 1964). A population of hair-like sensilla distributed over the surface of antennae is used to detect chemical signals (Keil 1984a, Larsson et al. 2002, Syed and Leal 2007, 2009, Takanashi et al. 2006). Several shapes of olfactory sensilla have been observed (Larsson et al. 2002, Syed and Leal 2007, 2009, Takanashi et al. 2006).

1.1.4.2 Sensilla

Under the microscope, pores can be observed on the olfactory sensillar cuticle through which the airborne odorants can enter into the sensilla to activate the receptors (Slifer 1961). Sensilla harbour odorant receptor neurons (ORNs), whose dendrites are housed in an aqueous fluid, the sensillum lymph, which forms a hydrophilic barrier for the hydrophobic airborne stimuli (Leal 2003). With the advance of molecular and cellular biology, the understanding of insect olfactory mechanisms has progressed. At least three major groups of proteins play pivotal roles in the dynamics, selectivity, and sensitivity of the insect olfactory system. They are odorant binding protein (OBPs) (Vogt and Riddiford 1981), odorant receptors (ORs) (Clyne et al. 1999, Vosshall et al. 1999), and odorant-degrading enzymes (ODEs) (Vogt and Riddiford

1981, Vogt, Riddiford, and Prestwich 1985). When hydrophobic semiochemicals reach the aqueous sensillum lymph through the pores, their lack of solubility prevents them from reaching the membrane-bound receptors. OBPs are involved in the first step of odorant reception, where they bind, solubilize and deliver the odorant molecules to the ORs (Leal 2003) (Figure 6). ORs are localized on the dendritic membrane of the olfactory sensilla (Sato et al. 2008, Wicher et al. 2008). ORs interact with odorant compounds and transduce the olfactory signals into electrical signals that travel to the brain to mediate various insect behaviours. After stimulation, the olfactory system could be reset by ODEs (Vogt and Riddiford 1981, Vogt, Riddiford, and Prestwich 1985, Ishida and Leal 2005), which can rapidly degrade odorants.

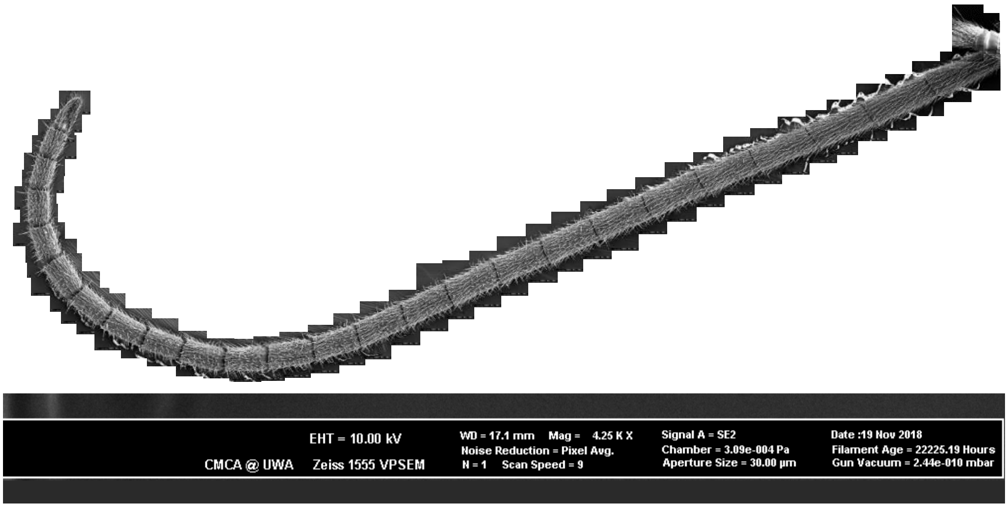

Figure 5. *D. Semiclausum* male antennae under a scanning electron microscope.

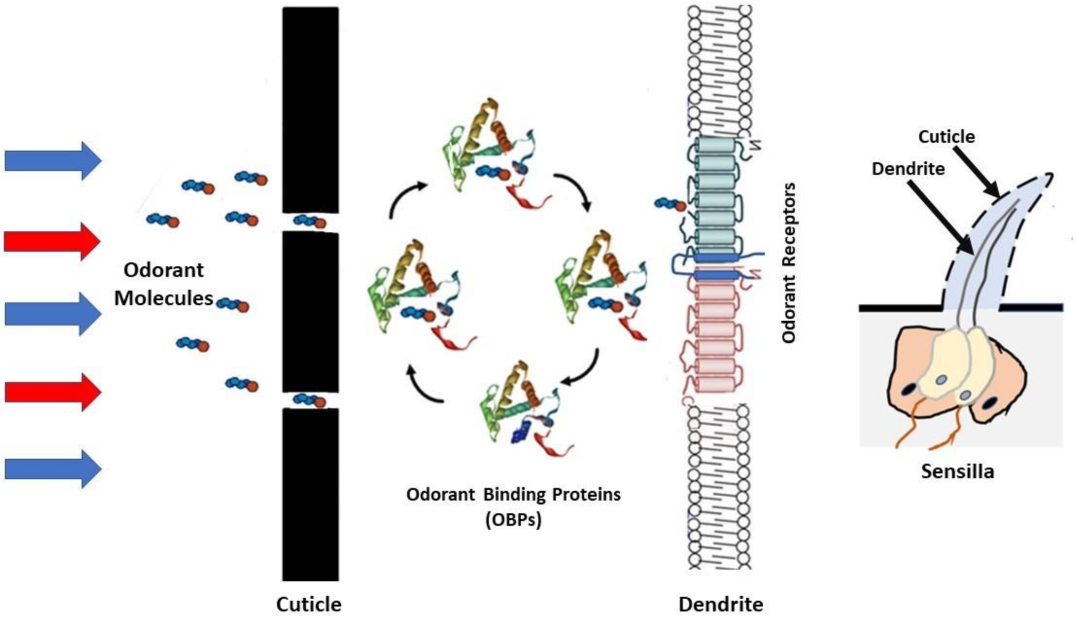

Figure 6. Molecular mechanism of insect olfaction.

1.1.4.3 Odorant binding Proteins (OBPs)

The first insect OBP was discovered at the beginning of the 1980s in the giant moth *Antheraea polyphemus* by using the tritium labelled specific pheromone (*E*, *Z*)-6, 11-hexadecadienyl acetate as a probe (Vogt and Riddiford 1981). Recently with the completion of the genome sequence of insects, including fruit fly, honeybee, mosquitoes, silkworm and beetle, more and more insect OBP genes were annotated, cloned and studied. OBPs are not synthesized by the ORNs themselves but rather produced in accessory cells associated with them and secreted into sensillum lymph surrounding the outer dendritic segment (Leal 2003).

The functional roles of insect OBPs have been addressed. LUSH is an OBP of the fruit fly *Drosophila melanogaster***.** Deletion of the LUSH gene suppresses *D. melanogaster* electrophysiological and behavioural response to the male pheromone 11-cis-vaccenyl acetate (cVA) (Xu et al. 2005). In another case, octanoic and hexanoic acids, two odorant compounds that originate from the *Morinda citrifolia*, act as oviposition attractants for *D. sechiella* but as repellents for *D. melanogaster* (Matsuo et al. 2007). Deleting OBP57d and OBP57e genes in *D. melanogaster* eliminates the avoidance behaviour while reinserting the orthologous genes of *D. sechiella* into *D. melanogaster* results in attraction to these two fatty acids (Matsuo et al. 2007). RNAi-mediated OBP genes silencing coupled with electrophysiological analyses have demonstrated the importance of OBPs in odorant recognition in two mosquito species (Biessmann et al. 2010, Pelletier, Guidolin, et al. 2010). By knocking down CquiOBP1 in *Cx. quinquefasciatus*, mosquitoes showed reduced antennal response to several oviposition attractants when compared to controls (Pelletier, Guidolin, et al. 2010). Meanwhile, after injecting AgamOBP1 double-strand RNA into *An. gambiae*, mosquitoes did not respond to indole, a key ligand for AgamOBP1 (Biessmann et al. 2010, Pelletier, Guidolin, et al. 2010). Combined, these studies show that OBPs are critical for the selectivity and sensitivity of the insect olfactory system.

1.1.4.4 Odorant receptors (ORs)

Insect OR genes were first identified in *D. melanogaster* in 1999, which led to the initial identification of members of the OR gene family (Clyne et al. 1999, Vosshall et al. 1999). A bioinformatic search of the completed *D. melanogaster* genome subsequently identified 60 OR genes that encode 62 OR proteins by alternative splicing (Clyne et al. 1999). Later, a family of 79 OR genes was identified bioinformatically in *A. gambiae* genome (Hill et al. 2002), 126 OR genes in *A. aegypti genome* (Bohbot et al. 2007), 158 putative OR genes in the *C. quinquefasciatus* genome (Pelletier, Hughes, et al. 2010), 49 putative OR genes from *B. mori*

(Anderson et al. 2009). The OR proteins are highly diverse, and no sequence similarity was identified between insect and mammalian odorant receptor genes, suggesting that insect ORs might not be GPCRs at all (Hallem, Dahanukar, and Carlson 2006).

The first functionally characterized insect odorant receptor was OR43a from *D. melanogaster*. Overexpression of OR43a in the antenna or heterologous expression in *Xenopus laevis* oocytes (Stortkuhl and Kettler 2001) allowed the identification of the ligands, cyclohexanone, cyclohexanol, benzaldehyde, and benzyl alcohol, that elicit OR43a response.

In vivo studies have been applied to functionally characterizing insect ORs. After BmorOR1 gene was disrupted by transcription activator-like effector nucleases (TALENs), male moths did not show ORN sensitivity to bombykol and corresponding pheromone-source searching behaviour (Sakurai et al. 2015). In response to silencing BmorOR1 in *B. mori* by RNAi, male adults spent significantly more time finding females than wild-type males (Zhang et al. 2018).

1.1.4.5 Other olfaction-related genes

Besides OBPs and ORs, several genes that have been reported are related to the insect olfactory system as well. For example, ionotropic receptors (IRs) (Liu et al. 2018), sensory neuron membrane proteins (SNMPs) (Zhang et al. 2020) and odorant degrading enzymes (ODEs) (Vogt and Riddiford 1981, Vogt, Riddiford, and Prestwich 1985). IRs, a variant subfamily of ionotropic glutamate receptors, work as ligand-gated ion channels in chemo-sensation (Liu et al. 2018, Rimal and Lee 2018). SNMPs, a subfamily of CD36 proteins, have been shown to play a critical role in pheromone detection in insects (Zhang et al. 2020). ODEs can degrade the odorant compounds after they activate ORs, clean the system (Vogt and Riddiford 1981, Vogt, Riddiford, and Prestwich 1985, Ishida and Leal 2005) for the new cycle of detection. In this study, OBPs and ORs are my focus because they are involved in the first step of odorant detection.

1.2 Knowledge gap

Though more and more attention has been paid to the DBM biological control using parasitoid wasps, limited studies have been conducted in their olfactory systems and chemical ecology, which will hinder the application of these natural enemies to control pests efficiently and effectively. Three major questions that need to be addressed are:

1. Are there any volatile compounds released from DBM-infested host plants which can initiate parasitoid wasps' response?
2. Do parasitoid wasps share similar olfactory proteins such as OBPs or ORs with DBM? May these proteins help wasps and DBM detect the same volatile compounds from plants and the environment?
3. Are the olfaction genes different between the specialist parasitoid wasp (e.g. *D. semiclausum*) and generalist parasitoid wasp (*T. Pretiosum*)? Will these differences be related to their different parasitic behaviours?

1.3 Research objectives:

For this project, my research objective is to improve our understanding of the olfactory systems of two parasitoid wasps, *D. semiclausum* and *T. Pretiosum*. Both are used broadly in DBM pest management. In this study, my specific research aims are:

1. To study if or what volatile compounds released from host plants (Canola) can attract DBM parasitoid wasp (e.g. *D. semiclausum*)
2. To identify, annotate and characterize the olfactory genes (OBPs and ORs) from DBM parasitoid wasps: *D. semiclausum* and *T. Pretiosum.*
3. To perform comparative studies on the olfactory genes (OBPs and ORs) between male and female wasps, between DBM and its parasitoid wasps, and between the specialist parasitoid wasp (*D. semiclausum*) and the generalist parasitoid wasp (*T. Pretiosum*). These comparative studies helped us identify the major candidate genes related to parasite behaviours of wasps.

The long-term goal is to improve our understanding of the olfactory systems of the parasitoid wasps and help develop more efficient and environmentally friendly insect control strategies for DBM.

Chapter 2

Electroantennogram responses of the parasitoid wasp, *Diadegma semiclausum*, to host-related odours

2.1 Abstract

An increase in insecticide resistance has been reported broadly in the diamondback moth (DBM) (*Plutella xylostella*), promoting interest in biological control using natural enemies. Parasitoid wasps have long been used as biocontrol agents of DBM, which use blends of volatiles released from attacked plants to localize hosts. Identifying and analysing these compounds and their specificity are the first key steps to understand the mechanisms of how natural enemies localize host insects within crops.

In this study, canola *Brassica napus*, seedlings were used as the host plant, which was consumed by *P. xylostella* to identify the volatile compounds by using gas chromatography-mass spectrometry (GC-MS). *Diadegma semiclausum* was studied as the DBM parasitoid wasp to examine its antennal responses to various volatile compounds. A scanning electron microscope (SEM) was used to investigate the olfactory sensilla of the male and female *D. semiclausum*. The results identified eight compounds that were of significant changes in their amounts from DBM-infested canola. Electroantennogram (EAG) results demonstrated a group of physiologically active compounds which can elicit antennal responses of male or female *D. semiclausum*, which might be candidate attractants for *D. semiclausum* to localize the DBM. Interestingly, male and female *D. semiclausum* showed different responses to certain tested compounds in EAG. SEM results revealed seven types of olfactory sensilla from *D. semiclausum* adult antennae.

This study identified candidate attractant compounds for *D. semiclausum* and improves our understanding of their olfactory systems, which will help optimize our biological control strategies to control *P. xylostella* in future.

2.2 Introduction

Plants generally release a wide range of organic compounds when they are attacked by insect herbivores, which can function as airborne semiochemicals for deterring, repelling or promoting interactions between plants and insect herbivores (Paré and Farag 2004). For example, *Heliothis virescens* have been reported to be repelled by herbivore-induced volatiles released from tobacco plants at night. These odorant cues may allow female *H. virescens* to avoid oviposition on previously damaged plants. On the other side, these compounds may be attractants of natural enemies to localize insect pests and protect plants (De Moraes, Mescher, and Tumlinson 2001). For example, sesquiterpene (E)-β-caryophyllene (EβC) is emitted in response to above- (Turlings et al. 1998) and below-ground injury (Rasmann et al. 2005) of maize, which serves as an attractant for natural enemies of maize pests (Rasmann et al. 2005, Köllner et al. 2008) and provides protection from herbivores with different modes and sites of attack (Köllner et al. 2008). (Rasmann et al. 2005) showed the attraction to the entomopathogenic nematode *Heterorhabditis megidis* (EβC), which is released by maize roots as a response to response to feeding by larvae of the beetle *Diabrotica virgifera virgifera*.

The chemicals released by damaged plants can be different due to the herbivorous insect species as well as the plant species. Volatile plant signals may also induce defence responses in neighbouring plants. Such semiochemicals that function in communication between and among species are emitted from a diverse group of plants and mediate key behaviours of insects. Therefore, chemical compounds released by plants regulate a variety of interactions between plants, feeding insects and their natural enemies.

To understand these interactions, the identification of the volatile compounds that play vital roles in triggering insect behaviours, especially attraction of natural enemies to plants for controlling herbivore pests, is essential (De Bruyne and Baker 2008, Riffell et al. 2009, Bruce and Pickett 2011). Although some attention has been paid to orientation and attraction of natural enemies to plant volatiles, insufficient studies have been carried out in this field (Rojas 1999, Del Socorro et al. 2010, Gregg, Socorro, and Henderson 2010, Del Socorro, Gregg, and Hawes 2010, Saveer et al. 2012) and that is probably due to the complexity of the odour blends released by plants, particularly in comparison with less complicated blends of, e.g., pheromones. From a practical perspective, unravelling the identity of non-pheromonal semiochemicals from host plants could provide sufficient insight for their future use in insect pest management (Schlyter 2012). Moreover, some plants of the same family such as *Brassica* *napus*, have greater developmental limitations for the parasitoid *D. semiclausum* than on *B. rapa* or *B. oleracea* (Dosdall et al. 2012).

The diamondback moth (*Plutella xylostella*) is a moth species of the family Plutellidae and genus Plutella. This small, greyish-brown moth has a cream-colored band that forms a diamond along its back. It was believed that this species may have been originated in Europe, South Africa, or the Mediterranean region, and has now spread worldwide (Wei et al. 2013) but a new evidence states its origin as South America (You et al. 2020) DBM are destructive as they feed on the leaves of cruciferous crops and plants that produce glucosinolates. Pesticides were used to kill DBM, but DBM have developed resistance to many of the common chemicals (Cai et al. 2020). For this reason, biological controls using natural enemies become increasing important.

Diadegma semiclausum is the most commonly found parasitoid of DBM in Australia, which is believed to aid in controlling DBM considerably. *Diadegma* spp. have been the most extensively studied and are the most used biological control agents in the world. However, how *D. semiclausum* are attracted to the damaged plants is still not clear. Do plant-derived compounds act as attractants to *D. semiclausum*? What are they? In regarding to answer these questions, canola *Brassica napus*, DBM and *D. semiclausum* were used in this study to investigate the chemical interactions among them. The aim of this study was to identify biologically active compounds in headspace volatiles of canola attacked by DBM, which may be the candidate compounds to attract *D. semiclausum* to DBM and DBM-infested plants. Identification of these compounds may help develop more environmentally friendly and efficient strategies to better use *D. semiclausum* in DBM management.

2.3 Materials and methods

2.3.1 Plants and insect culture

Canola plants (*Brassica napus* ATR Cobbler) were cultivated in the greenhouse at Murdoch University as described by (Akther 2019, Bilgi 2015). *P. xylostella* (DBM) colony was established with pupae received from the South Australian Research and Development Institute (SARDI). The colony was kept in (1×1×1.2 m^3) mesh cages inside the greenhouse at 25±2 °C. DBM colony was continuously enhanced with wild DBM adults that were captured using a specially designed UV light trap (Volka Lighting™, Australia). *D. semiclausum* pupae were purchased from Biological Services Agents (https://biologicalservices.com.au/) and kept in the lab at 25±1 °C, 70-80% (R.H.) and 16:8 h (L:D) photoperiod (See 2.3.4).

2.3.2 Plant headspace volatile collection and analysis

For the canola headspace volatile compound collection, the experiment was set up as in Figure 7. Various parameters amenable to headspace sampling were investigated preliminarily, including plant age (4, 6 and 8 true leaves), number of larvae (5, 10 and 15) on each plant, larval stage (3^{rd}. and 4^{th}. instars) and volatile absorption time (2, 4, 6 and 8 hours).

Preliminary results showed that a 4-hour collection could produce the best results because it balanced the signal intensity and sensitivity. When collection time was longer (e.g., 6 or 8 hours), the targeted gas chromatography (GC) analysis spike signals became harder to differentiate from the high background noise. On the other hand, when collection time was shorter (e.g., 2 hours), the signal peaks become extremely weak, especially for those trace amounts of compounds, resulting in problems to identify these compounds.

Various solid-phase microextraction (SPME) fibers were tested in this preliminarily experiment, and the 50/30 µm Divinylbenzene / Carboxen / Polydimethylsiloxane (DVB/CAR/PDMS) Stableflex fiber (Sigma-Aldrich, USA) with 2 cm coating was selected as it produced sound, stable and reliable results in this study. The SPME fiber assembly with 2 cm StableFlex fiber was activated at 270 °C for 30 minutes in accordance with the manufacturer's instructions, prior to the volatile organic compounds (VOCs) extraction.

Six-weeks old (6–8 leaves) canola plants were enclosed in a 3-litre glass cylinder equipped with GS septum for inserting the SPME fiber (Figure 7). To minimise the background noise, plants were isolated from the soil surface with an acrylic sheet covered with aluminium foil specially designed to surround the plant's stem gently. Artificial infestation was induced using ten 3rd and 4th *P. xylostella* instar larvae combinations and was left for feeding for four hours before collecting the VOCs. SPME fibers were inserted to absorb the headspace VOCs for 30 minutes. The fiber was transferred in a sealed glass tube to the lab for gas chromatography analysis. The fiber was injected into the GC for desorption of analytes, chromatographic separation, and subsequent detection of VOCs via mass spectrometry. The different parameters, including temperature, the mass of the sample, and the extraction time, were optimised in a univariate manner. Results of VOCs present in canola plants only and DBM infested canola plants were used in comparative studies. Each extraction and GC analysis was performed in triplicate

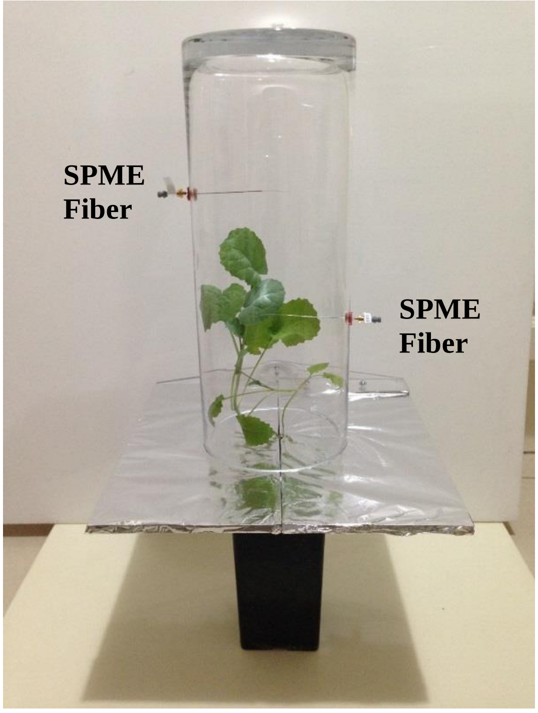

Figure 7. Volatile organic compounds collection from DBM infested canola plants by using SPME.

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2.3.3 Gas chromatography analysis

Gas Chromatograph 7820A coupled with a Flame Ionization Detector (GC-FID) (Agilent Technologies, USA) was used to optimize the parameters for GC analyse of the collected volatiles, which was equipped with a nonpolar column (HP-5MS 30 m × 0.25 mm × 0.25 µm) (Agilent Technologies, USA). At splitless mode, the inlet set point temperature was set as 250°C. The constant flow of carrier gas (Helium) was 1.1 mL/minute, and the pressure was 12.454 psi. The oven starting point was set to 40°C for five minutes, then increased at a rate of 5°C per minute and reached 250°C for five minutes, accumulating 52 minutes of total run time for one analysis. FID temperature was 280°C. To Identify the VOCs resulted from canola plants, GC-MS analysis was conducted using an Agilent Technologies 7820A instrument equipped with Agilent J&W mid-polarity column (HP-35 MS, 30 m x 0.25 mm x 0.25 µm and Agilent 5977E Mass Spectrometry Detector (MSD) (Agilent Technologies, USA) with the optimized parameters from GC-FID analysis above. At splitless mode injector, the inlet set point temperature was 250°C, and the detector temperature was set to 290°C. A constant flow of carrier gas (Helium) was 1.1 mL/minute. The oven starting point was set to 40°C for five minutes, then started to increase at a rate of 5°C per minute to settle at 250°C for 5 minutes, accumulating 52 minutes of total run time, the same as described for GC-FID.

Automatic Mass Spectral Deconvolution and Identification System (AMDIS-32) software coupled with NIST 2.2 mass spectra library were used for qualitative identification, taking in consideration the high match factor and the comparison of retention Kovat's index with retention index obtained from NIST library and the mass spectra shape.

2.3.4 Electroantennogram analysis (EAG)

D. semiclausum pupae were individually kept in a small screw-top, clear vial (Sigma-Aldrich Australia) covered with mesh. The external genitalia have been used in distinguishing the emerging male and female *D. semiclausum*, as the female adult has a very distinct ovipositor (Figure 8). At the end of the female abdomen, a slender ovipositor (terebra) around 0.8 mm long was observed, which is used to deposit the egg directly into the DBM larvae.

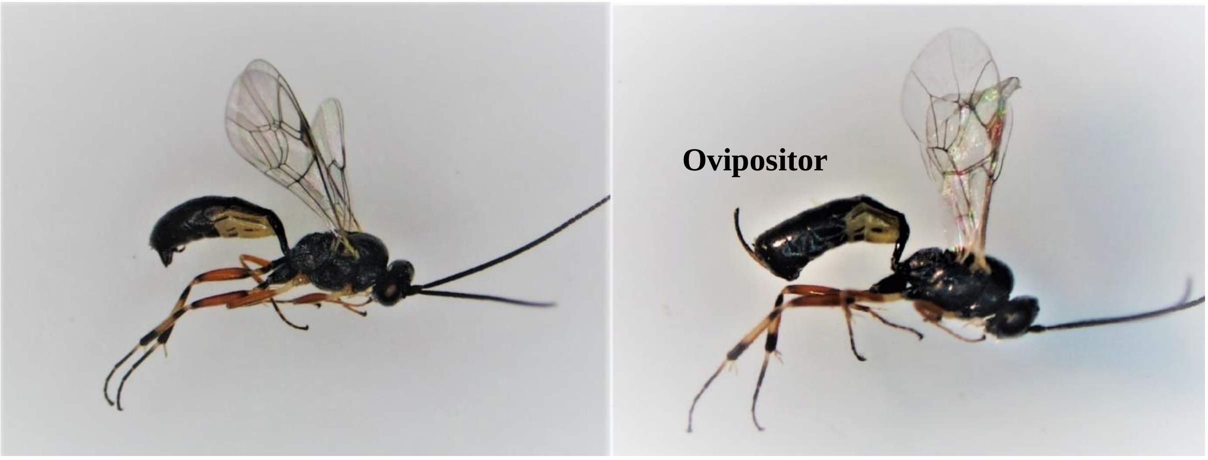


Figure 8. Male (Left) and female (Right) *D. semiclausum* adults.

D.semiclausum adults of 0-3 days old were individually put to anaesthesia using carbon dioxide (Purity >99.9%, Moisture <100ppm) for five minutes and then excised with a surgical scalped blade under an optical microscope. Heads were placed on the reference electrode (Syntech, Germany), and the distal end of the antennae (less than half a millimetre), which was cut to ensure good electrical contact, were carefully placed on the recording electrode and immersed in salt-free hypoallergenic gel (Parker Laboratories, Fairfield, USA). Stimulus Controller CS-55 (Syntech, Germany) with stimulus controller V.2.5 combined with IDAC4 Intelligent Data Acquisition Controller (Syntech, Germany) were used to record the electrophysiological responses. EAG signals were fed to a 10**X** amplifier and processed with a PC-based interface and software package (EAGPro V 2.0 Syntech, Germany).

A wide array of chemicals that are known to play various roles in insect behaviours were tested. That included 1-Octen-3-oL, Cis-11-Hexadecenal, Eucalyptol, Nonanal, Tridecane, Undecanal, (-)-ɑ-Pinene, (+)-ɑ-Pinene, (-)-Terpinen-4-ol, (+)-Terpinen-4-ol, (-)-trans-Pinocarveol, (1S)-(-)-Verbenone, 1-Butanol, 1-Nonanol, 1-Penten-3-one, 2-Ethylfuran, Cyclohexene oxide, Ethyl acetate, Octyl acetate, trans,trans-2,4-Hexadienal and Hexane as solvent/control treatment. All the chemicals were purchased from Sigma–Aldrich and were all ≥95% pure and prepared in hexane. These chemicals were selected because they have been reported related as general plant compounds (e.g., nonanal) or DBM pheromone (e.g., Cis-11-Hexadecenal). Pure chemicals were diluted 10 times to have a stock solution of 100 µg/µL, from which decadic dilutions were made. An 8 µl aliquot of each solution was applied to a filter paper strip (1×3 cm; Whatman No. 4, Fisher Scientific), and the solvent was evaporated in a fume hood before inserting the paper strip into 150 mm disposable glass pipette Pasteur (Rowe Scientific, Australia). A 500 ms pulse (5 mL/s) was delivered by stimulus controller CS-55 (Syntech, Germany) to deliver chemical stimulants to a humidified continuous filtered (through activated charcoal chamber) air flow at a rate of 10 mL/s over the EAG preparation. The chemicals were tested randomly, and the trigger delay was set to 10 seconds with one repeat cycle. Initial screening was performed using a 1:1000 (µg/µL) dose solution, and chemicals that elicited high EAG response (1-Octen-3-oL, Cis-11-Hexadecenal, Eucalyptol, Nonanal, Tridecane, and Undecanal) were selected for further dose-dependent response study. Recorded response graphs were translated into digital parameters (mV) using Microsoft Windows 10 Pro version 1903 Paint software.

2.3.5 Scanning electron microscopy (SEM)

To prepare *D. semiclausum* samples for the SEM examination, insects were preserved in 3% Glutaraldehyde in 0.025 M Phosphate buffer, pH 7.0 for 24 hours and then a standard protocol (Table 1) was used for fixation with Pelco Biowave microwave processor (Ted Pella Inc., USA). Critical point drying apparatus Polaron E3000 was used in the preparation process with a Critical point of CO_2 = 31.1 ˚C and 1071 psi. Dried samples were mounted on SEM aluminium stubs using 12 mm ProSciTech® double-sided conductive carbon tabs under Olympus SZH10 microscope (Olympus, Australia), and then sputtered with 10 nm gold using Polaron Sputter coater SC 7640 (Quorum Technologies, UK) with Argon gas (Pressure <1x10-2 bar, Voltage= 1 kv). Samples were examined and photographed under the Zeiss 1555 variable pressure field emission scanning electron microscope (VP-FESEM) (Zeiss, Germany), which was operated at 10 kV, high current, 10–12 mm working distance, and 30 µm aperture. Sample preparation and examining were conducted at the Centre for Microscopy, Characterization, and Analysis (CMCA) / University of Western Australia.

Table 1: Microwave fixation protocol

Step	Process	Wattage	Vacuum	Step repeats
1	Immersed in Glutaraldehyde for 2 minutes on - 2 minutes off - 2 minutes on	80	on	1
2	Buffer Wash in DI Water for 40 Seconds	250	off	4
3	50% Ethanol, 40 Seconds	250	off	1
4	70% Ethanol, 40 Seconds	250	off	1
5	95% Ethanol, 40 Seconds	250	off	1
6	100% Ethanol, 40 Seconds	250	off	2

2.3.6 Statistical analysis

In this study, ANOVA and post hoc test analysis were conducted using Microsoft Excel 360, MetaboAnalyst 4.0. and R-4.0.2 for Windows. The EAG response (-mV) values were directly utilized (not deleting the control values). For each test (one compound at one certain dose for one sex), over five insects were used for repetition. The bar represents the standard deviation.

2.4 Results

2.4.1 Identification of volatile compounds

In GC analysis, a number of major VOC compounds were obtained from both control and DBM-infested canola seedlings. However, some of these compounds might have been either released by canola, soil or originally present within the system, which are not my interesting compounds. Qualitative and quantitative variances of the chemical compounds were observed between control treatment (un-infested plants) and DBM-infested canola plants (Figure 9). Eight compounds showed statistically significant variation (Table 2), and they are strong candidates for attracting natural enemies of the DBM, such as *D. semiclausum*.

These eight identified compounds are 1-Penten-3-one, 2-Ethylfuran, 2-Hexenal, 7-Oxabicyclo [4.1.0] Heptane, trans, trans -2,4-Hexadienal, Eucalyptol, 1-Cyclohexene-1-carboxaldehyde, 2,6,6-trimethyl- and Tridecane (Figure 9).

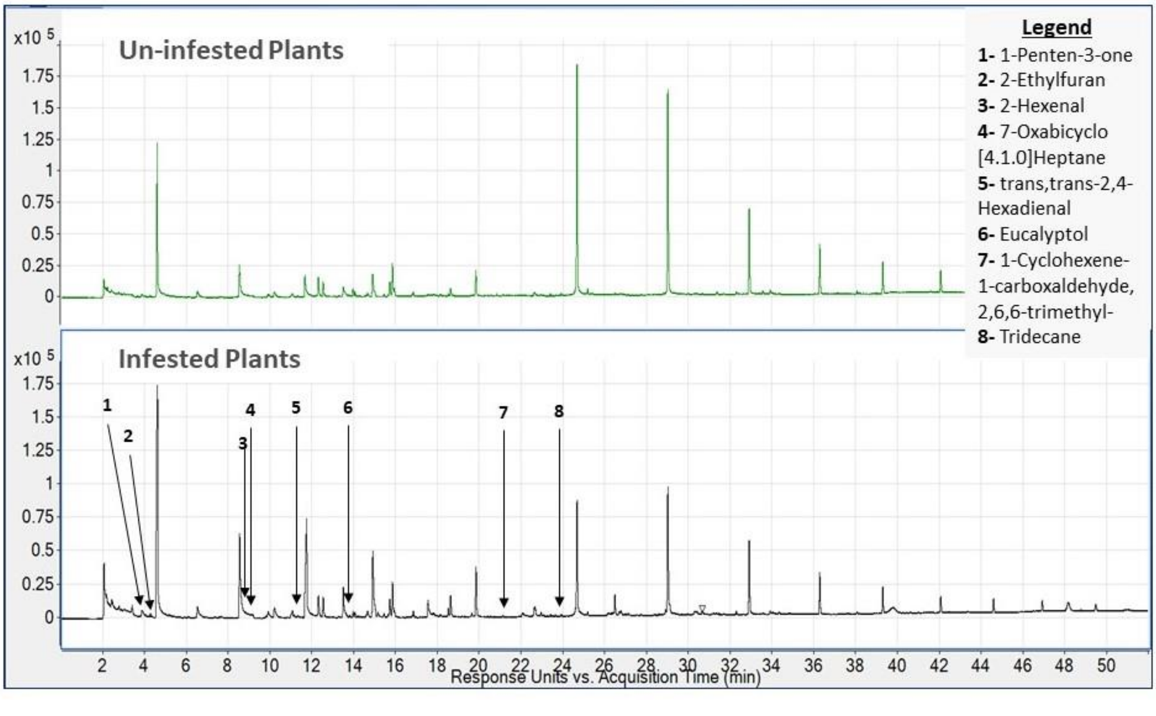


Figure 9. Gas chromatogram analysis of headspace volatile compounds extracted from (A) Uninfested, and (B) DBM-infested canola plants. Eight compounds showed significant amount differences comparing infested canola with un-infested canola, which are: 1-Penten-3-one, 2-Ethylfuran, 2-Hexenal, 7-Oxabicyclo [4.1.0] Heptane, trans,trans -2,4-Hexadienal, Eucalyptol, 1-Cyclohexene-1-carboxaldehyde, 2,6,6-trimethyl-, and

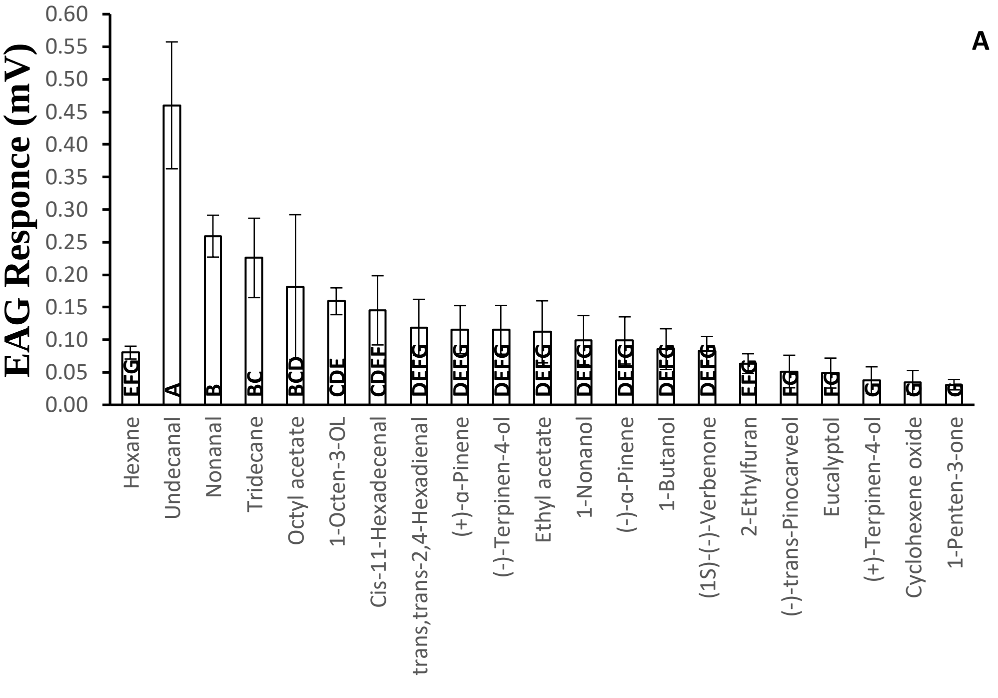
Eucalyptol and 1-Cyclohexene-1-carboxaldehyde, 2,6,6-trimethyl- are the first-time reported compounds in relation to canola infested by DBM. Both were not detected from the control canola but only detected from canola infested by DBM larvae. The amount of 2-Ethylfuran was reduced to 0.07 folds after DBM larval infestation. On the other hand, the amounts of 1-Penten-3-one, (E)-2-Hexenal, 7-Oxabicyclo[4.1.0]Heptane, (E,E)-2,4-Hexadienal and tridecane were increased by 1.19, 1.46, 1.23, 2.39 and 1.19 folds respectively after DBM larvae infested the canola plants (Table 2)

Table 2. List of volatile compounds identified from GC-MS analysis of the DBM-infested canola, which shows significant amount variance between infested and un-infested canola

	Retention Time (Minutes)	Chemical Compound	Formula	Fold Change
1	3.89	1-Penten-3-one	C_5H_8O	1.19 (± 0.05)
2	4.17	2-Ethyl-Furan	C_6H_8O	(-) 0.07 (± 0.05) (Slightly Reduced)
3	8.83	2-Hexenal	$C_6H_{10}O$	1.46 (± 0.05)
4	9.18	7-Oxabicyclo[4.1.0]Heptane	$C_6H_{10}O$	1.23 (± 0.05)
5	11.59	trans,trans-2,4-Hexadienal	C_6H_8O	2.39 (± 0.05)
6	13.98	Eucalyptol	$C_{10}H_{18}O$	Not detected Previously
7	21.59	1-Cyclohexene-1-carboxaldehyde, 2,6,6-trimethyl-	$C_{10}H_{16}O$	Not detected Previously
8	23.82	Tridecane	$C_{13}H_{28}$	1.19 (± 0.05)

2.4.2 Electroantennogram analysis (EAG)

A total of 20 compounds were utilized in the EAG analysis on both male and female adult *D. semiclausum* antennae. They are Undecanal, Nonanal, Tridecane, Octyl acetate, 1-Octen-3-OL, Cis-11-Hexadecenal, trans,trans-2,4-Hexadienal, (+)-ɑ-Pinene, (-)-Terpinen-4-ol, Ethyl acetate, 1-Nonanol, (-)-ɑ-Pinene, 1-Butanol, (1S)-(-)-Verbenone, 2-Ethylfuran, (-)-trans-Pinocarveol, Eucalyptol, (+)-Terpinen-4-ol, Cyclohexene oxide, 1-Penten-3-one in addition to the Hexane as a control. In these examined compounds, five compounds were identified from the DBM-infested canola seedlings, and they are Tridecane, trans, trans-2,4-Hexadienal, 2-Ethylfuran, Eucalyptol, and 1-Penten-3-one. The other three (2-Hexenal, (E)-, 7-Oxabicyclo[4.1.0]heptane and 1-Cyclohexene-1-carboxaldehyde, 2,6,6-trimethyl-) were not included because they were either not commercially available or have solubility issue in hexane which was used as a solvent and control in this study. The other selected 15 tested compounds are either plant-related compounds or DBM pheromone, which were available in our lab. For example, Cis-11-Hexadecenal is a major pheromone component for DBM.



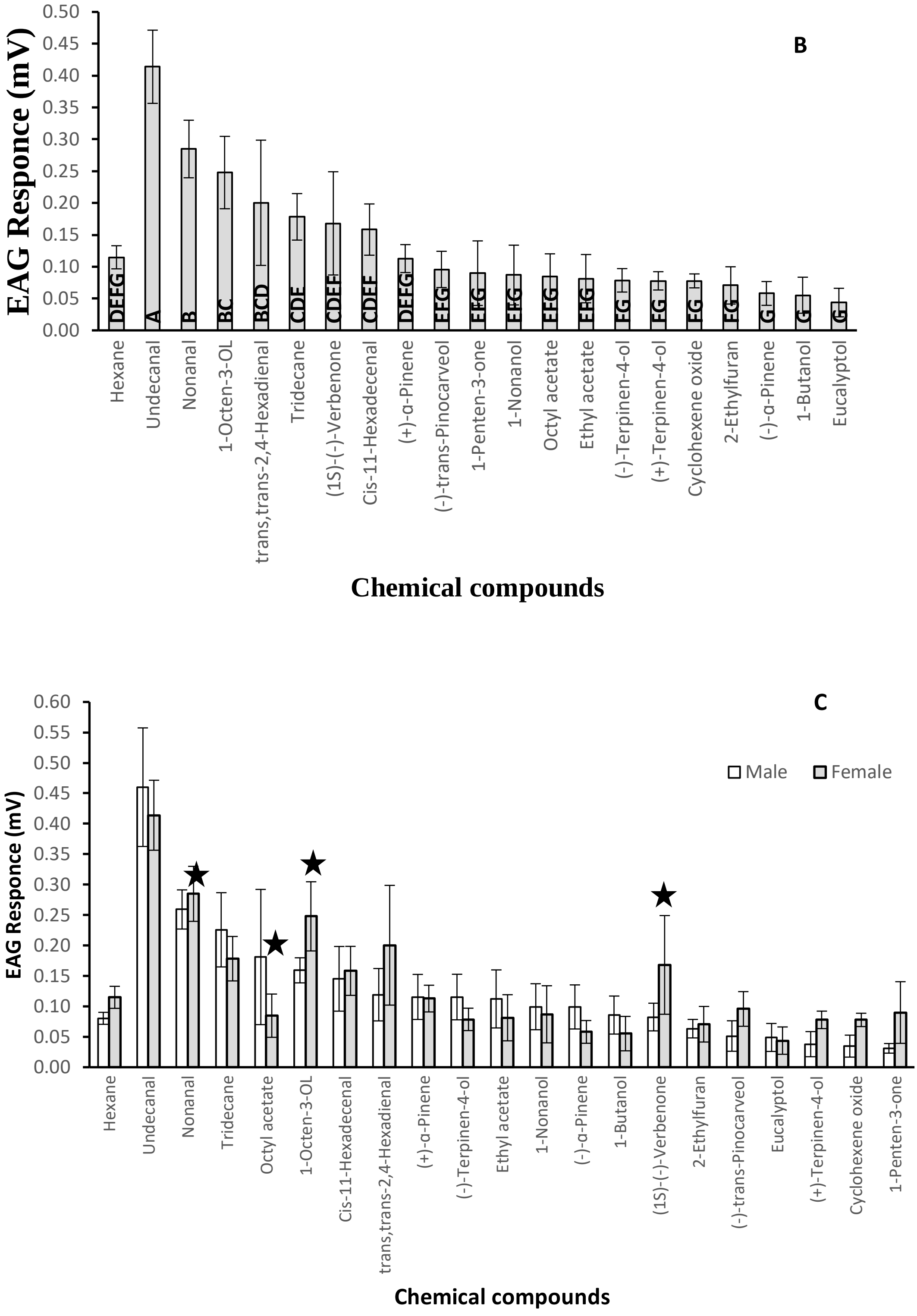


Figure 10. Electroantennography responses of *D. semiclausum* adults (A) males , (B) females and (C) both males and females at 1:1000 (µg/µL) dilution. The compounds that can initate signficantly different EAG responses between male and female *D. semiclausum* were highlighted using . (p<0.05).

For the male adult antennae, the Undecanal showed the highest EAG response. The response to the rest of the compounds graduated as follows: Nonanal, Tridecane, Octyl acetate, 1-Octen-3-OL, Cis-11-Hexadecenal, trans,trans-2,4-Hexadienal, (+)-ɑ-Pinene, (-)-Terpinen-4-ol, Ethyl acetate, 1-Nonanol, (-)-ɑ-Pinene, 1-Butanol, (1S)-(-)-Verbenone, 2-Ethylfuran, (-)-trans-Pinocarveol, Eucalyptol, (+)-Terpinen-4-ol, Cyclohexene oxide and 1-Penten-3-one respectively (Figure 10A). Several compounds such as 2-Ethylfuran and (-)-trans-Pinocarveol showed very similar responses as the control (hexane), suggesting these compounds may not initiate EAG responses. Furthermore, Eucalyptol, (+)-Terpinen-4-ol, Cyclohexene oxide and 1-Penten-3-one even showed lower responses than hexane, indicating they may inhibit antennae responses in the EAG study.

For the female adult antennae, the Undecanal also showed the highest EAG response. Followed by the Nonanal, 1-Octen-3-OL, trans,trans-2,4-Hexadienal, Tridecane, (1S)-(-)-Verbenone, Cis-11-Hexadecenal, (+)-ɑ-Pinene, (-)-trans-Pinocarveol, 1-Penten-3-one, 1-Nonanol, Octyl acetate, Ethyl acetate, (-)-Terpinen-4-ol, (+)-Terpinen-4-ol, Cyclohexene oxide, 2-Ethylfuran, (-)-ɑ-Pinene, 1-Butanol, and Eucalyptol, respectively (Figure 10B).

Interestingly, a number of compounds showed differences in the antennae responses between male and female adults (Figure 10C). For example, Octyl acetate, Tridecane, and Undecanal initiated higher EAG responses from male *D. semiclausum* antennae than female antennae. However, some compounds such as 1-Octen-3-OL, (1S) - (-)-Verbenone, trans, trans-2,4-Hexadienal, (+)-Terpinen-4-ol and Cyclohexene oxide, EAG responses from female *D. semiclausum* antennae was higher than male antennae response. Other compounds showed close responses from male and female *D. semiclausum* antennae, for example, Cis-11-Hexadecenal, 2-Ethylfuran, (+)-ɑ-Pinene and Eucalyptol. Three compounds showed significant differences between male and female antennae, and they are Octylacetate, 1-Octen-3-ol and (1S)-(-)-Verbenone (Figure 10C).

1-Octen-3-oL, Cis-11-Hexadecenal, Nonanal, Tridecane, Undecanal and Eucalyptol were selected and further evaluated for dose-dependent EAG response at 1:10 – 1:1000000 (Figure 11 and 12) because such as 1-Octen-3-oL, Cis-11-Hexadecenal, Nonanal, Tridecane, Undecanal can initiate good responses from antennae. Eucalyptol was also selected because it was one compound identified from DBM-infested canola. The compounds 1-Octen-3-ol, Cis-11-Hexadecenal, Eucalyptol and Nonanal showed dose-dependent responses from male and female *D. semiclausum* antennae (Figure 11 and 12). Interestingly, Tridecane showed the highest EAG responses at 1:1000 dilution for female but not male *D. semiclausum* antennae.

Undecanal showed the highest EAG responses at 1:100 dilution for both male and female *D. semiclausum* antennae (Figures 11 and 12).

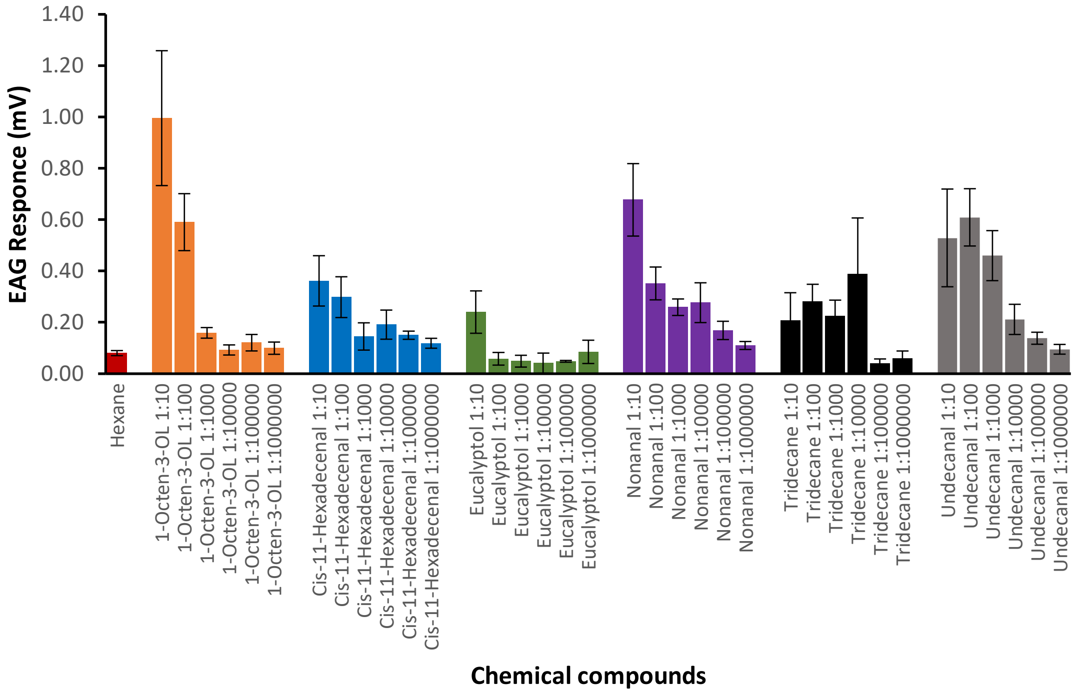


Figure 11. *D. semiclausum* (Hellen) male response to dilution series (µg/µL) of various chemical compounds including:1-octen-3-ol, cis-11-Hexadecenal, Eucylyptol, Nonanal, Tridecane and Undecanal.

The male and female *D. semiclausum* antennae responses to chiral compounds of α-Pinene and Terpinen-4-ol were also examined here. (-)-α-Pinene and (+)-α-Pinene showed similar responses from male adult antennae. However, (+)-α-Pinene can initiate much higher responses from female antennae than (-)-α-Pinene. The opposite phenomenon was observed from Terpinen-4-ol. Female adults showed similar responses to both (+)-Terpinen-4-ol and (-)-Terpinen-4-ol while male adults showed higher responses to (-)-Terpinen-4-ol than (+)-Terpinen-4-ol. (Figure 13), suggesting the insect antennae could differentiate the chiral compounds.

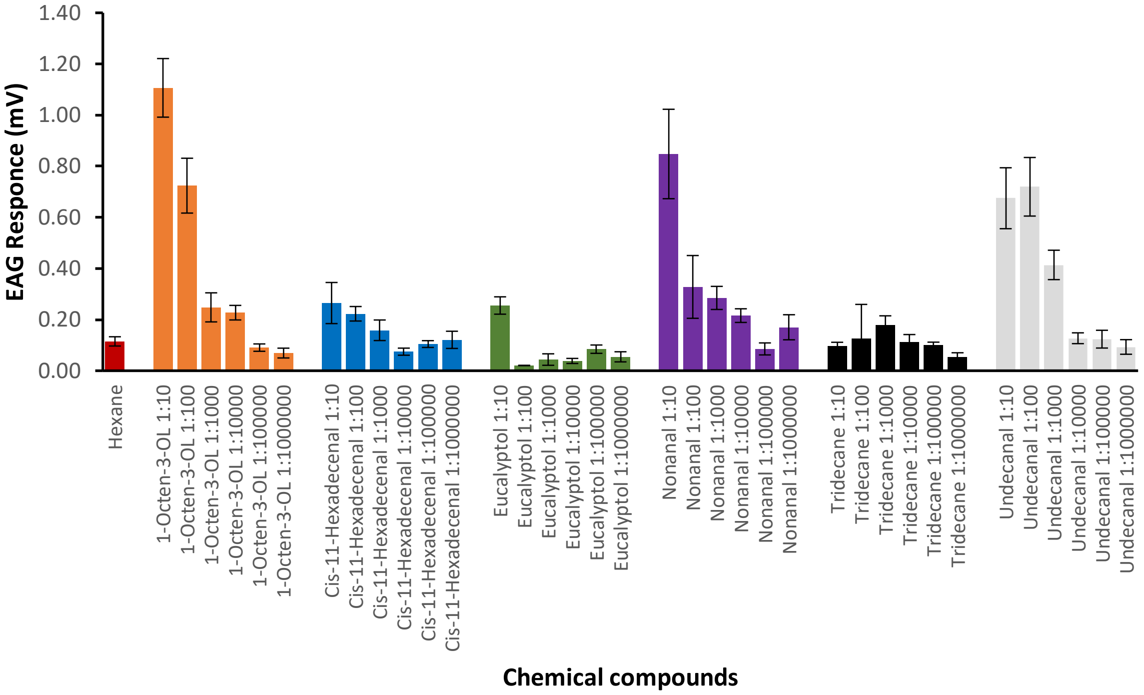


Figure 12. *D. semiclausum* (Hellen) female response to dilution series (µg/µL) of various chemical compounds including:1-octen-3-ol, cis-11-Hexadecenal, Eucylyptol, Nonanal, Tridecane and Undecanal.

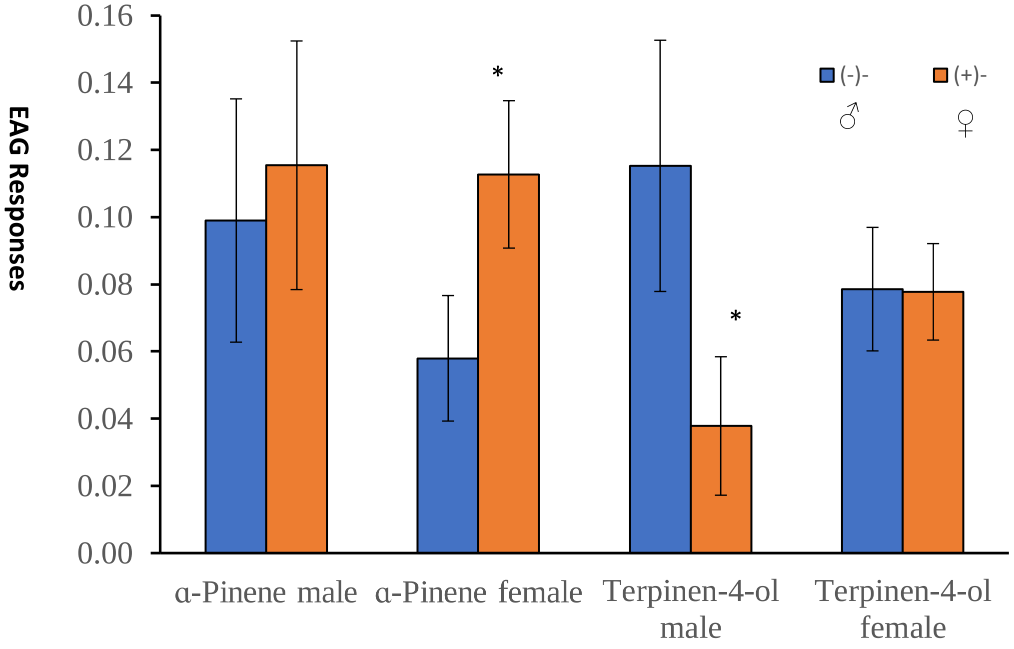


Figure 13. *D. semiclausum* (Hellen) male and female response to chiral compounds at 1:1000 dilution (µg/µL) of: (-)-ɑ-Pinene, (+)-ɑ-Pinene, (+)-Terpinen-4-ol and (-)-Terpinen-4-ol. Student t-test was applied to compare the responses between male and female antennae and * means p<0.05.

2.4.3 Antennae and sensilla

Different EAG responses to chemical compounds were observed between male and female *D. semiclausum* antennae, suggesting there may be differences in their antennae or sensilla. To examine these differences, an SEM analysis was conducted on both male and female *D. semiclausum* adult antennae. Both male and female antennae are filamentous, black, localized between the two compound eyes on the top of the head (Figure 14), and formed by five parts: Radical, Scape, Pedicel, Annellus and Flagellum. The knot is thick, and the base is born in the antennae fossa. The scape shape is like a bent egg cup with approximately 180 µm length and 120 µm diameter in its distal side and 78 µm in its proximal side. It sits on a radicle of 55 µm long by 80 µm in its widest part. The short cone-shaped pedicel measures 60 µm length and 98 µm (proximal end) to 68 µm (distal end) where the annulus sits. Anneellus length is 15 µm, and the width is 60 µm.

The flagella exhibited sexual dimorphism between females and males as the male antennal flagellum has two extra flagellomeres (25 in total), whereas the female flagellum has 23

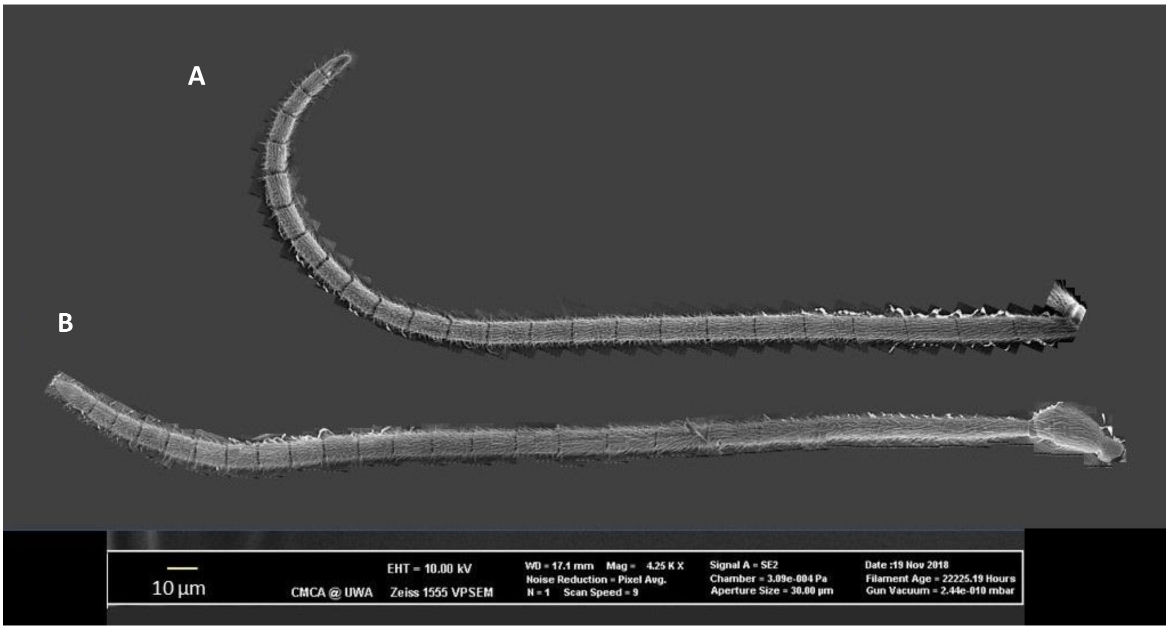


Figure 14. Scanning electron microscopy analysis of: A- Male (and) B- Female *D. semiclausum* (Hellen) at 4.25 K magnification.

flagellomeres (Figure 14). The total length of the male flagellum is about 3400 µm, and the total length of the female flagellum is about 3050 µm. All flagella are cylindrical except the apical flagella, which tends to be pointy with a round-tip (Finger shaped). Proximal flagellomere length is 205 µm. for males and 267 µ m. for females respectively, and it decreases gradually towards the apical flagellum, which is 105 µm for males and 138 µm. for females respectively. All male flagellomeres are longer than their counterparts of females except the first four flagellomeres. Flagellomeres are cylindrical in shape, and in males, almost every flagellomere is wider from the proximal side compared to the distal side by 1 to 3 µm. and that is extremely explicit in flagellomeres 1 and 2 whereas the difference is up to 11 µm. The same concept applies to female flagellomeres with more irregular differences. However, the diameter to the length ratio for male flagellomeres starts at 2.9 at the approximal flagellomere and decrease to 1.5 times at the tip flagellomeres. The same concept applies to the female flagellomeres, whereas the approximal ratio starts at 4.4 and ends at 1.3.

SEM study revealed that a total of seven different types of sensilla were identified on the antennae of both male and female *D. semiclausum* (Figure 15). Six of them, including Sensillum trichodeum (ST), Sensillum chaetium (SC1), Sensillum placodea (SP), Böhm's mane cylindrical sensor (BM), Sensillum cylindric (SC2) and Sensillum basiconca (SB), were previously described Sensillium Coeloconica (SCoe) was observed in this study for the first time (Table 3).

Sensilla trichodeum (ST) are the most widely distributed sensilla on the antennae of male and female *D. semiclausum*, which showed the highest density in the first flagellomere. Hair-like slender sensilla are present in clusters along the ventral surface of flagellomere. They are either straight at the base and taper toward the end or curved at the base and parallel along the antenna surface. Their lengths range from 33.7 to 34.5µm for both males and females

Sensillum chaetium (SC1) are upright, protrude and thorn shaped with a grooved surface. They are observed on each flagellomere and range from 7.5 to 8.0 µm. They are like the hair-shaped sensor but thicker and shorter. The end of SC1 is blunt and longer than the hair-shaped sensor.

Sensillum placodea (Sp) were plate-shaped sensilla in each flagellomere, slightly wider at both ends, narrower in the middle, and some curved in the middle. It has a plate-like outer wall with a ridge-like bulge

Böhm's mane cylindrical sensor (BM) is similar but shorter and sharper than the thorn-shaped sensor. It is thinner than the tapered sensor and without a base

Sensillum cylindric (SC2) is a cylindrical sensor and born in the concave, which is distributed between the plate-shaped sensors

Sensillum basiconca (SB) is Tapered in shape

Sensillium Coeloconica (SCoe) looked like a depression (Pit) surrounded by a groove on the surface and have what looks like three small pegs arising from the centres of the depression. The groove measured (2-3.7) µm in females and (4.2-4.3) µm in males.

Table 3. Sensilla types observed on the antennae of male and female *D. semiclausum*

	Sensilla Type	Short description	Observed in both sexes
1	Sensillum trichodeum (ST)	Hair-shaped Sensilla	Yes
2	Sensillum chaetium (SC1)	Thorn-shaped Sensilla	Yes
3	Sensillum placodea (SP)	Plate-shaped Sensilla	Yes

4	Böhm's mane cylindrical sensor (BM)	Similar to Sensillum chaetium but shorter and sharper	Yes
5	Cylindrical sensor (Sensillum cylindric) (SC2)	Cylindrical sensor	Yes
6	Conical sensor (Sensillum basiconca) (SB)	Tapered sensory	Yes
7	Sensillium Coeloconica (SCoe)	Pit pegs	Yes

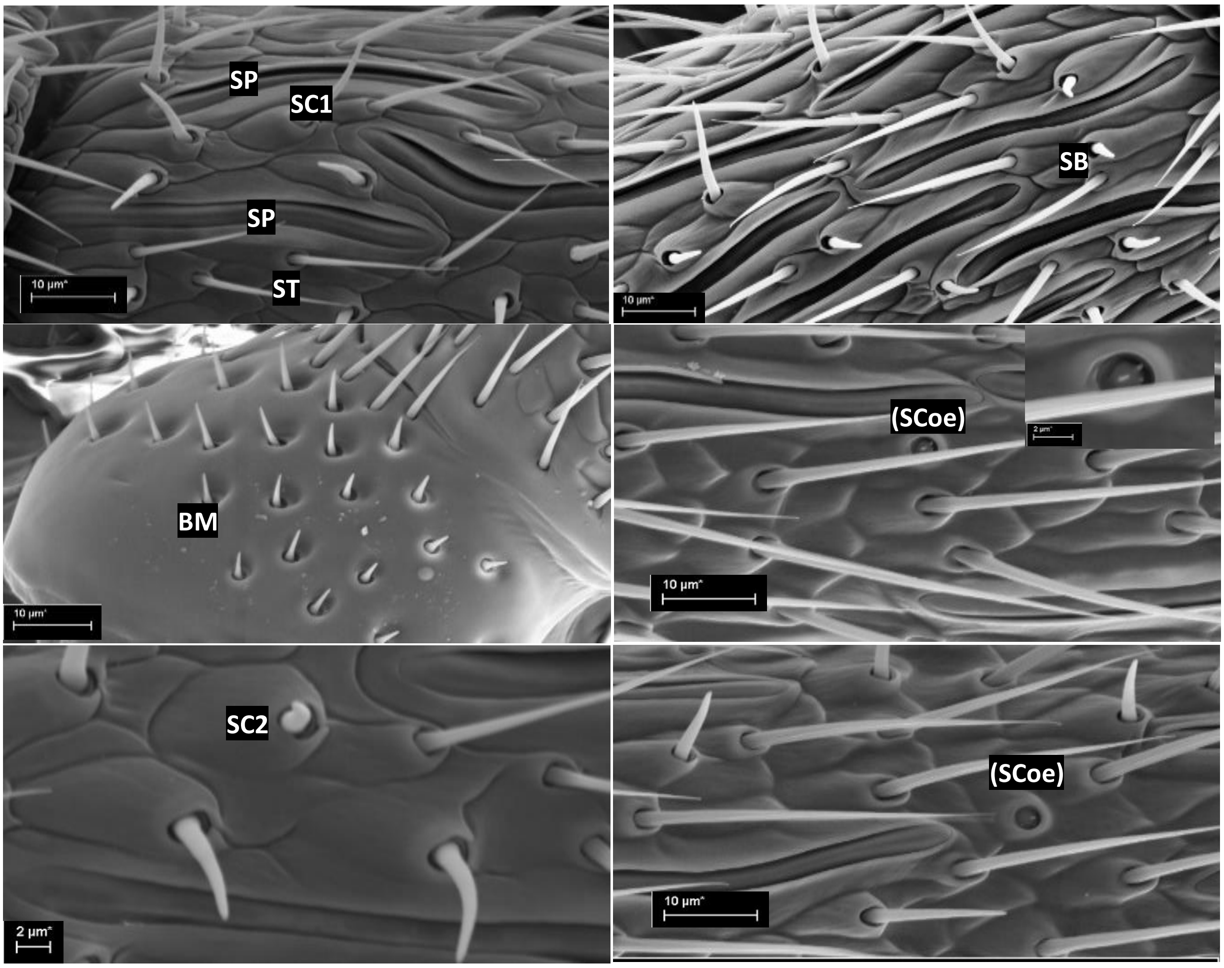


Figure 15. Scanning electron microcopy analysis of the *D. semiclausum* sensilla.

2.5 Discussion

This study aimed to identify the candidate attractants to guide *D. semiclausum* to DBM larvae and their damaged plants. Female *D. semiclausum* lay eggs into DBM larvae while males do not. Identifying the compounds that attract female *D. semiclausum* will help apply them as a lure in the field to attract *D. semiclausum* and make use of them to control DBM effectively and efficiently. Compared to chemical pesticides, this approach is more environmentally friendly and can overcome the increasing DBM resistance to chemical pesticides.

By using SPME and GC analysis, eight candidate compounds were identified significantly higher from DBM-infested canola seedlings. Most of these eight identified compounds have been reported as plant volatile compounds in previous studies. For example, 1-Penten-3-one was widely present in plant species (Jimenez et al. 2009, Xu and Barringer 2010). 2-Ethylfuran is a plant metabolite and a bacterial metabolite, which is also a constituent of numerous plant species such as coffee, tea, soybean and rice (Kashyap, Kumar, and Singh 2020). (E)-2-hexenal has been identified from Green leaf volatiles (GLVs) from *Brassica oleracea* subsp. *capitata* (L.) (E,E)-2,4-hexadienal (hexadienal) has been found in tomatoes, kiwi fruit, mangoes, potato chips, herbs and spices. 2,4-Hexadienal has been identified in numerous oxidized glyceridic oils, including canola, soybean, cottonseed, sunflower, sesame and palm oils (IARC 2013). Cyclohexene-1-carboxaldehyde, 2,6,6-trimethyl-(β-Cyclocitral) is a metabolite of the cyanobacteria of the genus Microcystis, which has a characteristic tobacco flavour (Jiittner 1984). It is one of the predominant volatile terpenoid compounds found in algal bloom water. However, some compounds are rarely reported from plants (Huang et al. 2010). For example, 7-Oxabicyclo[4.1.0]heptane. All these compounds may be candidate compounds for the canola plant to protect itself after DBM infestation and attract DBM natural enemies such as *D. semiclausum.*

In these eight compounds, some of them has been studied on *P. xylostella* responses and behaviours. For example, the performances of *P. xylostella* were affected by 1-penten-3-one treated *Arabidopsis* seedlings (Dong et al. 2016). Lower selection, growth rate and leaf consumption were observed on treated *Arabidopsis* seedlings. Furthermore, relative expression of defence-related genes in *Arabidopsis* seedlings were greatly up-regulated by 1-penten-3-one, suggesting that 1-penten-3-one functions as a gaseous signal that can induce defence response which is closely related to the jasmonite signalling pathway in *Arabidopsis* (Bruce 2015). On unmated male DBM, mixtures of (Z)-3-hexenyl acetate, (E)-2-hexenal, and (Z)-3-

hexen-1-ol with the pheromone induced attracting/arresting behaviour. In 80-100% of the males tested, the effect was significantly higher than the effect induced by the pheromone alone (Deng et al. 2004). The chemical composition and bioactivity of *Artemisia lavandulaefolia* DC (Asteraceae) essential oil on *P. xylostella* was measured. The principal compounds, including eucalyptol (35.60%) can result in LD50 contact toxicity of the essential oil to immature *P. xylostella* was estimated at 0.045 nL per larva, which also exhibited fumigant toxicity against *P. xylostella* adults with an LCH of 0.113 mg per L after 12 h and 80 to 100% repellence at a 1% v/v concentration.

Some compounds have been reported related to parasitoid of insect pests. For example, the green-leaf volatiles (E)-2-hexenal and (Z)-3-hexenyl acetate act as a specific indicator of the presence of *Acyrthosiphon pisum* for the parasitoid *Cotesia glomerata* (Shiojiri et al. 2006).

However, how these compounds interact with *D. semiclausum* is unknown. Therefore, an EAG analysis of the selected compounds to male and female *D. semiclausum* adults were performed. In the EAG analysis, Undecanal, also known as undecyl aldehyde, initiated the highest responses from both male and female wasps. Moreover, the response to Tridecane and 2,4-Hexadienal elicited strong responses compared to other tested compounds. The other two selected compounds (Eucalyptol and 2-Ethyl-Furan) did not initiate strong antennal responses may be related to the dilution tested here (1:1000). However, that does not mean they are not important. Chemical compounds have been reported that they do not initiate strong EAG responses from antennae alone, but they can activate behavioural responses.For example, DEET, the most successful insect repellent in the market. Though clearly repulsive behaviours were observed from DEET to many insect species, the EAG studies could not detect a strong response from the antennae. The reason may be the related odorant receptor is not highly expressed in the antennae (Stanczyk et al. 2013).

Nonanal, originating from various strains of *Pseudomonas sp.,* isolated from roots and stubbles of canola, exhibit high EAG responses as well. Generally, nonanal is a common volatile compound released from various plants broadly. It has been reported that nonanal is responsible for various essential physiological effects, including host-plant and host-seeking behaviours in some insects. Nonanal is attractive to various female insects in beahvioural bioassays. Male and female *D. semiclausum* showed differences in the antennal responses to certain tested compounds, for example, Octylacetate, 1-Octen-3-ol and (1S)-(-)-Verbenone, suggesting there should be differences in their olfactory systems. Only female D. semiclausum need to lay eggs into DBM larvae while males do not. D. semiclausum antennae and sensilla were investigated under SEM. However, we did not find significant differences on the antennae and sensilla between male and female *D. semiclausum* adult antennae. The seven types of sensilla were observed in both male and female antennae, suggesting the differences may exist at the molecular level. Sensillium Coeloconica (SCoe) was observed in this study for the first time, while the other six types of sensilla have been described before. Further studies such as single sensilla recording will be applied to study what kind of compounds can elicit this sensilla. Olfaction genes such as odorant receptors (ORs) or odorant binding proteins (OBPs) play critical roles in the odorant sensation in insect olfactory sensilla, so they need to be further studied and compared between male and female *D. semiclausum*.

Another interesting result is that a number of compounds that *D. semiclausum* detected can also be detected by DBM. For example, cis-11-Hexadecanal is a sex pheromone component of DBM. *D. semiclausum* can detect this compound and show strong EAG responses, suggesting that *D. semiclausum* also has a receptor for this compound and help *D. semiclausum* to localize the DBM for parasitism. DBM and *D. semiclausum*, which live in the same environment that is full of the same plant chemical compounds can use these semiochemicals around them to help detect each other and regulate their behaviours. Many plants attacked by herbivore insects emit leaf volatile organic compounds (VOCs) that attract the herbivore insects' natural enemies, such as parasitoids and predators (Dicke et al. 1990) to control the pests. It has been reported that insects and their natural enemies can detect the same odorant compounds by using their olfactory systems. These detections indicate they may possibly share certain conserved olfactory proteins like OBPs or ORs to help this process.

A large number of natural enemies have been widely used in the biological control of insect pests. However, only limited attention has been paid to their biology, ecology and behaviour. Without such knowledge, it is difficult to improve our understanding of the natural enemies and better use them in pest control. This study is the first to investigate *D. semiclausum* EAG responses to the volatile compounds released by DBM-infested canola. An improved understanding of *D. semiclausum* olfaction will help us use them more efficiently and effectively in the future.

Chapter 3

Identification and characterization of OBP and OR genes in the parasitoid wasp *Diadegma semiclausum* (Hellén) (Hymenoptera: Ichneumonidae)

Statement of Contribution

Title of Paper	Identification and characterisation of olfactory genes in the parasitoid wasp *Diadegma semiclausum* (Hellén) (Hymenoptera: Ichneumonidae).
Publication Status	Accepted
Publication Details	***Bulletin of Entomological Research***
Principal Author	
Name of Principal Author (Candidate)	Basman Al-Jalely
Contribution to the Paper	Methodology, Collected Data, Writing- Original Draft, Preparation, Conceptualization and Validation
Overall Percentage (100%)	85 %
Signature Bas	Date 24/08 /2021

Co-Author Contributions

By signing the statement of contribution, each author certifies that:

- ➢ The candidate's stated contribution to the publication is accurate (as detailed above).
- ➢ Permission is granted for the candidate to include the publication in the thesis
- ➢ The sum of all co-author contributions is equal to 100% less the candidate's stated Contribution

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Contribution to the Paper	Collaboration on data analysis
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Signature	Wei Xu Date 24 /08/2021

3.1 Abstract

Diadegma semiclausum is an important parasitoid wasp widely used in the biological control of the Diamondback moth, *Plutella xylostella*, one of the most destructive pests of cruciferous plants. Its behaviors, including mating, feeding, host-seeking and oviposition, are mainly guided by their olfactory systems, in which odorant binding proteins (OBPs) and odorant receptors (ORs) are the key components. In my previous studies, male and female *D. semiclausum* showed different antennae responses to various plant volatile compounds, suggesting the OBP or OR genes are expressed differently between male and female adults. Interestingly, *D. semiclausum* adults also showed responses to odorants that DBM respond to, for example, cis-11-Hexadecanal, indicating it may share some similar OBP or ORs with DBM.

To fully understand the molecular mechanism of the olfactory system of *D. semiclausum*, transcriptome sequencing was performed on the RNA samples purified from the male and female adult antennae. A total of 17 putative OBP and 67 OR genes were annotated from the transcriptome sequence and further studied by using phylogenetic and bioinformatics methods. The expression patterns of *D. semiclausum* OBPs between male and female antennae were analyzed using reverse transcription-polymerase chain reaction (RT-PCR) and quantitative real-time PCR (qRT-PCR). Six OBPs and twelve ORs were identified, showing significantly high expression in female than male adults. DsemOBP5 and 13 showed high similarity to PxylOBP31 and 23, respectively but no DsemORs showed similarity to PxylORs. This study advances our understanding of the chemosensory system of *D. semiclausum* at the molecular level and will help optimize our integrated pest management strategy for this species.

3.2 Introduction

Insect behaviours, including mating, foraging, host-seeking and oviposition, are guided by their olfactory systems (Krieger and Breer 1999). Antennae are the major insect olfactory organs, on which hair-like sensilla distributed over the surface are utilized by insects to detect chemical signals from the environment (Keil 1984b, a, Larsson et al. 2002). With the breakthrough of new technologies on next-generation sequencing, bioinformatics, RNA (Vogel et al. 2019), interference (RNAi), and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) (Lester et al. 2020), the understanding of insect olfactory mechanisms has progressed significantly in recent years. Odorant binding protein (OBPs) (Vogt and Riddiford 1981) and odorant receptors (ORs) (Clyne et al. 1999, Vosshall et al. 1999) have been reported playing pivotal roles in the dynamics, selectivity, and sensitivity of the insect chemosensory system.

OBPs are one group of proteins that are highly expressed in olfactory tissues, bind, solubilize and transport semiochemical molecules to the receptors (Leal 2003, Pelosi et al. 2018). The first insect OBP was identified from *Antheraea polyphemus* at the beginning of the 1980s by using the tritium labelled specific pheromone (*E*, *Z*)-6, 11-hexadecadienyl acetate as a probe (Vogt and Riddiford 1981). The functional roles of insect OBPs have been addressed by using *in vivo* technologies. For example, the deletion of a LUSH (OBP of the *Drosophila melanogaster*) gene suppresses *D. melanogaster* electrophysiological and behavioral response to the male pheromone 11-cis-vaccenyl acetate (cVA) (Xu et al. 2005). ORs are chemosensory receptors localized on the dendritic membrane inside the olfactory sensilla (Sato et al. 2008, Wicher et al. 2008), detect volatile compounds, and transduce the olfactory signals to insect brains to regulate behaviors. With the advances in the next-generation sequencing methods and the completion of the genome or transcriptome sequences of fruit fly, honeybee, mosquitoes, silkworm and beetle, more and more OBP and OR genes have been studied.

D. semiclausum is a parasitoid wasp, which specifically lays its eggs into the developing larvae of the diamondback moth (DBM), *Plutella xylostella*. DBM is a major agricultural pest that feed on cruciferous plant species such as canola, cabbage, cauliflower and broccoli. DBM is one of the most destructive insect pests because it can develop strong resistance to the common pesticides fast. *D. semiclausum* is the most commonly found parasitoid of DBM in Australia, which is believed considerable aids in controlling DBM. *Diadegma* spp. have been the most extensively studied and are the most used biological control agents in the world. For this reason, biological control using parasitoid wasp such as *D. semiclausum*, became more and more important in integrated pest management (IPM) strategies. In my previous studies, odorant compounds released from DBM damaged canola can initiate high electrophysiology responses from *D. semiclausum* antennae, for example, Tridecane. Male and female *D. semiclausum* antennae showed different EAG responses to various plant volatile compounds, for example,, 1-Octen-3-ol, suggesting OBP or OR genes are expressed differently between sexes. Interestingly, *D. semiclausum* antennae also showed EAG responses to odorants that DBM can detect, for example, cis-11-Hexadecanal, indicating the host pest (DBM) and the parasitoid wasp (*D. semiclausum*) may share similar OBP or ORs to detect the same compounds in their ecosystem. However, limited attention has been paid to *D. semiclausum* olfaction system. The olfactory basis of male and female behaviours in these parasitic wasps are still poorly investigated. The application of next-generation sequencing provides an efficient and comprehensive approach to examine the olfactory genes and their expression profiles in parasitoid wasps and shed light into the molecular mechanism of their olfactory system. In this study, RNA sequencing of *D. semiclausum* antennae were collected to obtain the transcriptome database for identifying and investigating the key olfactory genes such as OBP and OR genes. Phylogenetic, bioinformatics and molecular biology approaches were utilized to characterize these identified OBP and OR genes. An improved understanding of *D. semiclausum* olfactory system will assist in the development of more efficient and environmentally friendly pest-controlling strategies.

3.3 Materials and methods

3.3.1 RNA purification from adult insects

D. semiclausum pupae were sourced from Biological Services (https://biologicalservices.com.au/) and individually kept in a 7 ml screw top, clear vial (Sigma Aldrich, Australia) in the lab at 25± 1(°C), 70-80% (R.H.) and 16:8 h (L:D) photoperiod.1:10 sugar solution was provided to feed emerging adults.

Based on the presence/absence of the ovipositor, emerging adults were differentiated under a stereomicroscope (Figure 8). A total of 100 male and 100 female adults less than three days old were collected and put to anesthesia using carbon dioxide (Purity >99.9%) for five minutes. Then antennae were excised with a surgical scalpel blade under a stereomicroscope. All collected antennae were immediately stored in liquid nitrogen, and total RNA was purified using Qiagen RNeasy mini kit (Qiagen, USA) according to the manufacturer's protocol. The purified total RNA was quantified and quality controlled using NanoDrop ND-2000 (Thermo Scientific, USA) and stored in the -80 °C freezer in Western Australia State Agricultural and Biotechnology Centre (SABC). The purified total RNA samples were sent to BGI Hong Kong (https://www.bgi.com/us/) in dry ice for RNA sequencing.

3.3.2 Sequence assembly

The raw data received from BGI were pooled for the transcriptome assembly. All the raw RNA-Seq reads were analysed using: FastQC (Andrews et al. 2014) to check for reading quality. Overall, the raw reads displayed reasonable quality. The pooled reads were assembled as pair-ended reads using Trinity, version 2.4.0 (Grabherr et al. 2011). The minimum contigs length was set to 200 bp. The contigs were then annotated by using Swissprot protein database.

3.3.3 Gene annotation and bioinformatics analysis

Genes encoding OBPs and ORs in *D. semiclausum* were identified using BLAST searches from assembled transcriptome database with reported *D. melanogaster* and *Apis mellifera* OBPs and ORs as a query. Extensive manual curation was then performed on the *D. semiclausum* OBP and OR genes. The identified OBP and OR amino acid sequences were used for validation by NCBI blast based on the identity and similarity to orthologous genes from other insects. All identified *D. semiclausum* OBP and OR amino acid sequences are available in an online supporting FASTA text file (Supplementary data 1 and Supplementary Table 1). N-terminal signal peptides of DsemOBPs were predicted by using SignalP 5.0 (http://www.cbs.dtu.dk/services/SignalP). The calculated molecular weights (MW) and isoelectric points (pI) were obtained by using ExPASy proteomics server (http://www.expasy.org/tools/protparam.html) on the mature proteins (without signal peptides). The amino acid sequences of DsemOBPs were used to search for the best blast hit sequences from NCBI by using blastp (Supplementary Table 1).

3.3.4 Phylogenetics analysis

Encoded DsemOBP and DsemOR proteins were aligned using default settings for ClustalW in Geneious clustal alignment. Gap Opening Penalty (10.00) and Gap Extension Penalty (0.10) were used for multiple sequence clustal alignment with 30% Delay Divergent Cutoff. The DsemOBP and DsemOR amino acid sequences were used to create an entry file for phylogenetic analysis in Geneious with *A. mellifera* OBP (AmelOBP), *P. xylostella* OBP (PxylOBP), *A. mellifera* OR (AmelOR) and *P. xylostella* OR (PxylOR) sequences. Firstly, the amino acid sequences of OBPs and ORs were aligned by using Geneious alignment with default settings: Global alignment with free end gaps, Cost Matrix (Blosum62), Gap open penalty (12), Gap extension penalty (3) and Refinement iterations (2). A UGMA tree was then constructed using the default settings based on Jukes-Cantor Model with Bootstrap as resampling method, and 1000 replicates and 30% support threshold. To compare the OBPs and ORs between *A. mellifera* and *D. semiclausum*, a phylogenetic analysis was performed. The same method was used to study the OBP and OR genes between *D. semiclausum* with *P. xylostella* in order to give an insight into the interactions between the parasitoid and its host insects.

3.3.5 RT-PCR and quantitative RT-PCR

The cDNA templates were prepared from purified RNA samples using the SuperScript® VILO™ cDNA Synthesis Kit (Invitrogen, USA), according to the manufacturer's manual. RT-PCR was performed using gene-specific primers as Table 4, which were designed by using software http://bioinfo.ut.ee/primer3/. Reverse transcriptase PCR (RT-PCR) was performed using (*Taq* DNA Polymerase) (New England Biolabs, , Australia) as follows: 95 °C for 30 s; 41 cycles at 95 °C for 25 s, 55 °C for 30 s and 68 °C for 20 s; and final extension at 68 °C for 5 min then Hold at 10 ˚C. The PCR products were visualized using 1.0% agarose gel electrophoresis under 75 voltages supplied by (Bio-Rad 1000/500) power supply (Xu et al. 2017). The agarose gels were examined under Bio-Vision system (Vilber Lourmat, France).

Quantitative Real Time PCR (qRT-PCR) was performed using the same gene-specific primers in Table 4, and reference genes included DsemRPL8, DsemRPL13a and DsemORco. A 2-Step qPCR protocol was performed on Rotor-Gene Q-5 Plex (Qiagen, US) using Power SYBR® Green PCR Master mix (Thermo Fisher Scientific, Australia) following the protocol Bio-Rad CFX96 Real-Time PCR Detection System (Xu and Liao 2017). The qRT-PCR results were examined based on the Ct values for each gene. Statistical analysis was performed on the expression profiles between male and female adults using t-test in Excel 2016. The symbol * means $p < 0.05$, ** means p value < 0.01 while *** means p value <0.001.

3.3.6 *In silico* expression profiles of DsemORs

Using STAR aligner version 2.5.3a (Dobin et al. 2013), the retained reads were independently aligned against the assembled transcriptome library. Alignment parameters were set such that a maximum of two mismatches were allowed. Mismatches of two were used to accommodate potential base-calling errors. Only uniquely aligned reads were retained for analysis, while reads aligned to multiple loci were discarded. A count matrix was generated and loaded into R (version 3.5.1) for downstream statistical analysis. The transcripts were removed from analysis if less than 10 unique reads could be aligned. Fragments Per Kilobase Million (FPKM) values were used as the main criterion to identify differentially expressed (DE) genes.

3.4 Results

3.4.1 Identification of DsemOBPs

The transcriptome assembly of male and female *D. semiclausum* antennae consists of 84,668 contigs and has a total size of 123,007,582 bp, with a N50 of 2742 bp. A total of 33,295 transcripts were estimated. The maximum contig is 16148 bp, and the average size is 1452 bp. In total, 24,660 transcripts passed the quality control criteria and were evaluated for differential expression (DE) analysis.

Here, 17 DsemOBPs were identified from *D. semiclausum* transcriptome sequences, and 11 of them are full-length sequences, including DsemOBP6-13 and DsemOBP15-17. All the others are only partial sequences, either missing the N-terminal or C-terminal. In these 11 full-length DsemOBPs, no signal peptide was predicted from DsemOBP16 and DsemOBP17. The mature (without signal peptide) DsemOBPs with a full-length range from 108 to 231 amino acids, and their molecular weights range from 11926 to 27093 Da (Supplementary Table 1). The isoelectric points of most full-length DsemOBPs are below 7.0, but DsemOBP8 is above (pI 8.25). The amino acid and mRNA sequences, signal peptides, molecular weight, and other information of DsemOBPs were provided (Supplementary Table 1).

The newly identified DsemOBPs were aligned by using Geneious clustal alignment. The alignment of these DsemOBP amino acid sequences highlights the six conserved cysteine residues (Figure 16). Most of the *D. semiclausum* OBPs share the characteristic features of the classic OBP family: small size, presence of an N-terminal signal peptide sequence, as well as a highly conserved pattern of six cysteine residues called the “classic motif”.

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DsemOBP6  -------------------------------------------------------------------------------------MAVRNKMALRYSGWLVIVVCAAFLRNNRVDGAM-TMAQMETAATGFRNVCIPKSGADPAI-------VAGLRSGNFPE-DHNFQCYLKCVMAMLK
DsemOBP7  -------------------------------------------------------------------------------------MAVRNKMALRYSGWLVIVVCAAFLRNNRVDGAM-TMAQMETAATGFRNVCIPKSGADPAI-------VAGLRSGNFPE-DHNFQCYLKCVMAMLN
DsemOBP1  ------------------------------------------------------------------------------------------MFGRAATALLIVVSLYGFPDVSADLPDF-VTPEIIEMVQDDKVRCMDKFGTDQGM-------IDQVNNGNILN-DPKLTCYMHCLFESFG
DsemOBP3  ----------------------------------------------------------------------------------------------------------GLPDVSADLPDF-VTREIIEMVQDDKVRCMDKFGTDQGM-------IDQVNNGNILN-DPKLTCYMHCLFVSFG
DsemOBP2  ------------------------------------------------------------------------------------------------------FQVSLIFHQFSQLPDF-VTPEIIEMVQDDKVRCMDKFGTDQGM-------IDQVNNGNILN-DPKLTCYMHCLFESFG
DsemOBP4  ------------------------------------------------------------------------------------------------------FQVSLIFHQFSQLPDF-VTPEIIEMVQDDKVRCMDKFGTDQGM-------IDQVNNGNILN-DPKLTCYMHCLFESFG
DsemOBP9  ----------------------------------------------------------------------------------------------------MKIFAVVLLVCVAGSFAA-ISEEHKAKLRELKLACITETGVNAEV-------VENAKKGVLDESDPKLACFAACFLKKLG
DsemOBP12 ----------------------------------------------------------------------------------------------------MNRRFQSMQIFVFFFQAA-ISEEHKAKLRELKLACITETGVNAEV-------VENAKKGVLDESDPKLACFAACFLKKLG
DsemOBP11 ----------------------------------------------------------------------------------------------------MKIFAIAIAFCLVVAASA-LTDEQKTKLAGHRDHCVAETGIERAK-------VDAAKLGTFDETDDKLACFSACLLKRIG
DsemOBP10 MYRSVALVVLVVLASCVLGDPGKGRSQAWTKCMEESGIAKGSFKDTPWDDPKKKCFQACLMREHGHLKEDGTVDSEKMIADIPPDVEDRDRISAAITECSANQNDDQCETAHLVMKCL-HDKDAMTFPGGKKMHAAMKKCMQENG-------VEHKDFRELPPDDQKMKCFGACMMKEQG
DsemOBP14 ------------------------------------------------------------------------------------------------------------------MKCL-HDKDAMTFPGGKKMHAAMKKCMQENG-------VEHKDFRELPPDDQKMKCFGACMMKEQG
DsemOBP16 ----------------------------------------------------------------------MSLDEELFFGNSNKRSSGNTSRNGSNNGQSSNGNNGDNRRNDRSRQDS-VFNNRMTNPSNYDWMNERVNGMSNQNQYSNYHTSQSQNPDNNNNHRDRAECVTHCLFDELN
DsemOBP17 ----------------------------------------------------------------------MSLDEELFFGNSNKRSSGNTSRNGSNNGQSSNGNNGDNRRNDRSRQDS-VFNNRMTNPSNYDWMNERVNGMSNQNQYSNYHTSQSQNPDNNNNHRDRAECVTHCLFDELN
DsemOBP15 ----------------------------------------------------------------------------------------------------MKSCVILIAFCLVVAADSRTVKLTKEKLLEYRKYCEDEKDISRQA---------MKNDNGYYSAADRSGCITACISKRIG
DsemOBP5  ----------------------------------------------------------------------------------------------------MSIPILLGILILCVAKISAISDEMKQMAQVLHDACVEETGVEEKF--------IEQCRQGEFPEDANLKCYMKCLMTKVP
DsemOBP8  ----------------------------------------------------------------------------------------------MMKYLLVLGCLLQATLFCSANRPSFVSDTMINVASSVVNVCRARTGVDVAD--------IEAVRMGEWPETESLKCYMYCLWKQFG
DsemOBP13 -----------------------------------------------------------------------------------------------------MKVLVIFLAVPILAVSAALTPADIVEFGRARQRCGNEFTVDPTS--------LDRARAGETVNDPEFDCYMACILEGMH


DsemOBP6  TIKNNQPLLDPIIKQIKVM-----MPAELQDRNIGAARKCAALEFN---DDACVAAWQYIKCQYETDPEVFFFP------------------------------------------------------
DsemOBP7  TIKNNQPQQETIIKEIKVM-----MPAELQDRNIGAARKCAALEFN---DDACVAAWQYIK-------------------------------------------------------------------
DsemOBP1  VIDEDSGDFEYEMLLGFFP-----EDIQ--SQGRDILGGCA---------------------------------------------------------------------------------------
DsemOBP3  VIDEDSGDFEYEMLLGFFP-----EDIQ--SQGRDILGGCA---------------------------------------------------------------------------------------
DsemOBP2  VIDEDSGDFEYEMLLGFFP-----EDIQ--SQGRDILGGCA---------------------------------------------------------------------------------------
DsemOBP4  VVRNLNLDN-----------------------------------------------------------------------------------------------------------------------
DsemOBP9  FLTEN-GVFDETTFRAKIP-----ADIP-TEKVDAIVNKCKPMTG----ANACETGAKLLKCYIENKTISIFE-------------------------------------------------------
DsemOBP12 FLTEN-GVFDETTFRAKIP-----ADIP-TEKVDAIVNKCKPMTG----ANACETGAKLLKCYIENKTISIFE-------------------------------------------------------
DsemOBP11 IMNAD-GTFNEEATRAKIP-----SDIA-RDKAEEVITKCKDLTG----ATDCETGMKLMKCYMKNKTFSVLE-------------------------------------------------------
DsemOBP10 HLKED-GTVDSEKAVADLP-----PDVPDRDRVVAAMTECGAQSN----LHRFPAFLINSFVAY----------------------------------------------------------------
DsemOBP14 HLKED-GTVDSEKAVADLP-----PDVPDRDRVVAAMTECGAQKG----DNDCETAGLVWKCLEEKNALP----------------------------------------------------------
DsemOBP16 LVDQRGFPERGSVMEVMTRGV---QDPSLRDFIEESITECFHYLES---DTNNHEKCSFSNNLMNCFADKARDVRYLKMNIHRCSTFFSPRIPTKKKKPFVKIVRIYFSFFSIDLSDFFQRCEDWDNQ
DsemOBP17 LVDQRGFPERGSVMEVMTRGV---QDPSLRDFIEESITECFHYLES---DTNNHEKCSFSNNLMNCFADKARDR-----------------------------------------------CEDWDNQ
DsemOBP15 LMNTD-GSFNLEVARSTLPDIFHINNVQDFITAQNFITKCMNHTE----ANDCETGFQMIMCFMENIAPINPGNSA----------------------------------------------------
DsemOBP5  LFTED-GDIDEDFIIKIVP-------AEYREIAIPVIRGCGTIKG----ADLCETAFLTNKCWHSK--------------------------------------------------------------
DsemOBP8  MVDNK-KELDLKGMLTFFQR-----IPAYREEVRRAVSHCKVIARTSVSGDNCQYAYTFNRCYADLSPNTYYLF------------------------------------------------------
DsemOBP13 MMGPD-GKLDVNAAIDKVP------NAPFHDGLVGAINSCANQSG----RNNCDTARRLLACMQDQGVPSMFKV------------------------------------------------------.
```

Figure 16. The alignment of 17 DsemOBP amino acid sequences. The six conserved cysteines were highlighted in red color.

3.4.2 Phylogenetics analysis of DsemOBPs

The phylogenetic analysis between 17 DsemOBPs and 21 *A. mellifera* OBPs (AmelOBPs) showed that most OBPs form species-specific subfamily, as *D. semiclausum* specific OBPs or bee-specific OBPs (Figure 17A). Two groups of *D. semiclausum* specific OBPs were identified and labelled (Figure 17A), which may play specific roles in *D. semiclausum* biology and behaviours, for example, laying eggs to DBM larvae. Interestingly, a few OBPs were clustered together between these two species (Figure 17A). For example, DsemOBP5, DsemOBP8 and AmelOBP10 were clustered on the same branch. Similarly, DsemOBP6, DsemOBP7 and AmelOBP5 were clustered on the same branch, suggesting they may share similarities at the amino acid level and play the same functions between *D. semiclausum* and *A. mellifera*.

Furthermore, to study the chemosensory interactions between the host insect (DBM) and the parasitoid wasp (*D. semiclausum*), the phylogenetic analysis was performed between 17 DsemOBPs and 39 PxylOBPs (Cai et al. 2020). The results showed that most of OBPs are species-specific as well because *D. semiclausum* and *P. xylostella* belong to two completely different families: one is Hymenoptera, and the other is Lepidoptera. There is no DsemOBP clustered with *P. xylostella* PBP/GOBP subfamily (Figure 17 B), a conserved OBP group in Lepidoptera species. Interestingly, DsemOBP13 and PxylOBP23 were clustered together, which shared 26.52% identities at the amino acid level. DsemOBP5 and PxylOBP31 were clustered together, which showed 35.25% identity at the amino acid level.

3.4.3 Expression profiles of DsemOBPs

Expression profiles of DsemOBPs can help build the links between the genes and their functions. Here, 17 identified DsemOBPs were examined between male and female adult antennae by using RT-PCR. All 17 DsemOBPs were successfully amplified, and the band sizes are the same as expected in the RT-PCR analysis (Figure 18), suggesting the primers were designed properly. DsemOBP2 and DsemOBP5 were only amplified from male antennae, suggesting they may play the male-specific roles, for example, detecting sex pheromones released by females or localizing the females for mating. Other DsemOBPs were amplified in both male and female adult antennae, which may play the same roles between male and female adults, for example, detecting plant volatile compounds and assist the wasps to localize nectar sucking as energy resources.

To study the expression levels in a more accurate way, the qRT-PCR approach was applied by using *D. semiclausum RPL8* and *RPL13a* genes as reference genes (Figure 19). The results showed that there are clearly three types of expression levels of DsemOBPs. Type 1, male-specific or male-rich OBPs, means the OBP expression was significantly higher in male antennae than female antennae, including DsemOBP 1, 3, 4, 5 and 16 (Figure 19). Type 2 are the female-specific or female-rich OBPs, which are significantly highly expressed in female antennae comparing to male antennae, including DsemOBP 6, 7, 8, 9, 10 and 14. The other DsemOBPs, which did not show significant differences between males and females, belong to type 3 (Figure 19).

Further, another reference gene, *DsemOrco*, was used in this study to normalize the expression profiles of DsemOBPs and compare their expression levels between male and female antennae. *DsemRPL9* and *DsemRPL13a* are housekeeping genes that can be expressed anywhere through the whole body. *DsemOrco* is an antennae specific co-receptor gene, which assists other odorant receptors to function appropriately in the antennae (Benton et al. 2006). Therefore, it is believed that *Orco* is a more suitable reference gene to normalize the expression of the olfactory genes in the antennae (Pelletier and Leal 2009). The results (Figure 20) are quite similar as described using *RPL8* and *RPL13a* (Figure 19). DsemOBP6, 7, 8, 9, 10, 11, 14 showed significantly higher expression in female antennae than in male antennae, which may play important roles for female-specific behaviours such as oviposition on the DBM. DsemOBP6, 7, 8 and 10 showed nearly four times higher expression in male adult antennae than in female adult antennae. On the other hand, DsemOBP5 showed nearly twice higher expression in male adult antennae than in females, suggesting it may contribute to male-specific behaviours such as sex pheromone detection or mating behaviours (Figure 20).

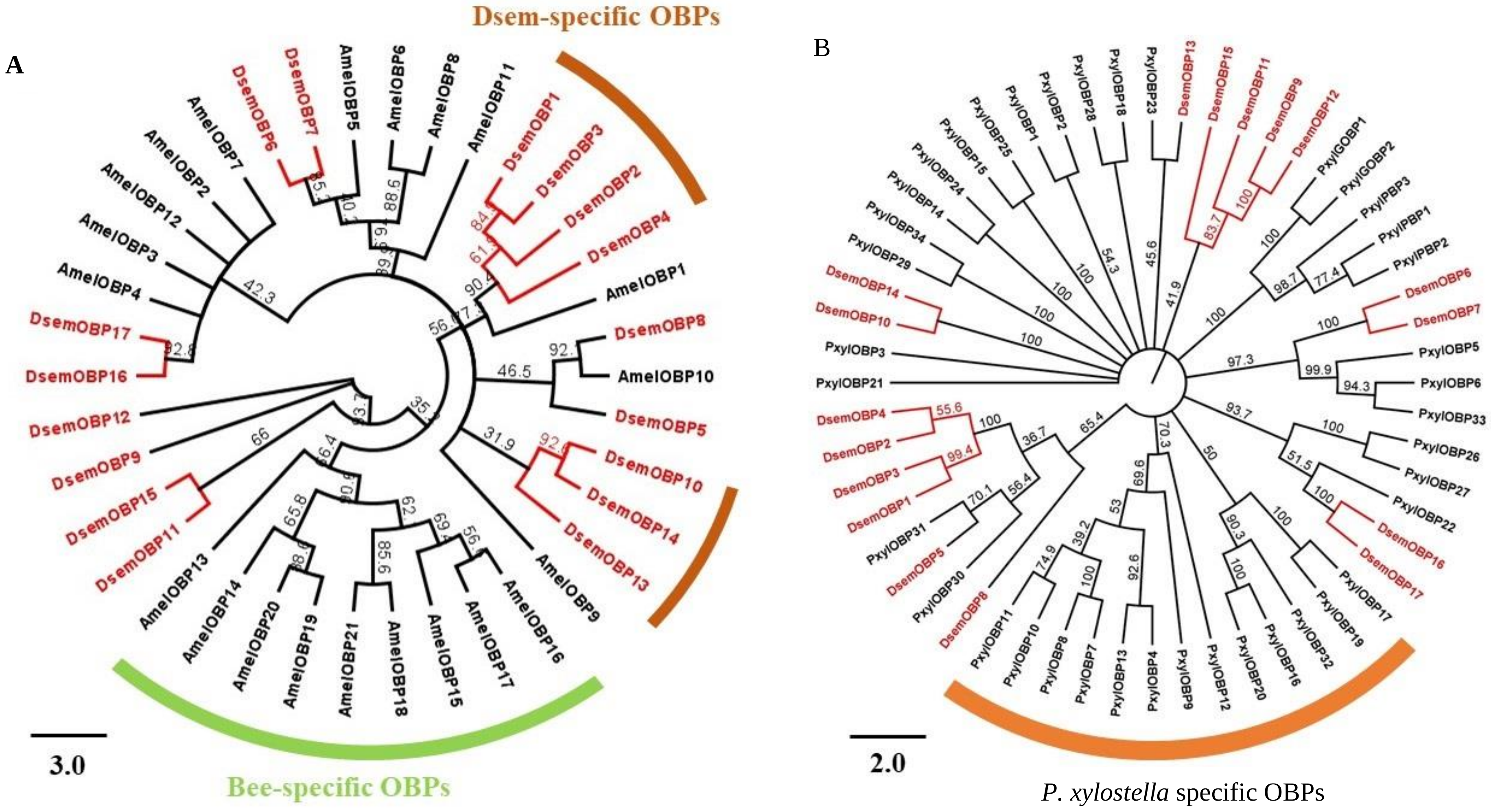


Figure 17: Phylogenetic analysis of *D. semiclausum* OBPs (DsemOBPs) and (A) *Apis mellifera* OBPs (AmelOBPs), (B) *Plutella xylostella* (PxylOBPs)

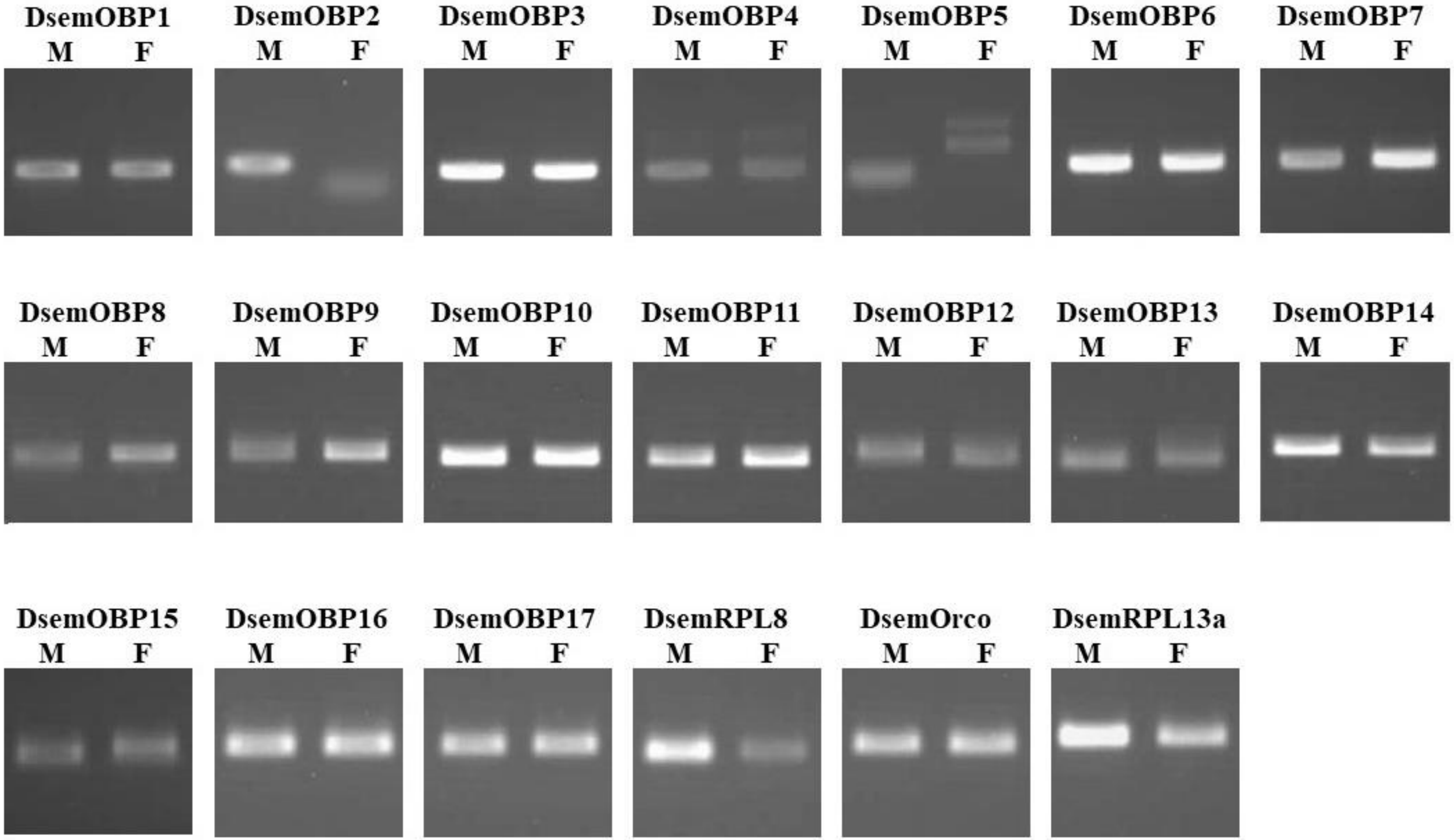


Figure 18. RT-PCR analysis of DsemOBPs between male (M) and female (F) adult antennae. DsemRPL8, DsemOrco and DsemRPL13a were used as control.

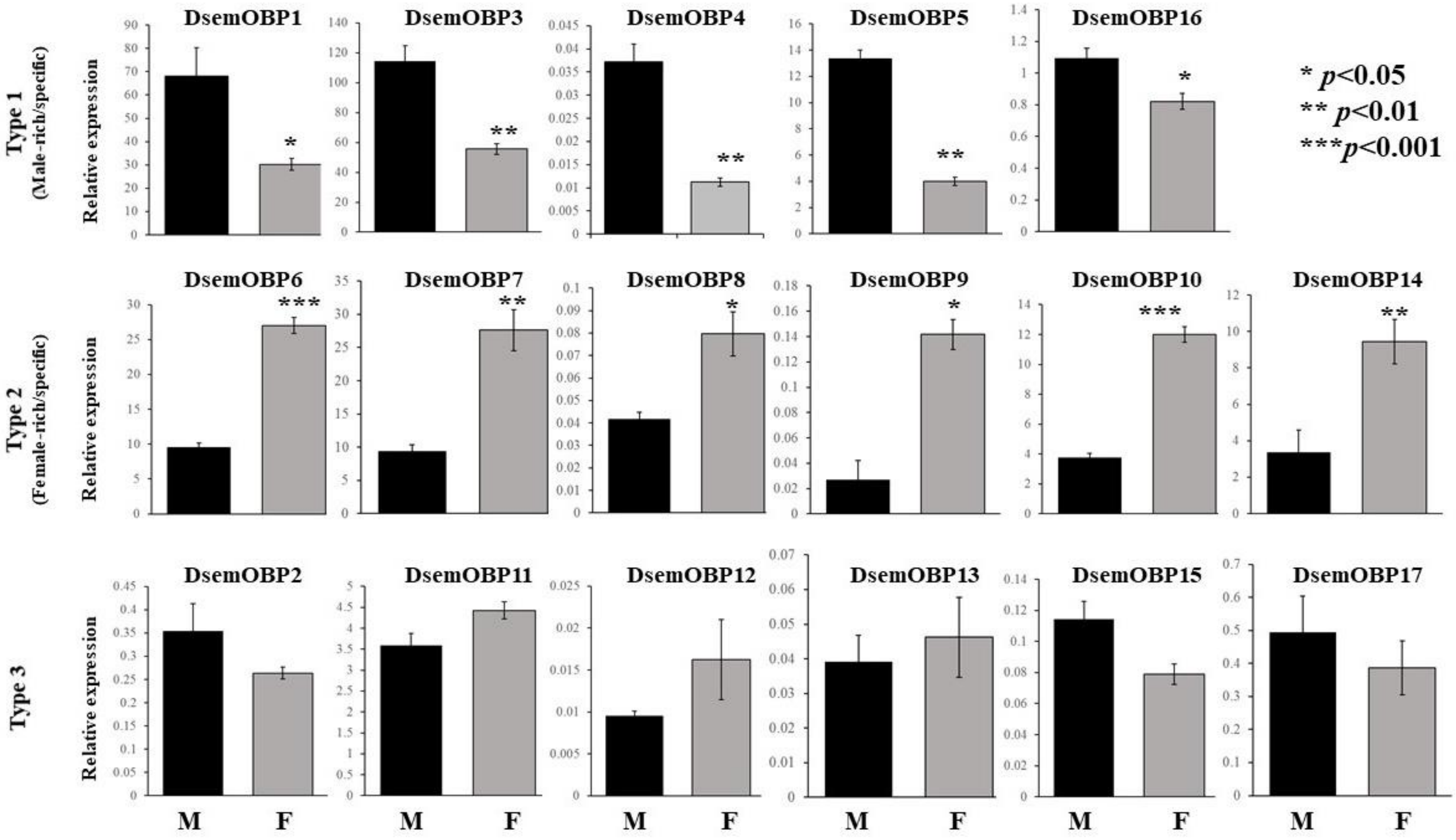


Figure 19. Relative expression profiles of 17 DsemOBPs in male (M) and female (F) antennae using quantitative RT-PCR. DsemRPL8 and DsemRPL13a genes were used as the reference genes for the normalization. * means p < 0.05, ** means p value < 0.01 while *** means p value <0.001

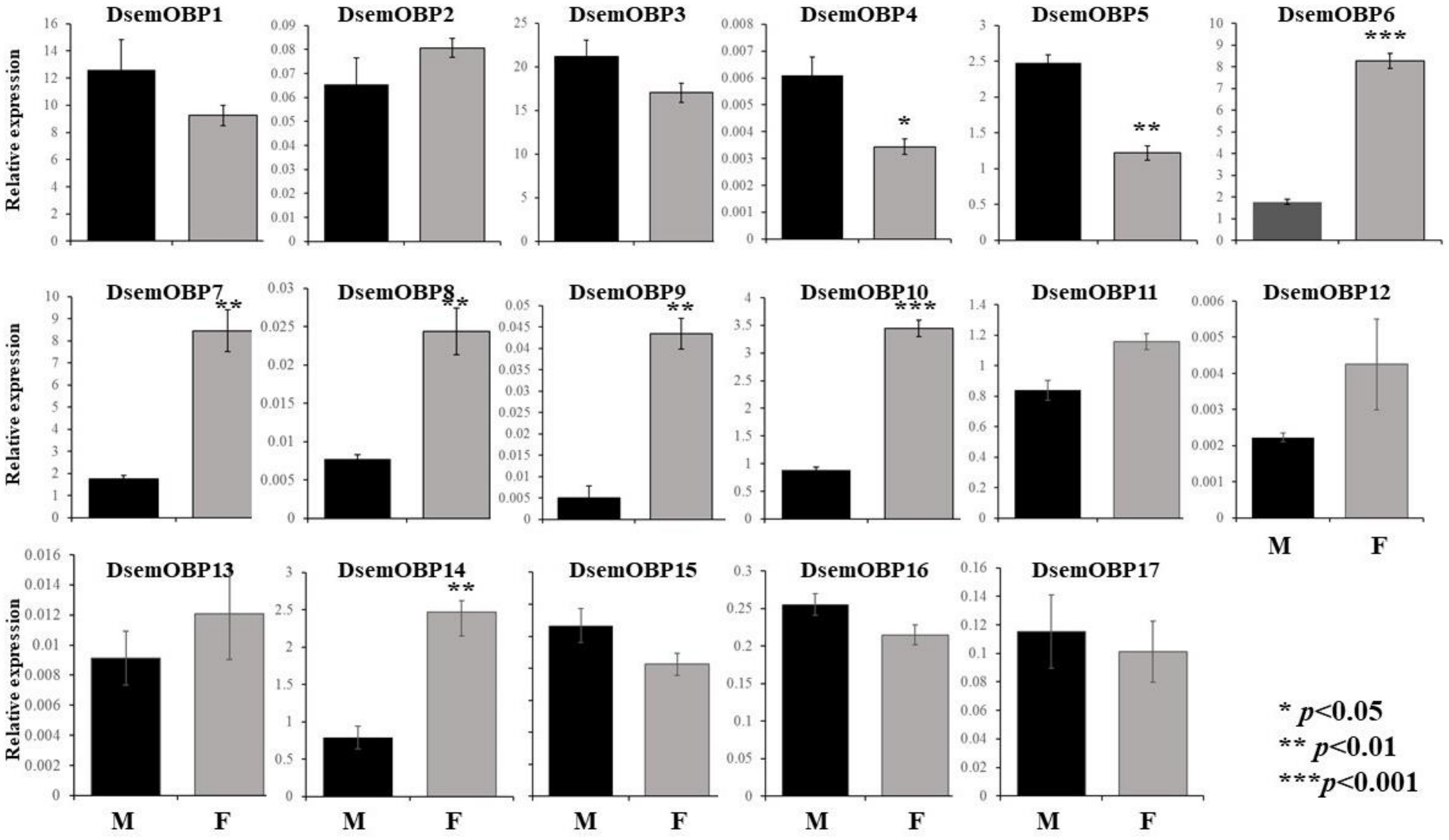


Figure 20. Relative expression profiles of 17 DsemOBPs using quantitative RT-PCR. Dsemorco gene were used as the reference gene for the normalization. * means p < 0.05, ** means p value < 0.01 while *** means p value <0.001.

3.4.4 Phylogenetic analysis and expression profiles of DsemORs

A total of 67 OR genes were identified from *D. semiclausum*, including DsemOrco gene. Phylogenetic analysis between DsemOR and *A. mellifera* ORs was performed, and the result showed that most ORs are species-specific (Figure 21A). It revealed the differences in the biology between *D. semiclausum,* and *A. mellifera*. *D. semiclausum* is a specific DBM larval parasitoid, and its ORs help *D. semiclausum* detect and localize *P. xylostella*. However, *A. mellifera* is an important pollinator and help plants to pollinate.

However, a few conserved ORs between *D. semiclausum* and *A. mellifera* were identified. DsemOR30, AmelOR1 and AmelOR3 are clustered together. DsemOR48, and AmelOR62 are clustered on the same branch. These ORs may support the wasps to find the nectar as an energy resource. Further, the phylogenetic analysis of the ORs from *D. semiclausum* and its host, *P. xylostella* was conducted (Figure 21B). Both two insects live in the same environment, so they are very likely to detect the same compounds to regulate their behaviours. The results showed that no ORs are conserved between these two species.

Using transcriptome sequencing data, a heatmap of the expression profile of the 67 DsemORs between male and female antennae was built (Figure 21C). A number of OR genes showed male antennae specific or rich expression, for example, DsemOR1, 4, 5, 6 and 48. They may play important roles in the reception of the sex pheromone and mating behaviours. Various OR genes showed female antennae specific or rich expression, including DsemOR2, 12, 16, 32 and 35, which may function for the female adults to detect the *P. xylostella* larvae for oviposition.

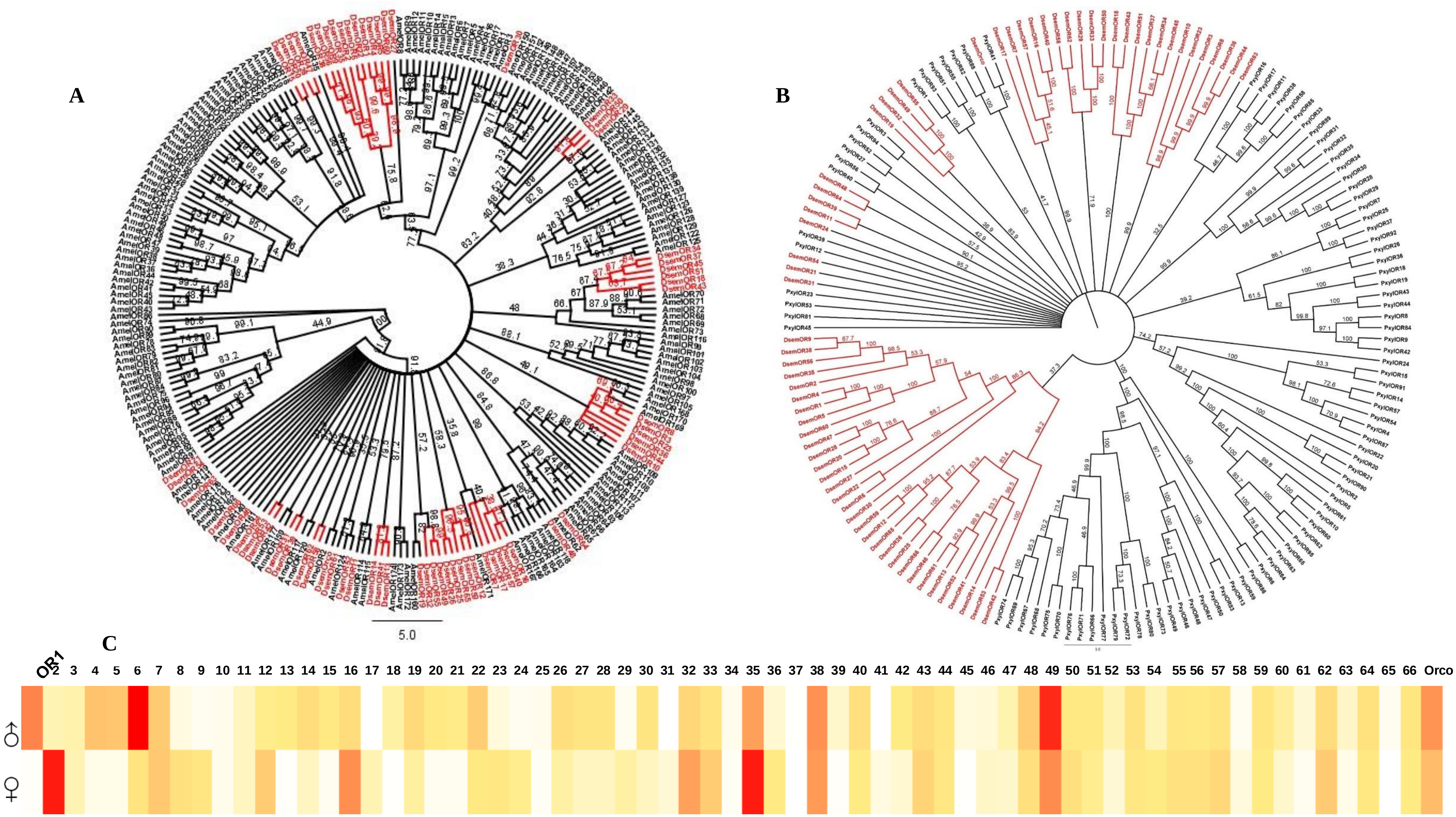


Figure 21. Phylogenetic analysis between DsemORs and: (A) AmelORs, (B) PxylORs, and (C) expression profile of DsemORs between male and female antennae

3.5 Discussion

Insects and their natural enemies live in the same ecosystem, so it is very likely they can detect the same semiochemicals around them to regulate their behaviours. For example, moths (*Heliothis virescens*) are repelled by herbivore-induced volatiles released from tobacco plants at night; such odour cues may allow females to avoid oviposition on previously damaged plants (Paré and Farag 2004). Electroantennographic recordings (EAG) and behavioural analysis consistently showed not only significantly higher responses to (**E**)-ß-farnesene than to structural related compounds but also adaptation to the alarm pheromone (Al Abassi et al. 2000, Verheggen et al. 2007, Verheggen et al. 2008, Kielty et al. 1996). All these studies demonstrated that insects and their natural enemies could detect certain same odorant compounds by using their olfactory systems, suggesting they may share certain conserved olfactory proteins such as OBPs or ORs. In my previous study, a number of compounds that elicit physiological or behavioural responses of DBM can also initiate *D. semiclausum* EAG responses. For example, cis-11-hexadecanal is a sex pheromone component of DBM. *D. semiclausum* can detect this compound, suggesting that *D. semiclausum* also has a receptor for cis-11-hexadecanal to help *D. semiclausum* to locate the DBM for parasitism. Therefore, *D. semiclausum* and *P. xylostella* are sound models for a comparative study between their OBPs and ORs. Since *D. semiclausum* lay eggs on *P. xylostella* larvae, it was hypothesized that they both detect the same compounds from plants and *P. xylostella*. Moreover, my previous studies showed that male and female *D. semiclausum* antennae exhibited different responses to the same compounds, suggesting their OBP or OR expression profiles are different. To perform an insightful study on these OBP and OR genes, the first step is to identify these genes.

Therefore, a transcriptome assembly was built using *D. semiclausum* adult antennae and identified 17 putative OBP and 67 OR genes. The numbers of ORs in Hymenoptera species are generally very high. For example, 177 OR genes have been identified from European honeybee (*A. mellifera*), and such a large number of ORs may be used to detect the odorants from various flowers for nectars and pollens. It is not surprising that a significantly lower number of OR genes (only 67) were identified from *D. semiclausum* because here, we only have transcriptome data on the antennae, not a genome sequence. Many OR genes with low expression in the antennae may not be identified by using transcriptome sequencing. Once the genome sequencing of *D. semiclausum* is available, more ORs will be identified. Moreover, *D. semiclausum* is a parasitoid wasp and spend immature (egg and larvae) stages in their host insects (DBM), which may decrease the number of ORs in the evolution since they do not need a robotic and sensitive olfactory system at the larvae stage.

Then, the *D. semiclausum* OBP and OR genes were compared with *P. xylostella* OBP and OR genes to investigate if they have certain similar olfactory genes. *P. xylostella* OBP and OR have been annotated and published (Cai et al. 2020, You et al. 2013), which provide us with an important database for this study. Insect hosts and their parasitoid wasps, which always dwell in the same ecosystem, may share similar OBPs or ORs to help detect the same volatile compounds from their environment. The results showed that certain DsemOBPs and PxylOBPs exhibit similarities but not ORs. *D. semiclausum* and *P. xylostella* are two totally different species: one is Hymenoptera, and the other is Lepidoptera. No conserved OR genes between these two species were identified, but DsemOBP13 and PxylOBP23 shared 26.52% identities at the amino acid level. DsemOBP5 and PxylOBP31 showed 35.25% identity. These DsemOBPs will be my targets for further examination; the questions including: which compounds do they bind? Do they bind the same compounds? After knock-down or knock-out of these genes, will it disrupt *D. semiclausum* parasite behaviours on *P. xylostella*?

No sequence similarity does not mean OBPs or ORs cannot detect the same compounds. For example, an endogenous OR in the fruit fly, *D. melanogaster,* is highly sensitive to the sex pheromone of the silkworm moth, bombykol (Syed et al. 2010). Intriguingly, the fruit fly detectors are more sensitive than the receptors of the silkworm moth, although its ecological significance is unknown. These two receptors showed extremely low similarities at the amino acid level.

RT-PCR, qPCR and *in silico* expression profiles were applied to identify the male or female-specific OBPs and ORs. Only females lay eggs on the host insect eggs, so the female-specific OBPs or ORs are more likely to play a significant role in the parasite behaviours. DsemOBP6-10, DsemOR2, DsemOR16, DsemOR32 and DsemOR35 are female-specific or female-rich OBPs, which are the candidate OBPs or ORs to assist in *D. semiclausum* oviposition behaviours. Meanwhile, a number of male-specific or male-rich OBPs were also identified, including DsemOR5, DsemOR1 and DsemOR4-6, which may contribute to the sensation of sex pheromones released by female adults.

3.6 Conclusion

In summary, 17 putative OBP, 67 OR and nine GR genes were identified from the transcriptome sequence of *D. semiclausum* adult antennae, which were further studied by phylogenetic and bioinformatics methods. The expression patterns of *D. semiclausum* OBPs identified a number of male-specific/rich and female-specific/rich OBPs and ORs. This study helps better understanding of the olfactory system of *D. semiclausum* at the molecular level and will help optimize our integrated pest management strategy for this species.

Table 4. DsemOBP primers designed for RT-PCR and qRT-PCR.

Primers	Sequences
DsemOBP1F	CGGCTTTACTCATCGTCGTG
DsemOBP1R	ATCTTCGTCGATCACACCGA
DsemOBP2F	CAATTTTCGCAGCTACCGGA
DsemOBP2R	ATCTTCGTCGATCACACCGA
DsemOBP3F	ACGACAAAGTACGATGCATGG
DsemOBP3R	AGCCTCCAAGAATATCGCGA
DsemOBP4F	CAATTTTCGCAGCTACCGGA
DsemOBP4R	TTCCTCACCACACCGAATGA
DsemOBP5F	ATGGCTCAAGTACTGCACGA
DsemOBP5R	ATCTTCGTCGATGTCACCGT
DsemOBP6F	GTGGCTGGTTTGAGAAGTGG
DsemOBP6R	AAGCTGCACATTTTCTCGCT
DsemOBP7F	GTGGCTGGTTTGAGAAGTGG
DsemOBP7R	AAGCTGCACATTTTCTCGCT
DsemOBP8F	TGGCCGGAGACAGAATCATT
DsemOBP8R	GTGTTTGGTGATAGGTCGGC
DsemOBP9F	CCGAGGAACACAAAGCGAAA
DsemOBP9R	TGTGGGAATATCGGCTGGAA
DsemOBP10F	GTTCTCGGAGACCCAGGAAA
DsemOBP10R	GATGACCGTGTTCTCGCATC
DsemOBP11F	AGTGCCTGTCTTTTGAAGCG
DsemOBP11R	TTCCAGTCTCACAGTCCGTC
DsemOBP12F	TTTTCAGGCCGCAATATCCG
DsemOBP12R	CAAGAAACACGCTGCAAAGC
DsemOBP13F	CGATTGTTACATGGCCTGCA
DsemOBP13R	TGCATACAGGCCAAAAGACG
DsemOBP14F	CGACAAAGACGCCATGACAT
DsemOBP14R	ACTGTTCCGTCCTCCTTGAG
DsemOBP15F	CTCGCTCTACACTTCCCGAT
DsemOBP15R	TCCGGGATTTATGGGAGCAA
DsemOBP16F	CCACACGTCGCAATCTCAAA
DsemOBP16R	TGAAATCGCGTAGACTCGGA
DsemOBP17F	GCGTGGCTCAGTAATGGAAG
DsemOBP17R	TTGTCCCAATCCTCGCATCT
DsemORCOF	TCGGTCACTTTCTTCACGGA
DsemORCOR	CGGACCCTCGGAATATGGAA
DsemRPL8F	GATAAGCACGTCCAGCCTTG
DsemRPL8R	CGTTATCGCTCACAACCCTG
DsemRPL13AF	CGTTTTCTGCGGTCATAGGG
DsemRPL13AR	CGAACAGCTCAACATCTCG

Chapter 4

Identification and characterization of OBP and OR genes in the parasitoid wasp *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae)

Statement of Contribution

Title of Paper	Identification and characterization of olfactory proteins in the parasitoid wasp *Trichogramma pretiosum* Riley
Publication Status	Under Revision
Publication Details	***Austral Entomology***
Principal Author	
Name of Principal Author (Candidate)	Basman Al-Jalely
Contribution to the Paper	Methodology, Collected Data, Writing- Original Draft Preparation, Conceptualization and Validation
Overall Percentage (100%)	90 %
Signature	Bas Date 24/08 /2021

Co-Author Contributions

By signing the statement of contribution, each author certifies that:

- The candidate's stated contribution to the publication is accurate (as detailed above).
- Permission is granted for the candidate to include the publication in the thesis
- The sum of all co-author contributions is equal to 100% less the candidate's stated Contribution

Name of Co-Author	Wei Xu
Contribution to the Paper	Supervision, Funding acquisition, Validation, Conceptualization
Overall Percentage (100%)	10 %
Signature	Wei Xu Date 24 /08/2021

4.1 Abstract

Trichogramma pretiosum Riley (Hymenoptera: Trichogrammatidae) is a tiny natural egg parasitoid of agricultural pest insects, which has been widely used in the biological control for *Plutella xylostella*, *Helicoverpa armigera*, *Spodoptera frugiperda* and *Ectomyelois ceratoniae*. However, limited studies have been conducted on *T. pretiosum* olfactory system, which is critical in regulating insect behaviours. In this study, *T. pretiosum* adult antennae were investigated under scanning electron microscopy (SEM). Four types of olfactory sensilla were observed, including chaetica sensilla (CS), trichoid sensilla (TS), faleate sensilla (FS) and placoid sensilla (PS). By using *T. pretiosum* genome sequence, 22 putative odorant binding proteins (OBPs) and 105 odorant receptors (ORs). The expression patterns of OBPs between *T. pretiosum* male and female adults were examined by reverse transcription-polymerase chain reaction (RT-PCR), and quantitative real-time PCR (qRT-PCR) approaches. Three female-specific OBPs (TpreOBP19, TpreOBP15 and TpreOBP3) were identified, which may play crucial roles in *T. pretiosum* host-seeking and oviposition behaviours. This study enriches our knowledge of *T. pretiosum* olfactory genes, improves our understanding of its olfactory system, and may help refine employing *T. pretiosum* in the integrated pest management schemes in future.

4.2 Introduction

Insect behaviours, including mating, foraging, host-finding and oviposition (Leal 2013) are guided by their olfactory systems. Hair-like olfactory sensilla distributed over the surface of antennae are utilized by insects to detect chemical signals from the environment (Keil 1984a, b, Larsson et al. 2002). With the advance of molecular and cellular biology, the understanding of insect olfactory mechanisms has extensively progressed. Several protein families have been reported playing pivotal roles in the dynamics, selectivity, and sensitivity of the insect olfactory system, including odorant binding protein (OBPs) (Vogt and Riddiford 1981), odorant receptors (ORs) (Clyne et al. 1999, Vosshall et al. 1999), ionotropic receptors (IRs) (Liu et al. 2018), sensory neuron membrane proteins (SNMPs) (Zhang et al. 2020) and odorant-degrading enzymes (ODEs) (Vogt and Riddiford 1981, Vogt, Riddiford, and Prestwich 1985). Hydrophobic odorants lack solubility, so it is difficult for them to pass through the aqueous sensillum lymph and reach the ORs. OBPs, one class of proteins that are highly expressed in antennae, bind, solubilize and deliver these bound semiochemical molecules to the receptors (Leal 2003, Pelosi et al. 2018). ORs are receptors localized on the dendritic membrane in insect olfactory sensilla, detecting odorant compounds and transducing these olfactory signals to brains to regulate behaviours (Fleischer et al. 2018). IRs, a variant subfamily of ionotropic glutamate receptors, work as ligand-gated ion channels in chemo-sensation (Liu et al. 2018, Rimal and Lee 2018). SNMPs, a subfamily of CD36 proteins, have been shown playing a critical role in pheromone detection in insects (Zhang et al. 2020). ODEs can degrade the odorant compounds after they activate ORs, clean the system (Vogt and Riddiford 1981, Vogt, Riddiford, and Prestwich 1985, Ishida and Leal 2005) for the new cycle of detection. OBPs and ORs are invovled in the first step of odorant sensation, so they are the major target proteins in this study.

The first insect OBP was discovered at the beginning of the 1980s in the giant moth *Antheraea polyphemus* using the tritium labelled specific pheromone (E, Z)-6, 11-Hexadecenyl acetate as a probe (Vogt and Riddiford 1981). The functional roles of insect OBPs have been addressed. LUSH is an OBP of the fruit fly *Drosophila melanogaster*. Deletion of the LUSH gene suppresses *D. melanogaster* electrophysiological and behavioural response to the male pheromone 11-cis-vaccenyl acetate (cVA) (Xu et al. 2005). *Bombyx. mori* pheromone binding protein 1 (BmorPBP1) is the first structurally investigated OBP using X-ray diffraction spectroscopy (Sandler et al. 2000) and nuclear magnetic resonance (NMR) techniques (Horst et al. 2001, Lee et al. 2002). Structures of other insect OBPs have been published, including

LUSH from *Drosophila melanogaster* (Kruse et al. 2003), Amel-ASP1 from *Apis mellifera* (Lartigue et al. 2004), ApolPBP1 from *Antheraea polyphemus* (Damberger et al. 2007), AgamOBP1 from *Anopheles gambiae (Wogulis et al. 2006)*, AaegOBP1 from *Aedes aegypti* (Leite et al. 2009). CquiOBP1 from *Culex quinquefasciatus* (Mao et al. 2010) and AtraPBP1 from *Amyelois transitella* (Xu et al. 2010). All these proteins are structurally homologous to BmorPBP1 with helices and three disulphide bridges connecting the helices in a similar manner.

In my previous studies, 17 OBPs and 67 ORs were identified from *Diadegma semiclausum*, an important parasitoid wasp widely used in biological control for the diamondback moth (DBM), *Plutella xylostella*. However, are similar OBPs, or ORs also expressed in other parasitoid wasps of DBM is unknown. The completion of the whole genome sequences of the *Trichogramma pretiosum* provides a platform for us to analyze olfactory genes in this minute parasitoid wasp (Lindsey et al. 2018). The polyphagous endoparasitoid wasp of insect eggs, *Trichogramma spp.*, is amongst many groups of egg parasitoids that are commonly utilized for biological control programs globally (Pereira et al. 2019) and the most researched and used as natural enemies in the world (Knutson 1998). *Trichogramma* wasps can parasitize and develop inside very small host eggs as maintaining the number of monoaminergic neurons can maintain cognitive and behavioural complexity and allow these smallest wasps to find suitable hosts (Van Der Woude and Smid 2017), including the *P. xylostella*, *Helicoverpa armigera* and *Ectomyelois ceratoniae*. While *D. semiclausum* is a specialist wasp and only lays eggs into DBM larvae, *T. pretiosum* is a generalist and widely used in integrated pest management (IPM) on various pest moth eggs. As olfactory systems are critical in regulating their behaviours, the comparative study on the olfactory genes between these two parasitoid wasps may shed light on their host-seeking behaviours.

In this study, the genome database of *T. pretiosum* was used to identify the key olfactory genes, including OBP and OR genes, which were further analyzed using bioinformatics, phylogenetics and molecular approaches. Improved knowledge of the olfactory system will assist in the development of more efficient and environmentally friendly pest-controlling strategies.

4.3 Materials and methods

4.3.1 Insects

T. pretiosum pupae were sourced from Bugs for Bugs™ (https://bugsforbugs.com.au/) and kept in the lab at 25± 1 (°C), 70-80% (R.H.) and 16:8 h (L:D) photoperiod. One hundred and thirty emerging adult pairs were collected immediately from supplied sheets using vacuum traps and anaesthetized using food-grade carbon dioxide (Purity>99.9%, Moisture<100ppm) for five minutes then sexed under a stereomicroscope using antennae structures as *T. pretiosum* body sizes are tiny (< 1 mm), and their antennae are strongly sexually dimorphic. One hundred males and 100 females were allocated for the molecular study, and the rest were allocated for the scanning electron microscopy examination.

4.3.2 RNA purification.

T. pretiosum adults for RNA purification were immediately stored in liquid nitrogen and then homogenized using a pestle. Total RNA was purified using the Qiagen RNeasy mini kit (Qiagen, USA) following the manufacturer's protocol. The purified total RNA was quantified, quality checked using NanoDrop™ ND-2000 (Thermo Scientific, USA) and stored at -80 °C in Western Australia State Agricultural and Biotechnology Centre (SABC).

4.3.3 Scanning electron microscopy (SEM)

To prepare the samples for the SEM, insects allocated for the scanning electron microscopy examination were preserved in 3% Glutaraldehyde in 0.025 M Phosphate buffer, pH 7.0 for 24 hours and then a standard protocol (Table 1) was used for fixation with Pelco Biowave processor. Critical point drying apparatus Polaron E3000 was used in the preparation process with a Critical point of CO_2 = 31.1 C˚ and 1071 psi. Dried samples were mounted on SEM stubs under Olympus SZH10 microscope then sputtered with 10nm gold (Polaron Sputter coater SC 7640 (Quorum Technologies, UK) with Argon gas) (Pressure $<1x10^{-2}$ mbar, Voltage= 1 kv). Samples were examined and photographed under the Zeiss 1555 VP-FESEM Scanning Electron Microscope operated at 10 kV, high current, 10–12 mm working distance, and 30 µm aperture. Sample preparation and examining were conducted at the Centre for Microscopy, Characterization, and Analysis (CMCA) / University of Western Australia.

4.3.4 Bioinformatic and phylogenetic analysis

Genes encoding for *T. pretiosum* OBPs and ORs in genome (https://i5k.nal.usda.gov/webapp/blast/ and https://www.ncbi.nlm.nih.gov/genome/14106) were identified using BLAST searches with reported *D. melanogaster* and *Apis mellifera* OBP and OR genes as a query as previously described (Xu et al. 2015). Extensive manual curation was then performed on the *T. pretiosum* genome according to exon/intron splice site of GT-AG (Chambon's) rule. The identified OBP and OR amino acid sequences were used for validation by NCBI blast based on the identity and similarity to orthologous genes from other insects. All identified *T. pretiosum* OBP and OR amino acid sequences are available in an online supporting file (Supplementary Table 2 and Supplementary Data 2).

Encoded TpreOBPs were aligned for clustal W in Geneious 8.0, a bioinformatics software for sequence data analysis using default settings. Gap Opening Penalty (10.00) and Gap Extension Penalty (0.10) were used for multiple sequence clustal alignment with 30% Delay Divergent Cutoff. N-terminal signal peptides of TpreOBPs were predicted using SignalP 5.0 (http://www.cbs.dtu.dk/services/SignalP). The calculated molecular weights (MW) and isoelectric points (pI) were obtained using the ExPASy proteomics server (http://www.expasy.org/tools/protparam.html). The amino acid sequences of TpreOBPs were used to search the best blast hit sequences from NCBI using blastp (Supplementary Table 2). The Exon-Intron graphics were generated with GSDS (http://gsds.cbi.pku.edu.cn/index.php).

The TpreOBP and TpreOR amino acid sequences were used to create an entry file for phylogenetic analysis in Geneious 8.0 with *D. semiclausum* or *P. xylostella* OBP and OR protein sequences. Firstly, the amino acid sequences were aligned using Geneious alignment with default settings: Global alignment with free end gaps, Cost Matrix (Blosum62), Gap open penalty (12), Gap extension penalty (3) and Refinement iterations (2). A UGMA tree was then constructed using the default settings based on Jukes-Cantor Model with Bootstrap as a resampling method, and 1000 replicates and a 30% support Threshold.

4.3.5 RT-PCR and quantitative RT-PCR

cDNA templates were prepared from purified total RNA samples using the SuperScript™ VILO™ cDNA Synthesis Kit (Invitrogen, USA), according to the manufacturer's protocol (Incubation at 42 C° was increased to 90 minutes instead of 60 minutes). Reverse transcriptase (RT)-PCR was performed using gene-specific primers (Table 5), which were designed using the Primer3web (version 4.1.0) software (http://bioinfo.ut.ee/primer3/). RT-PCR was performed using (*Taq* DNA Polymerase) (New England Biolabs, Australia) as follows: 95 °C for 30 s; 40 cycles at 95 °C for 25 s, 55 °C for 30 s and 68 °C for 20 s; and a final extension at 68 °C for 5 min then hold at 10 ˚C. The PCR products were analyzed using 1.0% agarose gel electrophoresis and 6× DNA Loading Dye (Bio-Rad, Australia) under 75 voltages as previously reported (Xu et al. 2017) with a 100 bp marker. The agarose gels were examined under the Bio-Vision system (Vilber Lourmat, France).

Quantitative Real-Time PCR (qRT-PCR) was performed using the same gene-specific primers in table 5 with reference genes GAPDH. A 2-Step qPCR protocol was performed on Rotor-Gene Q-5 Plex (Qiagen, US) using Power SYBR® Green PCR Master Mix (Thermo Fisher Scientific, Australia) following the protocol Bio-Rad CFX96 Real-Time PCR Detection System (Xu and Liao 2017). The qRT-PCR results were examined based on the Ct values for each gene and normalized by TpreGADPH gene. For each cDNA sample and primer set, reactions were run in triplicate, and average fluorescence Ct values were obtained. Relative expression levels were determined using the comparative $2^{-\Delta\Delta Ct}$ method for relative quantification (Pfaffl 2001). Statistical analysis was performed on the expression profiles between male and female adults using student t-test (SPSS). Three biological replicates were performed. The symbol * means $p < 0.05$, ** means p value < 0.01 while *** means p value <0.001.

4.4 Results

4.4.1 SEM antenna characterization

Both male and female antennae consist of an elongated scape (Sc) with basal radicle (R), pedicel (P) and flagellum (F). The flagellum is differentiated into basal anelli (ring segments), funicle and apical club (C), which are all apparent in the female antenna (Figure 22). The female club segment is broadest at its midpoint, slightly tapered, curved and is apically blunt. The apex is flattened on the dorsal surface and slightly curved on the ventral surface. The club is covered with numerous types of antennal sensilla on the surfaces.

The male antenna has a distinct thin first anellus. The second anellus and funicular segments are fused with the club to form an elongate tube-like structure called a club. The club is slightly curved with a blunt apex. The surface of the male's scape and pedicel are similar to that of the female antenna, but the surface of the club is more irregularly corrugated and covered with numerous relatively long sensilla.

Based on the shape, three types of sensilla were identified from male adult antennae, and they are chaetica sensilla (CS), trichoid sensilla (TS) and Placoid sensilla (PS) (Figure 22). However, in female adult antennae, four types of sensilla were identified, and they are chaetica sensilla, trichoid sensilla, Placoid sensilla and faleate sensilla (FS). The different structures of adults.

4.4.2 Identification of TpreOBPs

A total of 22 TpreOBPs were identified from *T. pretiosum* genome project, and all of them are full-length sequences. Using blast, all of them showed 66-99% identity at the amino acid level to known insect OBPs. For example, TpreOBP2 showed 99% identify to OBP2 (Sequence ID ANG08492.1) from *Trichogramma dendrolimi*. TpreOBP3 showed 97% identify to OBP3 (Sequence ID ASA40277.1) from *Trichogramma japonicum*.

No signal peptide was predicted from TpreOBP7, while all the other 21 TpreOBPs carry signal peptides. The mature (without signal peptide) TpreOBPs range from 109 to 153 amino acids, and their molecular weights range from 12, 279 to 16, 899 Da (Supplementary Table 2). The isoelectric points (pI) of most TpreOBPs are below 7.0 except TpreOBP1, TpreOBP14, TpreOBP18 and TpreOBP20, whose pIs are higher than 7.0. The amino acid and mRNA sequences, signal peptides, molecular weight, and other information of these OBPs were provided (Supplementary Table 2).

The newly identified TpreOBPs were aligned using the Geneious Clustal alignment (Xu and Liao 2017). The alignment of these TpreOBP amino acid sequences highlights the six conserved cysteine residues except TpreOBP7, which does not have the first conserved cysteine (Figure 23A). Most of the *T. pretiosum* OBPs share the characteristic features of the classic OBP family: small size, presence of an N-terminal signal peptide sequence, as well as a highly conserved pattern of six cysteine residues called the “classic motif” (Leal 2013).

The exon/intron structures of the TpreOBPs were shown in Figure 23B. A total of four different exon/intron structures were identified from TpreOBP genes, which consist of two exons, four exons, five exons and six exons, respectively. TpreOBP18 is the only OBP gene consisting of two exons. TpreOBP11 and 17 both contain four exons. TpreOBP2, 4, 6, 7, 8, 13, 14, 16, 20, 21, and 22 genes all contain five exons while TpreOBP1, 3, 5, 9, 10, 12, 15 and 19 all contain six exons.

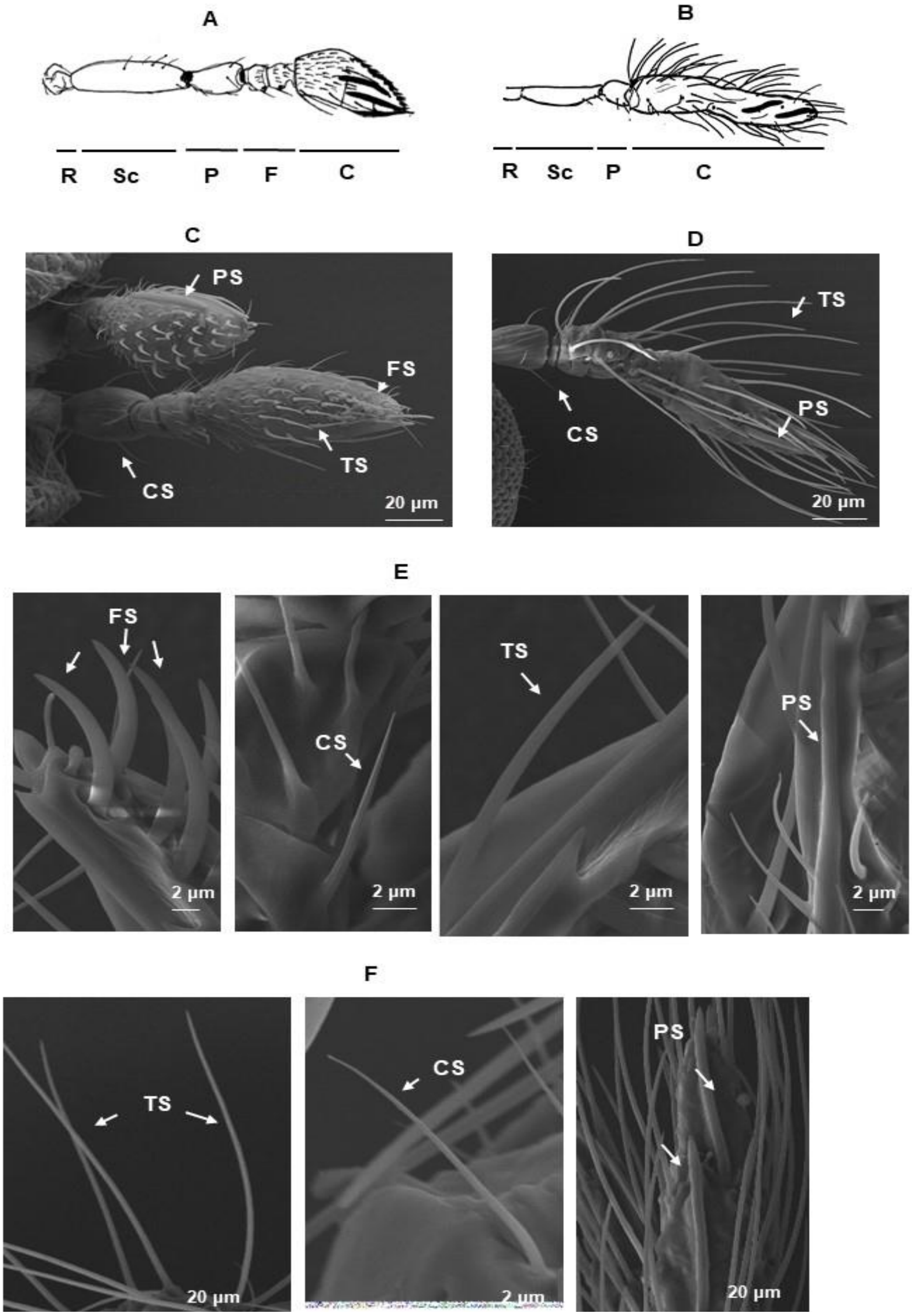
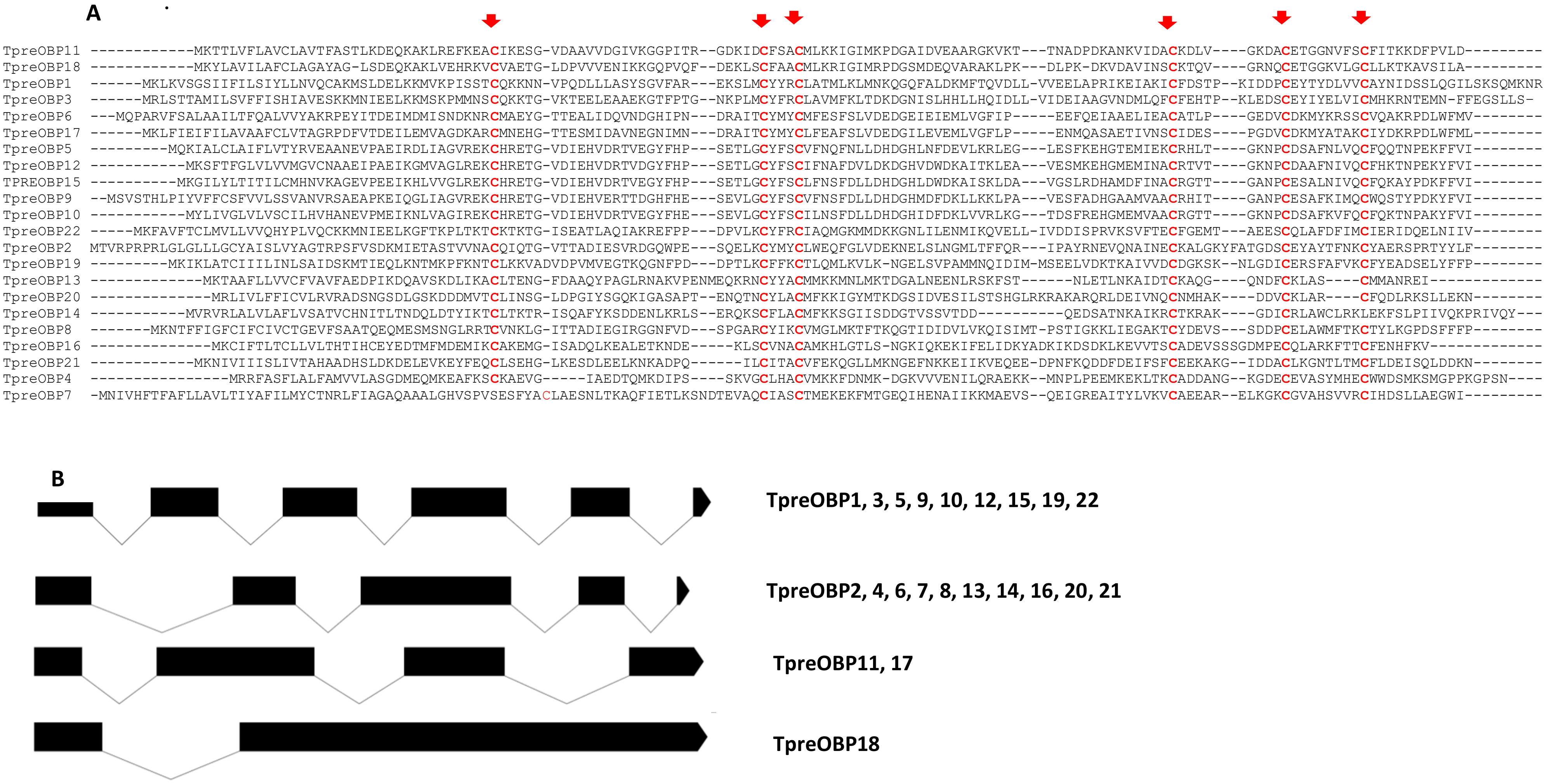


Figure 22. The scanning electron microscopy (SEM) analysis of *T. pretiuosum* female and male antennae and sensilla. A and **B**, the hand drawings of *T. pretiuosum* female (A) and male (B) antennae. **C** and **D**, SEM analysis of *T. pretiuosum* female (C) and male (D) antennae**. E** and **F,** SEM analysis of *T. pretiuosum* female (E) and male (F) olfactory sensilla on the antennae. Sc, elongated scape; R, basal radicle; P, pedicel; F, flagellum; and C, apical club. Various types of olfactory sensilla were observed including chaetica sensilla (CS), trichoid sensilla (TS), Placoid sensilla (PS) and faleate sensilla (FS).

4.4.3 Phylogenetic analysis of TpreOBPs

The phylogenetic analysis on OBPs was performed between *T. pretiosum* and *D. semiclausum*. The results revealed that most OBPs from *D. semiclausum* and *T. pretiosum* form species-specific subfamily (Figure 24A). *D. semiclausum* -specific OBPs were observed and labelled, which may play important roles for bees' behaviours as a specialist parasitoid wasp to DBM. *T. pretiosum*-specific OBPs were also identified (Figure 24A), which may help *T. pretiosum* localize host insect eggs as a generalist egg parasitoid. However, a few TpreOBPs were detected and clustered with DsemOBPs (Figure 24A). For example, TpreOBP2 and DsemOBP8 were clustered together on the same branch. TpreOBP6, TpreOBP17 and DsemOBP1-4 were clustered on the same branch as well. These TpreOBPs may play similar roles as those clustered DsemOBPs in olfaction. For example, detecting plant compounds for nectar or sugar feeding, which provide insects with critical energy resources or detecting the DBM to lay eggs on DBM.

Furthermore, a phylogenetic analysis was performed between TpreOBPs and PxylOBPs to find if there are conserved OBPs between the parasitoid wasp (*T. pretiosum*) and the host insect (*P. xylostella*). There is no TpreOBP clustered with *P. xylostella* PBP/GOBP subfamily (Figure 24B), a conserved OBP group in all lepidopteran species. Interestingly, TpreOBP2 and PxylOBP20 were clustered together, suggesting they may play the same roles in the host plant odorant detection, for example, plant compounds reception. Further clustal analysis of these two proteins showed they share 32.52% identity at the amino acid level.



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TpreOBP11 ------------MKTTLVFLAVCLAVTFASTLKDEQKAKLREFKEACIKESG-VDAAVVDGIVKGGPITR--GDKIDCFSACMLKKIGIMKPDGAIDVEAARGKVKT---TNADPDKANKVIDACKDLV----GKDACETGGNVFSCFITKKDFPVLD---------
TpreOBP18 ------------MKYLAVILAFCLAGAYAG-LSDEQKAKLVEHRKVCVAETG-LDPVVVENIKKGQPVQF--DEKLSCFAACMLKRIGIMRPDGSMDEQVARAKLPK---DLPK-DKVDAVINSCKTQV----GRNQCETGGKVLGCLLKTKAVSILA---------
TpreOBP1  ------MKLKVSGSIIFILSIYLLNVQCAKMSLDELKKMVKPISSTCQKKNN-VPQDLLLASYSGVFAR---EKSLMCYYRCLATMLKLMNKQGQFALDKMFTQVDLL-VVEELAPRIKEIAKICFDSTP--KIDDPCEYTYDLVVCAYNIDSSLQGILSKSQMKNR
TpreOBP3  ------MRLSTTAMILSVFFISHIAVESKKMNIEELKKMSKPMMNSCQKKTG-VKTEELEAAEKGTFPTG--NKPLMCYFRCLAVMFKLTDKDGNISLHHLLHQIDLL-VIDEIAAGVNDMLQFCFEHTP--KLEDSCEYIYELVICMHKRNTEMN-FFEGSLLS-
TpreOBP6  ---MQPARVFSALAAILTFQALVVYAKRPEYITDEIMDMISNDKNRCMAEYG-TTEALIDQVNDGHIPN---DRAITCYMYCMFESFSLVDEDGEIEIEMLVGFIP-----EEFQEIAAELIEACATLP----GEDVCDKMYKRSSCVQAKRPDLWFMV--------
TpreOBP17 ------MKLFIEIFILAVAAFCLVTAGRPDFVTDEILEMVAGDKARCMNEHG-TTESMIDAVNEGNIMN---DRAITCYMYCLFEAFSLVDEDGILEVEMLVGFLP-----ENMQASAETIVNSCIDES----PGDVCDKMYATAKCIYDKRPDLWFML--------
TpreOBP5  ---------MQKIALCLAIFLVTYRVEAANEVPAEIRDLIAGVREKCHRETG-VDIEHVDRTVEGYFHP---SETLGCYFSCVFNQFNLLDHDGHLNFDEVLKRLEG---LESFKEHGTEMIEKCRHLT----GKNPCDSAFNLVQCFQQTNPEKFFVI--------
TpreOBP12 -----------MKSFTFGLVLVVMGVCNAAEIPAEIKGMVAGLREKCHRETG-VDIEHVDRTVDGYFHP---SETLGCYFSCIFNAFDVLDKDGHVDWDKAITKLEA---VESMKEHGMEMINACRTVT----GKNPCDAAFNIVQCFHKTNPEKYFVI--------
TPREOBP15 ----------MKGILYLTITILCMHNVKAGEVPEEIKHLVVGLREKCHRETG-VDIEHVDRTVEGYFHP---SETLGCYFSCLFNSFDLLDHDGHLDWDKAISKLDA---VGSLRDHAMDFINACRGTT----GANPCESALNIVQCFQKAYPDKFFVI--------
TpreOBP9  --MSVSTHLPIYVFFCSFVVLSSVANVRSAEAPKEIQGLIAGVREKCHRETG-VDIEHVERTTDGHFHE---SEVLGCYFSCVFNSFDLLDHDGHMDFDKLLKKLPA---VESFADHGAAMVAACRHIT----GANPCESAFKIMQCWQSTYPDKYFVI--------
TpreOBP10 -----------MYLIVGLVLVSCILHVHANEVPMEIKNLVAGIREKCHRETG-VDIEHVDRTVEGYFHE---SEVLGCYFSCILNSFDLLDHDGHIDFDKLVVRLKG---TDSFREHGMEMVAACRGTT----GKNPCDSAFKVFQCFQKTNPAKYFVI--------
TpreOBP22 -----MKFAVFTCLMVLLVVQHYPLVQCKKMNIEELKGFTKPLTKTCKTKTG-ISEATLAQIAKREFPP---DPVLKCYFRCIAQMGKMMDKKGNLILENMIKQVELL-IVDDISPRVKSVFTECFGEMT---AEESCQLAFDFIMCIERIDQELNIIV--------
TpreOBP2  MTVRPRPRLGLGLLLGCYAISLVYAGTRPSFVSDKMIETASTVVNACQIQTG-VTTADIESVRDGQWPE---SQELKCYMYCLWEQFGLVDEKNELSLNGMLTFFQR---IPAYRNEVQNAINECKALGKYFATGDSCEYAYTFNKCYAERSPRTYYLF--------
TpreOBP19 ---------MKIKLATCIIILINLSAIDSKMTIEQLKNTMKPFKNTCLKKVADVDPVMVEGTKQGNFPD---DPTLKCFFKCTLQMLKVLK-NGELSVPAMMNQIDIM-MSEELVDKTKAIVVDCDGKSK--NLGDICERSFAFVKCFYEADSELYFFP--------
TpreOBP13 -------------MKTAAFLLVVCFVAVFAEDPIKDQAVSKDLIKACLTENG-FDAAQYPAGLRNAKVPENMEQKRNCYYACMMKKMNLMKTDGALNEENLRSKFST------NLETLNKAIDTCKAQG----QNDFCKLAS----CMMANREI-------------
TpreOBP20 --------------MRLIVLFFICVLRVRADSNGSDLGSKDDDMVTCLINSG-LDPGIYSGQKIGASAPT--ENQTNCYLACMFKKIGYMTKDGSIDVESILSTSHGLRKRAKARQRLDEIVNQCNMHAK----DDVCKLAR----CFQDLRKSLLEKN--------
TpreOBP14 -----------MVRVRLALVLAFLVSATVCHNITLTNDQLDTYIKTCLTKTR-ISQAFYKSDDENLKRLS--ERQKSCFLACMFKKSGIISDDGTVSSVTDD----------QEDSATNKAIKRCTKRAK----GDICRLAWCLRKLEKFSLPIIVQKPRIVQY---
TpreOBP8  -------MKNTFFIGFCIFCIVCTGEVFSAATQEQMESMSNGLRRTCVNKLG-ITTADIEGIRGGNFVD---SPGARCYIKCVMGLMKTFTKQGTIDIDVLVKQISIMT-PSTIGKKLIEGAKTCYDEVS---SDDPCELAWMFTKCTYLKGPDSFFFP--------
TpreOBP16 --------------MKCIFTLTCLLVLTHTIHCEYEDTMFMDEMIKCAKEMG-ISADQLKEALETKNDE-----KLSCVNACAMKHLGTLS-NGKIQKEKIFELIDKYADKIKDSDKLKEVVTSCADEVSSSGDMPECQLARKFTTCFENHFKV-------------
TpreOBP21 ------------MKNIVIIISLIVTAHAADHSLDKDELEVKEYFEQCLSEHG-LKESDLEELKNKADPQ------ILCITACVFEKQGLLMKNGEFNKKEIIKVEQEE-DPNFKQDDFDEIFSFCEEKAKG--IDDACLKGNTLTMCFLDEISQLDDKN--------
TpreOBP4  ----------------MRRFASFLALFAMVVLASGDMEQMKEAFKSCKAEVG-----IAEDTQMKDIPS----SKVGCLHACVMKKFDNMK-DGKVVVENILQRAEKK--MNPLPEEMKEKLTKCADDANG--KGDECEVASYMHECWWDSMKSMGPPKGPSN----
TpreOBP7  -MNIVHFTFAFLLAVLTIYAFILMYCTNRLFIAGAQAAALGHVSPVSESFYACLAESNLTKAQFIETLKSNDTEVAQCIASCTMEKEKFMTGEQIHENAIIKKMAEVS--QEIGREAITYLVKVCAEEAR--ELKGKCGVAHSVVRCIHDSLLAEGWI---------
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Figure 23. The alignment of 22 TpreOBP amino acids sequences (A) and their intron/exon gene structures (B). The six conserved cysteines were highlighted by red colour and arrows. Black blocks represent exons while the lines represent introns.

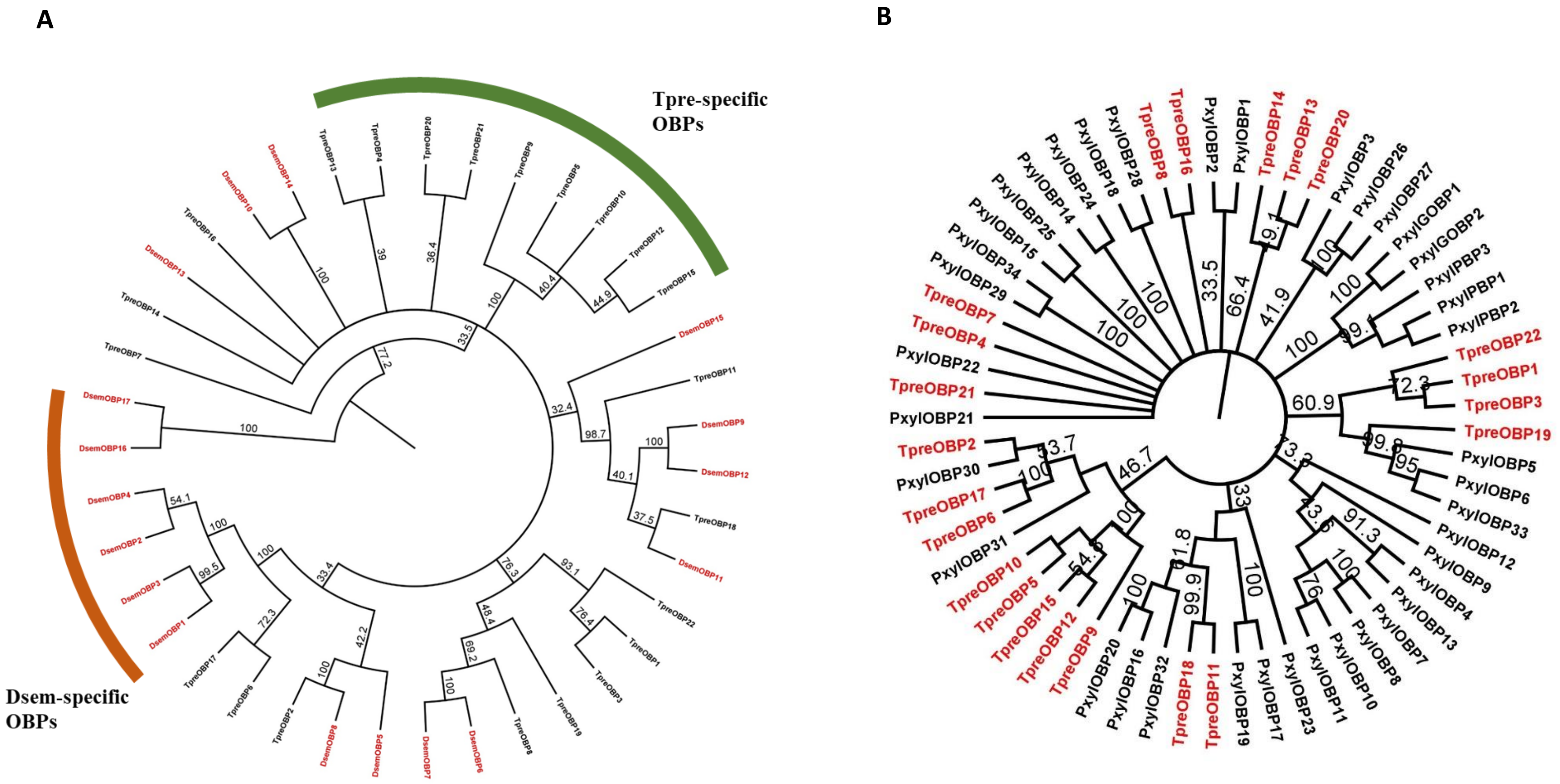

Figure 24. Phylogenetic analysis of TpreOBPs and DsemOBPs (A) and PxylOBPs (B).

4.4.4 Expression profiles of *T. pretiosum* OBPs

Expression profiles between male and female insects can help identify the female- and male-specific OBPs and build the links between the genes and their potential functions. Here, 22 TpreOBPs were examined between male and female adults by RT-PCR and qRT-PCR. To test the designed primers, RT-PCR products were analyzed using electrophoresis. All the 22 TpreOBPs were amplified, and their band sizes were exactly the same as expected in the RT-PCR (Figure 25). Interestingly, TpreOBP3 and 4 were only detected in females, not males. It suggests that they may be female-specific TpreOBPs and play female-specific roles such as detecting the host eggs of *P. xylostella* for oviposition. TpreOBP20 was only detected in male adults but not female adults, suggesting it may be a male-specific OBP and play a role for male-specific behaviours such as detecting the sex pheromone released from female *T. pretiosum* for mating. Most TpreOBPs were detected in both sexes, so they may play the role for detecting plant volatile compounds, which may assist the wasps to mate and feed on nectar.

To study the expression levels more accurately, the qRT-PCR approach was conducted to compare TpreOBP expression levels between male and female adults using TpreGADPH gene as a reference gene (Figure 26). The results were presented in three types: Type 1 are male-specific or male highly-expressed OBPs, including TpreOBP1, 5, 7, 8, 9, 10, 11, 12, 13, 14, 17, 20 and 22, which showed significantly higher expression in male adults than in female adults. For example, TpreOBP5 expression level showed ~65.8 times higher in males than females. Type 2 are female-specific or female-rich OBPs, including TpreOBP2, 3, 4, 6, 15, 16 and 19, which demonstrated significantly higher expression in the female than in the male. For example, TpreOBP3 expression showed ~23.2 times higher in the male than in the female. TpreOBP18 and TpreOBP21 did not show significant differences between males and females, so they belong to type 3.

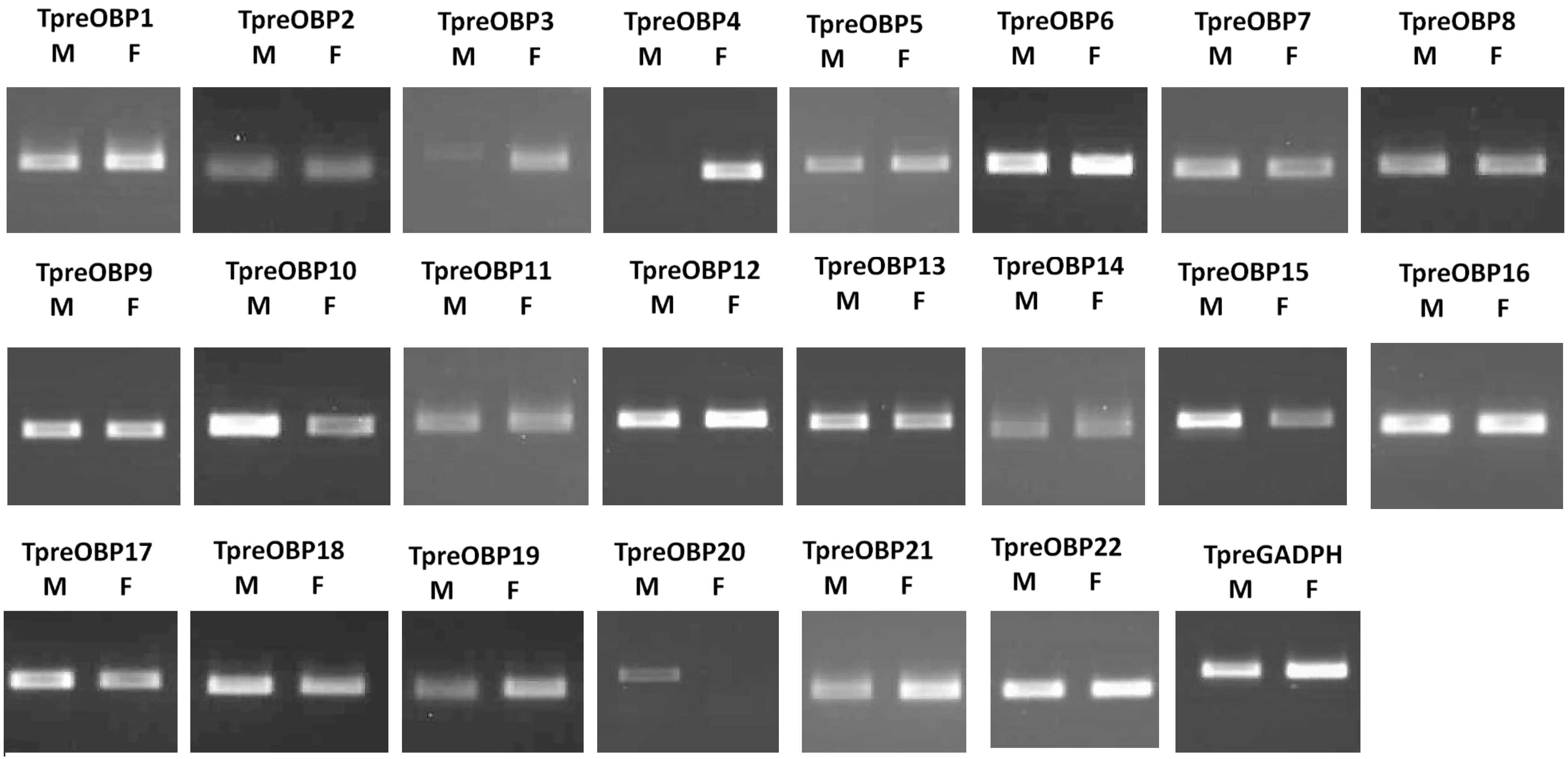

Figure 25. RT-PCR analysis of TpreOBPs between male (M) and female (F) adults. TpreGADPH was used as control.

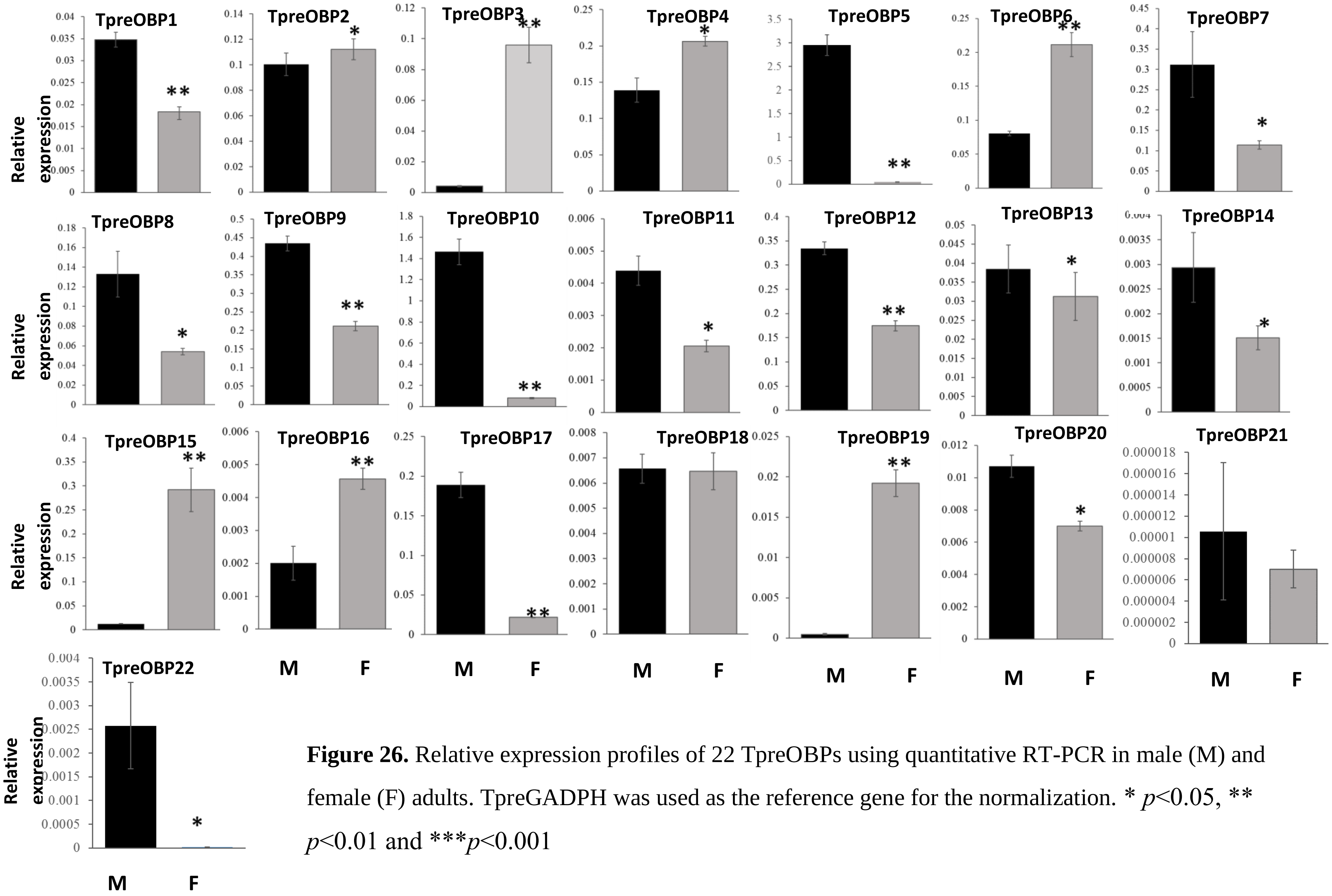

Figure 26. Relative expression profiles of 22 TpreOBPs using quantitative RT-PCR in male (M) and female (F) adults. TpreGADPH was used as the reference gene for the normalization. * $p<0.05$, ** $p<0.01$ and *** $p<0.001$

4.4.5 Identification of TpreORs

A total of 121 OR genes were identified from *T. pretiosum* genome, including TpreOrco gene, the co-receptor. The numbers of ORs in Hymenoptera species are generally large. For example, 177 OR genes have been identified from *A. mellifera* because they need a big “nose” to detect odorants from various flowers for nectar and pollen. In addition, its parasitoid lifestyle may result in a lower number of ORs because its egg and larvae stages were inside the host insect eggs. Only adults need to fly out to seek food, mating and host eggs for oviposition. The phylogenetic analysis between TpreOR and DsemORs showed that most ORs are species-specific (Figure 7A). Five groups of Dsem-specific ORs and eight groups of *T. pretiosum*-specific ORs were identified (Figure 7A). However, a few TpreORs and DsemORs were clustered together. For example, TpreOR39 and DsemOR30, TpreOR33 and DsemOR19, TpreOR77 and DsemOR63, suggesting that they may play the same roles between these two species. For example, detecting DBM related odorants for oviposition or flower odorants for nectar sucking. Further, we also performed a phylogenetic analysis between TpreORs and PxylORs (Figure 7B). The results showed that the ORs are very species-specific and did not cluster together except Orco.

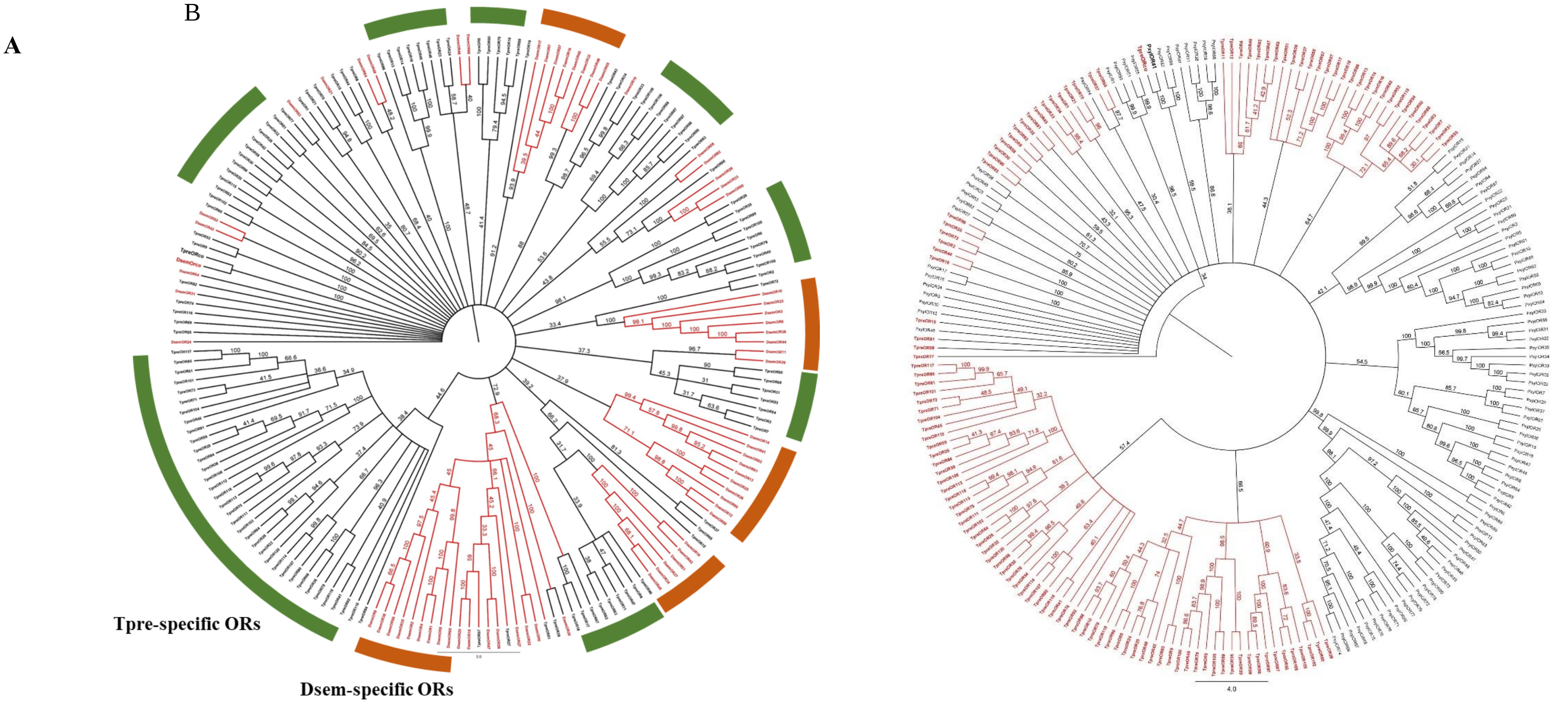

Figure 27. Phylogenetic trees between *T. pretiusum* ORs (TpreORs) and (A) *Dsem*ORs, (B) *P. xylostella* (PxylORs). TpreORs were marked in red colour.

4.6 Discussion

Insects and their natural enemies live in the same environment which is full of the same chemical compounds. They can use the semiochemicals around them to help detect each other and regulate their behaviours, for example, prey and flee. Many plants attacked by herbivore insects can also emit leaf volatile organic compounds (VOCs) that attract the herbivore insects' natural enemies, such as parasitoids and predators (Dicke et al. 1990), to control the pests. One example is that aphids can secrete droplets of sticky fluid in an attempt to keep parasitoids and predators away, which contains an alarm pheromone, (E)-ß-farnesene (Bowers et al. 1972, Wientjens, Lakwijk, and van der Marel 1973). Alarm pheromone may act as primer or releaser, thus eliciting conspecific physiological and behavioural responses, respectively. Parasitoids and predators, on the other hand, eavesdrop on aphid communication and utilize (E)-ß-farnesene as a kairomone, enhancing foraging behaviours (Hatano et al. 2008). All these studies demonstrated that insects and natural enemies could detect certain same odorant compounds by using their olfactory systems. These detections indicate they may possibly share certain conserved olfactory proteins like OBPs or ORs to help this process. It was reported that OBPs from aphids and their predators are highly conserved proteins. Purified recombinant OBPs from the English grain aphid, SaveOBP3, and the marmalade hoverfly, EbalOBP3, specifically bind (E)-ß-farnesene with apparent high affinity (Vandermoten et al. 2011). However, a following correction research (https://doi.org/10.1371/annotation/6fb8a803-8203-429c-b0d1-4d0d995c39e9) concluded that the "orthologs" from lady beetle (HaxyOBP3) and the hoverfly (EbalOBP3) are artefacts, probably derived from the template from the European grain aphid, *Sitobion avenae*. Whether there are conserved OBPs or ORs shared between insects and their natural enemies is still unknown. Here we used *T. pretiosum* and *P. xylostella* as models and performed a comparative study between their OBPs and ORs. Both two species are good models as serious insect pests and their effective natural enemies. It was hypothesized that since *T. pretiosum* lay eggs on the herbivore lepidopteran eggs, then they both should detect the same compounds from plants and the host insect ecosystem altogether. Another reason is both genome resources are available for us to explore as an ideal model system for the insect and the parasitoid (You et al. 2013).

Furthermore, *T. pretiosum* and *D. semiclausum* are both important parasitoid wasps, which are widely used in the biological control for *P. xylostella*. However, *T. pretiosum* is a natural egg parasitoid and can lay eggs to host insect eggs, including DBM. *D. semiclausum* lay eggs into the DBM larvae specifically and selectively. A comparative study on the olfactory systems of these two species will improve our understanding of their behaviours and biology.

Male and female *T. pretiosum* showed very different antennae structures. Especially, the faleate sensilla (FS) was only observed in females but not males, suggesting the significant differences between their olfactory systems, including the olfactory genes. Therefore, *T. pretiosum* olfactory genes, such as OBPs and ORs, were further investigated.

Firstly, *T. pretiosum* genome was utilized, and 22 OBP, as well as 121 OR genes, were identified. Then the *T. pretiosum* OBP and OR genes were compared with *P. xylostella* OBP and OR genes to investigate if they share certain conserved olfactory genes. They may share conserved OBPs or ORs to help detect volatile compounds. However, the results showed that most of OBPs or ORs are species-specific because *T. pretiosum* and *P. xylostella* are two totally different species: One is a Hymenopteran, and the other is Lepidopteran. Though many Hymenopteran species lay eggs into their Lepidopteran hosts, and they live in the same ecosystem, their olfactory genes are still extensively different during the long evolution. No conserved OR genes between these two species were identified, but we did find conserved OBPs like TpreOBP2 and PxylOBP20. These two OBPs will be my targets for further examination to answer the questions including: Which compounds can they bind? Are these compounds the same or different? After knock-down or knock-out of TpreOBP2, will it disrupt *T. pretiosum* parasite behaviours on localizing *P. xylostella* eggs?

Male or female-specific/rich OBPs were identified. Only females lay eggs on the host insect eggs, so the female-specific OBPs are more likely to play a significant role in the parasite behaviours. TpreOBP3, TpreOBP6, TpreOBP15 and TpreOBP19 are female-specific or female-rich OBPs, which are the candidate OBPs to assist in parasite behaviours. Meanwhile, several male-specific or male-rich OBPs were also identified, including TpreOBP1, TpreOBP5, TpreOBP7, TpreOBP8, TpreOBP9, TpreOBP10, TpreOBP12, TpreOBP17 and TpreOBP20, which may contribute to male-specific behaviours such as sex pheromone-seeking. These results help us linked to the functions of *T. pretiosum* OBPs for future functional characterization studies.

4.7 Conclusion

In summary, 22 putative OBP and 121 OR genes were identified from the genome sequence of *T. pretiosum*, which were further studied by phylogenetic and bioinformatics methods. The expression patterns of *T. pretiosum* OBPs were analyzed using RT-PCR and qRT-PCR between male and female adults. This study advances our understanding of the chemosensory system of *T. pretiosum* at the molecular level and provides a foundation for further research on the olfactory system in *T. pretiosum*.

Table 5. TpreOBP primers designed for RT-PCR and qRT-PCR.

Oligo	Sequences	Oligo	Sequences	Oligo	Sequences
TpreOBP1F	CCAATAAGTTCCACGTGCCA	**TpreOBP9F**	TAGCTGGAGTCCGGGAAAAG	**TpreOBP17F**	TGAACGAACACGGAACAACC
TpreOBP1R	CTAGGCGCCAATTCTTCGAC	**TpreOBP9R**	ATCCATGTGACCGTCGTGAT	**TpreOBP17R**	TCTCAGGAAGGAAACCGACC
TpreOBP2F	TCAACGCCTGCCAAATTCAG	**TpreOBP10F**	GAACTTGGTCGCGGGAATAC	**TpreOBP18F**	GAACATCAAGAAGGGCCAGC
TpreOBP2R	CACTCGTTGATGGCGTTCTG	**TpreOBP10R**	TGGTCCAACAGATCGAACGA	**TpreOBP18R**	TCTTGCAGCTGTTGATCACG
TpreOBP3F	TGAGGAATTGGAAGCTGCTG	**TpreOBP11F**	AAGCTTCGGGAGTTCAAGGA	**TpreOBP19F**	AGTTGCAGATGTAGATCCAGTT
TpreOBP3R	ACCCGCTGCAATCTCATCTA	**TpreOBP11R**	TTCGTCGTCTTCACTTTGCC	**TpreOBP19R**	TGATTCATCATTGCTGGAACTGA
TpreOBP4F	TCGAAAGTGGGTTGTCTCCA	**TpreOBP12F**	ACCTTCGGTCTTGTTCTCGT	**TpreOBP20F**	CAGCAAGGACGATGACATGG
TpreOBP4R	CTCGTCGCCTTTTCCGTTAG	**TpreOBP12R**	TTGTCCCAGTCTACGTGTCC	**TpreOBP20R**	TCCCGTCCTTGGTCATGTAG
TpreOBP5F	TGAAAAGATTGGAGGGTCTCGA	**TpreOBP13F**	CTGATCAAGGCTTGCCTCAC	**TpreOBP21F**	GCCGCCAACTATCGAGATTC
TpreOBP5R	TGACGAAGAATTTCTCAGGGTTG	**TpreOBP13R**	TGCACGTATCGATGGCTTTG	**TpreOBP21R**	ATGAAGTCTCTCAGCTCGGG
TpreOBP6F	GCGAAAAGGCCGGAATACAT	**TpreOBP14F**	CTTTCTCGCCTGCATGTTCA	**TpreOBP22F**	TTTCCACCTGATCCAGTGCT
TpreOBP6R	TCGTCGACCAAACTGAAGGA	**TpreOBP14R**	TTTTCCAGTTTGCGCAGACA	**TpreOBP22R**	CTGCCGTCATTTCTCCGAAG
TpreOBP7F	CGCTCAAGTCCAACGATACC	**TpreOBP15F**	CAATGTCAAGGCTGGTGAGG	**TpreGAPDHF**	TCAACGGCAACAAGATCACC
TpreOBP7R	AGAGTCGTGAATGCATCGGA	**TpreOBP15R**	AGAGATCCAACGGCATCCAA	**TpreGAPDHR**	CTTTGTGGCTTGGGTCGTAC
TpreOBP8F	TGTATTGTCTGCACGGGTGA	**TpreOBP16F**	CGCAGATCAATTGAAGGAAGC		
TpreOBP8R	CGCTCCAGGGGAATCTACAA	**TpreOBP16R**	TCCCCGCTGCTTGATACTTC		

Chapter 5

General discussion

An increase in insecticide resistance has been reported broadly in the diamondback moth (*P. xylostella*), promoting interest in biological control. However, how natural enemies localize host insect pests within plants is still not clear. Parasitoid wasps have been reported using blends of volatiles released from attacked plants to localize hosts for feeding and oviposition. In this process, olfactory systems play critical roles to guide parasitoid wasps to localize and parasite on the insect pests. Therefore, this project was initiated by using canola as host plants, *P. xylostella* as insect pest and parasitoid wasps, *Diadegma semiclausum* as natural enemies to explore the chemical interactions among them. Morphology, electrophysiology, molecular biology, phylogenetics and functional genomics technologies were applied to dissolve the mechanisms of olfactory systems of two parasitoid wasps.

Firstly, Gas chromatography-coupled with Mass Spectrometer (GC-MS) and solid-phase microextraction (SPME) were utilized to identify key chemical compounds released from canola seedlings damaged by *P. xylostella* larvae. Eight compounds were identified with significantly higher amounts than the healthy canola seedlings. They are 1-Penten-3-one, 2-Ethylfuran, 2-Hexenal, 7-Oxabicyclo [4.1.0] Heptane, trans, trans -2,4-Hexadienal, Eucalyptol, 1-Cyclohexene-1-carboxaldehyde, 2,6,6-trimethyl- and Tridecane. Five of them were further utilized, alongside other plant-derived chemical compounds, to investigate the antennal responses of *D. semiclausum* by electroantennogram (EAG). The results showed that a group of physiologically active compounds that can activate antennal responses of male or female *D. semiclausum* might be candidate attractants for *D. semiclausum*. Interestingly, male and female *D. semiclausum* antennae showed different responses to several tested compounds in EAG, suggesting the differences between male and female *D. semiclausum* olfactory systems. Under scanning electron microscopy, no significant differences were observed between male and female *D. semiclausum* antennae and olfactory sensilla. Interestingly, *P. xylostella* and *D. semiclausum* showed responses to some same compounds in EAG, for example, cis-11-hexadecanal, suggesting they may share similar odorant receptors (ORs) or odorant binding proteins (OBPs) in their olfactory systems. Insect behaviours are guided by their olfactory systems, in which OBPs and ORs are the key components. Moreover, there may be differences in the expression profiles of OBPs or ORs between male and female *D. semiclausum* antennae, which contributed to the different EAG responses to the odorants. To answer these questions, the OBP and OR genes in *D. semiclausum* were analysed.

First, a transcriptome sequencing was performed on RNA samples purified from *D. semiclausum* male and female adult antennae. Then 17 putative OBP and 67 OR genes were identified from the transcriptome sequence. These olfactory genes were further investigated by using phylogenetic, and bioinformatics methods and the results demonstrated a number of conserved OBP genes were shared between both *D. semiclausum* and *P. xylostella,* which may contribute to the EAG responses to the same compounds between these two species. The expression patterns of *D. semiclausum* OBPs were analysed using reverse transcription-polymerase chain reaction (RT-PCR) and quantitative real-time polymerase chain reaction (qRT-PCR) for the comparison between male and female antennae. A number of OBPs and ORs were identified, showing significant differences in expression levels between male and female antennae, which may play different roles in guiding male and female behaviours.

T. pretiosum and *D. semiclausum* are both important parasitoid wasps, which are widely used in the biological control of *P. xylostella*. However, *T. pretiosum* is an egg parasitoid that can lay its eggs into eggs of host insects such as *Plutella sp., Helicoverpa sp.* and *Spodopte*ra sp. *D. semiclausum* is a specialist that only lay its eggs into the *Plutella sp* larvae specifically and selectively. A comparative study on these two species will improve our understanding of their behaviours and biology. Therefore, *T. pretiosum* genome sequence was utilized, in which 22 putative OBP and 121 OR genes were identified and further compared to *D. semiclausum* OBPs and ORs. The identification of OBP and OR genes from *T. pretiosum* and *D. semiclausum* is the first step for future comparative studies on the olfactory system between these two species. The expression patterns of 22 *T. pretiosum* OBPs were analyzed using RT-PCR and qRT-PCR for the comparison between male and female adults. Through these tools, a number of candidates OBPs, that may play significant roles in the parasitoid's oviposition behaviour on *P. xylostella* eggs were identified.

In summary, this is a preliminary study of the chemical interactions among plants, pests and natural enemies and insect olfactory systems, which may help refine the application of these parasitoid wasps in the integrated pest management strategies in future.

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Supplementary resources

Supplementary Data 1 *D. semiclausum* OBP and OR amino acid sequences

>DsemOBP1

MFGRAATALLIVVSLYGFPDVSADLPDFVTPEIIEMVQDDKVRCMDKFGTDQGMIDQVNNGNILNDPKLTCYMHC
LFESFGVIDEDSGDFEYEMLLGFFPEDIQSQGRDILGGCA

>DsemOBP2

FQVSLIFHQFSQLPDFVTPEIIEMVQDDKVRCMDKFGTDQGMIDQVNNGNILNDPKLTCYMHCLFESFGVIDEDS
GDFEYEMLLGFFPEDIQSQGRDILGGCA

>DsemOBP3

GLPDVSADLPDFVTREIIEMVQDDKVRCMDKFGTDQGMIDQVNNGNILNDPKLTCYMHCLFVSFGVIDEDSGDFE
YEMLLGFFPEDIQSQGRDILGGCA

>DsemOBP4

FQVSLIFHQFSQLPDFVTPEIIEMVQDDKVRCMDKFGTDQGMIDQVNNGNILNDPKLTCYMHCLFESFGVVRNLN
LDN

>DsemOBP5

MSIPILLGILILCVAKISAISDEMKQMAQVLHDACVEETGVEEKFIEQCRQGEFPEDANLKCYMKCLMTKVPLFT
EDGDIDEDFIIKIVPAEYREIAIPVIRGCGTIKGADLCETAFLTNKCWHSK

>DsemOBP6

MAVRNKMALRYSGWLVIVVCAAFLRNNRVDGAMTMAQMETAATGFRNVCIPKSGADPAIVAGLRSGNFPEDHNFQ
CYLKCVMAMLKTIKNNQPLLDPIIKQIKVMMPAELQDRNIGAARKCAALEFNDDACVAAWQYIKCQYETDPEVFF
FP

>DsemOBP7

MAVRNKMALRYSGWLVIVVCAAFLRNNRVDGAMTMAQMETAATGFRNVCIPKSGADPAIVAGLRSGNFPEDHNFQ
CYLKCVMAMLNTIKNNQPQQETIIKEIKVMMPAELQDRNIGAARKCAALEFNDDACVAAWQYIK

>DsemOBP8

MMKYLLVLGCLLQATLFCSANRPSFVSDTMINVASSVVNVCRARTGVDVADIEAVRMGEWPETESLKCYMYCLWK
QFGMVDNKKELDLKGMLTFFQRIPAYREEVRRAVSHCKVIARTSVSGDNCQYAYTFNRCYADLSPNTYYLF

>DsemOBP9

MKIFAVVLLVCVAGSFAAISEEHKAKLRELKLACITETGVNAEVVENAKKGVLDESDPKLACFAACFLKKLGFLT
ENGVFDETTFRAKIPADIPTEKVDAIVNKCKPMTGANACETGAKLLKCYIENKTISIFE

>DsemOBP10

MYRSVALVVLVVLASCVLGDPGKGRSQAWTKCMEESGIAKGSFKDTPWDDPKKKCFQACLMREHGHLKEDGTVDS
EKMIADIPPDVEDRDRISAAITECSANQNDDQCETAHLVMKCLHDKDAMTFPGGKKMHAAMKKCMQENGVEHKDF
RELPPDDQKMKCFGACMMKEQGHLKEDGTVDSEKAVADLPPDVPDRDRVVAAMTECGAQSNLHRFPAFLINSFVA
Y

>DsemOBP11

MKIFAIAIAFCLVVAASALTDEQKTKLAGHRDHCVAETGIERAKVDAAKLGTFDETDDKLACFSACLLKRIGIMN
ADGTFNEEATRAKIPSDIARDKAEEVITKCKDLTGATDCETGMKLMKCYMKNKTFSVLE

>DsemOBP12

MNRRFQSMQIFVFFFQAAISEEHKAKLRELKLACITETGVNAEVVENAKKGVLDESDPKLACFAACFLKKLGFLT
ENGVFDETTFRAKIPADIPTEKVDAIVNKCKPMTGANACETGAKLLKCYIENKTISIFE

>DsemOBP13

MKVLVIFLAVPILAVSAALTPADIVEFGRARQRCGNEFTVDPTSLDRARAGETVNDPEFDCYMACILEGMHMMGP
DGKLDVNAAIDKVPNAPFHDGLVGAINSCANQSGRNNCDTARRLLACMQDQGVPSMFKV

>DsemOBP14

MKCLHDKDAMTFPGGKKMHAAMKKCMQENGVEHKDFRELPPDDQKMKCFGACMMKEQGHLKEDGTVDSEKAVADL
PPDVPDRDRVVAAMTECGAQKGDNDCETAGLVWKCLEEKNALP

>DsemOBP15

MKSCVILIAFCLVVAADSRTVKLTKEKLLEYRKYCEDEKDISRQAMKNDNGYYSAADRSGCITACISKRIGLMNT
DGSFNLEVARSTLPDIFHINNVQDFITAQNFITKCMNHTEANDCETGFQMIMCFMENIAPINPGNSA

>DsemOBP16

MSLDEELFFGNSNKRSSGNTSRNGSNNGQSSNGNNGDNRRNDRSRQDSVFNNRMTNPSNYDWMNERVNGMSNQNQ
YSNYHTSQSQNPDNNNNHRDRAECVTHCLFDELNLVDQRGFPERGSVMEVMTRGVQDPSLRDFIEESITECFHYL
ESDTNNHEKCSFSNNLMNCFADKARDVRYLKMNIHRCSTFFSPRIPTKKKKPFVKIVRIYFSFFSIDLSDFFQRC
EDWDNQ

>DsemOBP17

MSLDEELFFGNSNKRSSGNTSRNGSNNGQSSNGNNGDNRRNDRSRQDSVFNNRMTNPSNYDWMNERVNGMSNQNQ
YSNYHTSQSQNPDNNNNHRDRAECVTHCLFDELNLVDQRGFPERGSVMEVMTRGVQDPSLRDFIEESITECFHYL
ESDTNNHEKCSFSNNLMNCFADKARDRCEDWDNQ

>DsemOR1

MAMELEFKTRKLSLDITIENKDYKSDLNHAICYTRAVLTGLGIWPLLKKDSSKKELLSTALLIPTGMLLISYVLI
PETIYIALRVRSLTIFLKMLGPTAVAYGNFSKYCFFIRRRGLIKMCFEHIENDWKMAESAFDRKIMRRNAVIGRQ
VTVLCMVFRYASGFIYLTLMPLWRGRIARAHNLTTRPLPYPIYHLFVDTQSTPQYELVFTFSCVSSWVSFTVMIG
ACSSLAVFVAHTCGQIDIITTRLDTLFEKLDDDIDLLTDRVSFIIRCHVRVIRLAEAVESAFKDIFLTEVMAATI
IICLLEWRLTQWNTSDHVTICTYFIILISGLFDLFVQCYCGELLMEKCDTVGKATYLSEWWRMPPKHGQALIMII
ALAQIPRRLTAGGLVDLSLASFGAIIKTSLAYLNMLRTFNV-

>DsemOR2

RTVGRKSVKRELQHGCESFDAIYSLVFNLARDWPYVISDASRLDKFISKLLIPFPPLVISLLLVPLVSLPIYRNL
TPSQFILLLAPLSFRVSSVLKILILILRRDDIKFCIQHMETDWRRIETDEDREIMMKNLQIGRNLTIFCVLCRYS
GTILYNILPLFSPRKLNAMNETIRPITVPGIDIYVDLRAGHNYEIVVFISCISIFYVSAIICAICNLAAVLICHA
CGQIQIVTSRLERLVGQEDQEITDKGNLQRGIAFIVRIHVRVLRFASKIDQLLGELCLLEVVAATLVICLVAYNF
ITGQKNTGIIASISYTTILLSLSFNIFIFCHIGELLKEKCEAVGKAAYMTEWYRLPGKTGLTLVMFITMAHYPRR
LTAGRIIELSISTFGNIMRTSFGYLNMLRTMTE-

>DsemOR3

MERTRYFRTANQFNIATNLLSGNVFPLGDEKTSLSFFSLLWVVFVWLINVTYILSVMSGSLYFAELSTKEALKRS
GAIIALFFELTIPLVNLNLRRNAFRNLIRKYNVILIDSDDLKKFVLDTVEPYKRGLKIYTVACFIVTTAWKIEPI
FKIFNNDTFTYTDFTIPAYFPGEPFGKKMFTVGVIVQVLGACFINFGKISIDHYVGYFVAVLSAQYKFVRSQVAK
TLSQSSDHQQDELSIISALQNCVRHHGAVIETGQKLTQLLAFHIGAAYLTCIMKFCFLAFGFATFESANVEKASY
LVYAFACVVQIFLLCSSIQELFDESTSVTRDAFHENWHARSAAVKKIFCLMEMSNQMECRLSAYRIVDLAVPALA
VILSKSYSFCLLLLEIN-

>DsemOR4

MAMELEFKTRKLSLDITIENKDYKSDLNHAICYTRAVLTGLGIWPLLKKDSSKKELLSTALLIPTGMLLISYVLI
PETIYIALRVRSLTIFLKMLGPTAVAYGNFSKYCFFIRRRGLIKMCFEHIENDWKMAESAFDRKIMRRNAVIGRQ
VTVLCMVFRYASGFIYLTLMPLWRGRIARAHNLTTRPLPYPIYHLFVDTQSTPQYELVFTFSCVSSWVSFTVMIG
ACSSLAVFVAHTCGQIDIITTRLDTLFEKLDDDIDLLTDRVSFIIRCHVRVIRLAEAVESAFKDIFLTEVMAATI
IICLLEWRLTQWNTSDHVTICTYFIILISGLFDLFVQCYCGELLMEKCDTVGKATYLSEWWRMPPKHGQALIMII
ALAQIPRRLTAGGLVDLSLASFGAIIKTSVAYLNMIRTFDKN-

>DsemOR5

MAMELEFKTRKLSLDITIENKDYKSDLNHAICYTRAVLTGLGIWPLLKKDSSKKELLSTALLIPTGMLLISYVLI
PETIYIALRVRSLTIFLKMLGPTAVAYGNFSKYCFFIRRRGLIKMCFEHIENDWKMAESAFDRKIMRRNAVIGRQ
VTVLCMVFRYASGFIYLTLMPLWRGRIARAHNLTTRPLPYPIYHLFVDTQSTPQYELVFTFSCISSWVSYTVMIG
ACSSLAIFVAHTCGQIDIIMSRLDTLFEKLDDDIDLLTSRVSFIIRSHVRVIRLAEAVEAAFKEIFLTEVIASTV
IICLLEWRLTQWQTTDHVTICTYFIVLISALFNLFVHCASGELLMEKCDTVGRVTYLSEWWRMPPKHGQALILVI
ALAQIPRRLTAGGLMELSLASFGAIIKTSVAYLNMLRTFNV-

>DsemOR6

MNTDNSGNLEGETKASKKLKTQGTMEYSVRHLRWLLRLLGVWPLVTANPSQTEKLIGTIVRPIAVFYSTLVLFPM
FAEVAAQRAPREEMISLVAPLAYQAFNVMKHFFLLLRIKIISTSLRHMEHDWEQIDNDKDRKTMTKSIQQANQLA
IMITISMYVGGTLHNFILPALADSYLNELNETIRVLCFPGGDFFLDVQADVVFEIVFVTYWMSDMFVVTVSMALF
TLALILLRHSCGQIKILISNLGRLVDSDQEIELPAENTVAPLVRHHARLLVFSMHIEKILKEVCAMEVICDTLIL
CFLGYVLIMDISNYDLTTIFTYVMLLVTIAFNLYLYCQIGEVLKEEYESVGTAAYMIDWYKLPGKASLDLIMIIN
MSAQPRRLTAGGMMDLSRTSYVAIMKTSFAYLNMLRTMA-

>DsemOR7

MYQSKTQFLYFSSYDFNARSFCSRSLHEMIDQAQKYEKYANNIKRLFLLAGVSHGPAPYKFINYLLPVYLSTIVI
QMSMTVVYAADHITDMGVITKMMGMLISGFAGILKMSLYLWNRDEFSQMHDILETSFRNEFRNPDTRKVLLAPLN
FFYHSTIIMSRTFIALPVVFCIGPLLEIIAQCVEGADPIDYQLPFLAKFPWDPYSSLTVYVIHYIFMFICGMGVG
FISVSIDSAFGYYVFQIGAQFRVLSYRLENLKATDDYHALIKECVLRHRTLNQCREFLERVYGPIILFFMITSAI
SLCANVYQISKKNSDSIVISVIYIIAKLAQAFFYACFGSFLTSETESFRDAAYNSGWPGSGQTPFMTSILILLLW
KPLVLSACGFSTISLDMFVAILNTAMSYFFLLQTIDDSKQ-

>DsemOR8

MERTRYFRSANKFNVFVNNFSGNIFPLSEEKTSLALFMIIWVIFVWTVKITYFFTVLSGSLYFANITTQDALKKS
GAASSVCIELLIPVIYLNLRRDDLRNLIKKYNRILIDSDELKKLVLDTIEPYKKGLKFYIVTCIIAVASWSATPI

LSMSHNDQYTYADYAAPAYLPGAPFSANVFLAGITLQVVGSSFITIGKISIDIYISHLIAVLSAQYKYIRILMTK
ALSQENNREDESSVIRSLHKCIGHHCAVIEIGQILAEILALQIGATYITCIMKFCFLAFGVITFESANIEKAAYL
VYALACVLQIFLLCSCIQELLDVSTSVTEDAFHENWHGRSATVKKIFCIMEMSTQMGCRLSAYRIVDLVVPTMAV
ILSKSYSVCLLLLKVN-

>DsemOR9

MRMSFLNTEKKGIIISDKLKCIFNRKFVAREPRKKVTSGFSPRTLQSRLKLCSYRTMARHGNGIDSASWKNVKNK
NYKKNIKQTFQYTRLFFTISGIWPMITKYPSERVKYLSRLIILVCFVMIAFTFVPTASHMIFREKNSSVRIVLLG
PVGTSFASILKYTIILLRTNTIKKCVEQMKNDWMVVESVDEHAIMTSNANTGRKISVVCALFLYTAGVSFFAVVP
ILKGTKINEFNQTIRPLAYPGYDIFVDAQRTPTYEIIFFLIFVSAYVRYTVTIGIISLAAALVGHACGQIEIVML
RLNKLFDGIDEYLDKDELRERIAKIIKCHVNGFRFSARIDESFREACFVEIMSSTLVICLLEYSCMWTFLNRDAA
GVVTYVLLLTSYVFNVFIFCHIGELLQTQYETVGKSAYMIEWYRLPDKLGLSLVMVMAITGCPRKLTAGRMLDLS
IRNFGVIIRTTVTYLNMLRAMTTT-

>DsemOR10

MDFRTLNKYNVLANAVSGNFLPIHGEKTKFHFPSTFWGTFVWIVELTYITSWIIGNARYNPIAVAFQEASASWIL
AVELIIILTIHLNVHRGDLRDLIRRLNEILVDSDDFKRCLSKNVNPNVTYLKMYTVMAFGSTVLWAVTPIFQVFSR
DQFTYGDWTMTAYMPGEPFGPTAFACGVLFQVFGGCYLVLKKLSIDIYVSYFVTLLTTQYKYVREQLAKALHKEI
ETDRDEQSVIDALKKCVEHHCAVIQISRDFMKIVAINVGLTYIGSIFRFCFVSFAVVAFRGFYVYQFVFVLYGMG
VFAQVFILCLAAQQLLDASTTVTEDAFHENWHRNNKKVKKVFFTIELSNYMECKLTAYRTVDLIIPTLAVIMNKA
YSVFLLLQEMD-

>DsemOR11

RWRRTVIKSVLSAMAKDWAITRSDEERNVMLENAKYARFISYMSVALASGVMLAFSSVRAWTAPQERKFITNETK
LAIGMLYSAYFPYDTTQSRGFLPTWFSQFFLTILAMVGYSGPDSLIAMMTLHLCGQLATVRIALANIVDEECIKD
PSIYWKRLSSIVQRHEELNRCGELLEASFNVLLLMQMIFCTLTFCFQGFVTFTTLIGALTEDGELPVLQMTFLIV
HLFYTILHLFVYCWTGDLLVVESTALSYAPYESDWFNLSPAQAKALMMITNRSRIPLQMTAGKFVGFSLELFTSV
GPYKPFFQHHLLDQSFYHVFFSPVDHENIDGLSLGTTGYARLVFVV-

>DsemOR12

INKKVTSWQIWKLKAKDYIFRLVIYYGLILTHLSHPVLKKILQVMDPRNGSEPAPFPVLMKSRYLDTDVHYFSIL
PFYVVRLCAILIVILAADTLFIVQVQHGCAMFTVLGHQLQKVANQVTDDWATCSRPVEDPAFRYLTTCIKNHTKA
IEYSDLLESIYSPGFLVVVVLNMILISITLFQAALVAKEEPVQAFVFILATFGQIGHLFFLCYFSQKLMDHSQRV
REFLCEAEWYRTTLYAQKLFILIAMRTLVPCQLSAGKFLVMSLESFTM

>DsemOR13

MPSFFDHEYYKWNKISLACLGTWPEQSFFMRCLIPFIFCTNVISLIFVETRYVTNVWPNLDIFLEWLPAFCVLLT
SLLQLINGSIQHHKFATILNHIYSDWQRFSPSPHIKILDKYAARGCWITKTYFRWMYSACLCYLTMPMVMPKIIE
LFAPVNGSTGKIYLFYADYGVNSDDYYFSIFTHMFVESWLSIISIISNDAMYFVFAEHACALFEIVGENLKSVSS
NDTSAKDSEDDVNYKIICSSIDLHRKAIIFSALVDSCYNICLLAILGASLLALSVTGLQVLMHLDEPGELVRFVS
FAVAQIFHLYFNSVPGQKILDHSSSIFQYAYSVDWYDLSPRAQKLLILIMVRSQRPCQITAGKIYIMTMENYSAV
RNRKLFVLKIS-

>DsemOR14

SSDSMYFVFVEHACALFEIVRLNLTKLSNSVMDLNNRSKDSSYQIISDSVHLHRDAIFFASLIESSYTACFGGVI
VFCLLLLSVTGLQVLMHLNEPGNFIRFFIFGIAQIVHLYLNSVPGQKIMDYSLSISEYVYSVNWYNMNPRAQKML
LIIMQRSLTPCYITAGKIYIMSMENYSAVIQKSMSFFTV

>DsemOR15

MSEESTNLNGDGIPDRAVVIESNREYLRSIRTPKNTLSEKDYEYSTQMNRYFLKPIGIWPRRPETGICKKGLVNI
QICCCYFLICFLLVPCALHTFLIEKDPALQLKMIGPLSFCLMAIMKFSFLVYHRNDIYDCLKHIAVDWESIESSR
EREIMLDDAKFGRFVASLCAGFMYGGGFFYHTIMPLSCGVIVTPDNTTLRPLTYPVYDPFFDAQVSPTYDIVFIT
QWCSGFVMYTITIGACSIAAVFAIHACGQFKILVNRLNNLVDGDDQKSDSVSERMANIVELHVRTLNFVNHVEGV
LNEVCLVEFVGCTMNICFLGYYFMTEWERSETISTVTYCVLLVSFTFNIFIFCYIGEKLTEQSKRVGTMTYMIDW
YRLQHQKARGLILILVMSKYPARITAGKMAELSMTCFSNVLKTALTYLNLLRTVAS-

>DsemOR16

TNPIAIIRFIQVIVFTIYRESLVELHETLEKSFREDLQNVEYQPVLLSPIAYFDRPTLYLSLSTLGVITMYNLGP
IIGIIIQLSHGNAIIKHRTPFPSKYPLPESNGLYLTRYIIEVGASCAIGSITAATDALFGFYVFQMSSQLRVLSH
RMNKLTVADDHQRIIRECTIRRQLLARCRDKLQTIYGPIILWMLTTSAMTMCANIFQASQISMDKAALVFVYITM

KLMQCLLYGWFGLFLTTESEAYRDAIYSCGWPGCGDRLFVSNIKTMLMCRPLVLKAWQFYVISVDMFMAIMNTTA
SYFFLLRTLDEK-

>DsemOR17

MVDQMTSYRKYVRGIRGLLLFTGLWPSRKWAGTAYRFIPFINALVCFSLSCAVLNFCGDQLNNLELLTKGFGIVG
SYWTAVLKVSCFLINHKEVMDLHRTLEKYFEKSLANVEIREQIFGPLSIFVIPFYAITCLLFIATLAFFIAPVII
VCMQLSHDIRPLKFLLPYPGKYPYSFEGGSLLYYVHYAWQMLAATYLLCLTSGVDSLFGYYVFQMHAIFQLMNNR
VKSLGLTDQDNPTIVRECVEMHQMLAKCRDQLQMIYGPIVFWIFLTSAIILCSLIFQASQTGQLTVGRGVLFVTY
ISLKLLQALMYAWYGSVIVTESERFRDAVYGCEWPGSGDKFVMRNVLIMMCNKPLGITACKFLTISTNMFTTIVN
TTMSYFFLLQKLDESA-

>DsemOR18

MHTLQLNFRIFQYIGMWQPVMWSTGWKPLVYTCYSIYMILSFYMFGLSQFIELIVASHNINDFAHSSLFLAILAG
CGKCASIMKHRKDIIQLANVLKNDLSRPRNNDEMEIQIAFERDARSNTLWYTSVAGTTACLISLRSVLMDTPARN
MPFEGWLPFDRKLSGMVYWLVYTNQIIAHASFAMINCSFDTLVTGFVQSICAQMKILKYRIETLPEMTMVTENDS
FDGNANDVKSQEYFQSREAELLTGCIKHHVQIFQLANSFSDIFSNMVFFQIALSSLILCVSAYEISAMKLFSPEF
TATMMYLCCMTVQIFLYCFYGGELTLQSVGICDAVYRMDWSRLDIKTKKSILVIMLRSLRPITFICGNVITVSLD
SFNSIVKLSYSVFNVLQQQSDTA-

>DsemOR19

MSNVAVREQKEFDEAAKVLSWNRWLLSFLGIWPKSPSGFLFGLNFGYFCYHMLMEYLDLCLFIDNLEHVIENLTE
NMAFSQILIRMAMLKRYNKALGTVIEEVVKDFDSRKYKTIEECDAFIKYNKKSKLFVKLLSAFVVLTASSYYVKP
LTTEPPEDLSIVIPENATAPYILPYRFHVFYEIEDDQTYLYTYLSQLPFVFVSGFGQTAADCLMVTLVFHVCGQM
SILAMRISQVSSDADSCEEELRNIVKDHNRLLEMGTAVQEAFSQTLLGHLIGATSLVCILGYQVLTNYAKGQNAD
LATFVTFAFLVLLVLYAHCTVGESLITESLNVCNAFYDCKWYNMPPKKARVLIICMARSQKPLCLTAGKFGIFCL
STLTEVLKTAMGYLSVLRTLL-

>DsemOR20

MSEEAVSWHDEKTDRDVVTEPKKKYLHSIRTRKNSSFEKDYEYSTQMNRYFLKLIGIWPAGPEAGICEKVFIKFR
ICCCYFLMSYLLVPCVLFKFLAEKNPALKLKMIGPLSFVSMAMLKFTFLVIHRQNIYDCLEHITGDWANVTSSRE
RKIMLEDAKFGRFLASLCAGFMYGGGFFYHTILPLTIVIVTPDNVTLRPLTHPVYDPFFDSQSSPIYEIVFVTRW
CSACIAYTITIAACGIAAVFAIHACGQFKIIVSRLNNLIDGREQTCDSVDERMADIVELHLRTLNFVTNLEGVLN
EVCLVELIGCTMNICFLGYYFMAGWENRETIGTITYCVLLLSFTFNIFMFCYIGEKLTEQSKRVGTMTYMIDWYK
LQYAEARGLILILVISKYPARITAGKMSELSMTCFLNVLKTAFTYLNLLRTMAN-

>DsemOR21

CIMNVFTAGQFRMLQRKFELVKCTDGFDSPIKSFMEISLDNKSGNISRNGSAEKLYKCNETLQDCIQKHQTLIGF
VESVESMYTLVILGQVVVFSLLICLVSYQAVLADGEFSRRMIFIVHSAGTFTQLFMFTLTCDDLTSESKAVAEAA
YTARWYLCLSDPSRKTFGKDLLFVIMRARRSCCLTAGGFFPISLETFTTILSTAMSYLTLLRNGQAND-

>DsemOR22

MESNDSSDLEVETNVSEKLDSYGTMEYSVRHLRWLLRLLGVWPLVQENSSSLEKITSKIVRPIAVVYASSVLIPM
FAELFVQRASREELILLVAPLSYQACNVMKHFVLLLRVDIVKASFHHMETDWQMIDNDKERAIMMKRFKTANKVA
IVIIASVYVGGTLHNFVLPALADSQLNEFNETVRVLCFPGGDLLFDVQAKIVFEIVFLTYWISDMFVVTMSMALF
MLAIILLSHICGQIEIVESRLEALIDSDQDVDILLKNRVAFIIRSHARLLKFSMQMEDVLKEACSLEVISDTLII
CFLGYVSIADFDDNDFVTTMTYVMLVITISFNLFLYCEIGEIFKSQCQNVGKAAYMIDWYKLPAKTSLDIIMIIN
ASARPRELTAGGMLPICRVSFASIMKTSFAYLNMLRTMM-

>DsemOR23

MFWNSERTPYFQRVNRLNAFVNFFSGHFLPVSDDDEKLGYFNSLWMVFAWAVKIVYFVTAALGTLHFANLTVDQA
LKKSGAITVLSIEILVPFFYFHIRRKDLRKLIENYNLILIDSDQWRHCISRVVQYYNKGLKFYLVVGLVAAFFFP
AAPFFQIFDSEQFTYADFTVPAYLPGEPFGRGVFAAGVVIQIFGGCAIHVGAIGIYLYVIHYISLLVGQYKYVRI
RFAEAMCEKNGRKDDSCVITELRKCIQHHAAVINVGRRLIEILAFYVGMTYITCIMEFCLLAYGVMTFENAVVEM
ATYVSYIFCRILLVFMLCSSIQELVDASTSVTDDAFHENWYTRSKAVQQTFYIIQLSNQIECRISAYRIVDLVVP
TLALILSKSYSVCLLLLNVK-

>DsemOR24

MNLARKETRPSWNESVPVASSVRRHKPKNILSLVSTRIMNHFSPSSSEYDRLIKPIEIVSRIIAIWPVERDHTKL
KALLSRCHKICMICVAVIMTTAVGLDVTRHWNDLNEATECALIASAFALSIVRLVIYTIHENDMRYVLDTMRKDW

EESPPEDKLVLAEKCRWSFKLAKQFIISVGLALMLFMLTPMIEIVVFHSEDRILPFRGYFMRNQTSSPIYERLYV
VNVILGGLCGTTIASATSFNLVSTIHAAARFTVLQKKLEALKGDDADSEGAMVDCVKRHQNAIK-

>DsemOR25

MYFQYDWQKWDGEQEIQVFHEYAETGRKITTFYAAAVYATTVIFITQPVLPKIIDSLIYNGTQPGEFPFPVYYCQ
LDQQKYFFHIIALIYVGVSVIVVITIASDIMLIIHVQHACGVFAAIGQKLKRVGEEKKSIEGRDVDFENLTICID
RHNGAIEFADLLESLYSPCFFFVVGFNMILMSVTALQILTKSNEPEETLRFIVLVGAQITHLYIESYISQILMDH
SLSIHDHVHSGKWYNISLKARKLLGLMIMRSRTPCKLTAGKICTMSVETFGVVVKTSMSYFTLLRSMQ-

>DsemOR26

MDVFQRYYKLTEILMKSIGQWPYQTRLARRTFLGILIFLCTVQIVPQILAVIEFRDDSQIILESISPFLVDAASF
TKLVTYLCTSDMRLKMLNRVREDWKKWDGEREIEVLHEYAKAGKKMTTFYAVMVYVTTVLFITQPVLPKLIDTLF
SNGTKPGEFPFPIYHCQLDQQKYYFHIIAFTYVGASMVLIITIATDVMFIMHVQHVCGIFAAIGQKLKRVGEEES
CPAGRDINFENLATCIDRHNGAIEFAEHIENLYCPCFFFVVAFNMIIMSVTGLQILTKSNEPQETVRFIAFVGAQ
MIHLYIESYISQILMDHSLSIRDHIHSGKWYNISLKAQKLLGPMTMRCNTPCKLTAGKICTMSVETFGVVRDKNH
LNLLHDLKKKKTT-

>DsemOR27

MTCDLAPNKNYKEDFSYDMQLNRFFLHIIGIWPATSRRSKLLSKLSITISVLLLLFFYLPQSSYVFLVEEDSAKR
LEEFGPLSFTTMAIMRYAIMLWKLDRFSACIDVIANDWRQADDRICRPIMLRYARASKLFTKLYAMVMYGGVGFY
TWVLPFVNDHDKAHNSTDRPLPYPVEFFVIDAQTSPMYEIVFVGYSISMLGIVCTTTCGICTITVKLVMHACSQF
EIVIGFVNSLEDYTLDNKNKGSADSVAGNVHVEWRVAEIIRRHLYALAFVDLIEEILTEICLIDVVGGTLNICIV
GYYLLTEWNNSEAVGLVTYFMLWLTMVSSVFVFCFIGEHLSEQCERVGIAIYMTDWWKMKKDQMKAMILVIASSQ
TPVKFTAGKMATLSLFTFGSVMKASMAYLNALRTVM-

>DsemOR28

MDQNPLRGKERIEGLNMGKSNVNCDADIDYAVKMNRWLLKPLGVWPPTSHENWSLMGKFLNRSLVFLTTFLLGFI
LLPGSLYTFMREKKTEVRVKLIGALSFCVMAILKYISLLTGNAGVGSCVEHLINDWRYVSNPQDRHIMLSYAFFG
RFGTVICAFFMYGGGLFYAVVMPLLRGTIFIPEANLTVRPLAYPSYYVFFDPQIKPFYEIVFATHCCCAFVMHTI
ATAGCGLGVLFVMHACGQLEILIAWLNDLVESYSKGIFTLDDSIARIIRQHVRVLRFITSTEEVLRNICLVEMAG
CTLNLCLLGHYFLLEWEQSDAIAILTYTILLASFTFNILLFCYIGELLTEEVQKQSFSQFLNFNALVLLDFSFIE
RFVPLFFFLSIFFLTTVRENRWNDLHDGLVPFATEQEFRIYSNDCNFSTTFDNHCRTIGESVTRHILQCRENFGN
LFESFANCRCVKFDNYLRNYEKKIQSNPVLCINSQEKSHSPMTPVFFVIVSLKLQTRIYFSSNKSLMAIQTQKFC
RESFFLCVDHEMMHKFPQF-

>DsemOR29

FVFPDQPMPTFATYPFSIDSGIVMYLIFVHQSIVGLQSSAALTIDCQMAMLMWFAGARLEILAQEIRTCSGIKDF
RLFAKKHQRLLHYAERVSETMRYIALITTGVSGIASIMCCMQFVGNQPLMVQMQFGPVTIVGLTNLMICTWPAEN
MIRVCDSIGTAAYETSWFDMSPKLTKDIVMILQRSRQPVEVSIGGFLPALSFRFYSSFLSTVFSYFTTLRLVITD
DEK-

>DsemOR30

MEEILEGREIRDVERKDNKKYFNENYKNDIDYVVKIARTLLTPIGIYPLYGNDSVAAQIRARCQTIVVFGLMCFL
LVPHFIYTFFDAEDLSRLMKVIAAQIFSLLAIIKFFTMIINKSDLRYCLDELESHWRNVTCEEDREVMTNSAKIG
RFCTIAYLGLSYGGALPYHIVLPLVAERIVKDDNTTQIPLPYPSDYVFFVPEDSPGYEMLFVSQILISSIILSTN
CGVYSLIAIYVMHACCLFQVVQRQLATIFDYSRTLEELSQRLAVIVDNHIAAIRYAQRIEKALNVVFLSEMVGCT
VIICFLEFGVLKDWEDRKLIGVMTYAVLMTSIFVNVFIFSFTGDRLKEQSEKVGEFSYSLEWYDLPTNLAADLMF
IMIRASRPSTLTAGKVFDLSLQGFCEVVKTSAAYLNFLRAMVT-

>DsemOR31

AGDCENWEKDFDNVGIADQHNVRRVWSTIMDGTDARPDPRLAEGFLFDSYFPFSTNDLLIFLPIWLGQFFGTLLS
MASYSGPDCIVSMMVLHLCGQLRILRHSLKNIINERTIREPVLYRKNLKLIVNRHEQLNKDTQCSPPWSIEMVLP
F-

>DsemOR32

MEDDISEAKSLFEMNKQIFTIFGVWPVQPSNVRFIIWIVYILTFISTELVDLFDHFGDFEGIVLNLTETTLFIMI
FVKMNVMRFSETLVALMTRIIDGIDSKAFESPEELKTYLGYNRIAKTLSKFWLVMATASEGAYILKAYLPRAVSA
LKNETIPYVLAYRSRVFVDTSKPYNYWLISVCQGPVAMLPIYIAATIGYVFCLSLHVCGKLSVLVFRIKKLDLEL
IETDGSLRAAVFRDILKKHHEVLEMAEDINRVFGFVMLEELLMCTLLVGLTIYSGLENVNFEKSTMFLTYLCYGF

TVLAFICGYCIAGEYLITESRNLCDAYYQCQWYDVSESFKKQLVICMIRATKPIHLNAAGFYVFSLESFTSIVKS
AAGYISMLRT

>DsemOR33

MVQRVSLKEAIKFTRLSVRLVCTWPPSESATKWEIARENWAWCISVLTVLCLFFPLINAINQFIDNPLVITQTMC
YCSACIIILIKTMILRFHRKKCQDLIAEMEEFMANCCEHERQVLQNYVDKCWVYHMIVSFLNYFASICMIISPFV
IPDQKMPTAAVYPFSIESGIPMYLAYIHQSIVAFQTSASLTVDCQMAMLLWYACARLELLAIEIKNSSGIKEFGY
LIEKHDRLLLYAARVSQIMRYVAFTSACVCGIASIVCCLQLVSNQSIWVKLQFGPGSFVGLTNLMVSAWPAENVV
RVCNLIGTAIYEAPWVGMSPKLSRDIVMILQISQRPVIVSISGFLPALSFQFYTSFLSCVFSYFTTLRLVMLGAD
GKTS-

>DsemOR34

MHTLPLHFTILKLCGMWRPLPCLAGWKFVLYNCYTFFVVCIICAFVVSEFVELFGSFDSAEEFAHGSFLLLTMIG
ISGKIAILLKKRQEIIQLTIALKNDICGPRDDNEIQIHADSERDARWMASRYTALVLSSVFIMMFQSLTVDLPQR
QLFAQGWLPYAYNSTQIVFFLTYSHQAMAASTGAVINIGFDTLFPGLIMLTCSQIQTLKYRFEALPKILTRRKKL
LELDNSDECARENQTLESQLIADCIRHHIQIFQLADTLNSIFGFVVFLQFTVSSLVICVSVYEVSKVKVLSSEFA
STVLYLGSMLAQIFVFCFYGGELSLQVLYSRLLSSLSKCLHCF-

>DsemOR35

MQSPPKNKTNSTKPEIKANKNYEFDKRYMAQYTKWLLEILGVWPMVTNECSFKSKLLSKLSVTVCLLSMAFLLVP
TGLHISLRVRDLAGARVSIGYFSPCVASVLKYAFIIFHSKKIKLCIKHMETDWMNVSDDNDRAIMIKNAKIEHAI
TAASGLLLYSGGVFLHAIMPLIKGSSLNARNETIRPIVYPGYDLFVNPQITPTYEIIFCTICFSAFIRFTVTIAA
VHLTALFVTHACGQMEIVMSRLDKLFDGIDDVKDQIKVEQRLSFLVLCHVRGLRLSSIIDEALKEVCLVEVVTAT
LILCLLEYYVMDWRSLGLIQTITFSLLLVSYTYNIYMFCYIGELLKEQIQKVGKAAYMIEWYRIPRKRELSMIL

>DsemOR36

QEDELSVIRALQKCIGHHSAVIEIGQDLGDILALQIGTTYILCIFKLCFLAFGVITFGSANIEKITYVVYAIGCV
LQIFLLCSCVQELLDVSTSVTDDAFHENWHGRGYRVKKIFCIMEMSSQMGCRLSAYRIVDLVVPTVGVILSKSYS
VCLLLLEVN-

>DsemOR37

MKNPFCHDSFCFQRWMASRYTALVLSSVFIMMFQSLTVDLPQRQLFAQGWLPYAYNSTQIVFFLTYSHQAMAAST
GAVINIGFDTLFPGLIMLTCSQIQTLKYRFEALPKILTRRKKLLELDNSDECARENQTLESQLIADCIRHHIQIF
QLADTLNSIFGFVVFLQFTVSSLVICVSVYEVSKV

>DsemOR38

TMARNGNGTDFAICQRIRNDNYKSNIKRTFQYVRSFFTVLGFWPMISKIASRQTKCCSYLIIILLCFSMIAFTFVP
CASHMIFREKNAAVKLVLLGPVGAGFGAMLKYAMILFRASTIKLCVDQMENDWMIVERADEHAIMTNHANTGRKI
AVICTLFLYTGGMSLHAMVPILKGTKINEFNQTIRPLGYPGYDIFIDAQKTPTYEIIFFLICVSAYVRYTVTIGI
ISLAATFVGHACGQIEIIILRLNKLFDGIDEFLDKDVLRQRISDIIRCHVNGLRFSTTIDKSLREACLIEIMSST
LSICLLEYSCMWAFFNKDTVSIITYVMLLTSYIFNVFIFCHIGELLQNQYETVGKAAYMIEWYRLPDKLGLSLVM
V

>DsemOR39

MTFCFQGYVLSTTLFSGLTGDDGFPFLQMMFLTCFALFAMMHLFVYCWAVDLLVVESTSLSYAPYESEWYLLEPH
EARALMMFTYRARVPLQLTAGKFVGFSLELFTMVMHFIKTKVDQINPVKVVL-

>DsemOR40

MEEQLRKYESYARVVKNLLLISGLWPGRVSFKLFRLVVVFHSSLSIFTVCGIIYFVIGHITNLDLFTKGVGIAVS
FCLMLLKATVFTKYRPSLVELHETLEMNFRQDLANPEYQSVLLSTVAYFYHPTLFLSVSTLGLSTMYNLTPIISI
LIQLSQGNEIIKHRTPFPAKYPFPESNAFYFTRYIIEVGASYAIGAITAATDALFGFYIFQITSQIRVLGYRMKN
LTTADDYQKIIKECLIRREILVQCRDKLQDIYGPIVLWMLVTSAMVMCTNIYQASHMSMDKALIVCIYITMKLLQ
SLLYAWFGLCLTTESEDFRDSIYSCGWPGCGDKLFLSNIKTMLLCRSLVVKACQFYVISVEMFIAVCYNLSININ
VSGGRGAM-

>DsemOR41

MASFFDHPYYKWNKISLESIGIWPEQPFLKRCFLQIFVCINLWSIIIPELVYVKKTWPNFDVFLEWVPEFGVLLL
ANIQLLNVSFQRKKFIAMLDHIHSDWERHGDSPNAKVLHKYAAGGRWITKHYFGWMYSACLCYVMMPLVMPMILK
PPESVNGTAEPIYLYNADYFVNSDDYRGAILFHMFMESWLCIISIISNDAMYFIFVEHACALFGLVGEGLKRIHI
NDTIPDDSRDDINYRIICHCIEHHREAIIFTSLIESSYNLCLLAVIGVSLIMLSITGLQVLMHHEEPGQLIRFAS

FSIAQIFHLYLNSMPGQKILDHSLSIFDHAYSVNWHCLTPKAQKLLILMMVRSLRPCQITAGKIYVITMENYSAV
MQTSMSFFTVLSSVQ-

>DsemOR42

MPLIQHVLDPLGNNTKQEPLPVYYYRFDVQEYYYPIYAHAFITAWMFVASLVAADSIYVVLVENACAMFSLVSHR
LQKAVPTDQKYIDAGAKKSNDQAYNNFIDCIKRHKAAIKFADEVESSLSVSFLLTVGTNMIIISFTGTQLAESVG
ELNDVARHGAMIFAQMGHLYMENHYAQKLSDYSCDVYTKIWNSNWENTSLRTQKLLVFFIMRSLEPSVITIGGFY
VMSIDTFNWLLRTSAS

>DsemOR43

MHTLRLNFAILQFCGMWRPLVWSSGWKSMVYNFYTIFMVSSLYLFALSQFLGLVLSSENVKEFSHGSYMFLTVLA
ACGKCASVVKQRKGIIDITNTLTNHFCQPRNEDESKIQNDCERDARFNTFWYSVLGGTTCSLITLRSLIVDISER
NMPFKGWLPFDPKAGPEFGFWLAYFHQLIAHATTAILNSSFDTLVPGFMMQTCMQMKILKYRIESLPKVMEAKRT
LLREQNHEESLQKRQSQESELLTECIRHHIQIFQLANSVNEIFSNVVFLQFSLSSLILCVSVYETSTMRLFSSEF
TALMLYLCCMIVQIFIFCFYGGELTLQSVDMCDAVYQMDRSLLSTRTKKSLVMMMMRSLRPITFTCGNVITVSID
SFNKLIKLSYSVYNVLQRSGE

>DsemOR44

QEDELSVIRALQKCIGHHSAVIEIGQDLGDILALQIGVTYILCIFKLCFLAFGIITYESANIEKLTYVVYGTGCV
LQILLLCSCVQELLDVSTSVTDDAFHENWHGRGSRVKKIFCIMEMSSQMGCRLSAYRIVDLVVPTVGVILSKSYS
VCLLLLEVN-

>DsemOR45

MHTLPLHFTILKLCGMWRPLPCLAGWKFVLYNCYTFFVVCIICAFVVSEFVELFGSFDSAEEFAHGSFLLLTMIG
ISGKIAILLKKRQEIIQLTIALKNDICGPRDDNEIQIHADSERDARWMASRYTALVLSSVFIMMFQSLTVDLPQR
QLFAQGWLPYAYNSTQIVFFLTYSHQAMAASTGAVINIGFDTLFPGLIMLTCSQIQTLKYRFEALPKILTRRKKL
LELDNSDECARENQTLESQLIADCIRHHIQIFQLADTLNSIFGFVVFLQFTVSSLVICVSVYEVSKVKVLSSEFA
STVLYLGSMLAQIFVFCFYGGELSLQSMEICQAVYKMDWSSFGIKTKQSLVMIMLRAQQPITFVCYHVITLTLDA
FVSLVKLSYSAFNVISNDT-

>DsemOR46

LESRYYRVNANILRLIGQWPYEQRKCQKFHQILFHMSVMTVMLTQMKRLFEQIKTDWSNLHRQDEIEILKAYSEQ
GRKLSIIYIVSMYVTIAIYLTIPMMPKILDIILPLNESRPLTYLYRTEYFVDPEKYFYAIVLHTYTGTIITVSVV
AAADAMFNAYVQHVCGVLAIVGYRFENLVKIHDAQEDPSKRNDPCYQELVRCIDQHFQAAEYVMLLEKSYSMAYM
VQTIVSMILLSMSAVQLLMKFGERDEMIRMIQFNLAQFFHIFYNSWPGQKLLDQSVSLRNSVYSSEWYSLSMRSK
VLLKFIMMRTLAPLKITAGKLYDLSMENFGAVIRASASYFTVFASLR-

>DsemOR47

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LLPGSLYTFMREKKTEVRVKLIGALSFCVMAILKYISLLTGNAGVGYCVEHLINDWRYVSNPQDRHIMLSYAFFG
RFGTVICAFFMYGGGLFYAVVMPLLRGTIFIPEANLTVRPLAYPSYYVFFDPQIKPFYEIVFATHCCCAFVMHTI
ATAGCGLGVLFVMHACGQLEILIAWLNDLVESYSKGIFTLDDSIARIIRQHVRVLRFITSTEEVLRNICLVEMAG
CTLNLCLLGHYFLLEWEQSDAIAILTYTILLASFTFNILLFCYIGELLTEECEKIGGTTYMMDWYRLPPNKSLGF
ILTIATSQRPSTITAGRLVNLSLGTFCSVVKTSVTYLNLLRTVVV-

>DsemOR48

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ALGLSASLMYPFKVFYFDRESFVNTKIYDDQQQTIVKKKFLFPSVCLFNDSSTTVYDLVILNQFFQFIICVGVNL
TADSFFITIAYHLCGQFAILKQRLASFEKLDNETKTKREFGCLVARHKHLLMLTTSIEKSLNIIIFTQVVCNLIL
FNVAGINFLIYLKKKDNYSAVTHFGIISFTLTQTFMYACIGENVSSTIETITQSIYEGNWQTLPKNLSKDVIFLM
TFSNSTIYLTGGKLFRMKKSTFTQVLKTSISYLSVFNALVNDY-

>DsemOR49

MMDKYENARNLFKWIESFFSLLGTWPLRSTNARFFICITYFAIHFFFSQVYLYYVFGDLQAMVMNVTDTGLMLMV
SSRLITLKFSRKLGKILELTINFIDEKHFRNEQEWELYLHYNRLAKSYFKYVVPIALVTTVMFWLKPIQLRLVAA
LHNETKPYTLAYTTNYFFEVKDDTTFYLIWFYNFPMVFFAPWYTSSIGLLVSLMYHVSGRLAVLCNRIKNLSVKD
FQNEQAVRMVFRELAQTHLEISEFAKNVNAVFGPLLLNELFITTIIIGLTIYASLMSIDVANPVEIFSSSSYGLS
VLLLIYSSCAAGEVVMAESSNLYAAYYDFLWYDLSIICQKQLIICMIGASRPIQLSAAGFNTYSRFLFISILKTA
GGYTSLLRSMIEKQQ-

>DsemOR50

MVQRVSLKEAIKFTRLSVRLVCTWPPSESATKWEIARENWAWCISVLTVLCLFFPLINAINQFIDNPLVITQTMC
YCSACIIILIKTMILRFHRKKCQDLIAEMEEFMANCCEHERQVLQNYVDKCWVYHMIVSFLNYFASICMIISPFV
IPDQKMPTAAVYPFSIESGIPMYLAYIHQSIVAFQTSASLTVDCQMAMLLWYACARLELLAIEIKNSSGIKEFGY
FIEKHDRLLLYATRVSQIMRYIAFTSTCVCGIGSIVCCLQLVSNQSIWVKLQFGPVSFVGLTNLMVSAWPAENVV
RVCNLIGTAIYEAPWVGMSPKLSRDIVMILQISQRPVIVSISGFLPALSFQFYTSFLSCVLSYFTTLRLVMLGAD
EKTS-

>DsemOR51

CPAGLKFVLYNCYTFFIILLICTFVVSEIIELFGSFNSAEEFAHGSFMLLSMIGMSGKMMIMLRKRQEIIDLINS
LKNGICEPRDEVEIQIQADSERDARWTSSRYTAMTLSTAFIMVLQSMIVDIPERKLPFNGWLPYTYDSGQIIFLV
TYFHQATAACTGAAVNSAFDTLFPGLVVLTCAQMKILKYRLEVIPRIIETRKKSLDDEDSWGNDQALESELLADC
IRHHIKILQLADTLNRVFGFVIFLQFSVSTLVLCVSVYEVSKVQLLSAEFAGTVMYLGAMLVQIFVFCFYGGELS
LQVLYSRLLSSL-

>DsemOR52

MPSFFDHEYYKWNKISLACLGTWPEQSFFMRCLIPFIFCTNVISLIFVETRYVTNVWPNLDIFLEWLPAFCVLLT
SLLQLINGSIQHHKFATILNHIYSDWQRFSPSPHIKILDKYAARGCWITKTYFRWMYSACLCYLTMPMVMPKIIE
LFAPVNGSTGKIYLFYADYGVNSDDYYFSIFTHMFVESWLSIISIISNDAMYFVFAEHACALFEIVGFVEKKFYE
EKSQFLTNSM-

>DsemOR53

MKKARTANQSQSIPNSRRIMNSVKKAYYGKTLLLLSVFGVNPYQNRPSRFAAVFAYCGIACTQLSFKIAFVAQKW
PDVDIFIQQTPPILVDFISIFKMLNCAVNFNKIKDLFDQILFSADPLLSYEELDIIERHDEHCRKFTIVWFTYLV
SAATIFYAMPLIQHVLDPLGNNTKQEPLPVYYYRFDVQEYYYPIYAHAFITAWMFVASLVAADSIYVVLVENACA
MFSLVSHRLQKAVPTDQKYIDAGAKKSNDQAYNNFIDCIKRHKAAIKLVQSIPFNNNSDALELTFHRKFNELNTN
FHNIVQKYCFH-

>DsemOR54

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NKRYIVMVFVLCSSYALRPYILLLHYHWHEKLSNSSYDYSVTIYPTTYPLFTTDTMSKYIICVSWEQFVIVFVAL
YWMGSDIMFAQMTTHTAIQFRVLRHDLKYITTKADGQDSRIEEELIARLSRIARRHRDLYRHCEMIEQIYNPITF
LTMLLTAVNMCFCVFRLEKELSNKNWDEVIKYLFHAVTLIVQAIIYCGYADKLTYQSSLMADAAYDCQWVDRSEE
FKMLLHVIMMRSQKIFKCTAYGFFTVNLNQITVIANTAASYFALLKTVA-

>DsemOR55

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LRLTAALRNETKPYPLAYRVNYLFEIDTDRTFYLIWFYQAPQVYFAPWYCSSIGLELALVYHMTGQLSILSFRVR
NLTINDFANEQSARMVFQDLAQTHHDIVELAKGVNGVFGLLLLDELLISTILVGLTIYTTIVNVEQVDDILSSLS
YGLSVLLLIYSSCVAGEYLATESANLCAAYYECFWYDMPTTCKKQLLICMIGASKPIHLSAAGFYDYTMMLFLSI
VKTAGGYISLLRTMTEKQQE-

>DsemOR56

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IFREKNPAVRVVLLGPVGASFGAVLKYAIILFRSDTIKKCVEQIKNDWTSVESAEELAIMTRHANTGRGIAIICT
LFLYTGGMSFHAIVPILKGTRINEFNQTIRPLGYPGYDIFVDPQTTPTYEIIFFLICVSAHVRYTVTIGIISLAA
VFVTHACGQVQIVMLRLAKLFDGIDHRFDRDELRLRISRIINGHVNGLRLSATINDSLREVCLVEVMSSTLSICL
LEYSCMRAFFNNDTVSLITFFLLLLSYIFNVFIFCHIGQLLQEQYETVGKAAYMIEWYRLPDKLGLSLVMV

>DsemOR57

MVGKSNDKTAILVEKYILYQKALKKMLLFAGVWPKTDPSDFYRCLIWAHGFGSFLMLLAVARFCYEHATNLNLFL
TGLSLAIGFVTIIQKMICLTIYRNNLIEMHRTLAHTFVKDYSDVEMQPILMSPLSPFYRLSQIMMGTAWSGMAMY
WTVPLLLVIIQSSKGGVVKYLLPFPTSFPWPISANKFVYAGLYIFEVYTSTCLTIFTVAVDSLFGYYIFQISGQL
RTLSHRIRNLNSRDNHREVIVECVSRHRTLMRCQESLERIYGPIVLWLSVASAIVLCALIY

>DsemOR58

MGIERKSKLSGYLWYTRTSLKAVGLWPTGSDGILNFTYFLFVAGTFIAQVFQMLTNCWMNYTNFESAAESIGPMM
ALITSFGKMCIYRKERSRLGHLAISLDNCIKNGPLDVLEYLEDYAKISSLACGSLLIVSWLLTVVFLSAPILLNE
RVLPFASWYPFDYENSDIFYVIAYAHEALLTCLGCCTMSTEITFVWSIFHCCSRLRVLRHELSNLSDGKNNDDDT
ISHDISFKLSSLMDLHYEVLRDLVEINVAYTYITSVLFFAALIAICCAGLQITGSSVESSNWTAGLFLVLAMYYA

EQLLFYYLPGEIVAQEATGVALAAYSSGWETLDVKHRKTIGLIIMRSQLPPKLFVGQMIPLRLENYGAVSNSFDS
PQMQFCFSV-

>DsemOR59

MGVRSIKTRGESSIFFPSTVKFMTGKLNVYSHINTICQPFFVSINTNSGHLICCLNETVGNNRGGKNISLMMTAA
LATMTNLFQERYYIINKTLLSLIGGWPYTRQPVRRSALFFIVFLLAAGITPQAFTLREILNDWDVLVGTLLSWVM
NIGCLIKLATVIVNMKKIQILLEHVKIDWLSLVGQPELQILHEFAERGRQYSIAYGVIYYGLILTHLSHPVLKKI
LQVMDPRNGSEPAPFPVLMKSRYLDTDVHYFSILPFYVVRLCAILIVILAADTLFIVQVQHGCAMFTVLGHQLQK
VANQVTDDWATCSRPVEDPAFRYLTTCIKNHTKAIECEAEWYRTTLYAQKLFILIAMRTLVPCQLSAGKFLVMSL
ESFTMAIQTSISYFTLLLSMQ-

>DsemOR60

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FAKYCLIIHRRKFIKACFKHVQTDWEAVASVLDREIMKKNATVGRRVSLLSMGLMYSSGLAYLTLMPFWRAKAAR
TQNITIRPLPYPVYDLYVDSQATPQYEIIFSTSLLMGTVMFTVMVAACSLAAIFVSHVCGQIEIIMSRLDTLFDD
LDGNVQVFNTRITFIIRSHVRVLRLVDAVQIALKEILLAEVIASTLVICLLEWRLTQWRISDNVTLSTYWIVLVS
VTFNLFIFCYSGQLLIEQCKIVGRAAYLSDWWKMRGKSGQALILLIAISHIPRRLTAGGMLELSMASFGAVIRTS
VAYLNMI

>DsemOR61

MPSFFDHEYYKWNKISLACLGTWPEQSFFMRCLIPFIFCTNVISLIFVETRYVTNVWPNLDIFLEWLPAFCVLLT
SLLQLINGSIQHHKFATILNHIYSDWQRFSPSPHIKILDKYAARGCWITKTYFRWMYSACLCYLTMPMVMPKIIE
LFAPVNGSTGKIYLFYADYGVNSDDYYFSIFTHMFVESWLSIISIISNDAMYFVFAEHACALFEIVGENLKSVSS
NDTSAKDSEDDVNYKIICSSIDLHRKAIMFVNVIQKFYSHC-

>DsemOR62

MGIERKSKLSGYLWYTRTSLKAVGLWPTGSDGILNFTYFLFVAGTFIAQVFQMLTNCWMNYTNFESAAESIGPMM
ALITSFGKMCIYRKERSRLGHLAISLDNCIKNGPLDVLEYLEDYAKISSLACGSLLIVSWLLTVVFLSAPILLNE
RVLPFASWYPFDYENSDIFYVIAYAHEALLTCLGCCTMSTEITFVWSIFHCCSRLRVLRHELSNLSDGKNNDDDT
ISHDISFKLSSLMDLHYEVLRDLVEINVAYTYITSVLFFAALIAICCAGLQITGSSVESSNWTAGLFLVLAMYYA
EQLLFYYLPGEIVAQEATGVALAAYSSGWETLDVKHRKTIGLIIMRSQLPPKLFVGQMIPLRLENYGAFLTTTAS
YFTSIRAVA-

>DsemOR63

MDLSDSVAIRLTRFFMRFTGIWITHSRREHFLMELTTTYTISTIIISWQINGSDLWFCRADFQRAIYAACNVLTI
LLISIKLGMFAWRREKFVNLIKFCYQHFWEVQYDAGDMKIMKTCDIKCVLFVGLFTLFAWSTVCAYTAYPIVENM
GKNHSERIHAFPVRFSFIPATTTPYFELIFITET-

>DsemOR64

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ICFLQVTMMTSMIAEINTECSSISGIVEVISLISCSILSILKVTILSGCPSLLLEVISSAVEDWSNVKTPNARNI
MKKHAKLGRFVVLFQLGSGYVTTIPMIIGSLPFLVPQIDLNGTNSSVQFRPLPLRTTCVFGDMSDISYAIVFTMQ
ALQLLTTCTANVGIDVYFFGIVMHVCGQFRLLGQDLENFKSDLAETEQREILVQIIHRHVKLTRLAEHIEDSFNF
IILVQLSANAMQMCLMGIQMLLSLKSGNTVMIVNTIIIFYVMSLQLFLYSYAGDRLSSEMANLRVSAYFSPWYEM
PSTLARDVLFIMIRNQKNFNITGGKIYRIDIDNFKNLIKALGSSFSVLRIMFDA-

>DsemOR65

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PPFGIDIAFAIKLYTVVINAKKQMQKILEHIQIDWISLAGQPELRILQEYGEQGRQFTIAYAAGFYLTTMLFMMQ
PVIPKILQVMNPSNDSELAKYPLPANYYGIDTDANYFYLLPFTYVTTAIILTVIVAADTLFIVQVQHGCAMFSVL
GHKLRRMADKVTDDWAICIRPVEDPVFRHLTACIKNHNAVIECEAEWYRTTLYAQRLLTFISMRTLVPCRLTAGK
FSVMSLETFTSVVKTSMSYFTLLRSMQ-

>DsemOR66

MTHMDRDWRSRDDVTGTKVLHHWAERGRILAKGYALYLFIGGTLYVCAPLFPFFLDVVKPLNETRPREFLFRTEY
FVDQDQYFVPIFLHALTTTSISMVIVTACDTMYSAFIHYACAIFVELSQELEYPVKNKDDSFHVSKSDDNVYEHI
VKCVKKHKEVIEFCDFLESTYTVYNFGTVGLNIFLISFTGVQTVMKVDNPSLFVRYSIYVFGQIMHAFFGCWHGQ
LLIDHSEAILNSAYKSRWYASSFRSCRLLTLIMMRSTKPCQLTAGKLFSVSLQTFTTIIKTAMSYFTLLRSFQ-

>DsemOrco

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MMKFKQQGLVSDLMPNIRLMQISGHFMFNYYADGMKFMHKIYCVVHLFLVLLQFGLCLVNMAMESGDVDDLTANT
ITVLFFAHSIVKVAYFAVRSKLFYRTLGIWNNPNSHPLFAESNARYHAIALTKMRRLLFAVGGATALSVICWTGI
TFVGDSVKKVTDPVTNETTMVEIPRLMVRSFYPFDASHGMTHILVLVFQFYWLVFTMFDANSIDVLFCSWLLFAC
EQLQHLKAIMKPLMELSATLDTVVPNSNELFKAGSAEHLRDNGNNVQPPPSAQGDNMLDLDLRGIYSNRQDFTAT
FRPTAGTQFNGGVGPNGLTKKQEMLVRSAIKYWVERHKHVVRLVTAIGDAYGVALLLHMLITTISLTLLAYQATK
VNSVDVYAATTIGYFMYALGQVFLFCIFGNRLIEESSSVMEAAYSCHWYDGSEEAKTFVQIVCQQCQKAMSISGA
KFFTVSLDLFASVLGAVVTYFMVLVQLK-
```

Supplementary Table 1: *D. semiclausum* OBPs

Gene name	Full Length	Signal P	MW	pI	Expect	Ident	Sequence ID
DsemOBP1	No, missing C	NA	NA	NA	2.00E-34	59%	AZQ25006.1, odorant binding protein [*Aphidius gifuensis*]
DsemOBP2	No, missing N and C	NA	NA	NA	4.00E-34	59%	AZQ25006.1, odorant binding protein [*A. gifuensis*]
DsemOBP3	No, missing N and C	NA	NA	NA	5.00E-32	57%	AZQ25006.1, odorant binding protein [*A. gifuensis*]
DsemOBP4	No, missing N and C	NA	NA	NA	6.00E-21	59%	AZQ25006.1, odorant binding protein [*A. gifuensis*]
DsemOBP5	No, missing C	NA	NA	NA	3.00E-38	49%	XP_015601898.1, general odorant binding protein 83a [*Cephus cinctus*]
DsemOBP6	Yes	Y, 1-31	13495.73	5.78	3.00E-45	52%	ARN17857.1, odorant binding protein 1 [*C. cinctus*]
DsemOBP7	Yes	Y, 1-31	11926.84	6.79	1.00E-34	48%	ARN17857.1, odorant binding protein 1 [*C. cinctus*]
DsemOBP8	Yes	Y, 1-20	14552.67	8.25	6.00E-67	67%	XP_011641447.1, pheromone-binding protein-related protein 6-like isoform X1 [*Pogonomyrmex barbatus*]
DsemOBP9	yes	Y, 1-17	12752.83	5.92	8.00E-42	50%	XP_015603383.1, general odorant binding protein 56d-like [*C. cinctus*]
DsemOBP10	Yes	Y, 1-19	23093.18	5.38	2.00E-16	39%	ABM05970.1, odorant binding protein 3 [*Microplitis mediator*]
DsemOBP11	Yes	Y, 1-18	12779.65	5.73	2.00E-45	55%	XP_017795892.1, general odorant binding protein 56d-like [*Habropoda laboriosa*]

DsemOBP12	Yes	Y, 1-20	12481.51	5.92	6.00E-37	52%	XP_014604384.1, general odorant binding protein 56d-like [*Polistes canadensis*]
DsemOBP13	yes	Y, 1-17	12634.29	4.82	3.00E-32	41%	ANT46048.1, odorant binding protein 19 [*Microplitis mediator*]
DsemOBP14	No	NA	NA	NA	5.00E-19	44%	XP_015125081.1L, general odorant binding protein 69a [*Diachasma alloeum*]
DsemOBP15	yes	Y, 1-18	14045.94	5.66	1.00E-14	35%	XP_017753799.1, general odorant binding protein 56h-like [*Eufriesea mexicana*]
DsemOBP16	yes	No	27092.59	6.31	3.00E-30	43%	AQN78386.1, odorant binding protein 8 *[Meteorus pulchricornis*]
DsemOBP17	yes	No	21332.68	4.9	2.00E-36	44%	AQN78386.1, odorant binding protein 8 [*M. pulchricornis*]

Supplementary Table 2. ***T. pretiosum*** **OBPs.**

Gene name	Full Length	Signal Peptide	PI	Molecular Weight	mature AA	Expect	Ident	Sequence ID
TpreOBP1	Y	Y, 1-22	8.71	15032.73	132	2E-91	90%	ANG08491.1 odorant-binding protein 1 [*Trichogramma dendrolimi*]
TpreOBP2	Y	Y, 1-25	4.83	14599.41	127	2E-107	99%	ANG08492.1 odorant-binding protein 2 [*T. dendrolimi*]
TpreOBP3	Y	Y, 1-22	5.96	14983.56	130	5E-106	97%	ASA40277.1 OBP3 [*Trichogramma* japonicum]
TpreOBP4	Y	Y, 1-19	5.45	12776.75	114	8E-84	90%	ANG08494.1 odorant-binding protein 4 [*T. dendrolimi*]
TpreOBP5	Y	Y, 1-19	5.14	13830.53	120	2E-92	93%	ANG08495.1 odorant-binding protein 5 [*T. dendrolimi*]
TpreOBP6	Y	Y, 1-23	4.15	13825.72	120	6E-99	96%	ANG08496.1 odorant-binding protein 6 [*T. dendrolimi*]
TpreOBP7	Y	No	5.72	16898.69	153	4E-45	54%	CCD17854.1, putative odorant binding protein 85 [*Nasonia vitripennis*]
TpreOBP8	Y	Y, 1-22	6.46	13407.55	122	3E-98	97%	ANG08498.1, odorant-binding protein 8 [*T. dendrolimi*]
TpreOBP9	Y	Y, 1-27	5.36	13361.11	119	2E-103	99%	ANG08499.1, odorant-binding protein 9 [*T. dendrolimi*]

TpreOBP10	Y	Y, 1-18	5.63	13547.40	119	4E-90	93%	ANG08500.1, odorant-binding protein 10 [*T. dendrolimi*]
TpreOBP11	Y	Y, 1-17	7.48	12666.67	119	9E-88	98%	ANG08501.1, odorant-binding protein 11 [*T. dendrolimi*]
TpreOBP12	Y	Y, 1-18	5.27	13426.28	119	2E-96	98%	ANG08502.1, odorant-binding protein 12 [*T. dendrolimi*]
TpreOBP13	Y	Y, 1-17	7.8	12279.11	109	1E-53	66%	XP_014219837.1, uncharacterized protein LOC106647812 [*Copidosoma floridanum*]
TpreOBP14	Y	Y, 1-19	9.32	13439.53	117	1E-92	97%	ANG08504.1, odorant-binding protein 14 [*T. dendrolimi*]
TpreOBP15	Y	Y, 1-19	5.06	13402.08	119	2E-97	97%	ANG08505.1, odorant-binding protein 15 [*T. dendrolimi*]
TpreOBP16	Y	Y, 1-19	4.96	12914.79	114	4E-82	92%	ANG08506.1, odorant-binding protein 16 [*T. dendrolimi*]
TpreOBP17	Y	Y, 1-20	4.04	13483.34	120	4E-91	93%	ANG08507.1, odorant-binding protein 17 [*T. dendrolimi*]
TpreOBP18	Y	Y, 1-17	8.95	12678.93	117	2E-89	0.98	AZB49386.1, odorant-binding protein 5 [*Heortia vitessoides*]
TpreOBP19	Y	Y, 1-20	5.5	13980.46	123	2E-81	0.98	ANG08509.1, odorant-binding protein 19 [*T. dendrolimi*]

TpreOBP20	Y	Y, 1-16	7.75	13005.76	118	6E-68	0.99	ANG08510.1, odorant-binding protein 20, partial [*T. dendrolimi*]
TpreOBP22	Y	Y, 1-16	4.34	13959.62	121	2E-88	0.96	ANG08512.1, odorant-binding protein 22 [*T. dendrolimi*]
TpreOBP25	Y	Y, 1-23	6.53	14056.71	123	1E-82	0.82	ASA40280.1, OBP6 [*T. japonicum*]

Supplementary data 2: *T. pretiosum* OBP and OR amino acid sequences

```
>TpreOBP1

MKLKVSGSIIFILSIYLLNVQCAKMSLDELKKMVKPISSTCQKKNNVPQDLLLASYSGVFAREKSLMCYYRCLAT
MLKLMNKQGQFALDKMFTQVDLLVVEELAPRIKEIAKICFDSTPKIDDPCEYTYDLVVCAYNIDSSLQGILSKSQ
MKNR
```

```
>TpreOBP2

MTVRPRPRLGLGLLLGCYAISLVYAGTRPSFVSDKMIETASTVVNACQIQTGVTTADIESVRDGQWPESQELKCY
MYCLWEQFGLVDEKNELSLNGMLTFFQRIPAYRNEVQNAINECKALGKYFATGDSCEYAYTFNKCYAERSPRTYY
LF
```

```
>TpreOBP3

MRLSTTAMILSVFFISHIAVESKKMNIEELKKMSKPMMNSCQKKTGVKTEELEAAEKGTFPTGNKPLMCYFRCLA
VMFKLTDKDGNISLHHLLHQIDLLVIDEIAAGVNDMLQFCFEHTPKLEDSCEYIYELVICMHKRNTEMNFFEGSL
LS
```

```
>TpreOBP4

MRRFASFLALFAMVVLASGDMEQMKEAFKSCKAEVGIAEDTQMKDIPSSKVGCLHACVMKKFDNMKDGKVVVENI
LQRAEKKMNPLPEEMKEKLTKCADDANGKGDECEVASYMHECWWDSMKSMGPPKGPSN
```

```
>TpreOBP5

MQKIALCLAIFLVTYRVEAANEVPAEIRDLIAGVREKCHRETGVDIEHVDRTVEGYFHPSETLGCYFSCVFNQFN
LLDHDGHLNFDEVLKRLEGLESFKEHGTEMIEKCRHLTGKNPCDSAFNLVQCFQQTNPEKFFVI
```

```
>TpreOBP6

MQPARVFSALAAILTFQALVVYAKRPEYITDEIMDMISNDKNRCMAEYGTTEALIDQVNDGHIPNDRAITCYMYC
MFESFSLVDEDGEIEIEMLVGFIPEEFQEIAAELIEACATLPGEDVCDKMYKRSSCVQAKRPDLWFMV
```

```
>TpreOBP7

MNIVHFTFAFLLAVLTIYAFILMYCTNRLFIAGAQAAALGHVSPVSESFYACLAESNLTKAQFIETLKSNDTEVA
QCIASCTMEKEKFMTGEQIHENAIIKKMAEVSQEIGREAITYLVKVCAEEARELKGKCGVAHSVVRCIHDSLLAE
GWI
```

```
>TpreOBP8

MKNTFFIGFCIFCIVCTGEVFSAATQEQMESMSNGLRRTCVNKLGITTADIEGIRGGNFVDSPGARCYIKCVMGL
MKTFTKQGTIDIDVLVKQISIMTPSTIGKKLIEGAKTCYDEVSSDDPCELAWMFTKCTYLKGPDSFFFP
```

```
>TpreOBP9

MSVSTHLPIYVFFCSFVVLSSVANVRSAEAPKEIQGLIAGVREKCHRETGVDIEHVERTTDGHFHESEVLGCYFS
CVFNSFDLLDHDGHMDFDKLLKKLPAVESFADHGAAMVAACRHITGANPCESAFKIMQCWQSTYPDKYFVI
```

```
>TpreOBP10

MYLIVGLVLVSCILHVHANEVPMEIKNLVAGIREKCHRETGVDIEHVDRTVEGYFHESEVLGCYFSCILNSFDLL
DHDGHIDFDKLVVRLKGTDSFREHGMEMVAACRGTTGKNPCDSAFKVFQCFQKTNPAKYFVI
```

>TpreOBP11

MKTTLVFLAVCLAVTFASTLKDEQKAKLREFKEACIKESGVDAAVVDGIVKGGPITRGDKIDCFSACMLKKIGIM
KPDGAIDVEAARGKVKTTNADPDKANKVIDACKDLVGKDACETGGNVFSCFITKKDFPVLD

>TpreOBP12

MKSFTFGLVLVVMGVCNAAEIPAEIKGMVAGLREKCHRETGVDIEHVDRTVDGYFHPSETLGCYFSCIFNAFDVL
DKDGHVDWDKAITKLEAVESMKEHGMEMINACRTVTGKNPCDAAFNIVQCFHKTNPEKYFVI

>TpreOBP13

MKTAAFLLVVCFVAVFAEDPIKDQAVSKDLIKACLTENGFDAAQYPAGLRNAKVPENMEQKRNCYYACMMKKMNL
MKTDGALNEENLRSKFSTNLETLNKAIDTCKAQGQNDFCKLASCMMANREI

>TpreOBP14

MVRVRLALVLAFLVSATVCHNITLTNDQLDTYIKTCLTKTRISQAFYKSDDENLKRLSERQKSCFLACMFKKSGI
ISDDGTVSSVTDDQEDSATNKAIKRCTKRAKGDICRLAWCLRKLEKFSLPIIVQKPRIVQY

>TPREOBP15

MKGILYLTITILCMHNVKAGEVPEEIKHLVVGLREKCHRETGVDIEHVDRTVEGYFHPSETLGCYFSCLFNSFDL
LDHDGHLDWDKAISKLDAVGSLRDHAMDFINACRGTTGANPCESALNIVQCFQKAYPDKFFVI

>TpreOBP16

MKCIFTLTCLLVLTHTIHCEYEDTMFMDEMIKCAKEMGISADQLKEALETKNDEKLSCVNACAMKHLGTLSNGKI
QKEKIFELIDKYADKIKDSDKLKEVVTSCADEVSSSGDMPECQLARKFTTCFENHFKV

>TpreOBP17

MKLFIEIFILAVAAFCLVTAGRPDFVTDEILEMVAGDKARCMNEHGTTESMIDAVNEGNIMNDRAITCYMYCLFE
AFSLVDEDGILEVEMLVGFLPENMQASAETIVNSCIDESPGDVCDKMYATAKCIYDKRPDLWFML

>TpreOBP18

MKYLAVILAFCLAGAYAGLSDEQKAKLVEHRKVCVAETGLDPVVVENIKKGQPVQFDEKLSCFAACMLKRIGIMR
PDGSMDEQVARAKLPKDLPKDKVDAVINSCKTQVGRNQCETGGKVLGCLLKTKAVSILA

>TpreOBP19

MKIKLATCIIILINLSAIDSKMTIEQLKNTMKPFKNTCLKKVADVDPVMVEGTKQGNFPDDPTLKCFFKCTLQML
KVLKNGELSVPAMMNQIDIMMSEELVDKTKAIVVDCDGKSKNLGDICERSFAFVKCFYEADSELYFFP

>TpreOBP20

MRLIVLFFICVLRVRADSNGSDLGSKDDDMVTCLINSGLDPGIYSGQKIGASAPTENQTNCYLACMFKKIGYMTK
DGSIDVESILSTSHGLRKRAKARQRLDEIVNQCNMHAKDDVCKLARCFQDLRKSLLEKN

>TpreOBP21

MKNIVIIISLIVTAHAADHSLDKDELEVKEYFEQCLSEHGLKESDLEELKNKADPQILCITACVFEKQGLLMKNG
EFNKKEIIKVEQEEDPNFKQDDFDEIFSFCEEKAKGIDDACLKGNTLTMCFLDEISQLDDKN

>TpreOBP22

MKFAVFTCLMVLLVVQHYPLVQCKKMNIEELKGFTKPLTKTCKTKTGISEATLAQIAKREFPPDPVLKCYFRCIA
QMGKMMDKKGNLILENMIKQVELLIVDDISPRVKSVFTECFGEMTAEESCQLAFDFIMCIERIDQELNIIV

>TpreOR1

MDSYERSTSSYYWLFRIIGIVPYKNRRFNFIPAKVISMLIIAALFNYVFVFIFGTVAKAIKGTLTARFLSRTLPL
MSLWARLVILYSKRHYCNRTLHEFRQLWSRCADTGHENLLLLLRFNYKIRLLYIFYLANFFLFGLGAFLPTFYRR
LPPLQPNGTSRRILPFFWYTKVYDSPNYEIVYTIHCITYVCIVVSISACNVFPSFLFLVCSGFFRILRKRLNDLA
KIDWSTDTSYYTSLIELVNLHQDILSLSKRSVSLAQNLLLINLTSVTYNIAIIALEFIDPTSNRPRLLPILITLM
VQLFNIQWSAECIRQEVGRLKITRKKFKLFFICQRAKQSYLIADASYSALKLDASANSRYCIRMMIMRAQKPVEV
KAGGIYSMSFESLNRISGNIYTFYTVLRNVQT

>TpreOR2

MIKKLTEKFTKIDYDNFENYDPFLFLKFMYILCGIWPLDKSYYPNYARKIIHILPSLSMTGIFLIMMSAFFSPSV
STEDFIEIFSLQSAAITALTRMYFLLLRKPQVYSMIYHTIEMETKGPIQLLSKEECIIINKWRIIQDFLTKMLSV
GYLLGVIFYLIPPAFFRKLPFPVFIPENLCTEWYTRLYIIECIAIFLSCPAWVSLDLYLCIFLCQLCMHLELVYA
AVQDLRGKDRATLHTIIRRHSKVLMYGQKLCEVVHYIFFAICCSYGSFLIFGTITIGEISWKTHGALAIKNIVTL
VVCSSTIYLICYVGELLQDLSSRIGDGILLDNYAEHGSNRKYLKELEIMNDRCKLSLKIEYTPNMIVNMKLFTNI
MNYVVSTYIFVNTMFVNPV

>TpreOR3

MNLKIKAMSNNSAKTFIRHKQITNPAGSDGFEYIMAPCRFFLRLLGAWPDPLENDSWSSTLRILIVTTIMFLFAI
VSQTVKLFNCWDNLNAVAEILSNCNIPTSIATVKIVNIWYHRHVLKDMLSQVIDDWKLPRTEEELTLMWQNAKVS
RLLSIGCIFMTEATLLAQCMVGLWIPIFYTFQKSQLNSSVEWPLYMTGSFPYDTQKTPYYEFTIFGQLFSNVLAS
TSFSSSDSIFFILMLHLINQLSILKLSVTNLPQKIVTIQDRINFMNKFTSFHARHNQLWRFSLAVENTFNRMFLI
QMVPCIFGLGTQGYQLISNIIQEHTPLVELVFMIYFLVLFLFTIFTYCYVAELLRRQSLEISDAIFACNWRILKS
REIKLLAFVMARAQKPFEITVGKFANFSLQLYVRILKTSAGYLSMLLAVKEKIDT

>TpreOR4

MSDIKPSSDRDKEVPKNAKLVGFEKHLFPLLFSVLGASGIWRMSRVRHPLAVLLWKLYYLLAATSVFVLFFTISA
EQLINDEKSWREFFESLFLLLTIFNGMCKIVNVHLRRTRYLRIVSQLLQDSWLDLRDPEEVAIVEASKLDERFIV
RFNMTLVFFNNVSNAVGPLLDGNPGRDLMVDAYTPCRRSASLACYLIFYWYQVFGYNITSFVHIGCDGFFFDTVD
RICAHLKILERRLVKLPELVGAQKFESDEEKMQFEMDYVKECVRYHHSIFEVMRELRGTVHVAIIVQLLSSVIVL
CTSIYLLSCQPLFSPEFLQLFIYFNCALFQNFFYFWFGYKITVNTLHVSQALLDMNWWTLDVKTRKMLLFVMMRT
SNKVELFNSTIMILTPESFVKILKLSYSAFNILKQTS

>TpreOR5

MNPDEVFDNKYFRLNRTLLKQVSLWPYESTSTKVIKRIFIMVGFYSMSLPQMIRGIEEIRSDDPDPEIIIENLSG
FIYFHGVISKLITQMVTENKLKYLYEEISKDWKTITDKNEKAVLEKSAAVGHQLTIFYTGFVVLSAIFFVSITAF
VPVLLNHVLPGNQTYQKQICIYAEYFVDQEKYFYYIFTHTMVIGVMTVYVATAIDSVFVNCVQHVLGLFNIIKYR
LKEISRVYDSSVNNSIDLHFDVKRYLIDIILTHKKSLEFTNLIQSAYNECFFLLAGLIVAGLSAFTYVLSQNVNN
PLNFMRIWFLWFGVIVYMFFVNLPGQKLLNISEELLLAIYDSSWHKFPIKTRFLIQVMMLRCLKPCRLTAGPLIE
MNFASCSNILRTAFSYFTVVNSMNS

>TpreOR6

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MTYLCASMGFNSSVNGLLNNGALKKLFDSLKENWNDLQSDDERTIFATYANYGKMFTVGLSISYYLVILSYIVSA
LMPSMLHYYFTGNWTIPERNIFEAELFVDPVEYYWPLFIHGIIIAFVAIWILLAYDCFFIMIVLYCCGMFSVLLH
KIEQMDTELYNYSYDQQLALEKIEEIVTYHLKCLEFAQKVEDFFCIQYVIQLLINTIVISVCGSQVINIADESPL
DIFRYTYVAGSVIFRLSLTNVCGQSVHDRSLRVYEQLIHRNWYEYPIQIRKLFIVLYNRSVEPCNLTAGKMVNLN
LETFSKLMKTALSYCMMIIQTQ

>TpreOR7

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IYCYVAEKLNDRNSDLLVAVYECRWWALDKRDARSVQLIMERAQRPLQVTAGKFCVLSFRLFAIIGKTSLGYLSM
LLAVKER

>TpreOR8

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ATLGNETMVGMVRQLPLRTGCMFGNTRNGLYASLYALETLTIQTTAHGNIGSDCFIFSLMMHLCGQLELLRNRLS
TIGDGTSAESKEEEEEEERKKTGREIRERVERHSQLLELASGLNATLSGVLIIQLLLNAGLNLMLCIRIILALKSG
VALAAVRPLLAFGVLMLQLFLLCYASERLCQQHEALADSIYQSKWYELPIEQRRNLNFMAARSSKPIYLMAGHFY
AMNLENFKNILKASFSYFSILRIMFDAEEKNESTRPIFRCRLSVQACTVSISA

>TpreOR9

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QLPFIPLLLNVIMPLNESREAIIVINTDYSITPYTKSGHLWVHYSLTGTTTAAVFIATDATFLLVVFQILAIFEV
VKQRIRQAVLVAQESSEQKSYGILIKAVQLHKDAIQFLQLTDEANSLQFFAGLGGTIFMISFGSIALLIRMDAYA
DLFRVVILTMGYLFQLFILCLLGELVINASTELFNFPMLTDWHVLPIKSKKLILFFMGRTIRPSYYTAGGLYIMN
VQNFASIIKTAVSYIAVVLQFR

>TpreOR10

MDIFETNYFLINKKMLSLVGLWPYDDKATKNIKRSFFIAAIGVVTILPQFFGLRMKVLLDNMRKNWIKIVDKTER
AILIQYSERGFMISVGYMSYISLALIVYISTPFIPTLLDLVIPLNESRPKMHLLKGEFWFDADEHYWQIFQIDSI
SCIMASTVIMTVDSFYANCAEHCLGLFAIVKYRLSIPNKENLKKTDPRYEDVSYKWLVENIRLHSAVLEFSSMLN
YQIVHTTSLSVLVLMSLEKPLDFVRFSMLLIGLIIHLFYLCWPGQKLIDHSSGLFRDAYNSQWYDSSMRSRKLLA
IFILRCSKPCILTAGGICNMNFENFAVILKKSMSFFTVFSNIS

>TpreOR11

MQLFQKVIKQHLRLYDLVDLIQRTFNVPINGQFFTSYSVVSINIYLLTTFQTLSIHCLSVLLHLMCMLVEFLIYC
WAGNEITINHEKFRNSIFLINWTKLNLRSRKGLILIAARTVRPIVMSRNSIMDLSLQTFLKVLKLSYSAFNVVRR
AT

>TpreOR12

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LDSFVNILRISYAVLNFMRRNLVVCQTAGLLLTRQSFDEFNETFFVVLSTGFASFKGSCDLWNRAKIIRLVDMLS
EPFCLARCQRESAIQKTYDQMGRKIEIIMLCVVELAAVIFLFGPLINHSRELPYKVLLPYDLNDTLIFSLSYVHQ
TFSCILVTAGTMTNNALIVGFMMQLCSQLDILKLRFRKASDKIKEQLEKEVASGKLSNRSSRQMEVQIIRELAQH
HLHAYNLAETLCDTFYGIIVGEFCINSLVICVSVYKLSLNAGTIPEILFNALYLVCVTGEFFVYCYFGNEITYKS
TTLFEAIAAIDWNPMSKEFKKNIIFIMSRASKPIFISCGAYVYLTLESFMAVVKLSFSVFNVLKSTL

>TpreOR13

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AVSPFLGFNIRMISNITDYGDRHMLVQSYYPYDYSKSPIFELTFGSQLVAGIFIGMSVSVPDNYFAALVFHYSAQ
FEILGARVEQLIRDDCSLDAESHFFSENWPRLVDRHVHLMTMVSAIEQSFTFINLGQILCMSCMVCCLGFELSVS
SNVXGSINLQFTYGSDVQIFSGGEYKPSALQILTLVGTLIMMMSHTLVDCIACELLASNSSGVFHHISSGLGSNI
TRRKIKDIIPMLMTSKKPRKLTAGKMFNLNLASYCSILKSIAGYISMLIAVNQR

>TpreOR14

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VVAPLLGFDLRTSNNITDYPPGGRKLLVQSYYPFDYSASPTFELAYFLQLVGSFFVGMAVSIPDDYFGALILHAS
AQFNVLAARIDGLIDEVTTTRDCRGPAAVADRSDSSTINRRLGRLVDRHVHLYSMVLAIEKSFSLVLAGQIFCMS
VMVCCLGFQILSVLNNMSDKPTPLQMVTLIGTLFTMMLHTLIDCLASEALASQSTEIFYRICNSRWYELPYKSMR
CLVPMMLVSKTPRQIKTGKIINMSLATYCSIIKSTAGYISVLIAVSVK

>TpreOR15

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ETIECSLVVSALSMATIRLIVFNRNAREMFQVLDTMRQDWTVNYTSAEDRAVLRNRCRLSFKLAKIFISSVVITW
ALFSAMPYVEALHGHRRILPFRGYYFFDLDTASSPVYLGVYVMNFTLGAFGCSTIAAATSFSLIATIHASAKFAI
VKKKFETIDWNEPEQVKMCVQDHQNCIKYAENVETVINILALGQFVVSTGLICFAGFQVTTMMKDHGRLMKYSSF
LQAAIMELFIFSLSGQLMQTESLEIAEHAYNSQWIGCTKSINIRMVITRSRKACTITAGKFYDMSLESFLKVLSS
SFSYFTVLITVKDG

>TpreOR16

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NSAALTSVIKLFLLWKNRYVLEPAIRATEDDWSSVNGNNETTARNRFELESMTRQANRARIFTVSGYVIMFGCFA
GFVFTPLFGIGIRVVNNITDPLDGRFFPLQTYYPYDIASFPLYELTYASQLLAASFVGIAFSVPDNFFGALVFHA
CAQCEILNALLREVEQIADYCTLDLVEKEGERFHRHLRDFVRRHVQIIGFVDSIEQSFNVLVLVQVLCLSVVACF
LAFGVIQSIRSDDVNGLAIVQVVTLVATLLNLMIHTFVYCVACELLAQHSMSFFHTVYNFNWFCLRAKHSRPLLI
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>TpreOR17

MSEEAKKILTNEEGYSYAEQMTRCIMLPVGLWPVESDYPRFLRRFLIVGSVLVTLFMMIPLSIFICVDAPNITVK
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AKIVKTSAAYFNVLWNMTSNSIRA

>TpreOR18

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QHNYTRAYTLTISLLSMTYNIFVLCYYGQILMDKFEELGRSVYMSNWYKLSGSSARSVVLVLGWSNRPFRLTAGK

LITLSIESFAKVVELDCLSFDPIKYLKQLDILCNFCLPKKFGEDLCDSNMKDSPYSENVGCLFQCALEHDL

>TpreOR19

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LALRQGKYIRTYPVKIPFEYESGGLVHWIIYALEVAAGYYCWSVTVGVDTFFGFFTLHLVGELQLLSSRFADMKP
DKNHRKIIKELVDRHNLLIEAQRTVQKIFGLLSVWLAITCAFIICAIIFQATETKNMTAYKAVYLLCYAFLKLVQ
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>TpreOR20

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>TpreOR21

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>TpreOR22

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YFSDWYIMKPKLRSLLILTMARTLRPCYITACPSVPVDFNMVTVVFKTAMSYVAALISIQS

>TpreOR23

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IFMPLNESRPRQPLLIAEFFIDENKYFYSMTTYAYMTCLYGIIPLLGTDTFYMNCVHHICGMTVILSRRIKNNIN
GSKKELLRTKYQRTVDCIINHQSIIEFGDGINAMYNTSFFIIIFLNTTLLTFTGVAALIKFNEGNKYEDVVRFGM
FGVAEVFHLFCNNYMGQLVVNSGDEFRKNIFNSEWYQAPIKVRELVHFIQLRNSRPILMKAGIFPLCLPNFTVVL
KSSMSYFAFLQSTRY

>TpreOR24

MDLHNYFKYNEVLMSLCSQWPYQSVVKAKIIRGFFILQHISICIPEFIRFIELRNDLDNVVTCIIPIIYNIGVFI
KFLNAVRNMNKIKNIIETIKCDYTEIIDNVEVQILHKYASFGKFLTLGYISMCALTLILYLALPLSPVIVDKLNP
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HVEKSVNQYKGTILTWGQLHNMIRYGMFTAAQMFHLLFYCYQGHSVLDKSLTINDSINKSNWYGSSIRTRKLLTM
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>TpreOR25

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ICYPSQLLIDASNDIYFKCYTSKWYEYPVRTRRLLILMMTRAAEPCYMTIGPTVPLHFETASTIINTAMSYVTTL
VSLCAL

>TpreOR26

MIVELKKKMFMNFLKMCLAFLGGKMMLMLVCYPSQLMKDASENIGLACYCSSWYEFKPKVKSLLFLMMVRTLRPC
CLMACRGVPVNFSTVTFVEKTTMSYITALLSIQS

>TpreOR27

MSSVSWHSEYAVQMCRYFLRPIGLWVTNENSWREKFFNKLLTVSTFSLLLFLLIPCALHTFLEEPNIAVRMKLIG
PMSFAVMAIIKYSSLTRLTKRLEKCFKSVEEDWKSSDSAERKVLHRQAKIGRLLSIFSALLMYSGSFIFYHVIMP
VAAVQTFSANLEVAHDSLDINGSSSATNEKKLRILTFPTYEAWVNLDDEIAYQFVYLMQCLSGFVMDTITVGTCS
LAAVFVTHTCAQLEIVVEMSRNYVDSRKNDKTPKTSAERLTVLVKKHCRALKFATQIKDYLNGICFVEFIGCTAN
ICFVGYYCLTEWERKEPISMVTYFILVISFTLNIFIFCFIGEHLAEHCKQLDSVYMNIDWYKLPGKEVVDLLMII
AVSRRPVKLMAGSFADLTLITFSSVMKTAFTYFNLLRTII

>TpreOR28

MEIYNREYFMHSRFFLKLFGLWPFQSGKFNKIHQIMMMLPLGTLFIPHCAKAYETRHDFHIFFVCIISLLFFMHY
ITKFLYLSLTEKQFQRMLKKIDNDFSLFSDRSLQIIHDYSNSAQKFNMFYSIYLISSVITFNLGVFMPRALDLIM
PLNESRPLHPIRALRYYIDALDDSFYFVLFHGMFFDMVSIVVIVGFDTLLINCAQHACALFKIASMEIRDCTEEI
NQSNERVNLLTQRSMQKNIYHRKIVKAAIIHKHALEFFDVLESTYSLLNFFIIGISLSTVTLAEFAVNATDVMMR
FALLVAGQFLNILYQNYPGQLIKDHSLEVHAICCECQWYKDDVPDESKKLLVMIMLKSAKPSCLTAGGYFVLDLQ
NYLQIMKASLSYFAFLRSVN

>TpreOR29

MAGKFESIEKFYDQPFFALNKVTFRMSGWWPFQETKKRQMIWSFVWFCIVTVVIPEVIYLIQIIKDLTKVIECFM
ALTITYGAFTMAFNAWHNNDSLKKILEHIYSNWENLRDEQELRIFTDRATISRLVNIVYALAVFYNIMIHTVSPL
IPPVVDWFMIGNWTRPEKNLLEVEYLFLDPDRYYTLIYVHGAQAGFLVVFVIVTHDTFFMTITQHSCSMFTLLGH
RIRKMDEDILKRNRRHSYAMQRVKEIIFFHRDCLRFAQLLEDTMSVMFLFQLFPSVLMISVVGAQAMIRAQAIEE
LIKFGFIFISMIVRLFFICWCAQSIMDNSLVVMSYLANTRWYEYPESSKKLFQLMFMRCSRPSYLTAGNMIKTSM
SYLTMILQTQE

>TpreOR30

MKIPIIGKPLEISLKLTGFWNEEVNKVVLALMWSSVLTTMPFSVWVAIRMSKNPLMLFDNLSVLLAQVLLYSKLL
IFSYNRRLLRNLLREMESDFESDPQLLRYIELSGDDPRRFCKYDFFVYLGSSALFWVQIGFMYVAVPVEMREPVF
KVHYPFDYKSSPVYEVLVLTQVIQGMLQCCIQAFSESLLIALVSYVCAQIDTLFARMEEFSKMCVSDDKRNLPRL
SQPVYKQHIKVLNVFEKLNKIYFYVTFFQVFFTTVVICLSGFVVIVISDDPTILVKFAGYYFCSCWQIFSFCLAG
QRLLNKSDKISMKMYETIWYKTSIKEIHAVAFIIKRSQKPLMLSVAKSTELSMTTFTQIMKTSFSCLSVLRARYN

>TpreOR31

MSRTGFEECAGVTQWCLTSIGYWPVIHGKRHWFFQILLPIVTPTAMILFVIVPQTENIYRFRNDFSIVMDTLAVA
VVGCVLCLWKFLGLHSNQKDLQIIIENIELDWKTANEHEQIIMWKNAKKSRIVTTIILSSTIANVSNLLMGVIMG
CYYAEANRVGNGNIERPYYVLSHFDFDAQKSPIYELIVIGQFLGCFFASLIHTGYDGLFVFTILHFSGQLHNLRF
SVENVAKDCLRKKCTLKSLLRPLIRAHQRLDNFVMIIEKSFNQLFLGQILTSSFLICLQGYRFILLLSEVKTEVV
PEISFLISYFLSIILSIFMYCYMAEQLRIQNDELFKSIFKMEWYELLCKETKLLITFMSQSKSATRITIGKFAEF
SLEFFCKILKNVAGYISMLLALRDRFVHTDLILVE

>TpreOR32

MGQILELICMQGSVDEFSEVLYLTLTYVTLCLKLWNFLIHQEEMQEILDDLKSFHCEPKSTEEEDNCVEHHMLII
KLIRKIKAFFSQVIFFFFLSSLITLASSIYQLSKLKLASFEFFSIFFYFICILTQVFLYCWFGNELGYESRAITE
TYYMGNWLDLSNKDRRHIWFLNSVSRNSKNISFHGLCALSMETFLWIVKTSYAAFNLLQRFCEYLREKMDIIPAN
FRLLQFCGIWTETDSDKNILKSIWGFSLITVIFYFTIVQIIKLYFFLEDLEELIDVMFLTVTYILLCLKILNFIM

RRQSVLHLLKMFRHDMYKACSPEEEKILKMYSKSAYNMFRIILILSQSTGVFFCLLPFVTLDPENFELPFKTYQF
YDDETTLGFSVTYVIQLVALIFGIFINVSMDTMIYGFILLTSGQYELVSYRFQESISKNDEFLLKQTITHYGMVK
KTVKRIQTAFMIVIAPLFFLSSLTLCASIFQLSQNDVFTLEFLGFTMYLSCMLCQVFLYCKYGEELKSNELEFKN
NIYKSNWTSLQVNQQKLLTMMLILANNVEAISWKGQFTLSLDTFVWLMKTSYTAFNLIHKTSG

>TpreOR33

METLNVKENQKKLDKIARVFQWNRRLMSFLGLWPDSPNLLLFVLTFGYYSYDMFLEYMDLLVYIDRPQNVMLNLM
ENMAFTEIFVRILMLRVWNRQFGELLAAAEKDFEAKSYDTDEEVAKFVPFYAKAKSFMKLLISNTAFTATSFYVK
PLLGQLGPVMDYFGANGEPNSTLIFLLPYRFYVLYELDDAPTYFWTYGSYLPFVFISGFGQSAADCLMVTLVYHL
SGQLAVLSMRIEKIDGDAKELRRHVARHAKLLRMGKIIEEAFSAIEFGHLIGATSLVCLLGYQILVCLSIGEYSV
LVSLFAFIFLVLLVLYAHCTVGESLIAESNRVCEAYYNCNWIDMRPENARLLILCMARSQKPLRLTAGKFTDLCY
QTFTDSIKTSMGYLSVLRAVM

>TpreOR34

MERRKYRRRSYIYSLADCIYINQRKKQEKLRYCSRVFEWNELLLSVIGLYPGKFSFARFYLNLAYFTTAMGLEYL
DLVLSLGDFERVVLNLTENAAFTHIYAYTLSLWLGNREIGQLLGQVASDFAADYTDQEIATLRRYYFRTRVFIEF
LFVNLFTTASSYFMQPFTGQMDQILGYMRGSSANSSIVYQLPYRFHAFHRVDEPASYLWTSAAYAPFVLVTFFNQ
SSGECCLIALVYHVAAQMAVLASRIRGIEPGNDCTEQLANCLRRHARLLRMGQQINKVFSAMLLVHLTGIILLVC
LVGYQLLWCLANGEYALLPSFIVWMCLLLVSLYVHCTVGETLITESDRLHGAYYDCRWNEMPPSTARWLVLAMAR
SSRTLTLNAGSFSTLSLATYTSSLKASLGYLSVFRTVMQSES

>TpreOR35

MSGKFESIEEFYDQPFFSLNKFTFRMSGWWPFQETKKRQMIWSFVWFCIVTVVVPEVIYLIQIIKDLTKVIECFM
ALTITYAAFTMAFNAWHNNDSLKKILEHIHANWEDLQDEHELRIFSERAAVSRLLNVIYALMVFYNIMIHTVSPL
IPPAVDWFVSGNWSRPEKNLLEVEYLVLDPDEYYTLIYVHGAQAGFLVVFVIVTCDTFFMTITQHSCGMFMLLGY
RIRKMDEDIFEKRCSSSYATRRVKEIVFYHRDCLRFTQLLEATMSVMFLFQLFPTVIMISVVGAQAMIRAKAIEE
LIKFGFIFISMIFRLFFICWCSQNIMDSSLAVMSYLTNTRWYEYPESTKKLFTLMFMRCAKPSYLTAGNVFTLNF
VTYAGMIKTSMSYLTMILQTQESNL

>TpreOR36

MFKIIGHDMVHMHEKLELTSEPLQNEQIMEIINKRIRTNIQRHQETIELMINHFRLFKALQKQTNMKYFIIIILI
ISELSLAGFWSIVVLENNKLYALKMIATMIATMMILLYLIYSSEDLMNACDRLNQDCYDAKWYLFKVKERRLILL
MILRTANPCTLTAGPTVQMNYETSAIILKMVLSYLMAFYRIMGDGN

>TpreOR37

MRKIFHNLLQLQSIFGVWTPEIENNSTVNKLIYTVNRVLIFPMPILLIVGMILALLYNNLNEEDYLETVMMLLTV
LNNFLKAVGMRLGRKYLIKIIEITRCERMSRLHDPEEVLIEKNYSIFLRKFFNSLHIMIGATWFAWVLPILFQNK
NQRVLPVKVWTGFDGLSDQDFWWFFIPDVGFFLVAAIMALHHELVLTTVLLFTCSQFDILAHRIKNVARKATKIA
NSTHGYVESCESRLIEECVTHQLLIFK

>TpreOR38

MAVAFTIILIMVLVICYPSQLLLDASNDIYFKCYSSKWYEYPVKTRRLLILMMTKAAEPCCMTIGPTVPLNLETA
SIIINTAMSYVTTLVSLCAL

>TpreOR39

MPPSSSKVAASKSENEKIPYVNRDYLLDTEYVVKVAKTLLTPIGIWPRDGDDSPRSVTIFWMRIVAVFSLMLCLL
VPHFTWTFFKAEDLRKLMKIIAAMVFSSLAVLKYWNMIFTKKDIRACLETMEDHYRLVESEEARQIMLKNAKIGR
LFTVAYLSLSYGGALPYHIIMPLLQPRVLRQSDNSSMIPLPYPSEYVFFIVEDPPLYQIVFVGQILISSIILTTN
TGVYSLIACIVMHCCCLFEVTGHKLECLLDGRSYDKRAVRPDLVKRLVDIVDYHNEAIAYADTIENCLNIVMLSE
MGGCTLIICFLEYGILQDLEDADYLGMMTYGVLMTSIFVNVFILSFIGDKVREQSELIGNSMYSIQWMDLPNDFA
LKNVKFIIARANQPTRLTAGKLFDLSLQGFCDVAKTSMAYLNFLRTLEIT

>TpreOR40

MTLTRSSKDENPGFHWAFGLNRLSLKIIGIWPNEEDEDQAPKTVWKKLIALSVPLMVAGLILGIIVPQLYALNLV
WQDFSLVIDNLTTVCIAATSTIKLFLLWNSRKLFEPILQLASDHWRRGAQRDSSRVVMLRQARRAKLFTMSGYAI
MGVCFVGFMFTPFLGLSVRIVNNVTDDLASGSFLPFQTYYPFEFHETPVYEVVYASQVLTASFAGIGFSVPDNFF
GALAFHACAQCQLLSRMIRELPVVTMAGDKGGEFCGRLAAFVEHHLLVIRFVNLVEKAFNMIILVQTVSLTLIIC
FLIFGSINSLESDDNGAAIVQIFTLSGTALNLMIHMFIYCIASELLAEYSEGIADAVYSYDWHLLPSSYSRQLVI
IMIRTEFPLRFTAGKFFYLSLNAYLNIIKSSAGYISVLLAVQGG

>TpreOR41

MRIVNSDTNITSKFPIFKYKRHWMVINMSIEKMTHKDILNMNISEFCFKYNYWNTFMLYGMALWPKIPKKLKHAS
RLSIVITYTLLIMSGGKRFMRHYNEKQLNTTLYVDYIFQTMTAMTCLICYIASMTYDHLIYSCLGKVKDVFYEIS
RANARHRMENAYKRGVLMSTIYLVMIFGCFVGFLVSPVILPIFLNYIEHNNTHELALCIHIDLFINEEKYFLVYF
LVALYLVILTGMLCSVFNGFEIWVISVIIAIFEFIGHNLDSIIQDLRINGYKNDNSTEICNKLKISINYHQKSIK
IYEMLNHNSKVVLFTIMALLLFSLSVSGSEVVLQMKIDTATTARMAFIFFASLFLLIFISYPSQILVIASEDLRI
KCYACNWYEFPPKARLLLLMMLLRTSKPCVMTAGPGVPMNYETSSKISNMAMSFVTALISLNA

>TpreOR42

MDAVFDNPNYMISKFLLRFLGLWPTQSRLRKNLSFLAYTFIIFSLVIPIFFGMLNNKTDLVIIIEDITGILYLLS
IYTKYLSFYVFEERMIRVYNQLVKDLEDITIKEEKAILRKHARQGRFLSIIYVGYGAIATMVFNSTPYIPLILDK
VFPLNETRDLLFPYYADYYFIDAVEYHYTLFTFHGGMVICAATLGATAVDSMFVVNVKHNCGLFNIVCYRLENIG
KSALSKDHALPTAHASDEVVYREMKSVFISHRNSIESSQIIQETLSGSFLFIFAAAMAAIAMLVFDIMLNLQKPI
QIIRIGVLLVGVYLSIFYMNYIAQQIMNDSEKVNEAACNSYWYHCSPSAQKLVQLALLRSQAPIILSAGGVFDMN
LATFASMIKSSASYATVLLQMQQK

>TpreOR43

MTTTAATKERGPSIRYKNAFSEAKKLMSWNKYLMLPLGLWPSKPNDYIFVTFFCFFYYHLFLYHVVLLVSIRSFS
LMRIIGALMENVTMVQVFLRLYTMRRYNKEYGKILEEFGQDFSEDNYESNEEKRIFLSYNIQSKRFIRIVVISLG
LTAMLYFTKPLIRQLSTXKKNNKRKAFTYDLPYRIYFWYKIADLNIFILTYVSQIPLLYTIGFTQTAMDCLTLTL
VVHLCGQLGVLSERISKIDFINGTSKLIRAIKRHQELISTGLMLRKIYRVCLLGHFLGAAISICTLVYQLLMSIS
TGQKTNLVTFFVYGFLNIFRLYTHCWAGEYLIHESINVSNAFYQCEWYKLPVEDQKKIIFCIRRSQKALSLMAGN
FGHFSLVMFTSIMKSAMAYLSFLRNFI

>TpreOR44

MTFRLCVEALIGTLVNGASTQGAQFDSQYRLEEKEKRKDRVVGAAPMEIEAEYSKLTWPMRAVMSSISYWPGREQ
EEKDLGKRSSWSRLLHKCHRGVVNASMLVLTLGGSSEIVHLGRSADINDLIECCLIVSTAYLALLRVLVFATHAT
SMSRIVETMRNDWTDNYRDEADKALLRDRCLWYYKLASFYICSVIFAFASFTISPYMEIMLRKDDGPMFLPFRGY
YFFNLSSVSRTEFNGIYLLNSMAGFFACGTIAGASSFSLIAAVHGSAKFAIVQKHFESVEWTSRQQVKRCVRHHQ
DCIKFADDVEDSINILVLGQFVMSTCLLCLAGFQFTTMLRDRGRCMKYLSFLQAATTNLFLYSIAAQALQTESLE
VAEAIFRSKWIGSCSSYEIRMIIMRSRKACKITAGKFYELSLESFLKVLSSSFSYFTVLFTAKYDGV

>TpreOR45

MDFKYEYEVQKNKREACTQLKEAIKVVIQKHYRCIQLFETLNSVVRPVMLVAVVASCLNLAISGAWAIILMQINK
AEALKMFFGYWMAAISLIVTCIPSQFLSNASDDLLVKIYSIKWRNYPPKVRSMIVLLMTRGQKPFELTGGPTIVL
NFETCSKILKSTLSMIATFRSIYD

>TpreOR46

MNVNFAPIPFRVLKCCGLWRPLSWTSWKKMTYSGFSLVSLMIVIITALLVLIAVCQMSFNDDLFAENVFLMFALI
NATSKAANVLLFRGRFIALLNMVASERWSKLRSDEELEIRAKCDKTIRKISVYFTTAVFVAILLRVIAPLVDLFV
KGEIRLPVNAYCPCDIRNPSCYWMLYWQQAIGTGIATLIHAAKDCLICAFLLQTCSYLEILKRRIVAIPGIIASE
RNCGNSGNIQEMEKRLISGCVEDHDNIFLFSKILNSSLEVMLFGQIAVTLPNLCLSIYLLSTQNIASMDFVMTIQ
FFSAVVIELFFFCWYGNQVTLNSFDVEAAISTMDWTSLTVQSQKFLLQMMVRTARPILFRVGPIMDMNIDSFLSI
MKTSYSAFSVLQSTKE

>TpreOR47

MHSLVRDIIIQNVEYEILPYQFLLLTFWGIWYPQNWSLWAANIQKSYFVFISFLDIIICTEMLIFFINSFGTSNF
KLINFFFVSANITGVYKAIKLMLNRKMIREFLLTYFDADWRTPKDKIEQKIHDDINAKIKRVTLIYSVSMLGIVM
MKAMSPLTGSNSVSLPVEAWYPYKVEKTSWYWLTYLHQCILGSSAVCAHIGIDTLFMGLLLKTSYQLEVLKHRLR
SLNISLLSLNGKGSTLSIENEKMLIIECIKYHQRIYSFGKKLNDKFQDILIIILVVSSLPNICINIYALSTYTSRT
KIDIIATLFCTTSAFMQFFIACWFGNEITWNSINVRDALYDLDWTVFNLDSQKLFIFIMTRSMRPMQFKIGYLLS
LNLDSFIKIIKASYSSFNILQQTTH

>TpreOR48

MNVFKSEYYKINYYYLKLLGLWPNENHSRKKFKRITVIFLIVSLLIPQYIRLFEEWGRDVDIVIELIGSIFYFTG
SQMKYMSFVRVESKMKFIFEEISRHWNTLTDAKEQKLLRENGRYGRIIALGYIIPINIILVVYITVPLAPAVLDI
IDPLNESRPKAFPYFAEYFIDDQKYYFELTIHGWIVCILSVQIYATFDTTYQLCMQHVCALFSIVENRIREANKL
SWRGNSDSDHKIQMRDRSYERMIQAVVLHKEAMRFIGLIEECYTFVYSYVVLANTLLLSLTAVDTMLNFEKGNFK
QMIRLGMLYIGFSFHLLYNMNPGQNVIDSSVHIQEAAFHTNWYDSSSKTKQLLRIIMMRSWRPCKLTANGVVTLN
LETFAFVFKKSISYVAVIGSVR

>TpreOR49

MLNDQNSVDSFYDSTTYHLNKKCLILAGGWPFLNPRKRKTIWLAINFSLFIGFVAEIIYIGEIIDRTTEVINCLM
VIFCAILAFCLSINGAVKSHSMKRLLDSVRENWNDLQSDDERNIFSKYANYGKIFTIGLSVCYYLVLLCQCVSPL
IPPMLHYCITGNWTDPERNILDVEFFIDPVKYYWPIFIHGATSTVAAVWMLVAYDLFFVVVVLHCCGMFAVLRYK
IKQMDIMLYRYSYDQQLVLKKIEEIVMYHLKCLEFAQKIEDFFCIQCIIQILVNTIVISVCGSQVIQLADTSPQE
SFKFAFVGVSGTFRLAFFNVCGQIVNDQSLRVHDQLIYTTWYEYPKRARKLFVILYNRSAEPCNLTGAKMVNLNL
ATYSMLMKTAMSYFMMIIETQ

>TpreOR50

MSDDNLAGYEESISAIRFIARINGLWPFDKGARQYQCAVPACLIIFFIIIPQTTKAIYSRNDLDTVVEVLSTCEL
IEIVVLLKLFGLLYNKRDFQKLLTQVEDDWKISFEYEQKIMWSNARFSKLAAIFCVIATAGSVILHSFLFLLSVN
SINKSEYNNSTPVIYQLFIKSHFPFETQNSPIYEIICFSQFSAAFLSTFVFSTFDGYFFLSILHFSGQLYNLKYN
VYNLITQDSIQNKSFTRKLAVVVCRHRHIMSYIDLIENNFNLIFLLQIFSSTVVLCMQGYQFVLIISQGTRLFTS
IVFIIFFMCSSIISIFVYCYIAEIIRTESDNLLYAVYEIDWINLKSRDANLLLIFMSRLILPVKITVGKLVPFSL
EYFTTVMKTSAGYLSVLLAVAKD

>TpreOR51

MRLFPLFTNLIIIMSFSYNIFVYCYVGEKVSEQCSSVAKSIYRTNWYELPPLDARNLCYIMVLAQHPVQLTAGKI
MILSLENFTKFSKVSFNSLNMIRKILESTP

>TpreOR52

MNDNEKGFDECVGVVRAIMGLIGLWPMKEWKGFQKFQTIGVLLILFLCSLIPQVTQLLIGDNDFNTVIEILVIMV
LIEIVTIVKLFALWYNNEGMKHIAIQIWKDWQVSTESEMHVMKSNARKTKFISIFCISSSTASAMSYATQFFVAV
YTDEIYVNATHLRPYFLQAYFPFGSHYSPMYEIICCWQVIAALVSCLVFSSFDGFFIFSILHFSGQLRILSKRMR
NLVEEYHSEKSLFPKLLESVIRKHLFIIRNTDSIEHNFNKIFLTQIVITSFLICLQWYQLATILTDNGPIDVGNL
IFIICFVVGNMFSIFMLYFIAQKIHNESKRLLHSVYEMTWYELSPKYSRLLLILMNRLSLPIQITVGKFAPFSLE
YFAILVKTCAGYFSVLIAVKNKLNENSMIDWQSTKTQEQESSMYENARVVRKISMICCVLGFSSVTGHLMIRICQ
ELDFIPNYEKKREPMYLGAGLATLVYSGSHCLFVGLMLHLRGQVKNLSNTFRNNRTRINKHERQDSKLFKNFIKS
VVIRHNDLYDSTFICLLISSAMKIEKVFSKVFFVELLTCTIQLCLQVFLLMLHVSERSKGLHFQIIFMVVYVLHI
GTHIFICCYVADKLQDECLLFGNTIYNYEWYDLPPEDVRVLIIILRRSELPMQITAGKFVIFSLSLFAKILKTSG
GYLSMLLAVTDI

>TpreOR53

MANVKREKGFKVAMGIAESIMRFSGIWPGVEKPKINYTRFTFIPVMLMILVFVNIPQTIQLFYFDGNLSAILNVL
TMADVPISIALVKFLVTSYNHQTLNKLLVLLNDDWKHVRDASDIEIMWQKAKTSRKVSKICMVLSAGTVFAYSGR
MLYVLYISTLTNSEDNVDKQIVRPLYFSAKFFYDTQKTPNFEITWILQMIAAFLSALAFGSIDCLFISFILHLCG
QLVILQRAFEKIGSDDVLTDANFDYTIAKLIKKHNRINESVHYIETSFNKSILFQALSSSILFCFQGYLFIIILS
TAKDTEALIEVTFMLYFTTCFMFSVFIYCYVAEFLVDESLKLNYSIFYCKWYNLPVKKSRLLIMCLLRVRKPLQV
SAGKYVFLSLNLFCHIVRTSAGYISVLLAVREKLFIQ

>TpreOR54

MPDQADYSFAIGPCRTGLRIFGSWPDPIIPLTQLDIFRSIIVSLTILIFGFIPQLSMAIIVANARDWNGVIEILT
TATVPFVVSLTKFNVSCYQRNVLKTLTTMMKDDWNDYHLDADLKFMQENAGLGRKISQICLFLALSVVIPHCLLT
TVIYFVNWGEYGDLCLISYFPFETNRRPNYEIILVGQCFSLIFGASTHAIIDGFFSILVLHICSQFKILQRQLSQ
LIENCRDAQHEKSFFEMLPKIIDRHDQLNRFVSLIDDSFNLMFLAQIMATATSLCFQGYQLVMVTSASENGISVL
ELTQLVFFIGSYSSSLFVYCYVVEKLNYESYQLVNTIFSSGWYDLPSSVTKNLMLLMCRAQKPLEVTAGKFCYFS
LEFYCRILKSTGGYISMLLAVRDRLAEENQ

>TpreOR55

MIFNLNALGDFLHILEDDWKRYKTAKAEKELKLMEENAKTGRFIGLMFASFMYTAGLFFNTFIPIATMRAINRNY
QTETLRQSNDNITHVLPIPAKVLIYTVHSSVMTSSSPKYELLFLAQYFFAFLRYTIMVGICSIMAAYVLHVCGQL
DIVIMLLNQYIDNTNTDKTIHLDCTQRKKLSIIVTCHARALRLAARIEKTFNFMNLVDFIGCTFQICFTGFLLVM
TLGGKPLIWVTWALLLISFVFNIFIICHIGEYLTQKCQEIGEIAYSIKWYDFSSKRAMNLMNIMIISSSYPTRLT
AGKMVYLTMTTFSQVLSKDY

>TpreOR56

MYYTDSEVCAHIIDIVKFHKYIIKKIITIDKVFNVPWGFVIYQNLSCMGYGMLIISNLDNLVDYVRYASAVLLFL
IYFYNIFTRGQNIIDHSTAIADACFHCNWYGLSKRAMKLLLIIMMRSNKKCKLSAAGALELSCETFLLMFVKGVF
YYLFIARQFYK

>TpreOR57

MAKDWHRISNEEEKKILLENVKFARSMTIFCLIFMFGGGITYNTFLPYSKGEYLVDNVTHRYLAYPSYVGFFNPN
VQEFSNEIYLVEFGGSTIVICLLGYYLIVDIERMDTFGLLTHSCLFVSITFNIFIFCYVGEILTDKCNKIGQAVF
ESSWSELPPADVKKFILVLAVAQKPVFLTAGKMITLSIRSFTNVLKASATYLNMLRTLLVNNN

>TpreOR58

MLVLPFEIWFSIEHMNDIDKLMECFGILTGILGYGSKLLMLRLSWRSVSSLVQIIISDYEATREPALRAALLKNY
KIGTGVTKLLFGTYTSLAFFIPIENCIRIARNGYTPSLLYSVPTSYPMIKKTAKNHIMVNFVQLTQVIVAGSGHA
LCDVFFTVLVMHAAFKSLVLRTAIKRYFEVCTRQKKDLKVISDAMNRVILYHREFLKFTQIFEDAYCYVVFCQIF
CITLQTVGSGYILILLAEDQKMWSVEFLKYINFIMVDVFSIMAYCTAGEYYTHQSIKIYEQLCKSSWYDLSISEM
KSVLFLMIHTQKFVAITSGKFKNLQLDCCTSIMKGTYSYLSMFRATRNRD

>TpreOR59

MAMAFSVILVMVLVICYPSQLLIDASNDIYFKCYTSKWYEYPVRTRRLLILMMTRAAEPCCMTIGPTVPLNFETA
GTIINTAMSYVTTLVSLCAL

>TpreOR60

MDEIEKRYETLSKESLRFLGLWPTQEKSKRYKGFALVAFFMIKLTIPQFLCIIVNPDDKFIFFENTMILTFYIMV
SSKHGAAMVNFSKVKALMLSIDELKKPFTDPHELELMKKYEGQGFLLARIYAIIMLITVLVYNMVPFVPPFLDVI
YPLKSGPRPIILPYRGEFIWFKQEDYHYYVSFVAIFDVLIFLPVFVGIESVYTTTVREICGLFEIVCIRFQRQFS
RAEGSPTSEISDAVKLYNECVRCVQFLEGTYSITIFTTQITSLLIFSLCAIYILYVNNDVFNGLRFSTFGIAILI
HMGYYFGIGQLLINSSDKVHFSIYSSNWFLASAKLQRLLVMASTRTLYPCFITSGKLNPLNLESFGTILKRIAST
ISVMLAVL

>TpreOR61

MYNSWEEYLLCQCLLLLLLLGYYLKKTDFDYKCMVSESEENAHHFLTQSFKYFIQQHQRCIKLYEILYFLTERLM
FVIMLGSSALLIIAGSTMVVLMDSHVPMAIKMFVCCWAAMIVILFICVPGQLLSNAGHEFFIECYCVNWHGYPPK
ARVLLLLILVRTKKPLVLRAGFLADLSFETCSSVLKKAASFVTALRSIYTAF

>TpreOR62

MIIQSVDLGFHEFPMQFFIFTACGVWCPSDWSPSLKKLYKIFEIFQIIAATWFWLTMFMNLLLKNTESDLFYDNI
FALLVLTLAAYKKYFLLKNRKVISKVLSMCSDDSWYQPKNKQEAEIIERFENETRLVTELYALGISTALAIKAVI
PMLKSKSGLVLPIEVWYPYKTENIFIYLFSYLQQLIAGIPMICLQISVDSFFVCLVLRVVTQLNLLKYRMQFSSN
NFISKGQEVTTATGIHKNIPMIDTWLRICSRKHAKIYEFAESVHKCFKSIMAAQLIITIPCLCMTVFILAQFDEL
GFNFVDKFFCFATCLMQIFLYCWYGNEIILESLDVENSIYHMNWIALQSKTRKNILMMMLRARRPIKFTVRSYVV
NVASFIEIIKTSFSAFRLLQTMS

>TpreOR63

MLIKNSLEHQVLPVPFHLLTLWGVWCPENFNPSIKKVYSVFTIIVLAVQMSLTLGVSILFVMMISSKNFDLDIFF
MMTSLINGMYKALNIFYYRKRILSLLTRGFEDRWHVPRDDYEKYILEIYFNESWRTHLIYAVACLAGVFIKLIGP
MVKYDTDLKLPTPSWYPYNTNETVYFWVSYSQQMFVGGSIISMHIGADTLLSGMILQSCVQLQLLKHRLRSFSKH
CIDAHRYLGTKLSKSSIESVFLRQYICDHQLIYTYLKMINHDFSGWLLAMLAVVVPNICINVYLLSFQKIGMNVD
FVTTFGLLSISLFQIYLPCWYGNGVILHSTEITNAIFEMDWTDLSPFTRKALIIMMMRSLKPSKIETAHIIPINI
KALLKIMKTSYSFLSVLQQM

>TpreOR64

MSLSGFTMVIQLKRKMFLNALKMFVAYLGCKILLMCLCYPSQQMKNASENILIACYSNHWYEFKPKIRSLLLVMM
IRALRPCCLTACLGVQVNFSTVTYVVKTTMSYIAALMSVQV

>TpreOR65

MIIDTLNELFTLMTIAGAWRPLDQLSVKYRIYTVTRFVLLPLPFIIITGMILGLILNNSDKDEMYTTLLLMLTVE
NNTIRAVGLWMNRKHLVQLLGMLASPYAQPRGKSEIEIEKSYSEFLRKFLNLIYVWVGGTWLIWVSPPFFQKKDH
RNLPLTVWLPHEGPIPDRIFWWLWIPDAASFLISIILIIGHDISIATVMSFVSCQFDLLAHRVRCMTMEAKRRAH
RSSRSIEACEKEVIVENVNHHRYILEFADLLSKGYGCSMIFQFSNCFIQLTANTYLLASTTTIDVQLLMRLIFLS
CMFLQSSIYCYFGDHLTNKSRELTYALFTANWMDLCLRSKKDILFMTARTMYPVYIGKAFFIVLSLNSFVQILRI
SYGVFNVLRQT

>TpreOR66

MNFFQVFVVKFIAGMFEMLGRDMARIHEEIESSSAFMMNKQKIYKIINRKIRDNIRMHQEAIELFVGLREVSQKK
FLIIIVILILELCSTGIWFVIVIPYNKFYAAKMAFSMVTVVMILLYLIYSSEDIIYACNQLNSMCYDTKWYLLSV
KEKRLILFMILRTENPCTLMAGPTIFMNYETFSAILRFTLSYLMAFYRIMEQSSYLN

>TpreOR67

MSSKVKKVWKNEDGYLRAERMTRSILLSVGLWPLQKDYPRFLRNFLVAICVALSLFMMIPLGIFIYSDAPNISVR
LQLSAPLLFSALCMSKYANLMWKGPKIESCLRRMAEDWQNVKDPLERSTMLDYARRARSMTKFCMALTFTGGLFY
STVGPLSRAPIVVDNVTLHQLAYPGNFIFFNPRTRPAYDYVFALQSLGSVVRFSSTCGVCSIFIWFVMHISGRID
VLGSTIERAVDQLDNRMLKRIVDDQLNLYRLAKELGDIFNELCLVEFVGNTVLICLVGYYMIIALLQHNYTRAYT
LAISLSSMTYNLFILCYYGQILMDKFDELGRSIYMADWHKLSGSNARSIVLLLVRANRPFALTAGKIIVLSMASF
AKVIKTSATYFNVLWNITSHSMNAQ

>TpreOR68

MMSDTMENKELEAGIKMCRIEMEILGAWPDTKYNQWYLKFRYLVPIFLSVFFINIPQTRMLIIVKNNLDDLLEIL
TTADLIVFIALIKFSSILAKRKDLMVLLNKMRIDWNTADRNHKYEMRKSLIFSKLLMSCYNVCTSGTIIIYAASR
FLVLRQHNEETRCSNTTSMKPMFIKSKFFFETEYSPMFEIIWIGQFIAAFVAKMAFVTYDGFFIFSILHLSAQLI
NLKIDFRSLPVEESGQNFTQSLKCLVQKHWDLKKFRTLVEENFNQVFLVQMMCYSVTLCLQSYQIVTILTGESEK
NSATLEFIVIFTISNILSLFMYCYMGEKLSTESSGLHYAVYEIEWYRLKPSESKLLMIVMHGTKKPLVITAGKFT
NLSLSYFMQILKTASGYLSMLLAVQDRL

>TpreOR69

MFYNCTIGYLFLIAISQHQKVLIFTYLLSIMSVNMVLFLYCHAGQCLYNESTKLSDSLYHSCWYNLNPLQLKHQL
IFLTQSQICLRLTGKNICIFSYESFLNVIKSSMAYFSVLRKIL

>TpreOR70

MLLVQFQVSVLAYNSMYLVDGVNLIRDKRLNTEYVCQLMLIENICIRYLMFMLQRQNIARLMDKCQRLWTRLKSN
EVWSYVRPFERRVYFYRNFSLVISYLVIVLFVAGSQLTHLAPDRINGTARRKLPYSYYYDVQEDPDFSIISGIQG
LILCYTAIILAGIDTIAPFLIMLACGYSVTLKNRLLNMAHKDDKSTKVNNQLIYGDVIECAKFHREIMSYCQDIE
NHMRSFHMVIMICNVYNMSLIGIQILQNIEYFFQYSSLLAMHFMQLYLSQWAPDHLLHETKAIGNAAYFATLGHS
TYNHQANKILQIMMLRAHCKPVQLTAGGYIKLSMETFGKVNIFLFSIKTVEKFNAYTQFFSDDNQCGFYVYSCSK
FYFVKIYILNIIFIFLVIKQLEFHMHEII

>TpreOR71

MVILFDSNIYGAARMLVAYTCAMIAFMCICVPSQLMSNASNDFFTQGYCTNWYNYRPSMRINLMFIMTRAIKTFE
ARAGPMIDMNFESFSEVMKRSVSFIAAFRSLYAAYREKQRV

>TpreOR72

MTRLLHKVARSWPPDEKNHRHGILRKFLHVSPPIICIVLYIYNLSGFMTARTVEDILEVINFVSVFTTATGRNFL
LALTKPKIHALVSKTIEMEKTRRADELSSIQCQIMKKWRNIHDKLTKFSIYGVWVGALMYLIPPIFFSKLPFPGY
STKGLEESKWFYAIYFLQLLFACIFIPPWISVDFYVCTFLCTLCRELDMFYDAMQNIRDKDRSYLYRVIDRHSRI
LTYGMEVCDIVSYSFGVVHASYGVFLIFGTIAMTQINWSTHGGLAIRNIITMIVCASSLSLMCFIGDLIQDLSTR
LGDCIVLDNFLDRRKDRKYLKLLEIVHARSCCSLKIKYSPNMIVNMQMYSVTLNYILSIYTFALTVMA

>TpreOR73

MVFCWFAISFNNLQCITIGFIIAHIHCLRYYLNEIDIEFEQNEKRRNISEQKEVDRKLIQLIHQHQSCIKLYENL
DIMGKPLFFCSITTITFNLVCCGTAMVILLDSDFSGAAKMFIIYSCSIISFMLLCVPSQLLSNASYEFYKESNCI
NFHKYPPKTRVILLIMMMKISDTFVLRAGPIYELSFETFSVVISSFQLQLKSQN

>TpreOR74

MQSISSLLFTKRIKVREKNMKSMGCWVPSGTFVFEYLRVNEKLYRYNFLWPDQSTTLKFFGRSLFLLSLLTYQIP
QFMHSAINYQQQKITIHSAIENLLGTFLTFNLFIRYVLHMQHQETFKYIHVVLTKHLSDCDNYQEKLVMERIFSS
KTRHLQKIMIFFWTLLSMFILVSAPIPALINAITKKETLTKQLCFPAEYFVDFDSYFWLLYIHQLICIFFQCLTA
NAFDITYANNAHYIAAMFGVISYRLNRLNRFTRNPQLTSRQRDEYIGRELLEIIDKHNRVIDVTAKINRAYAPMK
MFSLMAMLMLIGEVGFMLAIDFGNVMTHMRFFIALAGLLLYLRFICWPGQTLIDASLSVFYSSYMNDWYLFTPKL
RYLVRGIMWRASKPCVITAGPFAVISSETFYWVIKSSYSYMSFLMKMDGESF

>TpreOR75

MLCTSGELYPNREDYYYFWLAYTTFLMFSIASIVVAQISLCIYFVSINLSKFEVVCYHLERLREMSDQSDENDPN
EIEKIVKHTCANHYETLNFFRLFQIFSRNVLAFLSTYVFVALSTTGTMILYAFKIKNTFLILEMIVSFLCILCFA
FLMGHIGQILCNASDNLFYQSYFCGWYNLPKESKKILHMMMTRCLTPCELQSAPLSFMKFNYENFNAVVNTSISY
MTVIASVM

>TpreOR76

MLEDQKTENEFFDSPEYHLNKKFMTVVGGWPYLSPKKRKTIWFVVNTFVVTVWVSELIHIIVIIDRTKEVIYCLM
AFLCTFLAICGSINAAVQPRSNKRLVDSLRDNWNNLRSDDERKIFATYAHYGKILSSVLAVCYYLVGMTYIVSPL
IPSALHYRITGDWIAPTRNVLEVEFFIDPVKYYWQLYVLLVHMCMGVVSLLLANDTFFFMVVLHCCGMFAVLRYK
VEQMDKEITFNGYSYNQQLAVKRIEEIVMYHLECLEFANKIEDFFCIQFIVQILVNTIIISVCGSQIIQLADQSL
RDSLLFAFLTSATIFRLTFFNVCGQGVYDHSLRVHDQLIHTTWYECPIKVRKLFIILYNRSAEPYNLTAAKMINL
NFVTCTQLLKTAMSYFMMIIQTP

>TpreOR77

MNRKSIDLSLNEAGYEYAIGYTRNLFLPFGIWPVRDYEESRGASRARAIANFLCLFILFFVVGPYFVQTFLIEKD
NQVRIKSTGACVFGITNVAKYLVFLLRGHRIGSCLAEMHLDWRSITNDPQRRLMLRNARAARLLTCCSFVFMFGG
GVPYVTVLPLSQPPLLSADNRTLLRHLSYPSYFGFFEPRVRPVYDLVFSVHFCFGILAFSLTTGLCSFIAMCTLH
VASRCAFVSAMYRELGRNFDRELLSRVVLQHRRIIA

>TpreOR78

MYMLENQCKSKTYLEHDEELRKAREGCDVVEECQLRNLEFLNRERCYVFRCRVDGRANLKRLELSCVPQVKIVLI
ALSGLINKNVPGHELVEATVYSGFLLCVLSIYALSFDDKLIAASSIMMEIWKEFREEDERSIIIEKLRQGFTISM
LYSVIMYTIFVGCSLQPEVKYRAISWISVNKTIEKDLFFPIYFTKQLKDNYYLALFVFVLGGLMLGTILSIFNYF
QTTLWKFIVASLLLIGLLNVLQLFAQQKYFIVIIVIIIELTLAGFWFLYVISYDKNYALKTVVWMIVAICNLFYI
IHPGQELINANNRLWGGCCNCKWYIFPAKERQLILLMMIRTSKPFLLTAGPIIPMSYETSAR

>TpreOR79

MELYDSRYFVINKKLQKLIGVWPFESRTRKNFCRTFGFIFMFIGVIPQVIALDVARRNKDFDKITQSFATFLFVL
AIYSKLVTSIMKEEQMIKLYEEIVNNWNQMKEKDDKKEMIKHAEIGRFMTICYAAYVFNALIGFITFPLLPSIMD
KISPLANGSRPRIYVLEGQYMVDREQYFGIIYILEAGVCIMMVVIFCTCDTAFCVCVEQSVGLMSVVKMRLKKAT
KYGSQWEKTDPNNTPYALVKKLIVFHQKVLTSVEIVESAYSFYLFALMGFNVFILSCGSLVIVANLDNPMETLRY
GMIFIGLMIHMFFLNLPGQRLLDASSDLHCNAYDNEWYECSEQTKQLLLFIMLKCKTPCIITAGKIVIMNIQNFG
ILAKSAASYFTVFASFR

>TpreOR80

MVQRHQFCLNITNSIQAMANPIYLSVMFSLMVCSSVAGCMMVIHFNNDTNFAIMMAFVYYVMTFEFTFGVCILSQK
LYDCNYNIFISCYDCGWYRLAPKMRKNLQLMMSISMEPSGIKCGPSLPFNFETYGNIVRLSASYLTALISILTLE

>TpreOR81

MYIMYYLKYGLILLDSWPGVDSAKLCNALVALSCASLCFQFWDAAAVFHDLDALLTNMETSIGVLSSVFKIVTFR
MQSASTKKMVKMSIEEHVEKNAANPNSKTGKSKSNNENAVKLILQILFVSYFVLGLSYPAVSLVSYALGSSEERV
FVLPSMYFIPSPRESPAFELLWIYQFVVVLFSVLGQCIADSSLIIILNLSIENDLRECSFYNFQEKEQNLVRYMLV
RAQNSNTLEIGKFGNLSLFSLTMVKLDYFNA

>TpreOR82

MNPEEAFSVYRSFLWFLGVWPLEEKSRYQRLRYYTAAFFQASFLLHTSIEICLNRSGVSDMVDVCLFFASAFLAL
IKHTYLHLHGRKIAYNLRSYMRDWRQPSEYGSAVMRSHFKMYRYQFIIYNSIGYIGTTLFLIRTLLLNYLKDRGQ
ARDDGPEFEYEFICKISFLSKPFLLRYHTGLLVLQYLQCMYVCSSGASTDCFFFGLLLHLAAQFKILNRRWHDFG
DASRDDQASFDELVARHQLLTKLGQHLEQSFSRVVLLQLLISVILICMSGCSILVSMMERDYVTMLISTNCVSFM
ITESFIYGYASDYLKSQSLALVDAVAACGWYDLERARRRDLAFVLMRALLPCSITAGKFFYVTHNTIVQLVKTSV
SYLSVLRMTIEHSRKADAN

>TpreOR83

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IESTVFTEATAVMIFSRWQRSRLESLFRLAEKQLAVKKRRVVRFYANYYAIVYLATLAFYVCVVMMYIILEKPKT
GNNELALSASYPFRLEDNPIKWLLWVNQAIVFVHAYIVANFDGIAVFLIFTCTDRLKQLDKHFRDSRSYEHLVAC
VREHNDVLALIKDTNRILRFMVLKTTAFFMSYVFGAGLQILNNTAQTVMIHQISILFLAYARLYLCAESANNMSA
AGRDIAMTVYSTSWYDETPKMSTAKTIIIQKCQREPVICVSGLMSALDRRYLRGIFSATISYLMTLRTIVGSK

>TpreOR84

MATQTTRAELAELSRQLGFEENHFYWLRIFLKFVFLCPVQPIVIKKFGRFIMHASFLFSIAITCARAYKEFYINE
IYPPMLAELTFQLITLVGVWCVYLQTIYSENLLNKICEFVTIDWLGIKDAEEKKIMKVACERGSRLIRIHFGFLL
PSLLGYALVPVALPYVLNPYLPENMTLERRLCAHVELFVDQDKYFYHILIFLIHMVILIMLIVSALDLAYTSCIA
YVMGKIIWIGDVFEKLGGIKISDQSPSSKRKMNVYVHQTVIGLIKRHQKCLEFSQMLNDICSPKFFISLVVLLNL
LSLSGSMAVVEISYDASSAAKMAVAFVMILIMVLVISYPSQLLIDASNDIYFKCYTSKWYEYPVRTRRLLILMMT
RAAEPCYMTIGPTVPLHFETASTIINTAMSYVTTLVSLCAL

>TpreOR85

MESVGDVVRVKEESKDTWPDAGDDQNFDSLDFDNFEARNVETFLGYKSSMVVLMDSDAVMAIRMLVISCATLTVM
LFMCVPGQFLSDASNELFTECYCVDWHGYHPKARMLLALILVRTMKPFILRAGFLADMSFETFSSIIKVTENTKE
YGFSSSLFIEDLFQVIASLMMIYVYPFMIWKYDRSYEKSNRFSLVITMLFIPITVTFAFAPLVIPILSTQFLPGN
QTFQKALPLHVEFFVDEEKYFYHLFALQFITIMIYFTIIGGLNTFLYSGVGFIVGELQYLQYYLEKTDSCYSDMV
SECRDIAYNFLTKSLVYFIQQHQRCIKLYEILYFVTESMMFVTMLGTSTSLIIACSAMVVLMDSDESMASKMLVL
CIASLSVILFICVPGQFLSDAGNELFTKCYCVDWHGYPPKARILLVLILVRTMKPMELKAGFLAVLSFETFSSVL

KKSMSFVTAFRSIYATL

>TpreOR86

MTKDPRASDIQHFEWAFGLNRFVFGLMGVWPKKRPNDDSNSNSSFMSTNVLVIPGMIVLLVGGLIAPQMYALSRI
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ITIFAVLCFVVTPFLGLSFRLINNRTDQVGRRRIHLPLQSVYPGDYLRSPYYELCYAAQMLGGCIVGMTIAATDN
FFAALTFHASARCRVLAERMAGLALIGIAREEEFFRALGASIREHVRIIRLVRTIERIFNQLLLTKLICMPLVVC
FVGLELIGSFGSESIRLTTLFAQVGALVTMVFHALIDCVACEVLMKYEHQPSRLRFRVVRFTRALCEMLHSDSHT
AQVSAETYGW

>TpreOR87

MSEKNQEELGLFDTEYWTITVKLQKVIGLYCFQSDRRNCISWIYVFVFTLSFMISMGVRLINEIGVHIEIVVENI
VGEMYLLAVFNKLVISVLKRKSFKKFYAKVADHWRMIEDAEELKIMTDNMRNGRDVVKLYSIFIIIGTTIFLSMP
IFSPLLDYVVPLKNATRPKALPYYAEYGVDIEEYYYPLIAQAVFGGVGTITVLVTFDLGFMMLSHYVIGLFALAK
YRLSKVNTLMRKIEERNGNPWKSNWPIPYILEAVNVHRQALAYVAELEDCFNIAWFITLILNMMMFGGGMAILLL
KNNPEDMFRYTMVLLAGFMHFYYIFLPGQEIINASEEVFDVCCACGWYNLSEKSKFLVQFIMVRSLQYSFLTGGK
MFPLSMETYCNVSKISKG

>TpreOR88

MTSAMLFYVIMPVAPTFFENVFSRMNETKSFDFVMKGEFPVNNMRDYYFEIFIFDMLVCVATVFVLGAVDSTYAA
CTEHCIGLFGLLKFRLVNLTPKISQNYSDMANSNYDQDDATYCINRNYTLLLSFFPRFAEILKTSYSLTFLILMG
ATVIYSSLVCTLILLKGDELKDRLRYSGIFVGLLIHLFYISWPGQKLIDHSTGLFDDAYTNKWYECNFRTKNLLR
IVRLRCLTPCQLTASDLYIMNFPNFAAVLKTSMSYMTVLASFI

>TpreOR89

MLRDQSCERDTFFNGPSYHLNRKGLILCGGWPYLNPRKRKIIWWTINLAIFTAWVPELIYIIEIFDDAQKVIYCT
MAFLISYLAFCASVNAVVNHNSMKQVLDSLKDNWNDLQNDEEREIFEKYANFGKIFSIGLSVCYFGNLSLYFVSP
LIPSMLHYRATGNWTTPEKNFLEVEYFVDPVKYYWHIYVHGAQAGSWVVCVLTAYDSFFIVLVQHCCGMFAVLGY
KIGKMDKEIHDYRHDQHSVVEKIEKIVMYHLHCLKFAKKIEDAFCMQMIQQIMINTIIISVCGSQAIKLADESKQ
ESFRFAYLACSTIFRLTIFNILGQSVYDQSLRNIHQLVRVSDGDEEIIHNPLQQKRRALQSHGCQDNLPESYYLF
EANENGDVLFHDDYPNVMRCLFLMIKAQAIIIRH

>TpreOR90

MGLSFGCFLSLRVHFHYLCRNKMSPNEVFDNKYFRHNRTLLKQVALWPYASTSSKVVKRIFIIVCFYSMSLPQAI
RGIEEIVSVNVNQEIVIENLTGFLYFHAVIAKVITQIVTEKRLKYLYEEISNDWKIITDKREKAVLEKSAAVGRN
LTLAYTGFVVISAILFISINAFVPVLLNYILPGNTTYQKQLCIYAEYFVDQEKYFYYIFTHTMIIGVMTVYVATT
IDCVFVCCVQHVVGLFNIIKYRLREISRIYDTAVNESIIDLHFDAKKYLINVVEIHKKTLDLAELIQNTYNECFF
LLTILIVAGLATFTYVLAENVHNPLNFMRIWCLWFGAIVYMFFVNLPGQKLLNISEELFLALYNSSWHKFPMKTR
FIIQVMMIRCLSPCRLTAGPLIEINFQSCSNVNVSSIKYLL

>TpreOR91

MLFEICDEREQTLLVDIRDQLGLTLLQWAVAYHLPNAILWVLKEKKEMDIFNNFMVEVIFQLLASLGLMFAYVSI
GAIKNKIQLVVARMWYDVTEDIQNIILVLVSKVVYIATIQAVLLITSCVIYALAPVTIPFFMTFISSGNQTFKIA
LPLHHEFLIDKDKYFYLIFTEQLLLLTDFGILVAGMNFFYCSHLVTIIGGFRGLEHLLKKLDDEYCYKISVGDKN
ANEWFNLQLKDFIRYHQAIIDFYKISNEVSSPIMFFLLLGSGCSIILALTSIVVATDTAMAATAKMVVGTLIAVI
LIIFICFPSQLLKNASEDLLLASYKLNYQNYPP

>TpreOR92

MVCKEKIHTCALLMGRMCDTNAWETQAIMFKPLKKGYRISIFQAVAMVILCTSFGISPIAIPSFLNRVLSGNQTH
EINLPLHTEMLLSEDEYFYQIFAAQLTSLFIYGLLISAINALQCLSVAYIIGEIRILEYLLHKMYLEYKFKNLVD
EKKRNKCIYEQLKYFVDTHRICIEFFNIVHDISNILIFGILIGATCIVVLTATSMIILMETDLTGSTKMLFAFCV
TMCAITFVCFPSQMLKNASDDLMFTCYKMNRQSYPAKIRKMLLFIMVRTVKPFVLTAGPTIELNLETCSTYYRLK
FKIAYKETFTPSGPGSLPKNVWGLQEPNNKAPYAKRGFLFY

>TpreOR93

MSEEIKLNEVDDFFDLHYFALNKKFQIFCGLWPLETGYMKYFKQGAMGAIILANLILFSHALGTFCGTNMDYCCE
NMLGVVYCITGFSKLVGISTTGGKFTGIYKMIARNWRETTDEIEYSILEKYARISKMLTRLYIVAFLMTGGVVTQ
APAIPLLLNYIIPLNESRQAIPIVNTDYSITPYTRFAHLWVHYSFTAVAVACVFIAIDATFVLIVFQILATLDVV
KQRIRQASSVESENAEQSYGILVKAVKLHKDAIEFLNLSNEANSLQFLVVLGGTIFNISFGSLAILSRAGAYADF
ARIGILTLGYLFQLFVLCLLGELIISSSSELFVIP

>TpreOR94

MDKRLENLKPGRLFSMESNIYYNFHLLRLMCIDPYMKESLRLPVNIIILTTYLLTILMTIVVLVKAIIKNETNIN
LIMEMSFTCIFTVDMMSYYIILLKYNKEEKYFYLLLAIVIVINALMIVESVLLLSANIMIIKYLEGLFDFMSYSV
NHMYDNLNPIMIKMSIKTSNKEIRRNIINLIKCYQQSIQIFNLFSTFTRYNYFIVVPQIALNIIFLGSKALLEIS
HDLQLFGRLIIICLASFLVLPVLIYPCQQLINANERFRNECYACKWYKFSPEARRLLLGLTFRASHYFVLKAGPI
ISMTMETCSTILKTSLTYLVTVNKIYSSFGEK

>TpreOR95

MAFHSKWTEKEQKFKNYILHVIAQSQHPVTIKANNLLPTLSLSFFSQYISIWPYDLTADKREIFIHQIRWWISFI
NVCILLVPLILGVYYFRHDNVKMTKTLSELTCLTEIFFNLIQSKLELKNFQIVFHEIGTFIKEANKNDTALLQKY
LNKYRDFQLLMGLTFILVAILFSLMPIVTRQSLPADAWYPFEIKSSIILICIYATQVLAIFQTAFGIFVDIMVAF
MLWFSAARFEMLEIELQKAVSELELKNCVHQHRKLIILTNKIKTAVKLIILKTNATMLLAVICGAFQLLHHESLE
VLIQFVMLVAAGCLRLYVSAKAADDLKENNDRFSRSILYTPVIRKSKSASKLSSLLTFFSQRPVVVSIPGVIKAY
TLQYYASFLSTTVTYFLHLRIILDE

>TpreOR96

MNELKLTREFDNHFFLTKICMIYIGLWPLDGACNDLKRNLKVFTGYFFVVSLILVELYDIYFYSSGSIEATAEIV
ITMTYAFGGLMKISHFLKEFGVFKDMWHTMHVDWTGVLGNDQERSYRSHVMRRITIKTRKYCRQYALLTLGAAVM
YMTMPFIGNQAHRVRKYPFFGRYYYDDQSDLVYILCYFSQVITGTFCATTNYALDTLFLICSYHMCAQLKILKHD
LIKMSKENVAQRLTCLIRRHQREIRNVKKLQKVFSNVGFWQLFVACVITCINIFKMLNSQALGDAIYKSGWLDFQ
GNYKRDLKFMIHRCQLPLTITAGKIYTLSIANFMEVIVNYIICQLSNQTCRL

>TpreOR97

MSSSVLEAKLERFIDISLKSFIVMRERNRKHQNNLKLFETEQWKITVGLQKISGLYCFQRDRINFISWLYTFLFT
SSFMTALGIRLVMEIGVNTEIVIENFLAELFMIVIIFKLGISVLQRKSFKSMYTKITDHWRMINNVEELKIMTKN
MNNVHVIAKVYALWCFFGMEFFLAMPVGYPLLDYVIPMKNRTRVVAFPCYVEYGLDPEKYYYPLMTQGFFGSLGC
VAVFAAFDLSYMMLTSYVIGLFALAKHRISKVNAIMQVMELRNVNALKSNWSIPYIVQAIEAHQLALAYVNDLED
NYNKAWFVSLVINVTTIGGAYVVILVKDTPEEIFRYSAMLIGAFVHFYFIFLPGQKIINASEEVFDACYAVNWYK
LSNKSKYLLKVIMTRSLRASYLTGGKMFSLSMDTYCRMLKTSLSCVTLLKRILEF

>TpreOR98

MMKDAEELVIMSKNMNQGHRIVKIYSLYCIVGMGCFIAMPIAYPLLDYVIPMENITRTMREPVYVEYGIDIEKYY
FPLMTQGVFGGLVCVSLFVAFDVSYMMLTNYVIGLFTLAKHRLRKINRLIWVMQQRNVNTKKSNWPIPYIVQAIE
THQLAVAYANDLEAKYNKAWFAPLLINITSIGGCFAILTVKDTLEEIFRYFAMLCGAFSHFYFIFLPGQLIINAS
EEVFHACYAVNWYNLSVKSKYLLKIIMIRSLRASYLTGGKMFSLSMETYCSMLKTSFSLIAILKRLM

>TpreOR99

MSERNRKQQNRLGLFQTEQWKITVGLQKISGLYCFQRDRINFICWLIIALFSLSFTIVLGIRLVMEIGVHNEIVI
ENILGELYMLIIFSKLGISVLKRKSFKCIYAKIADHWSMINNAEELDIVSRNMERGHGVVKIYSLYCIIGMGCFI
AMPITYPLLDYVIPMENKTRTMGEPAYVEYGLDPEKYYYPLMTQGFVGGLGCIAVFVAFDVSYMMLTNYVIGLFA
LAKHRLGKVNKLIKAMEQQNINVLKSNWPMPYIVQAIKTHQRALAYANDLEANYNKAWFITLVCDTATIAGCFAI
LKVKDTPEEIFRFFSLLFGAFIHFYFIFLPGQLMINASEEVFDACYAANWYKLSHKSKYLLKIIMIRSLRASYLT
GGKMFSLSMNTYCSVYAKDQLIVRRSSEEIVGFLKNILFFKFHV

>TpreOR100

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AFLCTYLSFVSSINAALKSHSMRRLIDSVRENWNDLKSDDERKIFATYANYGKLFTIGLTFAYYMVLLSYIVFPL
ITPSLRYLDTGNWTRQEKNILEVEYYVIDLQEYYWPIFVHGSLACMGAVWLMLAYDVLFGTVVQHCCGMFAVLRY
RIRQMDVILHNYSYDRQLALKKIEEIVMYHLACLEFAQKIEDFFCLQFIIQILTNTFVISVCGSQMHLQVIQLAD
TSLQESFRFLYLASATAFRLTFINVFGQSVYDHSLQIHDHLIYTNWYEYPVQVRKLFIILYNRSAEPCNLTAAKM
VNLNLDTYCKLFHIIKIIDQTREVIYCLMAFLCTYLAFVLSINAAVKSHSMRRLLDSVRENWHNLKNDDERKIFA
TYANYGRIFSIGLASAYYMVLLSFVVSPLITPALRYLDTGKWTRLERNVLEVEYLVIDPEEYYWTIFVHGSLASM

GSVWLVLAYDIMFGIVVLHCCSMAMKRSENFERILQVIQLADISLQESFRFAFLASATAFRLTFINVFGQSVYDH
SLRIHDHLIYTNWYEYPVQVRKLFIILYNRSAEPCNLTAAKMVNLNLDTYSKLMKTAMSYFMMIIQTL

>TpreOR101

MYIYPFLHWNYDKVILVVVTMKRILEENDALGRIIVKNSYEKSNAYSAYMSTLFIFMGTGFSIGPVAIPIFLTWI
QTSNETHEKSLPIHTEFFIDEDKYFYQLYAYQIVVILLYIILMGALSSFLFSAIGFVIGELHYLQNVLEESDVKY
KSKLRANKLLAHNFIVNKFKFVTHQHQNCITLYKNLNNITNSIMFVVIFGSSCIMVLSGSTMVILMSSDIGMAFK
MSIAYFGSMIIITMICVPSQLLSNSSDSLFMKSYSLSWDEYPPKARRTLVMILVRTIKPLALTAGSITELNLETC
SSIIKSSLSFVAGFRSLYTTV

>TpreOR102

MSNSHHQQQSVSNHHHHHHHHHHHHQQLQQSTLFYLSFPVIDQLARSKKNKAMDDIERRYEKISKFSLKSLGLWP
TQSKLKRYTGFTIFTLLVVKIVYPQIVNVLKHSDDTIIILETAVYVLFHIISLSKYVSTLLNLSKVKALMLSIYE
VRKNLTDHHELKLMKKYENEAYFLVRFYALYVLTGMLLHSLVPFAPPLLDAVYPLNNGTRGRLLPYRGDFVWFKQ
EDYHYEICVDFIVSAMIFWLLYAGIESIYTSTIKQICGLFAVVCYRFERKRKISSNVDRLSKQNFDEDYGLTSAE
IADAVKLYNECIKCVGFLEDTYSIPVLVTQISLLLDMSIVLFINEELLNGLRFGFFGSGMVLHLWYYFWIGQLVI
DSSSRVHFSV

>TpreOR103

MDALVDKQIKKDMFWSRFFLQSACLWPYTNRILKYAGRVLLIPSAIFMELCLLYGGYKNLRQDSLDIVYTFTNVI
ELLIGISIIPMYFITMSYDREFKLLLGDMMHFYSEEASAEERKIVQDVTRGTYHLSCLLAAFVCVFCAAYALAPH
SVWYHCAPVLLVPLLRHCLGHYRHHGNHSSGSSRALASTAVVFAVDQDKYYYPLFVYTTMNMIQLATVANALSAS
FLCSVAFIRAQFRIIVHRLTNLDSLVQREYLKRDLSIQDYIHQEFINLYLYHINSIRNLKLINMIGEKLLFIVSV
LCLLGLSFSGSMAVIFLRKAPLAALNFVMCFIVLLILSLMLNYVGQSVIDANNEVYHNCYHCGWYGFPVKTRPFV
FLMLMITRKACGLKSGPFTILQLSYENYSLILKSVISYITVMISVL

>TpreOR104

MTTIFQDNDTLGQKVILRSVKKFMTINSIITATFVYVSVNGATAPVVIPELLTWLLPGNKTFKKSLPIYWDFFMD
QHEYFYQIFVMQQILLILFSFTVTSLIQYEYLCTGFIIGEFHQLQVFEQLYKLNSPVMFVVIVGASCCIILSGTT
MVVLMNSSSAIAIKMLIVYCTALITVPIACIPSQLLSNACLDMFNKTYCINWYEYPPTARRILVLFLLRSMKTMV
FEAGKIIELNFETCSNVLSYFINWYKYPPKARLIVVLLLLRSMKTLVVEAGSIIQLNFETCSNISLLI

>TpreOR105

MLRDQNLETDKFFNGPLYHLNRKSLILVGGWPYLNPRKREIIWCTLNLAIFTAWVPELIYIIEIFDDTQKVIYCT
MAFLISYLAFCASVNAAVNHNSMKQVLDSLKDNWNDLQNDEEREIFEKYANFGKIFSIGLSACYTGNLSLYFVSP
LIPSMLHFRATGNWTTPERNFVEVEYFVDPVKYYWHIYVHGAQAGTWVVCLLMAYDSLFIVLVQHCCGMFAVLGY
VIKLTLENSYTIGMMDKEVHDHRHDQQLVVEKIEKIVMYHLHCLKFAKKIENSFCMQMIQQIMINTIIISVCGSQ
AIKLADESLQESLRYSFLAGTAIFRLTIFNILGQSVYDQSLRVHDYLMYTTWYEYPMETRKSFIILYKRSAKPCN
LTAAKIVCLNLITHTKLMKTAMTYFMMIIQTS

>TpreOR106

MDFEQLKSWNWVKFLEINAGCWPFQNPRKNLYIRVFSFMAIVTVYIPLAIKLMLSLDQYQEIVIENSLFLMIIHG
LFAKSIVLYLNEKQRGDLYREALSHLDAMDDEGEKEILRVSSESATKWMLTYLFTTITLGSFYHIIPLAHIHMNY
FLRANATPPRQLLHINVEYFMEDEDIYYYIIGHFLIVFVFGGILFFAHDLGFMALVKDNCALYEITCYRLRNCSS
RGSSDSRRSQGLADDEIHANVLRANDMYQLAMSITDRIEKAYNITWFVLLVQNMSSFGTCIMLLYIQEDPIEFIR
YITVVGSGLIHFQTIFYSGQKIIDSSQAIYHACFHTDWFTFPRRTRNLLIIMMMRSAKPCKLTGGKAFILCMSTM
RQFVLKGLVYFLVFAVE

>TpreOR107

MGFKLNQISNFSRKKDYLEKNDEIFKRIYELVQLQQFSIDCFETLNKFINPILVLGVLAVIIGCSASGCLMVMKF
NEDLNVAMKMGLIYSLCSEFLFVAAYPSQKIYDACNEIFDNCYCTDWYRYSIKNRKMIKLIMMRSSRHSCIRIGP
SKILNYDMSRKIIQLSLSYIVSLMSIYKL

>TpreOR108

MTEATKSIPIENTWDFTTQAELSELSRQLDFEENHFYWLKKFLKFVFLCPVQPKIIQKSGRAMMHSTFIFSITVT
CTKAYKELFVNEIYPPMLAELAFQLIVLSTTYIIYLQAIGMEKEVRIRIQSLKNPNDPNRMCEIITIDWLGINDP
EEKDIMRVVCERGSRLIFIHFGVLLPCFMGYAFAPVALPYILNRYLPENKTLKRSLCVHVEVFIDQDKYFNFILC
FVIHMIMLVLLITCALDLAYTSCITYIMGKIIWIGRVFEKLNEVNIYDQSPSLKQKANIYIHQTVIGLIKRHQKC
LDFSQTLNDACSPKFIIVVASLVILLSLSGSMAVVELSYDVSSAAKMTVAFTVLLVIVLVMCYPSQLMLDASHDI
YFKCYSSKWYEYPVRTRRLLILMMIKAAEPCCMTIGPTTPLNFNTAGKIIKTALSYVTTLVSLCAL

>TpreOR109

MDELARMFEAKETWRYIILIEKLAGCWPYQYPRVNSIVRVFNGVMLVSLLVPIVCRLLIELNCVDMDIQLVIENT
FVSTILSGVLIKFIALYINEKQREEVYQKMTFKTLKITNNKETQVMNYHSRAAPLFLTKILLCVGLTTMMFETIP
LANMNLEYYLQSNLTFKIKQLPMYAEYFVDQEKYFYALLFHMFVSSWVAVFINISFDAAFVLIVHNHLALYKIVS
MRLREASKNDDDNVCYCKTISYEHETRGNIINAIEIHQTAIEITNTIEKAFSVPLFLLTVQNMSTFGGLLVLVMT
TVRDPVELFRYFVTFAGVLSYFSLFAVLGEKVITNSTAIFSESYTTNWKNHSKPLTALFRIIMMRSMKPVDIKGG
SLVLGMQTLGQFFQQSFLYILFFRLFRRPA

>TpreOR110

MKRIDYQFDLRVFTIEETKKDWICSELKFIIHHHHNLLQLHKLAQKLLSSIFLVLLMGIAFDLVLIGTWMVILLD
KEVIEALKMIMAFLIIQFTVFTMCIPSQLMSNASYDLFVQNYSTNWYKYPPQARRMLVLLIAGTTRNFEIKAGPI
FIMTFETCSSLIKLFQELKKDNVDSNTVVECALQISVYFRHLQGYIFISTNFDMVLKFLAIMNHIFQNNSKLGQK
IIMHRVDKALNLSTLQSVAFILMCISYVITPFTFQTFLSWIFPEHYMKRIDYQFDLRVFTIEETKKDWICAELKF
IIHHHHNLLQLHKLAQKLLSSIFLVLLMGIAFDLVLIGTWMVILLDKEVIEALKMIMAFLIIQFTVFTMCIPSQL
MSNASYDLFVQNYSTNWYKYPPQARRMLVLLIAGTTRNFEIKAGPIFIMTFETCSSIFQSSMSYIATFKTMYTAF

Q

>TpreOR111

MINADLEQELMNSLLTDPRVAVNLSKKEIGDIVNKFMEENYCWVKKTLQMAFLWPYDSAKRKYAGRFFILFNIVH
AFLSNTWTVTHKTFRKPIDFEFLIANLFEWMVVLVVLMAYTLMILKGLDMQIFFKLSTLDMVNIIEQDERIELEK
CIRRQYNLSRIFGVLIAVSFVGFAIMPGLFPVIGEFLIPYLDGNFTFKKTLCTTADLILDRDDYFYLWYFYTWVY
MAVQATVVISIAMSFLYIVSYTEGKFNIVGLGLHKLYEFTSADNFVNNKKVDDKIHEMMREVHINHFRCIEYCNH
VNRISRSLLFILWSLILASLSFTGSMSVILIHSNLIVFLEMSVSFGCAVSLGFFISYSGQIVTDASGSLYEKAYN
CGWYHFPKRSIVYVQLMLVRCIRPCELNAGPSVIRLNYESFSVIMNTAMSYITVMVSLV

>TpreOR112

MCEVVTIDWLGISDPEEKDTMKVVCKRGSRLIFIHFGVLLPVFACYALGPVALPYVLNPYLPENMTLQKTLCVHV
ELFIDQEKYFYYILAFSIHILTLVMLIICALDLAYTSCITYVMGKIIWIGYVFEKLCEIKTSNQNLSAKRKIDIY
VHQTVIGLIKRHQKCLEFSQTLNDACSPKFIIVMSGFMICLSLSGSMAVVEISYDAISAAKMAVSFVLILILVIV
ICYPSQLLIDASNEIYFKCYCSKWYEYPVRTRKLLILMMTRAAEPCCMTIGPTMPLNFETAGVVSNDRHKISF

>TpreOR113

MRFCDKIQYIGSKLLMVTSIYVFVGLSFTGTLILFSLSVKNYALLVEMIISFFVVIIMAFLISHFGQIVINASDD
IFYNCYFCGWYKFPSNAKPYVIMMMVRALSPYEFNSGPFTVLKLNYENFNTIVNTAVPYMTVMASLM

>TpreOR114

MYCIIIPVLLPVTANFILPGNHTYAKQLSAHIELFLDEEIYFYNYFVMILITCYTAQVLISSWGIIYVSSAAYLV
AKFRFMGFKLKQILILSEGEDFLEKNDEIFKHVSQLVQLHQYCINYCDVLNSITSRMFGLGTLHTIMAFVISGYV
IVMEYERDKNLALKMFFVYILCSEFLLVTSHPSQMIFNSSGDIFETCYSTDWYKYSIKTRKMIHLILIRSSRPSC
MKIGPSKYSNYETSGQLIRLSLSYIVSLMSINEQ

>TpreOR115

MGGNKGFSYAFGFCNVTLFIVGLWPKLKKSEKKDWLSIISFIFSLCIIVIFINIAQTTKLIIIWGDFVEMINNIS
TANLPIMVAVIKMFIFQYYKNVLGCLLSKAMIDWQSTKTQEQESSMYENARVVRKISMICCVLGFSSVTGHLMIR
ICQELDFIPNYEKKREPMVSSYFPYDYKSSPTYEITWFFQYLGAGLATLVYSGSHCLFVGLMLHLRGQVKNLSNT
FRNNRTRINKHERQDSKLFKNFIKSVVIRHNDLYE

>TpreOR116

MEQTIQLDLETIENFYSNEYFKVCKILNCMAGLWPYKGYMDKLLRRVIVSIVILGILFVPYLRGVKKWCGKNLAA
CSEDIAGTVFASGVFLKYLVTFINEKKLIQVYEEVARNHLALDDPAEQAIMAKWSMDGKIKYMGYLGYLTLAGVS
FSQMIIVPQMLDLVVPLNESRQRVTITMADFGIDSDQYFYTIYVTYCVVSVVSFFVLTSIDTMYTAIIHQILGIF
NVVKYRLLNVQTANFGLNKGNDKTGRDISSLNVISAIRLHHKSLQFIDLIESTYRYCFLILIFICVIFLSFGSIT
VLEAVNELYNFLRLGIVLFGVAVHLFYLSWPGQLVIDESSDVFRATYESTWYDMPKRTQVLIDIMMMRCRQPCSL
TAGGLYVMSFENYGKIIKTTGSYITVLASFRG

>TpreOR117

MKPFILRAGFLADMNFETFSSIIKITENTKESGFRSTLFIEDIFQVIVSLMMMYVYPFMIWKYDRIVMTVVHMKR
VLENNDKLGQAMVKKSYEKSNRFSLFITMLFIPITVTFAIAPLVIPILSTQFLPGNQTFQKALPLHEEFFVDEEK
YFYYLFALQFLTIMIYCTIIEGLNTLLYSGVGFIVGELQYLQYYLEKTDSCYNDMVSECKDIAYNFLTKSLVYFI

EQHQKCIKLYEILYLLTESIMFVSMLGCSTSLIIACSGMVIFMDSDVSMALKMLVLCIATLSVILFICIPGQFLS
DAGDELFTKCYCVDWHGYPPKARILLVLILLRTMKPLQLKAGFLAVLSFETFSSVLKKSMSFVTAFRSIYATL

>TpreOR118

MFKKLFTIIYLPEIPGLVELDKNYGETLIFLFHTTFYVTQPPINWEFAIQNLFELSFVLMMFSMYFILGNMQREI
HDYTSMTVNFWDSITDSDQKEYFEKCEYIGRKVLMILSTYVLVALSFTGTMILFSLSVNNYFLLLEMIISFFSII
FTAFLTAHFGQIVINASDDIFYDCYFCGWYKFPSNTKPYIIMMMIRALSPCELKAGPSAVLTMNYENFSAVSYDQ
FHVQFNKLKPI

>TpreOR119

MELFNQIASPLMIVILTLVSMCLVISAFWMVILSKTDTAETFRMFCAFSLANISLFFICLPSQIVVDTNTYVYNS
CYSCNWYDYPTKSRKYFQLFILRLSKTLVVPAGPLAKLNFETYGNIIKTSLSYTTALMSIYL

>TpreOR120
MSVGVYGGIVVLVISCLPSQLINDSSSEIGLACYRSNWYQWEPKIRLAYLLVMIRTSSPCGWKGTKFFENDDAFF
GYILLYEITVSIFFLTFISLSIGSYIFYSYLIIIRFQQISKKFETLQLDQLNTYNLELSDDKIQVDVIATIKKHD
KCINYRSNWYQWEPKVRLAYLLVMIRTSSPYEWKAGPIFVLNFEMVAFALVQFKKGFVTDALQMGFGFLSCIITL
VLLCIPSQFIIDVSDKVGHACYFNTNWYEWKPKDRLVIRRTLMRTLYPCGWKAGPSITLNLRTAAFVIKTAMSYV
MTMISIYA

>TpreORco

MMKMKQQGLVADLLPNIRVMQFSGHFMFNYYNEGLKFPHRVFCIVSFLLILVQYSMMGINLAMEVGDPDDMAANT
ITMLFFIHPIVKTIYFAARSKTFYKTLGIWNNPNTHPLFAESDAHYHSVAVQKMRKLATFVGAATIFTLFAWTTI
TFFEDSVKTVVDKETNETTIIPIPRLPIRAWYPFNSMKGFGHIMAFVYQFYYLAMCLTLSISVDVLFCSWLIFAC
EQIMHLKAIMKPLMELSATLDTVVPNSGDLFKAGSDDHLRDTNGVQPAGDGIVDSDVRGIYSNRQDFTATFRPTA
GTNFNGNVGPNGLTKKQEMLVRSAIKYWVERHKHVVKLVTAIGDTYGVALLLHMLATTITLTLLAYQATKINGVN
PYGATVIGYLLYTLGQVFHFCIFGNRLIEESSSVMEAAYSCHWYDGSEEAKTFVQIVCQQCQKAMSISGAKFFTV
SLDLFASVLGAVVTYFMVLVQLK

الحمد لله من قبل و من بعد