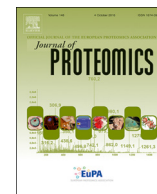




Contents lists available at ScienceDirect

Journal of Proteomics

journal homepage: www.elsevier.com/locate/jprot

Proteomic analysis of chemosensory organs in the honey bee parasite *Varroa destructor*: A comprehensive examination of the potential carriers for semiochemicals

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ARTICLE INFO

Keywords:

OBP-like

Niemann-pick type C2

Forelegs

Mouth parts

Olfaction

ABSTRACT

We have performed a proteomic analysis on chemosensory organs of *Varroa destructor*, the honey bee mite, in order to identify putative soluble carriers for pheromones and other olfactory cues emitted by the host. In particular, we have analysed forelegs, mouthparts (palps, chelicera and hypostome) and the second pair of legs (as control tissue) in reproductive and phoretic stages of the *Varroa* life cycle. We identified 958 *Varroa* proteins, most of them common to the different organs and stages. Sequence analysis shows that four proteins can be assigned to the odorant-binding protein (OBP)-like class, which bear some similarity to insect OBPs, but so far have only been reported in some Chelicerata. In addition, we have detected the presence of two proteins belonging to the Niemann-Pick family, type C2 (NPC2), which have also been suggested as semiochemical carriers.

Biological significance: The mite *Varroa destructor* is the major parasite of the honey bee and is responsible for great economical losses. The biochemical tools used by *Varroa* to detect semiochemicals produced by the host are still largely unknown. This work contributes to understand the molecular basis of olfaction in *Varroa* and, more generally, how detection of semiochemicals has evolved in terrestrial non-hexapod Arthropoda. Moreover, the identification of molecular carriers involved in olfaction can contribute to the development of control strategies for this important parasite.

1. Introduction

One of the main threats to honey bee colonies [1] worldwide is the mite *Varroa destructor* (hereon referred to as “*Varroa*”). Females of this ectoparasite are transmitted between hives by foraging bees, and once in the hive they settle in the bee larval cells and lay eggs. The newborn *Varroa*, generally one male and four females for each cell, feed on the honey bee larvae and, once the females leave the cell, spread in the hive by adhering to adult bees.

Communication between *Varroa* individuals as well as their interactions with honey bees are mediated by chemical signals. Some cuticular hydrocarbons of bee larvae as well as 2-hydroxyhexanoic acid, a component of brood food, have been reported as attractants for mites in their reproductive stage [2–5]. Once inside the cells, a blend of three

fatty acid methyl esters produced by the bee pupae regulates laying of unfertilized (male) and fertilized (female) eggs [6] by *Varroa*, and induce the reproductive maturation of young *Varroa* [7]. Mature female mites attract males with a cocktail of three fatty acids (palmitic, stearic, and oleic) and their ethyl esters [8]. While in their phoretic stage, the *Varroa* are repelled by geraniol and nerolic acid [9], as well as by (Z)-8-heptadecene [10], which are all produced by the foragers; for this reason, the mites tend to parasitize nurse bees.

Compared to insects, chemical communication in other arthropods, particularly Chelicerata, is poorly understood. Most of the studies are focused on morphology [11] and electrophysiology [12–14] while several papers report on the identification of putative semiochemicals [8,9,15–18]. Gustation and olfaction take place in sensilla, which are located on mouthparts and forelegs in ticks and mites. In *Varroa* the

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main olfactory organ, referred to as pit organ, is located on forelegs and presents nine olfactory hairs, which are morphologically similar to insect sensilla basiconica [19,20]. Furthermore, electrophysiological experiments have clearly demonstrated that the forelegs of *Varroa* respond to chemical stimuli [21,22].

Only preliminary information is available on *Varroa*'s biochemical tools (receptors and carrier proteins) for chemosensing. Based on genome and transcriptome projects, ionotropic receptors (IRs), gustatory receptors (GRs) and sensory neuron membrane proteins (SNMP) have been identified in some ticks and mites [23–26], but chelicerates lack homologs of the typical insect olfactory receptor family [27,28].

Odorant-binding proteins (OBPs), which act as carriers of odorants and pheromones in the sensillar lymph of insects, are absent in Chelicerata [27].

The presence of CSPs also seems questionable. A single sequence reported in the tick *I. scapularis* [25] turned out to be identical with a CSP of the mosquito *Culex quinquefasciatus* (acc. XP_001844693), indicating a result of contamination. Furthermore, the two CSPs reported in a transcriptome study of the mite *Tyrophagus putrescentiae* [29] are very similar to CSPs of Diptera (around 80% identity), leaving the possibility of contamination an open question. Therefore, in the absence of OBPs and CSPs, other carrier proteins are likely to be present in the chemosensing systems of Chelicerata.

A third family of proteins possibly acting as semiochemical carriers in insects include the NPC2 (Niemann-Pick proteins of type C2) proteins [30–32]. This family is well represented in Chelicerata with a variable number of genes [31,33], depending on the species. In particular, in the tick *Ixodes scapularis*, a dozen genes have been identified and one of the encoded proteins was detected by immunocytochemistry experiments in chemosensilla of this species [34]. Members of the NPC2 family have been also found in the tick *Amblyomma americanum* [35] and eight transcripts encoding such proteins have recently been reported in a transcriptome project in *Varroa* chemosensory organs [26]. For NPC2 proteins, a function of semiochemical carriers seems to be well supported by their ligand-binding properties as well as by their localization in chemosensilla [30,32,34]. Moreover, three-dimensional structures of NPC2 members both from vertebrates and insects are available, some of them containing hydrophobic ligands inside their binding pockets [30,36].

Another class of soluble proteins has been proposed as semiochemical carriers in the tick *A. americanum* [35] and in two spider species [24], as well as in *Varroa* [26]. Given some structural similarity with insect OBPs, these proteins have been named as “OBP-like.” Sequence identity values with insect OBPs are generally low (around 15% or less) and the pattern of six cysteines, a typical signature of most insect OBPs, is not fully conserved. Some OBP-like proteins of Chelicerata contain four cysteines in a pattern resembling that of insect C-minus OBPs, but other members present six cysteines, although in positions different from those of classic OBPs of insects [35]. Binding data and cellular localization are still needed to support their putative role in chemosensing.

In this work we report the results of a proteomic analysis on chemosensory organs of *Varroa* to better understand chemical communication in this economically devastating species. In particular, knowledge of the molecular mechanisms used by the mites to follow chemical signals from the larval bees could provide the basis for alternative strategies to control the population of the parasite inside the hive.

2. Material and methods

2.1. Sample collection

Adult mites were collected at two different stages: “reproductive mites” from drone larvae and “phoretic mites” from young adult bees, foragers, or adult drones. Specimens were kept at -20°C until

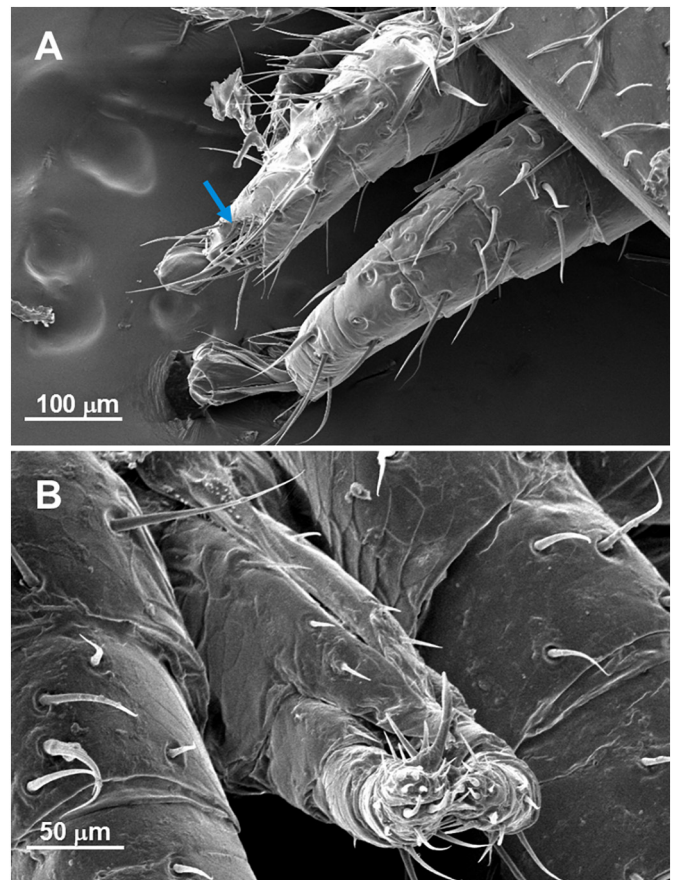


Fig. 1. SEM of legs and mouth parts of *Varroa*. Scanning electron microscope images of forelegs and second pair of legs of an adult female coated with gold (panel A) and ventral view of mouth parts of an adult female coated with graphite and gold (panel B). The chemosensory pit organ is visible on the foreleg tarsi (red arrow). Mouth parts include hypostome, chelicera and pedipalps. The pictures have been taken through a ZEISS EVO MA 15, at MEMA (Centro di Servizi di Microscopia Elettronica e Microanalisi, University of Firenze), using the signals produced by secondary electrons, accelerated at 10 KV, with a resolution of 1024×768 nm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

dissection. Reproductive mites were collected from frames containing exclusively drone brood, produced by workers after excluding the queen from that part of the frame, in an apiary located in Certaldo (Firenze). Phoretic mites were collected from adult bees in the experimental apiary at the Department of Biology, University of Firenze. Foragers and drones were collected with a net in front of the hive, while young bees were obtained from brood frames temporarily removed from the hive.

Dissections were performed on ice and three appendages were isolated: forelegs, bearing the tarsal organ; mouthparts, containing palps, chelicera and hypostome; and the second pair of legs, to be used as control (Fig. 1).

Three biological replicates for each appendage were prepared for “reproductive mites” and for “phoretic mites” from young bees, while a single pool was prepared for “phoretic mites” from foragers or drones, which are more difficult to collect; protein extracted from these latter samples were divided into three aliquots (technical replicates) before enzymatic digestion. The organs were dissected from 35 reproductive and phoretic *Varroa* on young bees, from 50 phoretic *Varroa* on foragers or drones.

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