Evolution of insect olfactory genes

Dissertation
zur Erlangung des akademischen Grades
„doctor rerum naturalium“ (Dr. rer. nat.)

vorgelegt dem Rat der Biologisch-Pharmazeutischen Fakultät
der Friedrich-Schiller-Universität Jena

von Dipl. Biol. Christine Mißbach

geboren am 21.04.1983 in Eisenach

Gutachter

1. Prof. Bill S. Hansson, Jena / Deutschland

2. Prof. Rolf G. Beutel, Jena / Deutschland

3. Assoc. Prof. Dr. Richard Benton, Lausanne / Schweiz

Tag der öffentlichen Verteidigung

4. Juli 2014

Die Natur offenbart sich hier in ihrer ganzen Größe.
Augen und Gedanken schwelgen.
Der Dichter kann es besingen, der Maler in reichen Bildern darstellen,
aber den Duft der Wirklichkeit, der dem Betrachter auf ewig in die Sinne dringt
und darin bleibt, können sie nicht wiedergeben.

Hans Christian Andersen (1805-1875)

Table of Contents

Introduction

Chemoreception is the ability of organisms to detect chemicals in their environment (reviewed in Bargmann 2006). This ability is common to all living organisms, including bacteria, protozoans, fungi, plants as well as animals, and is thus viewed from an evolutionary perspective as one of the oldest senses (Zhou and Chen 2009). While chemosensation involves both taste and smell, most animals rely on olfaction as the principal chemosensory modality (Saghatelyan et al. 2003). The power to detect and discriminate odor molecules is often strongly connected to the survival and the reproductive success of the organism. This strong selective pressure has led to a highly sensitive olfactory system across many animal phyla (Hildebrand and Shepherd 1997). Although there is an evolutionary convergence towards a conserved organization of signaling pathways in vertebrate and invertebrate olfactory systems (Hildebrand and Shepherd 1997), the involved receptor gene families are often evolutionarily unrelated.

Olfaction: Same odor molecules – different receptors

The identity of olfactory receptors was first determined in vertebrates (Buck and Axel 1991) using the rat *Rattus norvegicus* (Rodentia: Muridae) as the model system and building upon three assumptions: First, biochemical evidence had implicated that G proteins are involved in olfactory signal transduction (Pace et al. 1985); therefore, vertebrate odorant receptors are likely G protein–coupled receptors. Second, odorant receptors likely encode a large gene family, because a high number of receptors are required to detect and discriminate the immense number of chemically different molecules. Third, the presence of odorant receptors should be restricted to the olfactory tissues. These three assumptions led to the subsequent identification of as many as 1000 G protein–coupled seven-transmembrane proteins in the rat genome (Zhang et al. 2007). Further studies have shown that these odorant receptors are present in vertebrate species ranging from fish to humans (reviewed in Mombaerts 1999).

A similar number of chemosensory receptors, with about 1300 receptor genes and 400 pseudogenes, have been identified in the nematode *Caenorhabditis elegans* (Rabditita: Rabditidae) (Robertson and Thomas 2006). These receptors are seven-transmembrane domain proteins with no sequence homology to vertebrate olfactory receptor genes, and moreover they have very limited sequence similarity to each other (Troemel et al. 1995).

Olfactory receptors of insects and other arthropods

The first insect olfactory receptors (ORs) were discovered in the genome of the vinegar fly *Drosophila melanogaster* (Diptera: Drosophilidae) (Clyne et al. 1999, Gao and Chess 1999, Vosshall et al. 1999, Clyne et al. 2000). Similar to G-protein coupled receptors, ORs are multitransmembrane domain proteins, but have an inverted topology (Benton et al. 2006, Lundin et al. 2007). Insect ORs have been suggested to be distantly related to the gustatory receptors (GRs) of arthropods, constituting a single highly expanded branch within the arthropod chemoreceptor superfamily (Robertson et al., 2003). This is also supported by the presence of a signature motif in the carboxyl terminus in some members across the superfamily (Scott et al. 2001).

Insect ORs are heteromeric odor-gated ion channels (Sato et al. 2008, Wicher et al. 2008) formed by at least one ligand specific OR and the OR coreceptor, named Orco (Vosshall et al. 1999, Elmore et al. 2003, Krieger et al. 2003, Larsson et al. 2004, Vosshall ad Hansson 2011). The number of functional OR genes varies from 10 in the human body louse *Pediculus humanus humanus* (Phthiraptera: Pediculidae) (Kirkness et al. 2010) to about 350 OR genes in ants (Zhou et al. 2012a). Interestingly, the sequences of OR genes reveal almost no overall sequence identity, even within the same insect order (Krieger et al. 2003). Nevertheless, Orco is highly conserved among insects. Homologues were found in almost all investigated species, including lepidopterans, dipterans, coleopterans, hymenopterans and hemipterans (Krieger et al. 2003, Pitts et al. 2004, Smadja et al. 2009). However, even though highly conserved within insects, no Orco-coding genes have been identified outside of the Insecta, neither in the genome of the crustacean *Daphnia pulex* (Onychura: Daphniidae) (Peñalva-Arana et al. 2009) nor the genome of the chelicerate *Ixodes ricinus* (Ixodida: Ixodidae) (Vieira and Rozas 2011). Furthermore, OR genes are also absent, suggesting that Orco and ORs are specific to insects.

IRs – the insect´s second nose?

A second receptor family involved in insect chemosensation are the ionotropic receptors (IRs) (Benton et al. 2009). These IRs are ligand gated ion channels derived from the ionotropic glutamate receptor family (iGluRs, Croset et al. 2010). iGluRs are structurally and functionally conserved in most animals (Tikhonov and Magazanik 2009), mediating chemical communication between neurons at synapses (Mayer 2006, Gereau and Swanson 2008, Sobolevsky et al. 2009). However, over evolutionary time the IR lineage was coopted into chemosensation, detecting chemical signals from the external environment (Croset et al. 2010).

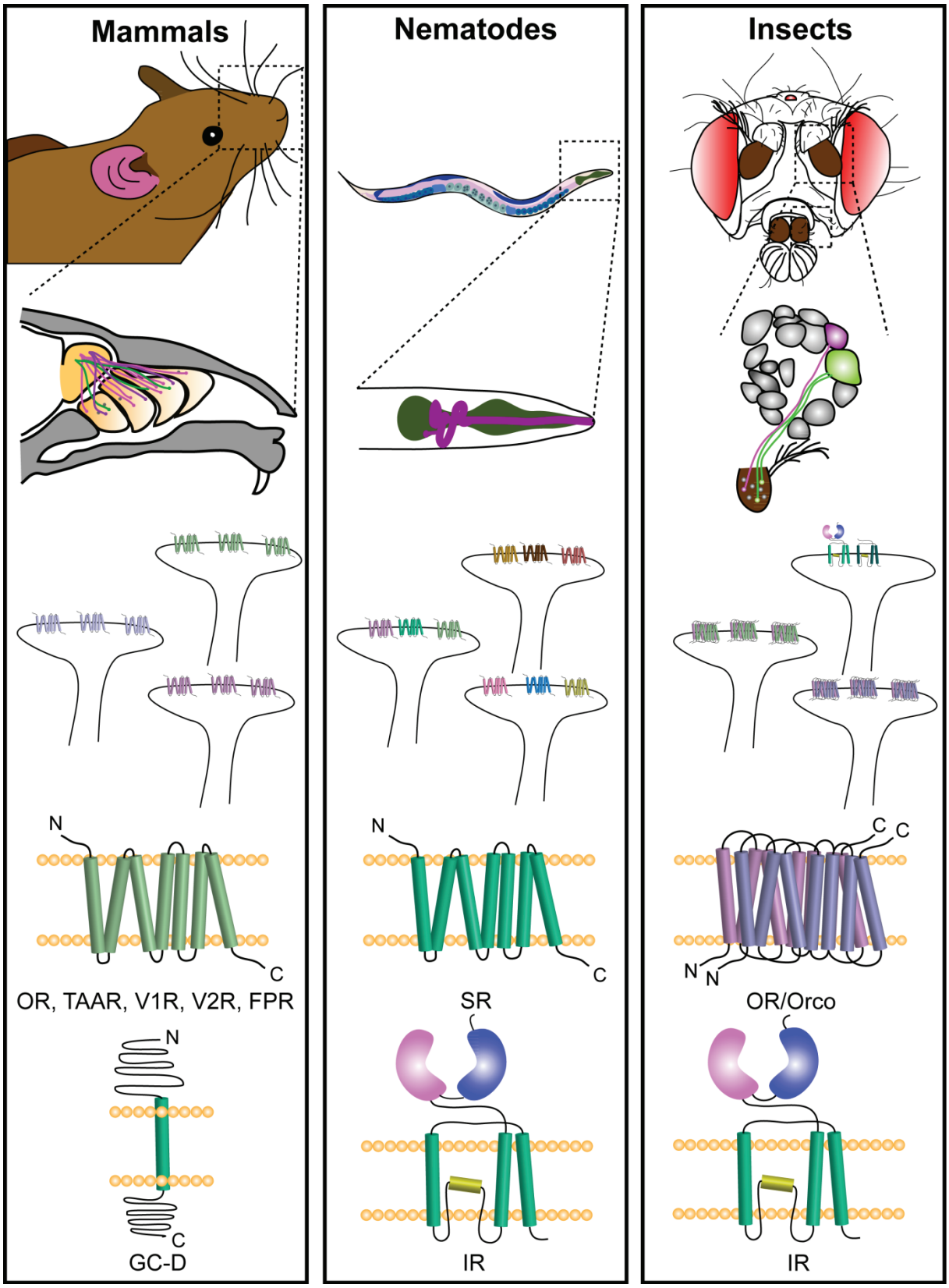


Figure 1. Molecular mechanisms of odor detection in mammals, nematods and insects
From top to down: 1) Localization of the main olfactory tissue in the mouse *Mus musculus* (left), the nematode *Caenorhabditis elegans* (middle) and the vinegar fly *Drosophila melanogaster* (right); 2) Connections of sensory neurons to first olfactory brain centers. Different colors of neurons used for the mouse and the fly denote to the expression of different receptors; 3) Schemes of olfactory cilia. Different receptors are symbolized by different colors; 4) Schemes of the different receptor types. Note the inverted topology of insect ORs compared to mammalian and nematode G protein-coupled receptors. Modified from Bargmann 2006.

Figure 1. Abbreviations: OR – olfactory receptor (same name, but unrelated in vertebrates and insects), TAAR – trace amine-associated receptor, V1R – vomeronasal receptor type 1, V2R - vomeronasal receptor type 2, FPR – formyl peptide receptor, GC-D – receptor guanylyl cyclase type D, SR – chemosensory recetor, IR – ionotropic receptor, Orco – olfactory receptor coreceptor.

IRs are present in chemosensory tissues across protostomes (Croset et al. 2010), including molluscs, nematodes, crustaceans and insects.

Based on expression profiles in *D. melanogaster* the IR family is further subdivided into the "antennal" and "divergent" IRs (Benton et al. 2009). Out of 66 identified IR genes 16 genes are expressed in the antenna of *D. melanogaster* and were therefore termed "antennal" IRs (Benton et al. 2009, Croset et al. 2010). Antennal IRs act in combinations of up to three subunits, which includes individual odor-specific receptors and one or two of the broadly expressed coreceptors IR25a, IR8a and IR76b (Abuin et al. 2011).

Distinct populations of insect sensilla express different receptor types

The main olfactory organs of insects are the antennae and the maxillary palps. Both are covered with a high number of small sensory structures, called sensilla. Insect olfactory sensilla can be mainly categorized into two fundamentally different types: Single-walled sensilla with pore tubules (sw-wp sensilla) and double-walled sensilla with spoke channels (dw-wp sensilla) (Steinbrecht 1969, Altner 1977, Altner & Prillinger 1980). Both sensillum types are present side by side in most insect orders. The sw-wp sensilla include for example basiconic and trichoid sensilla, whereas coeloconic sensilla are dw-wp sensilla. Moreover, the dw-wp olfactory sensilla are preferentially tuned to more polar, hydrophilic stimuli (e.g. short chain acids and amines), while the sw-wp sensilla are involved in the detection of more nonpolar, hydrophobic stimuli (Altner 1977).

There is some evidence that insect ORs and IRs are expressed in olfactory sensory neuron (OSN) populations of a distinct sensillum type (Benton et al. 2009). In *D. melanogaster* antennal IRs are the functional receptor type of OSNs in dw-wp coeloconic sensilla, and ORs are predominantly expressed in OSNs housed in sw-wp basiconic and trichoid sensilla (Hallem et al. 2004, Silbering et al. 2011). The previous literature concerning IR expression beyond *D. melanogaster* is incomplete, thus overarching trends could not be established. In Chapter I and Chapter III this dissertation will present some work on IR expression outside *D. melanogaster*.

Insect odorant binding proteins

ORs are expressed in the dendritic membrane of OSNs (Elmore and Smith 2001, Dobritsa et al 2003). Therefore these receptors are exposed to the aqueous environment of the sensillum lymph, while their ligands are primarily hydrophobic. The aqueous solubility of hydrophobic odorants is thought to be greatly enhanced by odorant binding proteins (OBP), which are present at high concentrations in the sensillum lymph (Pelosi 1994). Although several studies have demonstrated selective binding of odorants and/or pheromones to different OBPs (Danty et al. 1999, Plettner et al. 2000, Pophof 2002, 2004, Zhou et al. 2004), the exact function of OBPs is not well understood. It is currently believed that OBPs participate in the solubilization and transportation process of odorants through the lymph (Figure 2, Vogt et al. 1991, Pelosi 1994, Pophof 2004, Prestwich et al. 1995, Tsuchihara et al. 2005, Grosse-Wilde et al. 2006), that they mediate sensitivity (Gomez-Diaz et al. 2013) and protect odors from degradation by odorant-degrading enzymes (Chertems et al. 2012, Gomez-Diaz et al. 2013). Since different OBPs are present in a particular olfactory sensillum type, it is likely that OBPs also play a role in olfactory coding (Hakmat-Scafe et al. 1997). Similar to ORs, OBPs

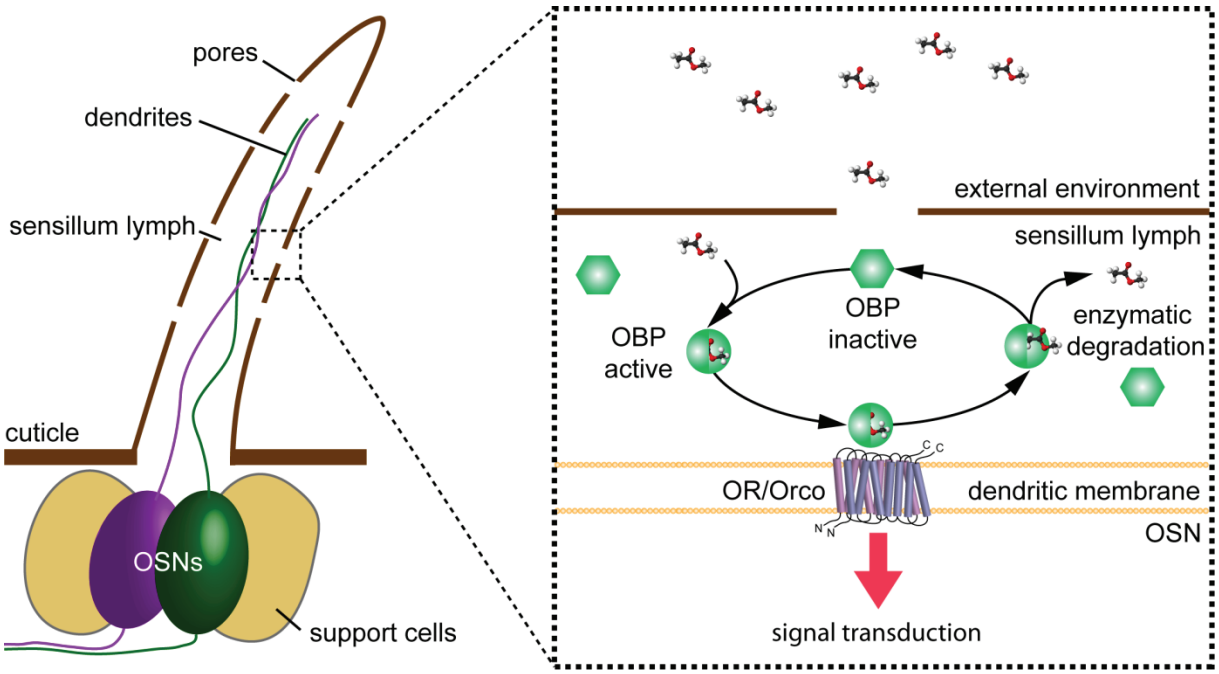


Figure 2. Possible functions of OBPs in insect olfaction
A: Schematic representation of the general structure of a single-walled olfactory sensillum. The dendrites of the olfactory sensory neurons (OSNs) extend into the sensillum shaft where they are surrounded by the sensillum lymh. This lymph is secreted by special support cells and contains the odorant binding proteins (OBPs).
B: Simplified functional scheme of perireceptor events in the insect olfactory pathway. Odor molecules enter the sensillum through small pores in the sensillum wall. Odor molecules are taken up by OBPs. OBPs transport the molecules through the sensillum lymph to the OSN dendrites where the odors bind to ligand specific receptors (OR). OBPs also help to protect odors from early degradation. Modified from Sánchez-Gracia et al. (2009).

were only identified in insect species, but not in genomes of other arthropods (Vieira and Rozas 2011).

Evolution of insect ORs and OBPs

Terrestrial hexapods originally derived from an aquatic crustacean ancestor, probably in the Early Ordovician period (Rota-Stabelli et al. 2013). The transition from sea to land meant that molecules needed to be detected in the gas phase instead of in an aqueous environment.

Therefore, the olfactory system of this hexapodan ancestor had to adapt to terrestrial conditions and to adapt to the detection of volatile, air-borne chemical signals. One proposed hypothesis has been that the ORs and OBPs that are found in insects are a direct adaptation to the terrestrial lifestyle (Robertson et al. 2003, Vieira and Rozas 2011, Krång et al. 2012). Furthermore, the contemporal origin of both gene families suggests that both families may have coevolved (Vieira and Rozas 2011).

However, all studies on insect olfactory gene families published thus far have focused on neopteran insects (Paleoneoptera, Paraneoptera and Holometabola, see Figure 3). Evolutionary older insect taxa, such as the primary wingless insects or the first flying insect orders, were not investigated for their olfactory gene equipment, although their study could help to understand the evolution of insect ORs and OBPs, especially with respect to testing the validity of the hypotheses discussed above.

Olfactory capabilities of Archaeognatha and Zygentoma

Archaeognatha and Zygentoma are considered to be among the oldest extant insects lineages likely having arisen in the early to late Devonian period (Grimaldi 2001, Mendes, 2002, Engel and Grimaldi 2004). The species within these two early insect lineages appear to be quite similar and were previously joined together in the order Thysanura (Koch 2001, Regier et al. 2004, Grimaldi and Engel 2005). However, more recent molecular, and combined morphological and molecular studies support a basal split of Archaeognatha and a sister group relationship of Zygentoma and Pterygota (see Figure 3, Hennig 1981, Kristensen 1997, Regier et al. 2010; reviewed in Carapelli et al. 2006). According to the fossil record, Archaeognatha evolved about 390 million years ago (Labandeira et al. 1988). Several ferns, moss and club moss species as well as the first terrestrial fungi are also reported from this time period (Cacales-Miñana and Cleal 2011). As even today bristletails mainly use algae, lichens and moss as a food source (Sturm 1955) and their enemy spectrum has not changed (Sturm and Machida 2001), their olfactory system might still display some ancient characteristics.

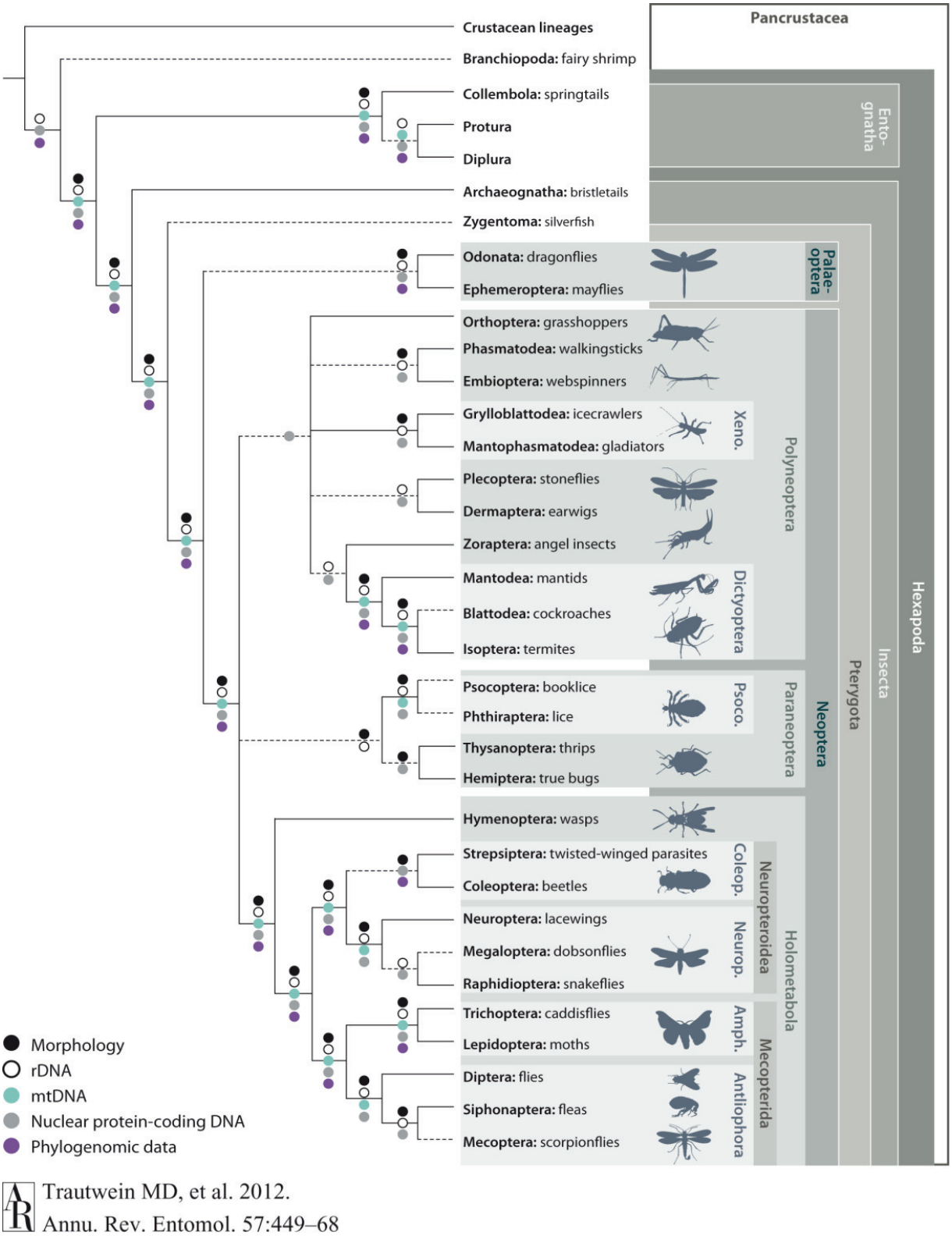


Figure 3. Insect relationships based on a review of recent literature (Trautwein et al. 2012)
The most accepted hypothesis about the higher-level phylogeny is a close evolutionary relationship between Hexapoda and Crustacea, maybe even with a position of the Hexapoda within the Crustacea. Within the Insecta *senso stricto*, the earliest lineages are the Archaeognatha (jumping bristletails) and the Zygentoma (silverfishes/firebrats), two groups of primarily wingless insects. The present thesis is mainly focused on two species belonging to either of these two taxa. Dashed lines indicate tenuously supported relationships or possible nonmonophyly (in the case of terminal branches). The tree is taken from Trautwein et al. (2012).

So far, only a few morphological studies have been conducted from both of these two insect taxa to address the organisation of the olfactory system. Both insect taxa possess really long and filiform antennae (Figure 4) that are covered with several sensillum types. Putative olfactory sensilla were previously described for both Archaeognatha (*Machilis* sp.: Berg and Schmidt 1997; several species: Bockhorst 1988; *Lepismachilis y-signata*: Missbach et al. 2011) and Zygentoma (*Lepisma saccharina*: Berg and Schmidt 1997; *Thermobia domestica*: Adel 1984). The olfactory function of sensilla was proposed based on ultrastructural investigation, but no previous electrophysiological studies on putative olfactory sensilla had been done in either of the two groups. This dissertation will present in Chapter I the first electrophysiological study on antennal olfactory sensilla of Archaeogatha and Zygentoma.

The olfactory information perceived in the periphery is transmitted by the antennal nerve to the antennal lobe (AL), which acts as the first olfactory processing center. Within the insect AL, the OSNs synapse onto projection and interneuron terminals in spherical and dense synaptic regions called glomeruli (Homberg 1994, 2005, Hansson and Anton 2000, Schachtner et al. 2005, Vosshall and Stocker 2007). In contrast to all other insects studied so far, the glomeruli of Archaeognatha appear irregular and more or less elongate in shape (Missbach et al. 2011), while more spherical shaped glomeruli are present in the AL of Zygentoma (Schachtner et al. 2005). In both Archaeognatha and Zygentoma the number of glomeruli is quite small when compared to other insects. Since the number of glomeruli is roughly considered to correspond to the number of receptor proteins expressed in distinct subpopulations of OSNs, the expected number of receptors for of both taxa could be estimated to be around 10 (Missbach et al. 2011, Dweck pers. comm.).

After a first processing in the AL, the olfactory information is transferred by projection neurons to higher brain centers, such as the mushroom bodies, the inferior protocerebrum and the lateral horn (Strausfeld et al.1998, 2009, Anton and Homberg 1999, Hansson and Anton 2000, Galizia and Rössler, 2010). Interestingly, mushroom bodies are found in Zygentoma, but are completely absent in Archaeognatha (Farris 2005, Strausfeld et al. 2009, Missbach et al. 2011). Besides other functions, the mushroom bodies play an essential role in odor discrimination and in the formation of an olfactory memory (Heisenberg, 2003). The absence of real mushroom bodies in Archaeognatha is often seen as a plesiomorphic character that Archaeognatha share with their crustacean relatives (Strausfeld et al. 2009). Moreover, in Archaeognatha the projection neurons extend out to the lateral protocerebrum where they provide an extensive volume of layered neuropil, the architecture of which is reminiscent of protocerebral olfactory neuropils of the eumalacostracan olfactory systems (Strausfeld 2009).

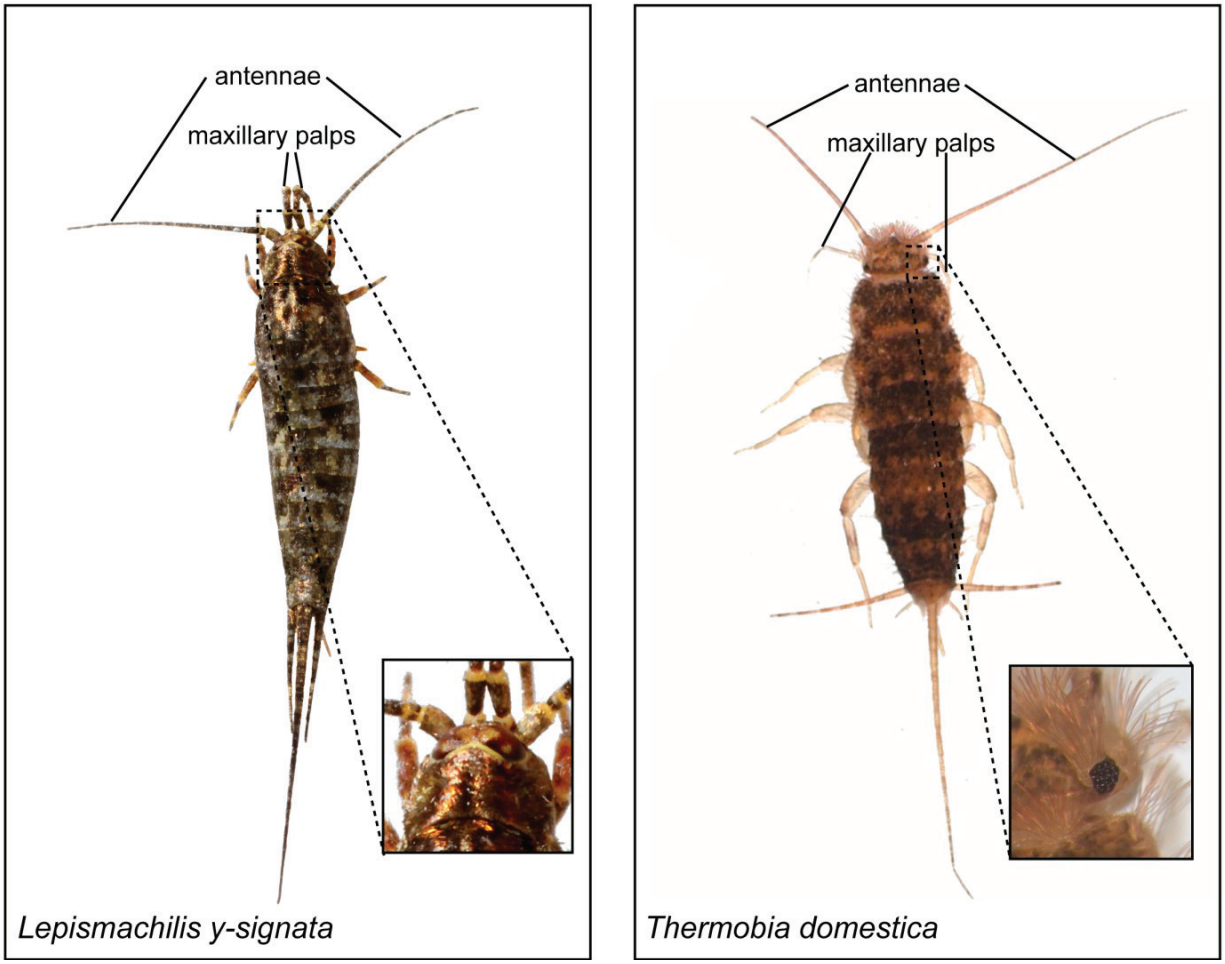


Figure 4. Main experimental organisms: *Lepismachilis y-signata* (Archaeognatha: Machilidae) and *Thermobia domestica* (Zygentoma: Lepismatidae)
The habitus of both animals is quite similar. Both insects are apterygote and have very long filliform antennae. Archaeognatha in general have very large compound eyes that are often converged on the backside of the head, whereas in Zygentoma compound eyes are reduced or absent making other senses even more important for these animals.

Therefore, on this level the olfactory system of Archaeognatha again appears to share features with that of crustaceans, whereas the zygentoman system appears more similar to flying insects.

In general, the investigation of Archaeognatha and Zygentoma, when compared to data from close relatives such as flying insects and crustaceans, will lead to novel insights into the evolution of the insect olfactory system, as well as further insights into the identity and evolution of the receptors and other olfactory gene families that are crucial for insect olfaction (discussed in Chapter I and Chapter II).

Overview of Manuscripts

Chapter I

Evolution of insect olfactory receptors

Christine Missbach, Hany K. M. Dweck, Heiko Vogel, Andreas Vilcinskas, Marcus C. Stensmyr, Bill S. Hansson and Ewald Grosse-Wilde

eLIFE 2014; 3:e2115
Publiziert (doi:10.7554/eLife.02115)

In the first chapter I present an analysis of the olfactory capabilities of *Lepismachilis y-signata* (Archaeognatha: Machilidae) and *Thermobia domestica* (Zygentoma: Lepismatidae), as well as a third pterygote species *Phyllium siccifolium* (Phasmatodea: Phylliidae) for comparison. Single sensillum recordings demonstrated that both wingless species detect a wide range of different odors, however possess a lower number of distinct functional neurons than *P. siccifolium*. Deep antennal transcriptome sequencing led to the identification of the evolutionary ancient ionotropic receptors (IRs), but not the insect specific olfactory receptors (ORs) in both wingless insects. In contrast to *L. y-signata*, where neither ORs nor their conserved coreceptor were present, Orco was identified in *T. domestica*, where it existed in multiple independent variants. Altogether the data of this chapter suggests that in wingless insects other receptors than ORs are involved in odor detection, and that the full OR/Orco complex evolved in a stepwise manner after insects colonized land.

Built on an idea conceived by all authors.
designed experiments: C. Missbach (50%), E. Grosse-Wilde, H. Dweck, M. Stensmyr, B. Hansson
bioinformatic analysis: C. Missbach (90%), E. Grosse-Wilde
performed and analyzed molecular experiments: C. Missbach (90%), E. Grosse-Wilde
performed and analyzed SSR-experiments: H. Dweck, M. Stensmyr
wrote the manuscript: C. Missbach (60%), E. Grosse-Wilde, H. Dweck, H. Vogel, B. Hansson

Chapter II

Identification of Odorant Binding Proteins and Chemosensory Proteins in Antennal Transcriptomes of the jumping bristletail *Lepismachilis y-signata* and the firebrat *Thermobia domestica:* Evidence for an independent OBP-OR origin

Christine Missbach, Heiko Vogel, Bill S. Hansson and Ewald Grosse-Wilde

Chemical Senses, submitted January 18, 2014
In Überarbeitung

In Chapter II we analyzed the transcriptomes of *Lepismachilis y-signata* (Archaeognatha: Machilidae) and *Thermobia domestica* (Zygentoma: Lepismatidae) for the presence of odorant binding proteins (OBPs) and chemosensory proteins (CSPs). While previous studies have identified CSPs across arthropods, OBPs are insect specific. Their assumed function in cooperation with ORs led to the suggestion that ORs and OBPs coevolved. Together with the results of Chapter I we showed that OBPs were likely present in the last common ancestor of insects and therefor evolved independently of ORs.

Built on an idea conceived by all authors.
Bioinformatics analysis: C. Missbach (90%), E. Grosse-Wilde
Wrote the manuscript: C. Missbach (70%), E. Grosse-Wilde, H. Vogel, B. Hansson

Chapter III

Variant Ionotropic Receptors Are Expressed in Olfactory Sensory Neurons of Coeloconic Sensilla on the Antenna of the Desert Locust (*Schistocerca gregaria*)

Mei Guo, Jürgen Krieger, Ewald Große-Wilde, Christine Mißbach, Long Zhang and Heinz Breer

International Journal of Biological Sciences 2014; 10(1):1-14
Publiziert (doi: 10.7150/ijbs.7624)

In the third chapter we have identified and localized the coreceptors of ionotropic receptors, SgreIR8a and SgreIR25a, in the antenna of the desert locust *Schistocerca gregaria* (Caelifera: Acrididae). Both receptors are expressed in antennae of all five nymphal stages and in adults. *In situ* hybridization experiments revealed expression of SgreIR8a and SgreIR25a in olfactory sensory neurons of coeloconic sensilla. Additionally SgreIR25a was found in neurons of some chaetic sensilla. Double FISH experiments demonstrated that cells expressing SgreIR8a or SgreIR25a do not express ubiquitous coreceptor of olfactory receptors. Hence we found a complementary localization of IRs and ORs.

Built on an idea conceived by all authors.
designed experiments: M Guo, J Krieger
bioinformatic analysis: M. Guo, J. Krieger, C. Missbach (20%), E. Grosse-Wilde
performed and analyzed molecular experiments: M. Guo
wrote the manuscript: M. Guo, J. Krieger

Additional Manuscript

During the production of this thesis I have contributed to an additional manuscript that did not exactly follow the aim of the thesis.

Title and authors of this manuscript are as follow:

Olfactory coding in five moth species from two families

Sonja Bisch-Knaden, Mikael A. Carlsson, Yuki Sugimoto, Marco Schubert, Christine Missbach, Silke Sachse, Bill S. Hansson.

The Journal of Experimental Biology, accepted January 16, 2012

Chapter I

Evolution of insect olfactory receptors

Christine Missbach, Hany K. M. Dweck, Heiko Vogel, Andreas Vilcinskas, Marcus C. Stensmyr, Bill S. Hansson and Ewald Grosse-Wilde

eLIFE elifesciences.org

RESEARCH ARTICLE

Evolution of insect olfactory receptors

Christine Missbach[1]*, Hany KM Dweck[1], Heiko Vogel[2], Andreas Vilcinskas[3], Marcus C Stensmyr[4], Bill S Hansson[1†], Ewald Grosse-Wilde[1*†]

[1]Department of Evolutionary Neuroethology, Max Planck Institute for Chemical Ecology, Jena, Germany; [2]Department of Entomology, Max Planck Institute for Chemical Ecology, Jena, Germany; [3]Institute of Phytopathology and Applied Zoology, Justus-Liebig-Universität Gießen, Gießen, Germany; [4]Department of Biology, Lund University, Lund, Sweden

Abstract The olfactory sense detects a plethora of behaviorally relevant odor molecules; gene families involved in olfaction exhibit high diversity in different animal phyla. Insects detect volatile molecules using olfactory (OR) or ionotropic receptors (IR) and in some cases gustatory receptors (GRs). While IRs are expressed in olfactory organs across Protostomia, ORs have been hypothesized to be an adaptation to a terrestrial insect lifestyle. We investigated the olfactory system of the primary wingless bristletail *Lepismachilis y-signata* (Archaeognatha), the firebrat *Thermobia domestica* (Zygentoma) and the neopteran leaf insect *Phyllium siccifolium* (Phasmatodea). ORs and the olfactory coreceptor (Orco) are with very high probability lacking in *Lepismachilis*; in *Thermobia* we have identified three Orco candidates, and in *Phyllium* a fully developed OR/Orco-based system. We suggest that ORs did not arise as an adaptation to a terrestrial lifestyle, but evolved later in insect evolution, with Orco being present before the appearance of ORs.
DOI: 10.7554/eLife.02115.001

***For correspondence:** cmissbach@ice.mpg.de (CM); Grosse-Wilde@ice.mpg.de (EG-W)

†These authors contributed equally to this work as senior authors.

Competing interests: The authors declare that no competing interests exist.

Funding: See page 19

Received: 18 December 2013
Accepted: 17 February 2014
Published: 26 March 2014

Reviewing editor: Gáspár Jékély, Max Planck Institute for Developmental Biology, Germany

Introduction

All living organisms, including bacteria, protozoans, fungi, plants, and animals, detect chemicals in their environment. The sensitivity and chemical range of animal olfactory systems is remarkable, enabling animals to detect and discriminate between thousands of different odor molecules. Although there is a striking evolutionary convergence towards a conserved organization of signaling pathways in vertebrate and invertebrate olfactory systems (***Hildebrand and Shepherd, 1997***), the involved receptor gene families evolved independently. The molecular identity of olfactory receptors was first unraveled in vertebrates (***Buck and Axel, 1991***). In mammals, as many as 1000 heterotrimeric GTP-binding protein (or G protein)-coupled receptors are considered to be employed in olfactory discrimination (***Buck and Axel, 1991***). A similar number of chemoreceptors, with about 1300 receptor genes and 400 pseudogenes, have been hypothesized for *Caenorhabditis elegans* (***Robertson and Thomas, 2006***).

All data on insect olfactory receptors are based on studies investigating the neopteran insects (overview of insect order relationship is given in ***Figure 1***). The identity of receptors involved in olfaction in the evolutionarily more ancient apterygote insects (Archaeognatha, Zygentoma) and paleopteran insects (Odonata and Ephemeroptera) is thus completely unknown. In neopteran insects (Polyneoptera, Paraneoptera, and Holometabola) most volatile stimuli are recognized by members of the olfactory receptor family (ORs). ORs are multitransmembrane domain proteins unrelated to nematode or vertebrate olfactory receptors (***Mombaerts, 1999***; ***Robertson, 2001***; ***Hill et al., 2002***), displaying a distinct membrane topology (***Benton et al., 2006***; ***Lundin et al., 2007***). The number of functional OR genes varies from 10 in the human body louse *Pediculus humanus humanus* (***Kirkness et al., 2010***) to about 60 in *Drosophila melanogaster* (***Clyne et al., 1999***; ***Gao and Chess, 1999***; ***Vosshall et al., 1999***) and up to 350 OR genes in ants (***Zhou et al., 2012***). ORs have been suggested

eLife digest Detecting chemical cues can be a matter of life or death for insects, and many employ three families of receptor proteins to detect a broad range of odors. Members of one of these receptor families, the olfactory receptors, form a complex with another protein, the olfactory coreceptor that is essential for both positioning and stabilizing the receptor, as well as the actual function.

Crustaceans share a common ancestor with insects, and since they do not have olfactory receptors it has been proposed that these receptors evolved when prehistoric insects moved from the sea to live on land. According to this idea, olfactory receptors evolved because these ancestors needed to be able to detect odor molecules floating in the air rather than dissolved in water.

Previous research on insect olfactory receptors has focused on insects with wings. Missbach et al. have now used a wide range of techniques to investigate how evolutionarily older wingless insect groups detect scents. As all investigated groups evolved from a common ancestor at different times these experiments allow tracking of the historical development of olfactory receptors.

In the wingless species that is more closely related to the flying insects there was evidence of the presence of multiple coreceptors but not the olfactory receptors themselves. In the most basal insects no evidence for any part of the olfactory receptor-based system was found. This indicates that the main olfactory receptors evolved independently of the coreceptor long after the migration of insects from water to land. Missbach et al. suggest that olfactory receptors instead developed far later, around the time when vascular plants spread and insects developed the ability to fly.

DOI: 10.7554/eLife.02115.002

to be distantly related to the gustatory receptors of arthropods, with some proteins containing a signature motif in the carboxyl terminus (***Scott et al., 2001***).

Insect olfactory receptors function as heteromultimers composed of at least one ligand-specific OR and the coreceptor Orco (***Vosshall et al., 1999***; ***Elmore et al., 2003***; ***Krieger et al., 2003***; ***Larsson et al., 2004***; ***Sato et al., 2008***; ***Wicher et al., 2008***). Interestingly, while Orco (***Vosshall and Hansson, 2011***) is highly conserved among insects, the sequences of other olfactory receptor genes exhibit very little sequence similarity even within the same insect order (***Krieger et al., 2003***), complicating their identification. So far, Orco homologues have been identified in Lepidoptera, Diptera, Coleoptera, Hymenoptera, Hemiptera (***Krieger et al., 2003***; ***Pitts et al., 2004***; ***Smadja et al., 2009***), and Orthoptera (***Yang et al., 2012***). Neither Orco nor ORs are present in the genome of the crustacean *Daphnia pulex*, indicating that ORs are insect specific. However, GRs were found in Crustacea, just as in insects (***Peñalva-Arana et al., 2009***).

A second receptor family, the variant ionotropic glutamate receptors (IRs), is also involved in insect chemosensation (***Benton et al., 2009***). IRs act in combinations of up to three subunits; individual odor-specific receptors and one or two of the broadly expressed coreceptors IR25a, IR8a, and IR76b (***Abuin et al., 2011***). IRs are present in olfactory tissues across the Protostomia (***Croset et al., 2010***), for example two conserved members of this group were described in the *Daphnia* genome (***Croset et al., 2010***) and the coreceptor IR25a homologue is expressed in many, if not all mature OSNs of the American lobster *Homarus americanus* (***Hollins et al., 2003***) and the spiny lobster *Panulirus argus* (***Tadesse et al., 2011***). Since crustaceans are the closest relatives of insects (***Friedrich and Tautz, 1995***; ***Boore et al., 1998***; ***Regier et al., 2010***), IRs are most likely the ancient type of insect olfactory receptor.

But when and why did insect ORs evolve? Hexapods derived from an aquatic crustacean ancestor, probably in the Early Ordovician, approximately 483 mya (***Rota-Stabelli et al., 2013***). The transition from sea to land meant that molecules needed to be detected in gas phase instead of aquatic solution. Therefore, the olfactory system of a hexapod ancestor had to adapt to the terrestrial conditions and detection of volatile, air-borne chemicals. One proposed hypothesis has been that Orco and ORs of the insect type are an adaptation to this terrestrial lifestyle (***Robertson et al., 2003***; ***Krång et al., 2012***). To reconstruct an evolutionary scenario for insect ORs, we investigated species belonging to different ancient insect orders, including Archaeognatha (jumping bristletails) and Zygentoma (silverfishes and firebrats), and a neopteran insect belonging to the Phasmatodea (leaf and stick insects) as so far not analyzed control group using morphological, electrophysiological and molecular techniques.

eLIFE Research article Genomics and evolutionary biology

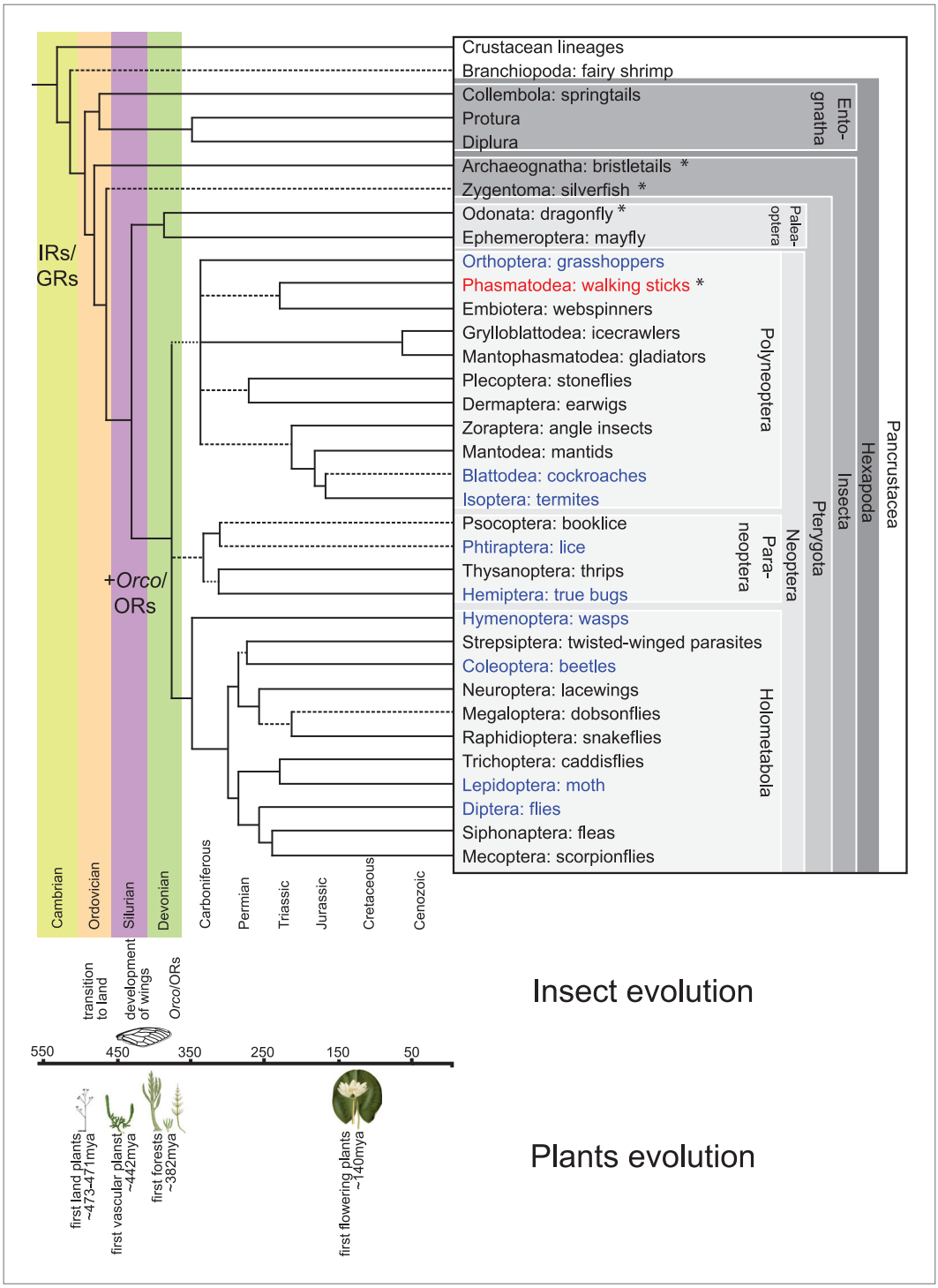


Figure 1. Hexapodan phylogeny. Phylogeny was adapted from ***Trautwein et al. (2012)***. Timescale was adjusted for higher level taxa based on ***Rota-Stabelli et al. (2013)***, for Holometabola according to ***Wiegmann et al. (2009)*** and the remaining groups based on their fossil record (http://insects.about.com/od/evolution/a/Timeline-of-Fossil-Insects-by-Order.htm), in order to correlate important events in plant and insect evolution with the emergence of insect olfactory receptors. IRs and GRs are known to be much older than insects (***Peñalva-Arana et al., 2009***; ***Croset et al., 2010***), however, ORs and Orco have evolved during the evolution of insects and cannot be found outside the insect clade (***Peñalva-Arana et al., 2009***). Insects with a described OR/Orco-based olfactory system

Figure 1. Continued on next page

Figure 1. Continued

were highlighted in blue, whereas species were *Orco* was described in this study were colored in red. All orders investigated in this study are labeled by an asterisk. Our data suggests the evolution of the coreceptor Orco after the bristletails split from its last common ancestor with the remaining insects. However, an olfactory system that relies both on ORs and *Orco* seems to have evolved after the emergence of wings.

DOI: 10.7554/eLife.02115.003

Results

Our first step was to analyze the evolutionary ancestry of the insect olfactory system by assessing its complexity in each of three non-holometabolan insects.

To correlate OSN responses with type of sensillum (with pores and grooves) identified in SEM studies of the antennae, we investigated the morphological and physiological characteristics of olfactory sensilla and their olfactory sensory neurons.

Morphology and physiology

On the antennae of *L. y-signata* the only putative olfactory sensilla were porous olfactory basiconic sensilla (***Figure 2B–E***). These sensilla were arranged in a pattern that is highly stereotypical between antennal modules composed of 5–12 annuli, with annuli typically containing zero-to-four *Sensilla basiconica* (***Missbach et al., 2011***). Responses to all tested chemical classes of odors, including acids, alcohols, aldehydes, esters, and ketones, were recorded from OSNs housed in these sensilla using the single sensillum recoding measurements (SSR) (***Figure 3***, uppermost heat map). Based on the response profile, spontaneous activity, and colocalization inside the same sensillum, we identified 12 OSN types, present in five functional basiconic sensillum types. Out of the 12 OSN types, only seven responded to odors tested; two exclusively to acids, while five responded with a similar activity rate to acids or amines and to other odors. OSNs belonging to this second class were broadly tuned and exhibited relatively low spiking activity. In general, OSN classes displayed a low baseline activity with about 1 to 7 spikes/s, with Lys-ab2A that had a spontaneous activity of more than 25 spikes/s as the only exception. Only rarely was an increase in spiking rate of more than 60 spikes per second recorded, even for the best identified ligands (***Figure 3—source data 1***). No responses were obtained for ammonia or pyridine. Coeloconic-like sensilla, s-shaped trichoid sensilla, and chaetic sensilla did not display any morphological features indicating olfactory function and did also not respond to any odor tested (***Missbach et al., 2011***; data not shown). In conclusion, 7 OSN types that were all housed in basiconic sensilla responded to a wide spectrum of odor molecules.

The morphology of the zygentoman antenna and its sensilla was similar to that of *L. y-signata*, with the presence of grooved sensilla as the only exception (***Figure 2G***; ***Adel, 1984***; ***Berg and Schmidt, 1997***). Five different functional types of olfactory sensilla were present (***Figure 3***: three porous, two grooved *s. basiconica*, the latter are indicated by blue caption). In contrast to *L. y-signata*, a nascent functional and spatial separation of the detection of amines and acids, and ketones and alcohols appeared in *T. domestica*. The former primarily elicited responses in OSNs of grooved sensilla, while less polar ones were mainly detected by porous sensilla. However, most of the OSNs in porous sensilla exhibited broad tuning and responded to at least one of the tested acids or amines as well.

We then turned to a neopteran insect. Unlike the other analyzed species, the leaf insect *P. siccifolium* displayed a strong sexual antennal dimorphism, with males having very long antennae covered with trichoid sensilla (***Figure 2L***), and the females very short antennae without trichoid sensilla (***Figure 2K***). In comparison to the wingless insects, the response repertoire of the leaf insect was much more diverse, with a total of 23 different functional sensillum types as identified by SSR recordings (***Figure 3***). No responses were obtained from trichoid sensilla, but since they were only present on the male antennae they could be involved in detection of an unknown volatile pheromone. In all cases, reported detection of volatile pheromones in insects is dependent on very specific ORs. Taken together these data suggest that leaf insects have a much broader response repertoire with a higher number of different OSN types than the more basal species we analyzed; apparently the number of olfactory receptors has increased. It also seems likely that at least the leaf insect makes use of ORs in odorant detection.

An antennal and maxillary palp transcriptome

We generated expansive antennal transcriptome datasets of the three insect species, employing a bioinformatics-based approach to identify Orco, ORs, GR, and IRs. In a second transcriptome of

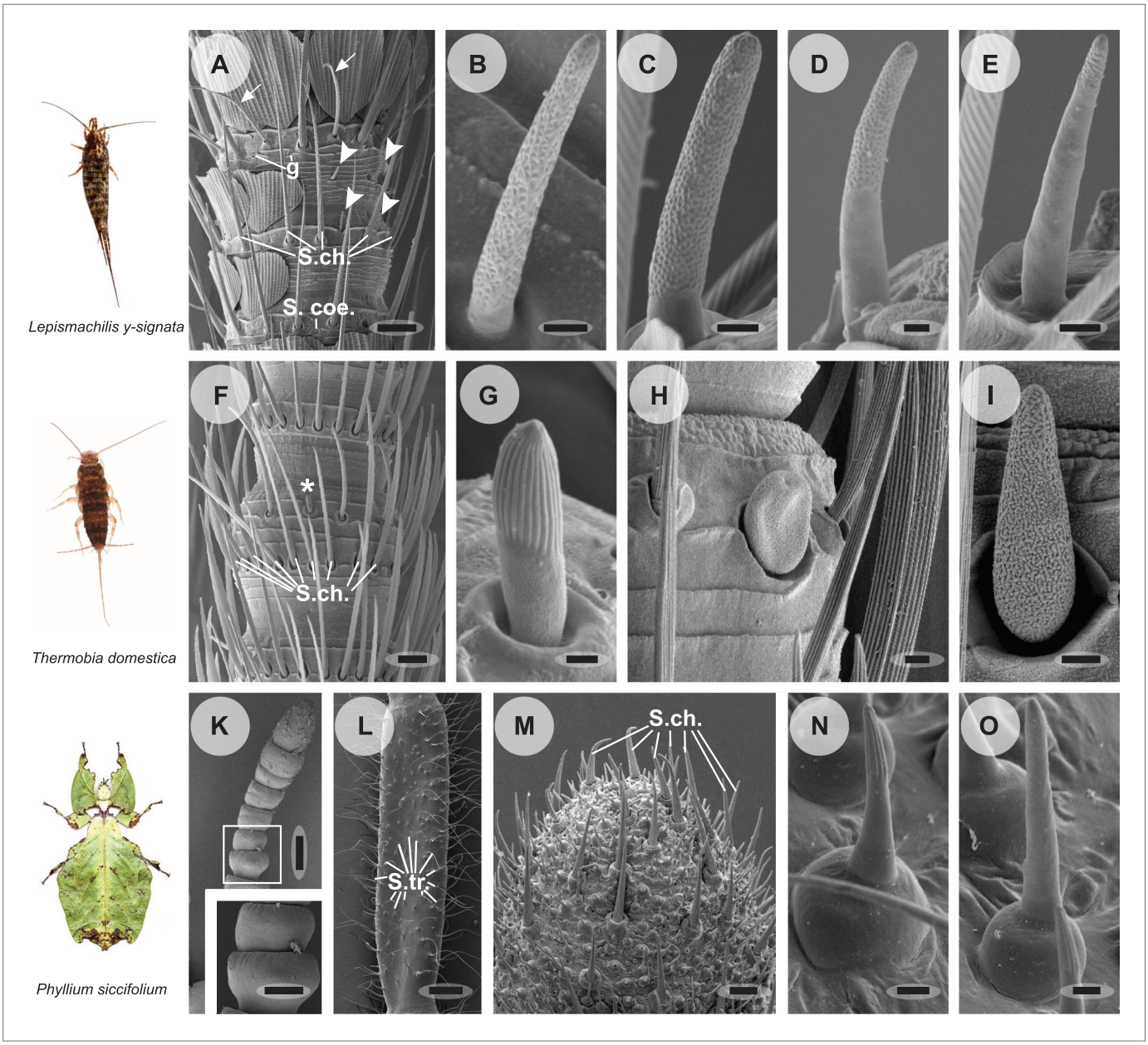


Figure 2. Olfactory sensilla on the antennae of *L. y-signata* (A–E), *T. domestica* (F–I) and *P. siccifolium* (K–O). Animals are depicted next to the corresponding antennal SEM images. (**A**) Detailed view of the antennae of *L. y-signata*. The proximal part of the antennae is not only covered with sensilla, but also scales. Glands (g) are highly abundant on the antennae. Many mechanosensory sensilla (S.ch.: Sensilla chaetica) were arranged in circles on the antennal segments. On some antennal segments gustatory sensilla (arrows) can be found between the S.ch (for further information read ***Missbach et al., 2011***). Very rarely zero to four olfactory Sensilla basiconica were identified per segment, in a mostly redundant pattern on the antennae with similar numbers of olfactory sensilla and sensilla types on each antennal segment. Antennal segments are separated by antennal breaking points. The pattern of sensilla is modulated by increasing the number of annuli of a segment through molting. (**B**–**E**) Different morphological types of basiconic sensilla. No grooved sensilla/olfactory coeloconic sensilla were identified on the antennae. Only small pegs surrounded by a cuticular wall (s. coe.; referred as coeloconica-like sensillum, ***Bockhorst 1988***) were located on the antennae. These sensilla are not olfactory (for detailed external morphology see ***Missbach et al., 2011***). (**F**) Detailed view of the antennae of *T. domestica*. The antennal organization is similar to the bristletail, with antennal breaking points and lifelong molting. The most abundant sensilla on the antennae again are mechanosensory S.ch.; beside those gustatory and olfactory sensilla are distributed in a species-specific modular manner over the antennae. (**G**) In contrast to *L. y-signata*, grooved sensilla can be found on the antennae of *T. domestica*. (**H** and **I**) Different morphological types of basiconic sensilla. (**K** and **L**) Gender specific differences between a female (**K**) and a male (**L**) antennae of *P. siccifolium*. Female antennae are short and lack trichoid sensilla (S.tri.). They more or less lack sensilla on the proximal annuli, only the last

Figure 2. Continued on next page

Figure 2. Continued

two annuli are covered with a high number of olfactory and also some mechanosensory sensilla (S.ch.). (**M**) Male antennal tip. Similar to the distal female antennal annuli the highest density of sensilla can be found on the last annuli. (**N** and **O**) Both grooved and pored sensilla can be found on these segments. Scale bars: **A**: 50 μm; **B**, **C**, **D**, **E**, **H**, **I**, **N**, **O**: 2 μm; **F**: 100 μm; **G**: 1 μm; **K**, **L**: 200 μm; **M**: 20 μm.

DOI: 10.7554/eLife.02115.004

L. y-signata also maxillary palp RNA was included. In total 99'504'815 reads were generated for the two *L. y-signata* chemosensory transcriptomes, out of which 77'060'687 were paired end reads. In addition to the transcriptomes of chemosensory tissues, we sequenced pooled RNA of whole bodies and heads resulting in 25'242'666 reads. This data set was analyzed separately. 27'704'231 and 30'762'777 reads were generated for antennae of *T. domestica* and *P. siccifolium*, respectively (detailed information about transcriptomes and assembly parameters can be obtained from the 'Material and methods' section and ***Table 1***).

No ORs or Orco were found in the transcriptome of *L. y-signata*

The transcriptome data sets were manually screened for genes encoding proteins putatively involved in insect olfaction, including ORs, Orco, GRs, and IRs (number of identified contigs are given in ***Table 2***).

Neither OR- nor Orco-coding transcripts were identified in the transcriptomes of *L. y-signata* using BLAST and HMM domain profile searches as described in the 'Material and methods' section. Custom HMMR-profiles directed against conserved regions of Orco proteins also failed to identify any Orco-related sequences in the bristletail transcriptome. We discovered five GR candidates. MSA analysis of these together with ORs and GRs of various insect species and the *Daphnia* GRs always confirmed the position of the *L. y-signata* GR candidates within the GR and not the OR family (***Figure 4A***, ***Figure 4—source data 1***, ***Figure 4—source data 2***, ***Figure 4—source data 3***, ***Figure 4—source data 4***, ***Figure 4—source data 5***). Since expression levels of gustatory receptors are very low even in gustatory tissue (***Clyne et al., 2000***; ***Scott et al., 2001***), we argue that ORs or at least Orco should be represented in the large, sensory tissue-specific transcriptome data set of *L. y-signata* if they are indeed part of the olfactory system in the species.

The three Orco-paralogues of *T. domestica*

In contrast to *L. y-signata*, three different Orco-related sequences were identified in the transcriptome of *T. domestica*. All candidates were cloned as full-length coding sequences using RACE-PCR. The three sequences displayed different similarities to the Orco sequence of *D. melanogaster*, one sequence shared 45.8%, one 35.1%, and the third 24.4% sequence similarity at the amino acid level. Orco was the protein most similar to all three Orco candidate sequences (***Figures 4B and 5***), although some of the key amino acids of the coreceptor are substituted at least in TdomOrco3 (***Wicher et al., 2008***; ***Sargsyan et al., 2011***; ***Nakagawa et al., 2012***; ***Kumar et al., 2013***; highlighted in alignment ***Figure 5***). Apart from the Orco variants, no OR-related sequences were identified, but 9 contigs for GR candidates were found that were assigned to seven GRs, including three candidates close to full length or full length and four additional fragments (***Table 2*** and ***Figure 4A***).

Normal OR/*Orco* in the leaf insect

In the transcriptome data set of *P. siccifolium*, both various OR-related sequences and a single *Orco* sequence were detected (***Table 2***). The exact number of OR genes was hard to ascertain since some of the contigs were too short and did not show sufficient sequence overlap in a multiple sequence alignment (MSA) to be confidently identified as independent. However, in total, we identified 30 gene fragments coding ORs, indicating that the transcriptomic approach chosen was applicable to our question, successfully identifying both Orco and ORs in *P. siccifolium*.

Orco expression in *T. domestica*

Considering that for all other insects analyzed so far one Orco is the norm, the appearance of three *Orco* candidates in *T. domestica* is highly unusual. We thus assessed the expression of the three candidates in different tissues using RT-PCR. For all three Orco types expression was limited to the antenna (***Figure 6***). To further assess the expression, we used in situ hybridization employing an antisense probe of one of the coreceptors. This led to staining of single cells below one or two basiconic sensilla of an antennal subsegment (***Figure 7***), suggesting that TdomOrco1 might indeed be expressed in OSNs. However, only one neuron per sensillum was stained. No signals were obtained when using a sense probe for TdomOrco1 (***Figure 7—figure supplement 1***).

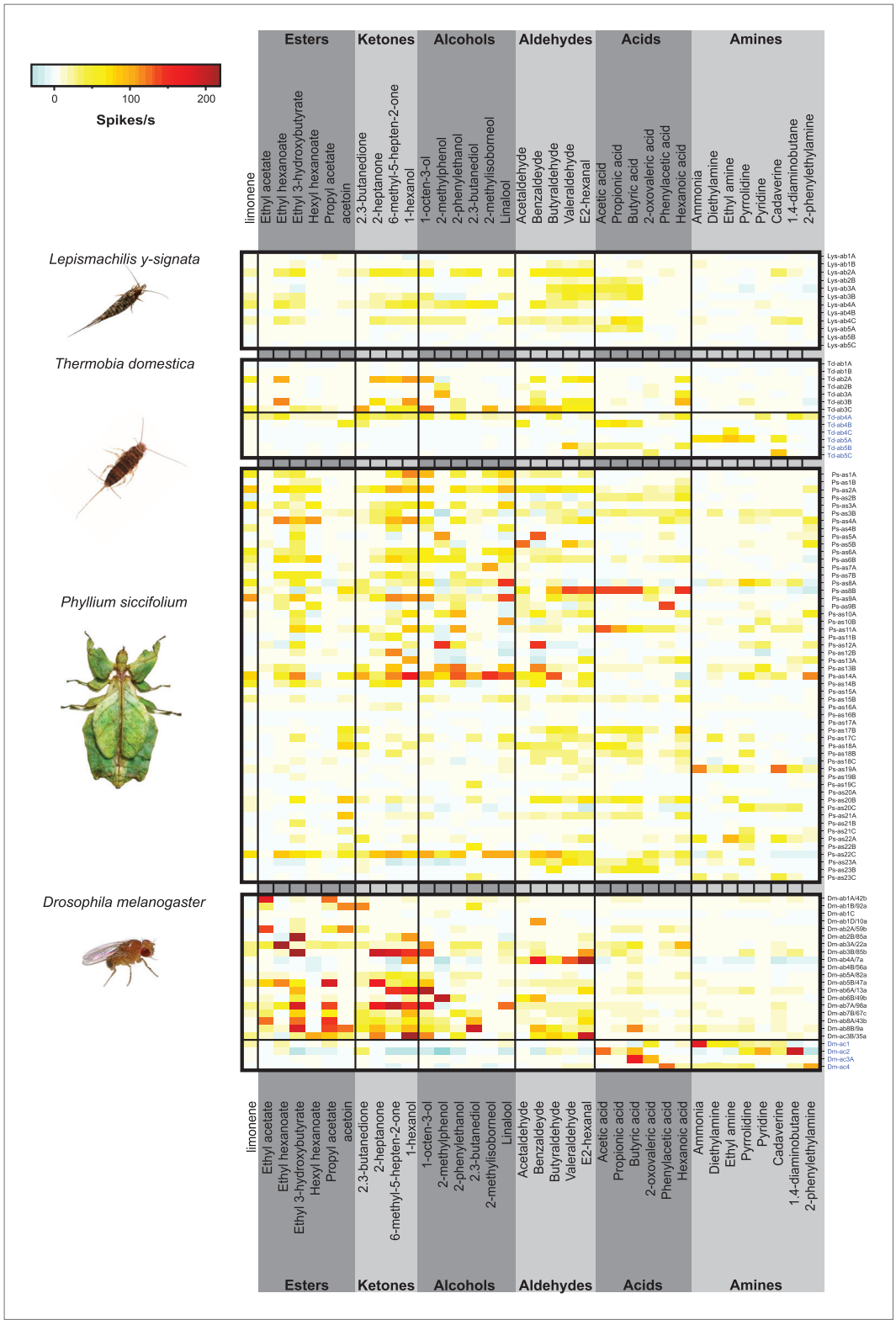


Figure 3. Color coded response profiles of *L. y-signata*, *T. domestica*, *P. siccifolium* and *D. melanogaster*. Spikes are sorted by neurons, with the exception of ac1, ac2, and ac4 of *D. melanogaster* where spike sorting was not possible. Means over 5 to 23 recordings were used as basis for visualization (source data are given in

Figure 3. Continued on next page

Figure 3. Continued

Figure 3—source data 1). The same color code was used for all species, ranging from highest to lowest encountered change in activity. Neurons in grooved sensilla are indicated by blue letters (ac). For *L. y-signata* responses to odors were only obtained from neurons in porous sensilla (ab). A separation between porous and grooved sensilla was not possible for *P. siccifolium*. Sensilla were classified as antennal sensillum (as). *L. y-signata* neurons are mostly broadly tuned with comparable low change in spiking activity. For *P. siccifolium* a total of 23 different functional sensillum types were identified in SSR recordings (in comparison five in *L. y-signata*, five in *T. domestica*) suggesting that leaf insects have a broader response repertoire.

DOI: 10.7554/eLife.02115.005

The following source data are available for figure 3:

Source data 1. Excel file of mean responses and baseline firing rate of the different OSN classes of *L.y-signata*, *T. domestica*, *P.siccifolium*, and *D. melanogaster*.

DOI: 10.7554/eLife.02115.006

Only IRs in *L-y-signata*

As none of the experiments gave a hint for the existence of any OR or Orco-related sequence in the bristletail transcriptome, we focused on the second olfactory receptor family of insects, the IRs. Although we could not identify any OR sequences in the transcriptome, a high number of putative glutamate receptor coding contigs was identified (***Table 2***). However, only five candidate iGluRs and 14 candidate IRs appeared to be real unigenes, possessing at least two of the three transmembrane domains. Some candidate sequences were extended in 3′-direction using RACE-PCR with antennal cDNA as template, allowing verification of unigene status and antennal expression. In MSA and phylogenetic analysis, the identified IRs grouped with DmelIRs (***Croset et al., 2010***). Among the identified putative LsigIRs were orthologues of the *D. melanogaster* coreceptors IR25a and IR8a, as well as one receptor similar to IR76b (***Figure 8A***, ***Figure 8—source data 1***, ***Figure 8—source data 2***, ***Figure 8—source data 3***, ***Figure 8—source data 4***, ***Figure 8—source data 5***). As in other IRs (***Benton et al., 2009***) one or several key amino acids in the predicted glutamate binding domains were absent in the non-coreceptor IR candidates and LsigIR76b (***Figure 8B***). 7 out of 14 LsigIRs group close to a cluster of *D. pulex* IRs and the antennal IRs IR21a and IR68a of *D. melanogaster*, with no clear relationship to one or the other. None of the *Lepismachilis* IR candidates grouped with the 'divergent' *Drosophila* IRs.

We then performed fluorescent *in situ* hybridization with RNA probes directed against the IR coreceptor candidates (***Figure 9***). Antisense probes of IR25a and IR8a led to labeling of one to three OSNs underneath basiconic sensilla (***Figure 9—figure supplement 1***). In control experiments with sense probes, or without any probe, no staining was obtained (***Figure 9—figure supplement 2***). The pattern of expression of IR coreceptors in OSNs of *L. y-signata* indicates that most OSNs are covered by this gene family.

All experiments thus indicate that the olfactory system of this species employs other receptors like IRs or GRs, with no ORs or Orco present.

Discussion

Insects provide us with an excellent opportunity to study groups of animals that have retained ancestral characteristics and understand how the specific building blocks in olfaction have evolved in both insects and other animals. Consequently, we selected insects at crucial positions of the phylogenetic tree with a functional olfactory system adapted to terrestrial conditions and detection of volatile chemicals. This species collection provides an excellent model to study the early evolution of the insect olfactory system.

To address which receptors are involved in odor detection in these insects and in basal insects in general, we applied several different approaches. Based on our transcriptome data sets, we suggest a stepwise evolution of the Orco/OR complex with Orco having evolved in the lineage of Dicondylia (Zygentoma + Pterygota) and the functional complex of Orco and ORs emerging within the pterygote insects (this study, ***Clyne et al., 1999***; ***Gao and Chess, 1999***; ***Smadja et al., 2009***; ***Vosshall et al., 1999***; ***Robertson and Wanner, 2006***; ***Kirkness et al., 2010***). Although it is impossible to completely rule out the presence of ORs, none of our extensive experiments led to the identification of either ORs or Orco in the bristletail *L. y-signata*. The well-established conservation of the *Orco*

Table 1. Technical overview of transcriptomes (study accession: PRJEB5093, study unique name: ena-STUDY-MPI CE-12-12-2013-15:03:23:860-31)

Organism	Sequencing technique	Number of reads	Number of contigs above 400 bp	N50	Average length of contigs	Tissue	Sample accession	Secundary accession	Sample unique name
Lepismachilis y-signata	HiSeq2000 (Illumina)	22'444'128	68'984	1'179	1'000	antennae and palps	ERS384175	SAMEA2276780	Lysig1
	HiSeq2500 (Illumina)	77'060'687 paired end				antennae	ERS384176	SAMEA2276781	Lysig2
	HiSeq2000 (Illumina)	25'242'666	37'860		857	heads, whole bodies	ERS399748	SAMEA2342071	LysigMix1
Thermobia domestica	HiSeq2500 (Illumina)	27'704'231 paired end	31'172	1'349	1'070	antennae	ERS384177	SAMEA2276782	Tdom1
Phyllium siccifolium	HiSeq2500 (Illumina)	30'762'777 paired end	34'653	1'890	1'305	antennae	ERS384178	SAMEA2276783	Psic1

DOI: 10.7554/eLife.02115.007

Table 2. Number of candidate contigs (not unigenes) for the different gene families identified in the transcriptomes of the different species

Organism	Orco	ORs	GRs	IRs
Lepismachilis y-signata	–	–	7 (5 above 400 bp)	17 (16 above 400 bp)
Thermobia domestica	6 (1 above 400 bp)	–	9 (3 above 400 bp)	19 (9 above 400 bp)
Phyllium siccifolium	1 (1 above 400 bp)	30 (16 above 400 bp)	6 (2 above 400 bp)	32 (19 above 400 bp)

DOI: 10.7554/eLife.02115.008

coding gene through evolution suggests that it is highly unlikely that we missed it. We did, however, identify a number of IRs, including the IR coreceptors IR25a, IR8a, and IR76b in the *L. y-signata* antennal transcriptome. FISH allowed us to visualize expression of the IR co-receptors in a large number of OSNs associated with basiconic sensilla. Based on these results we propose that the olfactory system of *L. y-signata* is not based on ORs.

In insects, different sensillum types house OSNs typically responding to different sets of odors. In *D. melanogaster* IRs are the functional receptor type of OSNs in double-walled coeloconic sensilla, and ORs are predominantly expressed in OSNs housed in single-walled basiconic and trichoid sensilla (***Hallem et al., 2004***; ***Silbering et al., 2011***). It follows that this organization cannot exist with just one sensillum type present, as is the case in Archaeognatha (***Berg and Schmidt, 1997***; ***Missbach et al., 2011***) and older hexapod taxa as the Collembola (***Altner and Prillinger, 1980***). The oldest insect taxon where double-walled sensilla were investigated is Zygentoma, which have both single-walled basiconic sensilla with pores and double-walled sensilla with spoke channels (***Berg and Schmidt, 1997***). Coeloconic sensilla differ dramatically from the single-walled trichoid and basiconic types in both wall structure and in internal environment. The coeloconic structure has been thought to be a prerequisite for IR function (***Benton et al., 2009***; ***Guo et al., 2014***). However, in the Archaeognatha we find that IRs are most likely located in OSNs of Sensilla basiconica. IRs might thus have evolved in a single-walled sensillum and did not find their modern, coeloconic environment until neopteran insects evolved.

In the bristletail *L. y-signata,* we found that many of the OSNs are very broadly tuned, responding to volatiles with several different functional groups at higher doses. However, broadly tuned receptors might not have high affinities. By counting and integrating molecules over longer times, OSNs could include even low-probability binding events in generating their response (***Firestein, 2001***). This might also mean that the system does not have a high temporal resolution, which seems to be a fair trade-off for a walking insect that lives in its substrate.

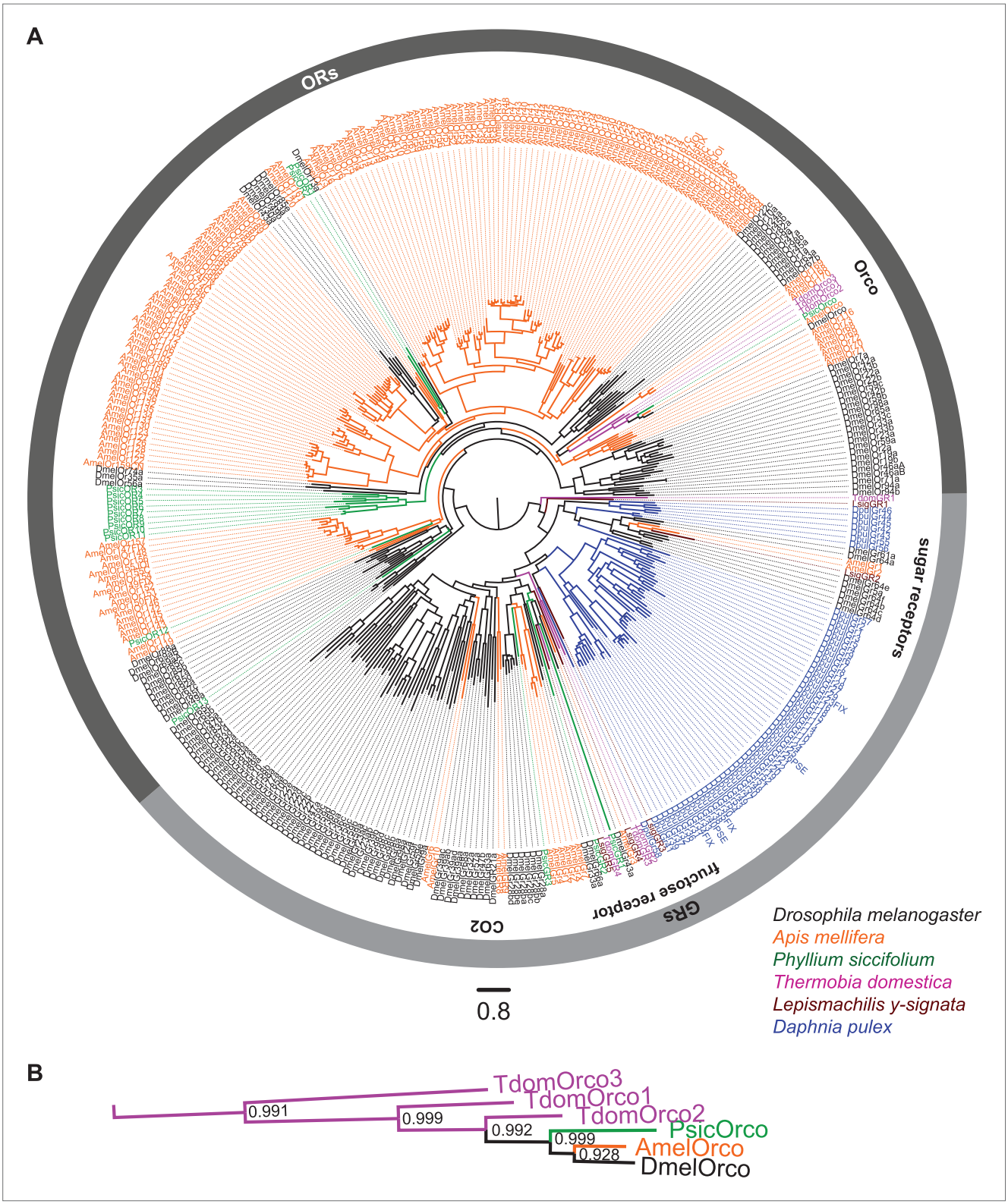


Figure 4. ORs and GRs of *L. y-signata*, *T. domestica*, and *P. siccifolium*. (**A**) Dendrogram displaying the relationship of identified OR and GR candidates of *L. y-signata*, *T. domestica*, and *P. siccifolium* to *D. melanogaster* (***Clyne et al., 1999***; ***Gao and Chess, 1999***; ***Vosshall et al., 1999***) and *Apis mellifera* (***Robertson and Wanner, 2006***) GR and OR proteins, and *Daphnia pulex* GRs (***Peñalva-Arana et al., 2009***). The dendrogram was determined by

Figure 4. Continued on next page

Figure 4. Continued

maximum likelihood analysis of a MAFFT-Alignment using FastTree2. All *L. y- signata* candidates group within the GRs. Only candidates with a translated amino acid sequence longer than 120 amino acids and overlap in multiple sequence alignment were taken for analysis, since ORs and GRs are highly divergent and only unigenes should be included in the analysis (all candidate OR and GR sequences of *L. y-signata*, *T. domestica* and *P. siccifolium* are given in ***Figure 4—source data 1*** for amino acids and ***Figure 4—source data 2*** for nucleotide sequences). For *T. domestica*, we identified three different variant Orco types that were included in the analysis as full length translated amino acid sequences. (**B**) Blow-up of the dendrogram showing the support values for the coreceptor subgroup. The whole group is well supported.

DOI: 10.7554/eLife.02115.009

The following source data are available for figure 4:

Source data 1. Amino acid sequences of putative olfactory and gustatory receptors of *L. y-signata*, *T. domestica*, and *P. siccifolium*.

DOI: 10.7554/eLife.02115.010

Source data 2. Nucleotide sequences of putative olfactory and gustatory receptors of *L. y-signata*, *T. domestica*, and *P. siccifolium*.

DOI: 10.7554/eLife.02115.011

Source data 3. MAFFT-alignment of OR and GR candidates of *L. y-signata*, *T. domestica*, *P. siccifolium* and *D. melanogaster* (***Clyne et al., 1999***, ***Gao and Chess, 1999***, ***Vosshall et al., 1999***) and *Apis mellifera* (***Robertson and Wanner, 2006***) GR and OR proteins, as well as *Daphnia pulex* GRs done.

DOI: 10.7554/eLife.02115.012

Source data 4. FastTree file resulting from the MSA of ***Figure 4—source data 3*** (can be opened with FigTree).

DOI: 10.7554/eLife.02115.013

Source data 5. Tree file resulting from the MSA of ***Figure 4—source data 3*** containing node support values (can be opened e.g., with Adobe Illustrator).

DOI: 10.7554/eLife.02115.014

The response spectrum of *Drosophila* IRs is much narrower than the responses we find in the bristletail. If IRs are the only olfactory receptor type in basal insects they should exhibit a broader spectrum of possible ligands, including acids, aldehydes, alcohols, but also esters and ketones, as revealed in our physiological measurements. One additional observation in the bristletail is that many of those neurons have a broad overlap in their response spectra. One hypothesis to explain an IR-based olfactory system in *L. y-signata* would be very broad tuning of single receptors, another that the selectivity of OSNs could be regulated by combinations of different IRs.

In *D. melanogaster*, one conserved IR (IR64a) is expressed in different subpopulations of sensilla in the third chamber of the sacculus (***Silbering et al., 2011***). Corresponding OSNs are activated either by free protons or organic acids and many other odors, including esters, alcohols, and ketones (***Ai et al., 2010***). Expression of this IR together with IR8a is both necessary and sufficient for sensitivity towards organic acids and other odors, but probably requires a different, until now unknown cofactor to mediate the specific response of OSNs to inorganic acids and CO_2 (***Ai et al., 2010***).

Alternatively, GR candidates could account for part of the non-neopteran olfactory setup, especially since it has been shown that GRs can add to the olfactory repertoire (***Tauxe et al., 2013***). Putative contact chemosensory sensilla are highly abundant on the antennae of *L. y-signata* (***Missbach et al., 2011***) and *T. domestica* (***Adel, 1984***). Both detection of sugars/amino acids (shown for *T. domestica*: ***Hansen-Delkeskamp, 2001***) and a proposed contact-pheromone (***Fröhlich and Lu, 2013***) likely involve GRs, indicating that involvement of the limited set of GRs beyond this scope is unlikely.

However, these data do not explain the presence of three different Orco variants in the firebrat. So far only one Orco orthologue has been identified in each studied insect species (e.g., ***Krieger et al., 2003***; ***Pitts et al., 2004***; ***Smadja et al., 2009***; ***Yang et al., 2012***). All *T. domestica* variants were found to be expressed in antennae, suggesting their involvement in chemosensation. TdomOrco3 even has an amino acid exchange of a functional important residue from asparagine to glutamic acid at position 466. This residue was demonstrated as critical for the ion channel function in *D. melanogaster*, where substitution of D466 with amino acids other than glutamic acid resulted in a substantial reduction in channel activity, but substitution to glutamic acid leads to an increase in sensitivity of the heteromeric receptor complex (***Kumar et al., 2013***). Additionally, this residue is highly conserved across insects (***Kumar et al., 2013***) including two of the three *T. domestica* Orcos (this study).

While the antennal expression argues for a potential involvement in chemosensation, the existence of three Orco types remains mysterious. It will be part of future studies to investigate if the Orco candidates form heterodimers with other receptors like GRs or with each other to build functional receptors or if they fulfill a channel function in other processes than olfaction.

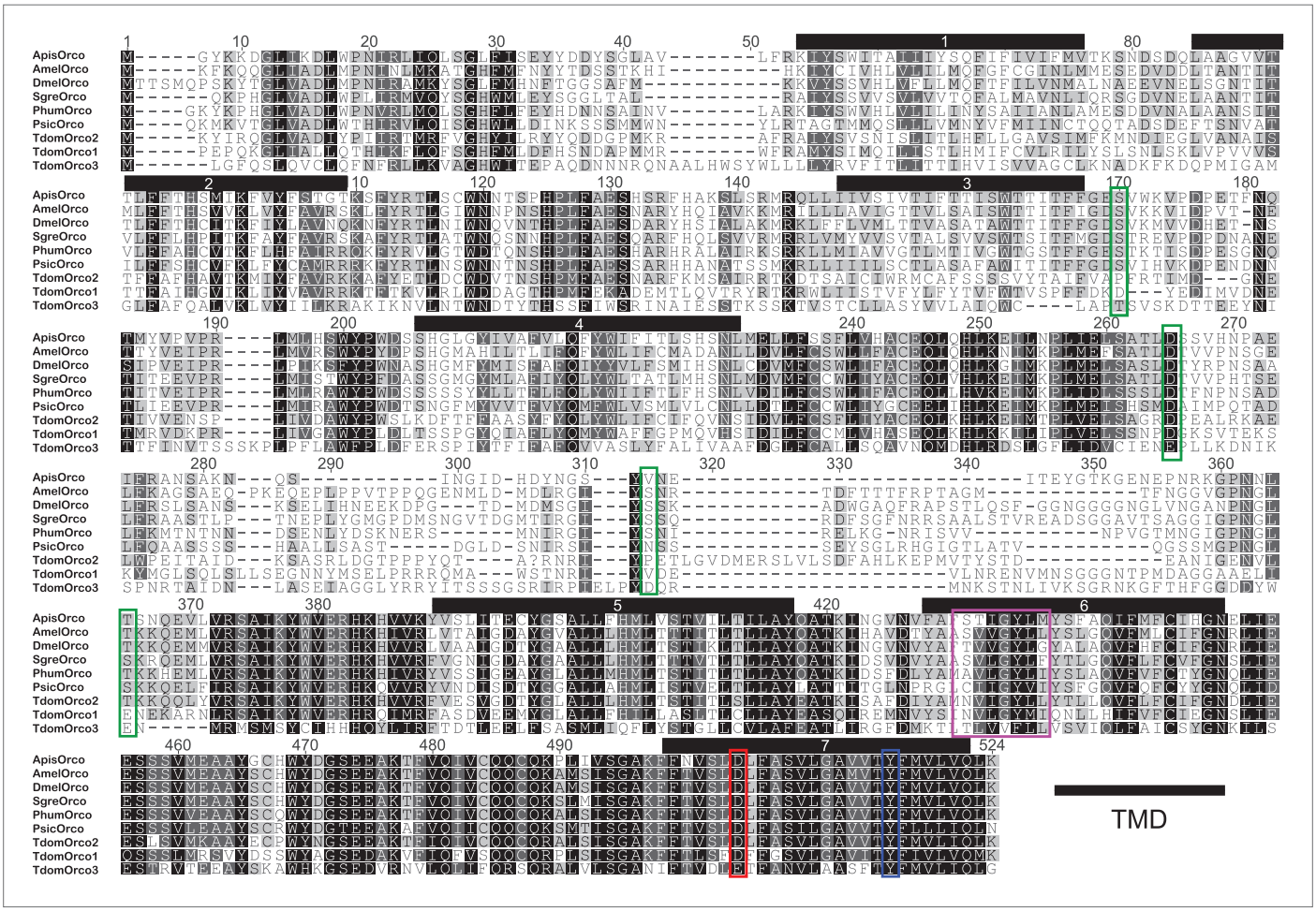


Figure 5. Multiple sequence alignment of *T. domestica* Orcos. Alignment of *T. domestica Orcos* with Orcos of *Acyrthosiphon pisum* (GI:328723530), *A. mellifera* (GI:201023349), *D. melanogaster* (GI:24644231), *Schistocerca gregaria* (GI:371444780), *Pediculus humanus corporis* (GI:242009783), *P. siccifolium* (this study). Important amino acids are highlighted in colored boxes (purple: effect on ion permeability, ***Wicher et al., 2008***; green: phosphorylation sites for PKC of DmelOrco, ***Sargsyan et al., 2010***; blue: affect spontaneous and evoked action potentials in receptor complex, ***Nakagawa et al., 2012***; red: important residue for channel activity, ***Kumar et al., 2013***).

DOI: 10.7554/eLife.02115.015

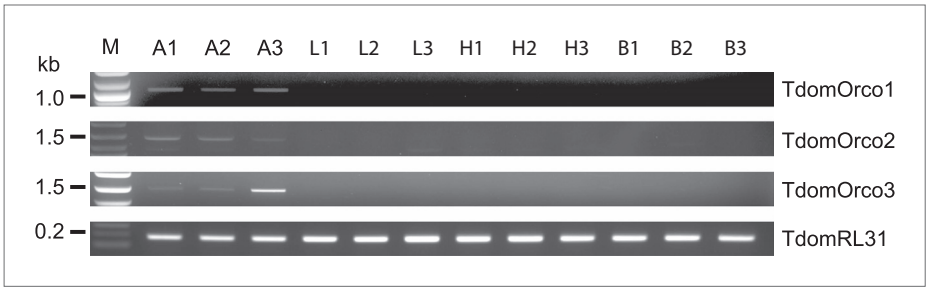


Figure 6. Expression of *T. domestica* Orcos. Using RT-PCR Orco expression was detected in the antennae (A) of *T. domestica*, but not in legs (L), heads without antennae and palps (H), and bodies (B). Primer sequences are given in ***Figure 6—source data 1***.

DOI: 10.7554/eLife.02115.016

The following source data are available for figure 6:

Source data 1. Primers and their properties used in this study.

DOI: 10.7554/eLife.02115.017

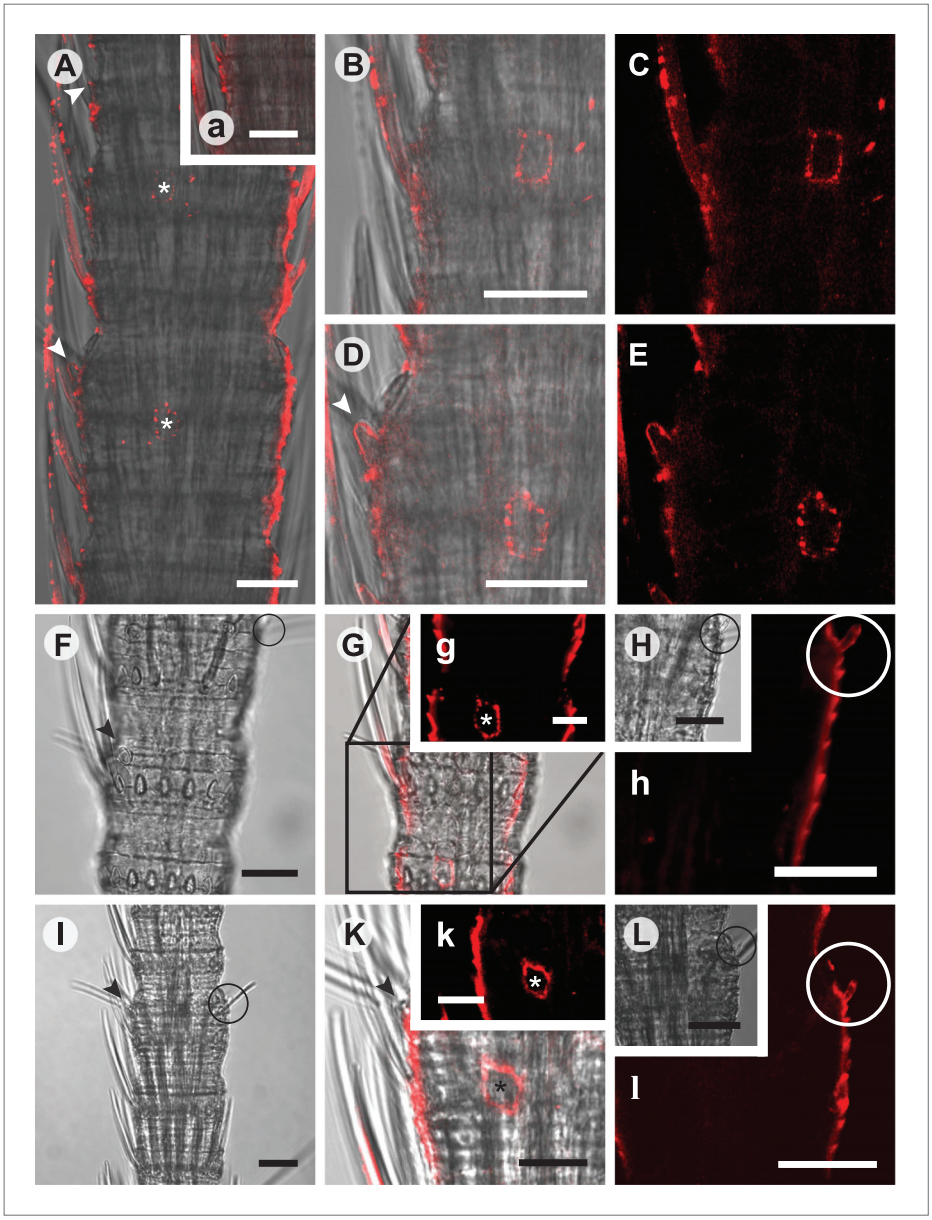


Figure 7. *In situ* hybridization on whole mount antennae of *T. domestica* using a Dig-labeled TdomOrco1 antisense probe. (**A**) Part of a *T. domestica* antenna. Combined image of fluorescent and transmitted light channel taken with cLSM. The positions of pored sensilla are indicated by arrowheads, with the upper sensillum displayed in the small box in the upper right corner. Labeled cell bodies are assigned by asterisks. (**B**–**E**) Single confocal planes through the antenna. Only a single soma close to each pored sensillum is labeled suggesting that only one neuron per sensillum expresses this Orco variant. In **B** and **D** some precipitate is visible. (**C** and **E**) Same image section as **B** and **D**, but without transmitted light. (**F**) Transmitted light image of a part of a second antenna. Location of a pored sensillum is again assigned by an arrowhead. A grooved sensillum indicated by a black circle is situated on the opposite side of the antenna. (**G**) Same part of the antenna taken with transmitted light and fluorescent channel. Again only one soma is labeled close to a pored sensillum. g: Only the Dig signal. Cuticle shows a strong autofluorescence on both sides. **H**, h: No signal was obtained close to a grooved sensillum. (**I**) Part of another antenna with a pored and a grooved sensillum on the same annulus. **K**, k: Image section from the part of the antenna close to the pored sensillum. A single soma is labeled by the probe. k: Only the fluorescent signal. **L**, l: No soma was labeled close to the grooved sensillum. For sense controls view ***Figure 7—figure supplement 1***. Scale bars **A**–**F**, **H**, **I**, **L**: 20 µm; g, **K**, k: 10 µm.

DOI: 10.7554/eLife.02115.018

Figure 7. Continued on next page

Figure 7. Continued

The following figure supplements are available for figure 7:

Figure supplement 1. *In situ* hybridization on the antenna of *T. domestica* using sense probes directed against the TdomOrco1.

DOI: 10.7554/eLife.02115.019

Altogether our data suggests that ORs evolved in insects after the emergence of Archaeognatha and Zygentoma, and therefore long after insects transitioned to a terrestrial lifestyle. At the time when flying insects occurred, the vegetation on earth was rapidly spreading and diversifying. ORs might not only increase the diversity of detected chemicals, but also allow the olfactory system to rapidly assess airborne odors. This is especially important for insects for which stimulus contact is very short and a fast response time is critical (***Getahun et al., 2012***). The oldest flying insect orders Odonta (dragonflies and damselflies) and Ephemeroptera (mayflies) were traditionally considered to be anosmic, lacking both a glomerular antennal lobe and mushroom body calyces (***Strausfeld et al., 1998***; ***Farris, 2005***). Recent studies have shown that at least dragonflies have an aerial sense of smell (***Rebora et al., 2012***). However the small antennae and the low number of olfactory sensilla will make it even more challenging to identify putative ORs and Orco in antennal transcriptomes. ORs were definitely present in the last common ancestor of 'hemi'- and holometabolan insects at least 318–300 million years ago, with *Orco* present in both groups (this study, ***Krieger et al., 2003***; ***Pitts et al., 2004***; ***Smadja et al., 2009***; ***Yang et al. 2012***). The increasing dispersion of vascular plants together with the development of wings and a secondary wing articulation opened new and wider ranges of habitats and ecological niches for insects and the receptors to find them.

Material and methods

Animals

Different stages and sexes of *Lepismachilis y-signata* were collected at several locations around Jena (Germany). Animals were kept under normal light conditions and room temperature, in plastic boxes with paper towel on the ground, covered with bark with lichens, dried grassroots, and dead leaves of maple (*Acer campestre*, Sapindaceae). The boxes were moistened twice a week.

Firebrats of the species *Thermobia domestica* were obtained from a colony of the Botanical garden of Friedrich-Schiller University of Jena. Animals were maintained in a plastic container with paper towel on the bottom and egg cartons filled with cotton at around 25°C and 50–75% humidity, and were fed fish food (Zierfischflocke, TFH-Haimerl, Roding, Germany).

Different stages and sexes of *Phyllium siccifolium* were provided by the Institute of Systematic Zoology and Evolutionary Biology of the Friedrich-Schiller University of Jena. Animals were kept in a big gaze cage at 25°C and normal light cycle feeding on blackberry leaves. The substrate was moistened every second day.

Physiology

Odorants

Pure odorants were diluted (10^{-2}) in hexane or in water as appropriate. Diluted odors (10 µl) were pipetted onto a small piece of filter paper (~1 cm^2) and placed inside a glass Pasteur pipette. For odorant application, a stimulus controller was used (Stimulus Controller CS-55, Syntech, Hilversum, The Netherlands).

Single sensillum recordings (SSR)

Adult animals were immobilized and the antennae were placed in a stable position. Sensilla were localized at 1000x magnification and the extracellular analog signals originating from the OSNs were detected by inserting a tungsten wire electrode in the base of a sensillum. The reference electrode was inserted into the eye or the body. Signals were amplified (10x; Syntech Universal AC/DC Probe), sampled (10,667.0. samples/s), and filtered (100–3000 Hz with 50/60 Hz suppression) via USB-IDAC connection to a computer (Syntech). Action potentials were extracted as digital spikes from the analog signal according to top–top amplitudes using Syntech Auto Spike 32 software. Neuron activities were recorded for 10 s, starting 2 s before a stimulation period of 0.5 s. Responses of individual neurons were calculated as the increase (or decrease) in the action potential frequency (spikes/s) relative to the

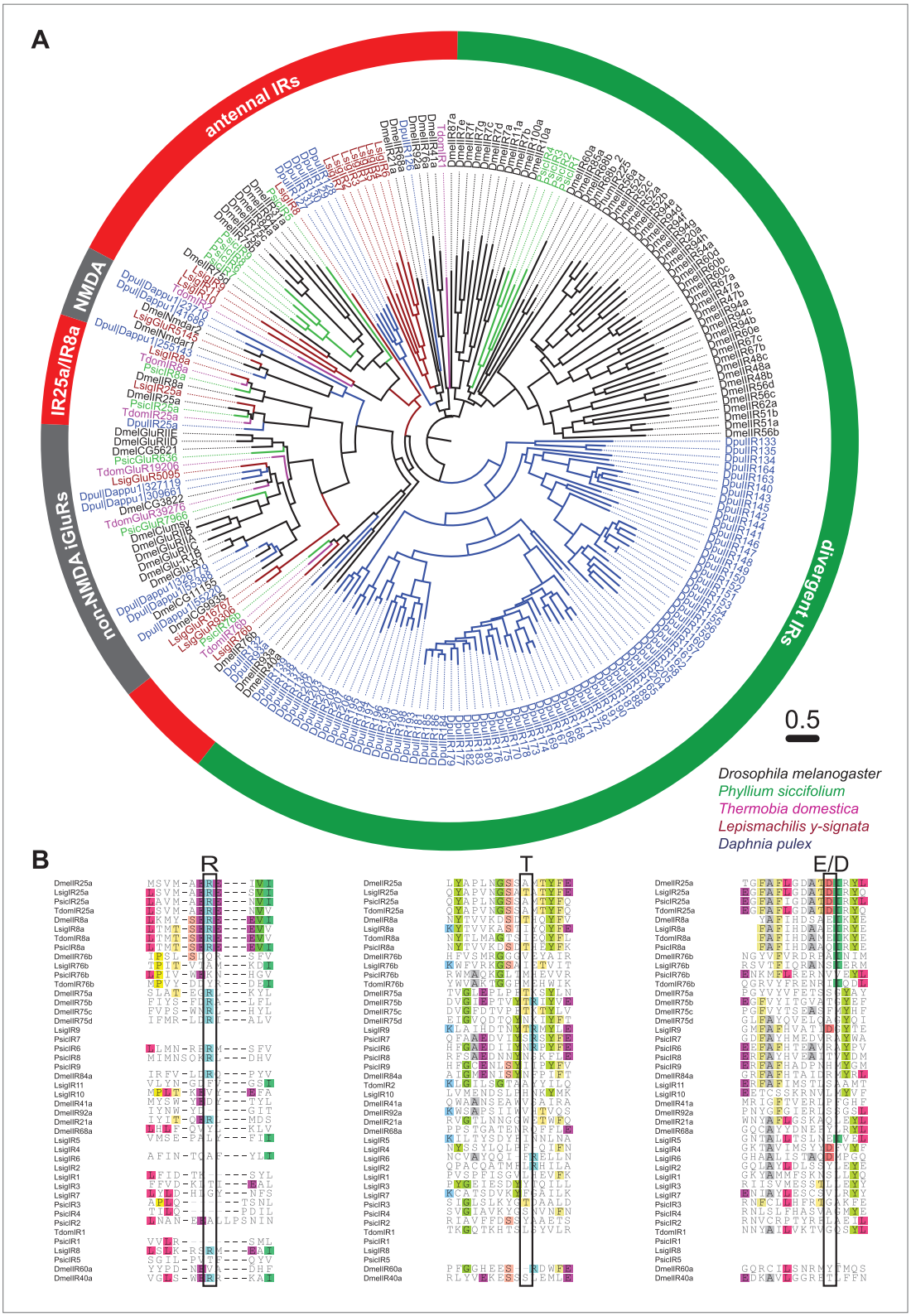


Figure 8. Ionotropic glutamate receptors of *L. y-signata*, *T. domestica*, and *P. siccifolium*. (**A**) Analysis of the relationship between *L. y-signata*, *T. domestica*, *P. siccifolium*, *D. melanogaster* and *D. pulex* iGluRs and IRs (*D. melanogaster* and *D. pulex* sequences were sequences taken from ***Croset et al., 2010***). Amino acid sequences were aligned using the MAFFT alignment tool plug-in in Geneious Pro 5.0.4 (BLOSUM72, gap open

Figure 8. Continued on next page

Figure 8. Continued

penalty: 1.53, offset value: 0.123, E-INS-i settings). The dendrogram was generated using maximum likelihood analysis with FastTree2. (All candidate IR sequences of *L. y-signata*, *T. domestica*, and *P. siccifolium* are given in ***Figure 8—source data 1*** for amino acids and ***Figure 8—source data 2*** for nucleotide sequences) (**B**) Excerpts of the alignment showing the predicted glutamate binding domains and key amino acids. Mutations in one or several of the key amino acids are a structural feature to distinguish between iGluRs and IRs, although they can be present in the coreceptors.
DOI: 10.7554/eLife.02115.020

The following source data are available for figure 8:

Source data 1. Amino acid sequences of putative variant ionotropic glutamate receptors of *L. y-signata*, *T. domestica*, and *P. siccifolium*.
DOI: 10.7554/eLife.02115.021

Source data 2. Nucleotide sequences of putative variant ionotropic glutamate receptors of *L. y-signata*, *T. domestica*, and *P. siccifolium*.
DOI: 10.7554/eLife.02115.022

Source data 3. MAFFT amino acid alignment of iGluR and IR candidates of *L. y-signata*, *T. domestica*, *P. siccifolium*, *D. melanogaster*, and *D. pulex* (*D. melanogaster* and *D. pulex* sequences were sequences taken from ***Croset et al., 2010***).
DOI: 10.7554/eLife.02115.023

Source data 4. FastTree file resulting from the MSA of ***Figure 4—source data 3*** (can be opened with FigTree).
DOI: 10.7554/eLife.02115.024

Source data 5. Tree file resulting from the MSA of ***Figure 8—source data 3*** containing node support values.
DOI: 10.7554/eLife.02115.025

pre-stimulus frequency. Sensilla were classified as basiconic, coeloconic, or trichoid based on morphological criteria. Further subdivision of distinct sensillum types was based on response profiles of all the OSNs housed within, independently from their possible olfactory receptor.

SEM

Male and female antennae were cut at the base and fixed in glutaraldehyde. Antennae were dehydrated in an ascending ethanol series (70%, 80%, 90%, 96%, 3 × 100% ethanol, 10 min each), critical point dried (BAL-TEC CPD 030, Bal-Tec Union Ltd., Liechtenstein), mounted on aluminum stubs with adhesive film and sputter coated with gold on a BAL-TEC SCD005 (Bal-Tec, Balzers, Liechtenstein). Micrographs were taken with a LEO 1450 VP scanning electron microscope (Zeiss, Wetzlar, Germany).

Molecular Biology and bioinformatics

RNA extraction

Antennae and maxillary palps were cut off close to the base and were transferred to Eppendorf cups chilled over liquid nitrogen. RNA of different tissues, respectively antennae, palps, heads, whole bodies and juveniles (unscaled juvenile stadia) was isolated using TRIzol isolation following the manufacturer's instructions, but replacing chloroform with 1-bromo-3-chloro-propane. Total RNA was dissolved in RNase free water and total RNA quality and quantity measured using an Agilent Bioanalyzer (Agilent Technologies, Santa Clara, USA).

Transcriptome sequencing

RNASeq was performed for *L. y-signata* RNA using the HiSeq 2000 (TruSeq SBS v5) Sequencing System from Illumina, utilizing the single read 100 bp (+7 index) technology at Eurofins MWG/Operon (Berlin). The resulting 22'444'128 reads were filtered for vector and linker sequences, as well as contaminants by Eurofins. A second RNASeq run for deeper sequencing was done using the HiSeq2500 at the Max Planck Genome centre in Cologne, resulting in 77'060'687 paired end reads of 100bp. Additionally to the transcriptomes of *L. y-signata* chemosensory tissues, a pooled transcriptome of whole body and head RNA was generated at Eurofins MWG/Operon (Berlin) using single read 100 bp (+7 index) technology.

Both *T. domestica* and *P. siccifolium* RNA was sequenced using the HighSeq2500 Sequencing system generating 27'704'231 paired end reads for *T. domestica* and 30'762'777 paired end reads of *P. siccifolium*. Before sequencing rRNA depletion was performed at the Max Planck Genome centre. Since the depletion did not work out for *L. y-signata*, a much deeper sequencing was performed in the second sequencing run as described above.

Bioinformatics

Removal of duplicate reads and de novo assembly was performed with CLC Genomics Workbench 5.5 (CLCbio, Copenhagen, Denmark). Sequence databases were generated in Geneious Pro 5.0.4 (Biomatters, Auckland, New Zealand). Within these databases, we manually tBLASTn searched for

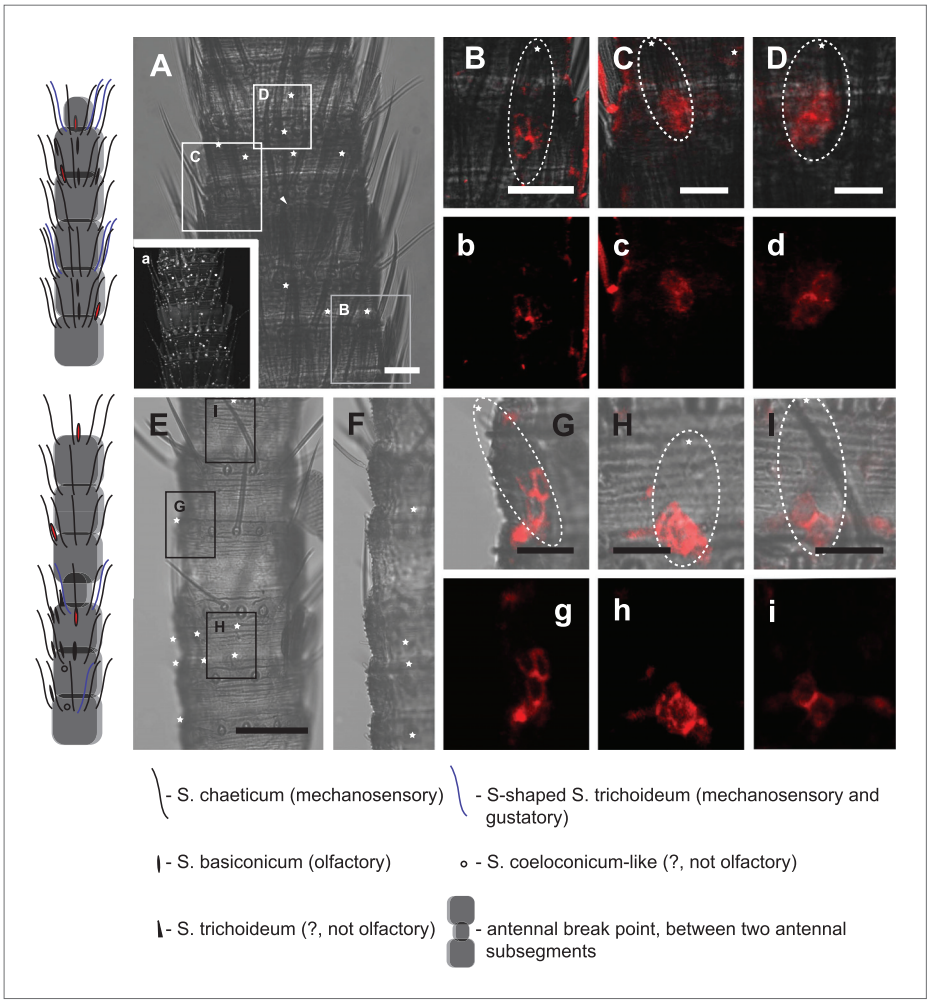


Figure 9. *In situ* hybridization on the antenna of *L. y-signata* using a Dig-labeled LsigIR25a antisense probe. On the left: schematic drawings of the position of the different sensillum types on the particular antennal subsegment. The legend for the sensillum types is given below the confocal images. (**A**–**D**) Labeling of somata in a subsegment of an antenna. Mostly two somata were labeled with the probe. The positions of the somata were in line with the positions of basiconic sensilla, but not gustatory and mechanosensory sensilla. Ultrastructural investigation of basiconic sensilla of *Machilis* sp. (Archaeognatha) and *Lepisma saccharina* (Zygentoma) suggests that the sensory neurons are located in a distance of at least 25 µm from the sensillum base in the extension of the sensillum (***Berg and Schmidt, 1997***). Therefore, we concluded that the labeled somata correspond to neurons housed in basiconic sensilla. These sensilla were colored red in the drawing on the left. (**A**) Transmitted light overview with asterisks labeling basiconic sensilla. Image sections given in **B**–**D** are indicated by white boxes and the corresponding letters. a: Projection of confocal planes recorded with Argon laser at a wavelength of 488 nm to identify the position of basiconic sensilla. (**B**–**D**) Overlaid transmitted light and fluorescent images of labeled somata. b–d: Images without transmitted light channel. (**E**–**I**) Labeling of somata in a second antenna. Parts of two antennal segments that are separated by an antennal break point. The break point can be recognized by a thinner segment on the distal part of the antennae or by a special trichoid sensillum that is only present on the segment proximal to a breaking point. (**E** and **F**) Transmitted light images of the antenna. **E** is more from the top. Image sections given in **G**–**I** are indicated by white boxes and the corresponding letters. **F** is more central plane. Asterisks denote the location of a basiconic sensillum. (**G**–**I**) Overlaid confocal images of labeled neurons. Images are projections of three confocal planes. On some positions the cuticle is given a background signal. g–i: Images without transmitted light channel. Scale bars: **A**–**C**, **G**–**I**: 20 µm, **E**: 50 µm, **D**: 10 µm.

DOI: 10.7554/eLife.02115.026

Figure 9. Continued on next page

Figure 9. Continued

The following figure supplements are available for figure 9:

Figure supplement 1. *In situ* hybridization on the antenna of *L. y-signata* using an antisense probe directed against the IR coreceptor IR8a.

DOI: 10.7554/eLife.02115.027

Figure supplement 2. *In situ* hybridization on the antenna of *L. y-signata* using sense probes directed against the IR coreceptors IR25a, IR8a.

DOI: 10.7554/eLife.02115.028

olfactory receptors (ORs), antennal ionotropic receptors (IRs), and gustatory receptors (CSPs). Templates for manual searches were the published amino acid sequences of the respective gene families of *Drosophila melanogaster*, *Bombyx mori*, *Pediculus humanus*, *Apis mellifera*, *Acyrthosiphon pisum*, and *Daphnia pulex*, as well as identified sequences of *L. y-signata*, *T. domestica*, and *P. siccifolium*.

Contigs with similarity to a member of these gene families were edited and subject to personal scrutiny of blast results, as well as further analysis. ORFs were identified and translated into amino acid sequence in Geneious Pro 5.0.4. Alignments with other members of the respective gene families were carried out using MAFFT (E-INS-I parameter set; ***Katoh et al., 2005***). Dendrograms were calculated using maximum likelihood analysis with FastTree2 (***Price et al., 2009***; ***Liu et al., 2011***) and displayed and edited with FigTree (http://tree.bio.ed.ac.uk/software/figtree). Candidates were named with the abbreviation for the gene family and ascending numbers with the exception of coreceptors, where a clear homology could be assigned. The body transcriptome of *L. y-signata* was independently screened for both ORs and Orco-related sequences.

Gene Ontology (GO) annotation was performed with Blast2GO (http://www.blast2go.com/b2ghome, ***Conesa et al., 2005***).

HMMR-design

HMMER v3.0 (***Eddy, 2011***) was used to construct HMM profiles based on a multiple sequence alignment of Orco sequences of *D. melanogaster*, *Apis mellifera*, *Tribolium castaneum*, and *Manduca sexta* resulting in three local HMM (83bDom_1: VKHQGLVADLMPNIRLMQMVGHFMFNYYS,

83bDom_4: TVEIPRLMIKSWYPWDAMHGM,

83bDom_5: DVMFCSWLLFACEQLQHLKAIMKPLMELSASLDTYRPNS) profiles and a global HMM profile. Profiles were used to search online against nr (http://hmmer.janelia.org/search/phmmer) to test the quality of the generated HMM profiles. Profiles were used subsequently to screen the antennal and maxillary palp transcriptome database of *L. y-signata* using the command line version of HMMER.

cDNA synthesis for RT-PCR

SuperScript III First-Strand Synthesis System (Invitrogen, Life Technology, Grand Island, USA) was used for cDNA synthesis according to the manufacturer's instructions, including a DNAse digestion step.

Receptor cloning

To validate and extend candidate sequences total RNA was purified using the Poly(A)Purist MAG Kit (Ambion, Life Technologies, Grand Island, USA). Synthesis of cDNA was performed using the SMARTer RACE cDNA Amplification Kit (Clontech, Mountain View, USA). Gene-specific primers were designed against receptor candidates (Primer3 v.0.4.0, Whitehead Institute for Biomedical Research and Oligo Calc version 3.26). RACE-PCR amplification was done according to the manufacturer's instructions.

FISH

Biotin- and digoxigenin (DIG)-labeled sense and antisense probes targeting candidates were prepared using a T7/Sp6-Polymerase (ROCHE, Berlin, Germany) as per manufacturer's instructions, a Biotin RNA Labeling Mix 10x conc. (ROCHE) or DIG RNA Labeling Mix 10x conc. (ROCHE), and incubating 3 hr at 37°C. RNA was precipitated and washed once with 70% ethanol, dissolved in water and finally diluted in hybridization buffer. Probes were fragmented to a length of about 600 nucleotides (***Angerer and Angerer, 1992***).

Antennae of adult *L. y-signata* and *T. domestica* were cut off, shortly dipped in distilled water with Triton X-100 (Sigma Aldrich, St. Louis, USA) and fixed for 24 hr in 4% PFA (ROTH, Karlsruhe, Germany) in 1 M $NaHCO_3$ (Sigma Aldrich, pH 9.5). The antennae were washed in 1xPBS containing

0,03% TritonX100 and incubated in 0.2 M HCl (0.03% TritonX100) for 10 min. Afterwards, antennae were rinsed twice in 1xPBS (1% TritonX100) and autoclaved distilled water. After incubation in 2xSSC (3 M NaCl, ROTH; 0.3 M $C_6H_5Na_3O_7$*$2H_2O$, Sigma; pH 7.1) at 70°C a treatment with Proteinase K (1U/ml Proteinase Buffer) at 37°C for 30 min followed. The antennae were thoroughly washed in PBS and fixed again for 20 min. Fixative was washed away with PBS and antennae pre-hybridized in Hybridization Buffer for 8 hr at 55°C. Hybridization was performed at 55°C for 2 to 3 days. DIG-labeled probes were detected using an anti-DIG-conjugated antibody in combination with HNPP/FastRed (HNPP Fluorescent Detection Set, Roche), biotin-labeled probe using a TSATM Flouresin System. Preparations were analyzed using a Zeiss LSM510 Meta (Zeiss, Jena, Germany).
Due to the modular organization of the antenna, with compartments of a size varying between 5 and 12 annuli, and to the repetitive pattern of olfactory sensilla between the compartments, we did not need to map labeling of neurons along the whole antenna.

Image processing

Contrast and false color images were optimized in Zeiss LSM Image Browser (Version 4,0,0,157). Further image processing, including cutting and image mode conversion was done in Adobe Photoshop CS4, figures were prepared in Adobe Illustrator CS4.

Acknowledgements

We thank Christin Grossmann and Sascha Bucks for technical assistance in sample preparation and receptor cloning. The authors thank Renate Kaiser and Sandor Nietzsche (both Electron Microscopy Center Jena) for help with the scanning electron microscope, Richard Reinhardt and Liza Czaja (both Max Planck Genome Center) for support in transcriptome sequencing. We also thank Shannon Olsson and Dieter Wicher for critical comments on the manuscript.

Additional information

Funding

Funder	Author
Max Planck Society	Bill S Hansson

The funder had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

Author contributions

CM, Conception and design, Acquisition of data, Analysis and interpretation of data, Drafting or revising the article; HKMD, Conception and design, Acquisition of data, Analysis and interpretation of data; HV, MCS, Analysis and interpretation of data, Drafting or revising the article; AV, Drafting or revising the article, Contributed unpublished essential data or reagents; BSH, Conception and design, Drafting or revising the article; EG-W, Conception and design, Analysis and interpretation of data, Drafting or revising the article

Additional files

Major dataset

The following dataset was generated:

Author(s)	Year	Dataset title	Dataset ID and/or URL	Database, license, and accessibility information
Missbach C, Dweck HKM, Vogel H, Vilcinskas A, Stensmyr MC, Hansson BS, and Grosse-Wilde E	2014	Evolution of insect olfactory receptors - RNAseq	PRJEB5093; http://www.ebi.ac.uk/ena/data/view/PRJEB5093	Publicly available at the European Nucleotide Archive (http://www.ebi.ac.uk/ena/).

References

Adel T. 1984. Sensilla and sensilla pattern on the antenna of *Thermobia domestica* and *Lepisma saccharina* (Insecta: Zygentoma). *Braunschweiger Naturkundliche Schriften* **2**:191–218.

Ai M, Min S, Grosjean Y, Leblanc C, Bell R, Benton R, Suh GS. 2010. Acid sensing by the *Drosophila* olfactory system. *Nature* **468**:691–695. doi: 10.1038/nature09537.

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

Altner H, Prillinger L. 1980. Ultrastructure of invertebrate chemo-, thermo- and hygroreceptors and its functional significance. *International Review of Cytology* **67**:69–139. doi: 10.1016/S0074-7696.

Angerer LM, Angerer RC. 1992. *In situ* hybridization to cellular RNA with radiolabeled RNA probes. In: Wilkinson DG, editor. *In Situ Hybridization. A practical approach*. Oxford: IRL Press. p. 15–32.

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

Benton R, Sachse S, Michnick SW, Vosshall LB. 2006. A typical membrane topology and heteromeric function of *Drosophila* odorant receptors in vivo. *PLOS Biology* **4**:e20. doi: 10.1371/journal.pbio.0040020.

Berg J, Schmidt K. 1997. Comparative morphology and moulting of Sensilla basiconica of *Lepisma saccharina* Linnaeus (Zygentoma: Lepismatidae) and *Machilis* sp. (Archaeognatha: Machilidae). *International Journal of Insect Morphology & Embryology* **26**:161–172. doi: 10.1016/S0020-7322(97)00018-4.

Bockhorst KG. 1988. Patterns of sensilla on the antennae of some Machilidae (Insecta, Archaeognatha). *Braunschweiger Naturkundliche Schriften* **3**:137–170.

Boore JL, Lavrov DV, Brown WM. 1998. Gene translocation links insects and crustaceans. *Nature* **392**:667–668. doi: 10.1038/33577.

Buck L, Axel R. 1991. A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. *Cell* **65**:175–187. doi: 10.1016/0092-8674(91)90418-X.

Clyne PJ, Warr CG, Freeman MR, Lessing D, Kim J, Carlson JR. 1999. A novel family of divergent seven-transmembrane proteins: candidate odorant receptors in *Drosophila*. *Neuron* **22**:327–338. doi: 10.1016/S0896-6273(00)81093-4.

Clyne PJ, Warr CG, Carlson JR. 2000. Candidate taste receptors in *Drosophila*. *Science* **287**:1830–1834. doi: 10.1126/science.287.5459.1830.

Conesa A, Götz S, García-Gómez JM, Terol J, Talón M, Robles M. 2005. Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. *Bioinformatics* **21**:3674–3676. doi: 10.1093/bioinformatics/bti610.

Croset V, Rytz R, Cummins SF, Budd A, Brawand D, Kaessmann H, Gibson TJ, Benton R. 2010. Ancient protostome origin of chemosensory ionotropic glutamate receptors and the evolution of insect taste and olfaction. *PLOS Genetics* **6**:e1001064. doi: 10.1371/journal.pgen.1001064.

Eddy SR. 2011. Accelerated profile HMM searches. *PLOS Computational Biology* **7**:e1002195. doi: 10.1371/journal.pcbi.1002195.

Elmore T, Ignell R, Carlson JR, Smith DP. 2003. Targeted mutation of a *Drosophila* odor receptor defines receptor requirement in a novel class of sensillum. *Journal of Neuroscience* **23**:9906–9912.

Farris SM. 2005. Evolution of insect mushroom bodies: old clues, new insights. *Arthropod Structure & Development* **34**:211–234. doi: 10.1016/j.asd.2005.01.008.

Firestein S. 2001. How the olfactory system makes sense of scents. *Nature* **413**:211–218. doi: 10.1038/35093026.

Friedrich M, Tautz D. 1995. Ribosomal DNA phylogeny of the major extant arthropod classes and the evolution of myriapods. *Nature* **376**:165–167. doi: 10.1038/376165a0.

Fröhlich A, Lu Z. 2013. The "rosette-like" structures in the cuticle of *Petrobius brevistylis* are the openings of epidermal glands. *Arthropod Structure & Development* **42**:89–94. doi: 10.1016/j.asd.2012.10.001.

Gao Q, Chess A. 1999. Identification of candidate *Drosophila* olfactory receptors from genomic DNA sequence. *Genomics* **60**:31–39. doi: 10.1006/geno.1999.5894.

Getahun MN, Wicher D, Hansson BS, Olsson SB. 2012. Temporal response dynamics of *Drosophila* olfactory sensory neurons depends on receptor type and response polarity. *Frontiers in Cellular Neuroscience* **6**:1–11. doi: 10.3389/fncel.2012.00054.

Guo M, Krieger J, Grosse-Wilde E, Mißbach C, Zhang L, Breer H. 2014. Variant ionotropic receptors are expressed in olfactory sensory neurons of coeloconic sensilla on the antenna of the desert locust (*Schistocerca gregaria*). *International Journal of Biological Sciences* **10**:1–14. doi: 10.7150/ijbs.7624.

Hallem EA, Ho MG, Carlson JR. 2004. The molecular basis of odor coding in the *Drosophila* antenna. *Cell* **117**:965–979. doi: 10.1016/j.cell.2004.05.012.

Hansen-Delkeskamp E. 2001. Responsiveness of antennal taste hairs of the apterygotan insect, *Thermobia domestica* (Zygentoma); an electrophysiological investigation. *Journal of Insect Physiology* **47**:689–697. doi: 10.1016/S0022-1910(00)00159-1.

Hildebrand JG, Shepherd GM. 1997. Mechanisms of olfactory discrimination: converging evidence for common principles across phyla. *Annual Review of Neuroscience* **20**:595–631. doi: 10.1146/annurev.neuro.20.1.595.

Hill CA, Fox AN, Pitts RJ, Kent LB, Tan PL, Chrystal MA, Cravchik A, Collins FH, Robertson HM, Zwiebel LJ. 2002. G protein coupled receptors in *Anopheles gambiae*. *Science* **298**:176–178. doi: 10.1126/science.1076196.

Hollins B, Hardin D, Gimelbrant AA, McClintock TS. 2003. Olfactory-enriched transcripts are cell-specific markers in the lobster olfactory organ. *The Journal of Comparative Neurology* **455**:125–138. doi: 10.1002/cne.10489.

Katoh K, Kuma K, Toh H, Miyata T. 2005. MAFFT version 5: improvement in accuracy of multiple sequence alignment. *Nucleic Acids Research* **33**:511–518. doi: 10.1093/nar/gki198.

Kirkness EF, Haas BJ, Sun W, Braig HR, Perotti MA, Clark JM, Lee SH, Robertson HM, Kennedy RC, Elhaik E, Gerlach D, Kriventseva EV, Elsik CG, Graur D, Hill CA, Veenstra JA, Walenz B, Tubío JM, Ribeiro JM, Rozas J, Johnston JS, Reese JT, Popadic A, Tojo M, Raoult D, Reed DL, Tomoyasu Y, Kraus E, Mittapalli O, Margam VM, Li HM, Meyer JM, Johnson RM, Romero-Severson J, Vanzee JP, Alvarez-Ponce D, Vieira FG, Aguadé M, Guirao-Rico S, Anzola JM, Yoon KS, Strycharz JP, Unger MF, Christley S, Lobo NF, Seufferheld MJ, Wang N, Dasch GA, Struchiner CJ, Madey G, Hannick LI, Bidwell S, Joardar V, Caler E, Shao R, Barker SC, Cameron S, Bruggner RV, Regier A, Johnson J, Viswanathan L, Utterback TR, Sutton GG, Lawson D, Waterhouse RM, Venter JC, Strausberg RL, Berenbaum MR, Collins FH, Zdobnov EM, Pittendrigh BR. 2010. Genome sequences of the human body louse and its primary endosymbiont provide insights into the permanent parasitic lifestyle. *Proceedings of the National Academy of Sciences of the United States of America* **107**:12168–12173. doi: 10.1073/pnas.1003379107.

Krång A-S, Knaden M, Steck K, Hansson BS. 2012. Transition from sea to land: olfactory function and constraints in the terrestrial hermit crab *Coenobita clypeatus*. *Proceedings Biological Sciences/The Royal Society* **279**: 3510–3519. doi: 10.1098/rspb.2012.0596.

Krieger J, Klink O, Mohl C, Raming K, Breer H. 2003. A candidate olfactory receptor subtype highly conserved across different insect orders. *Journal of Comparative Physiology A, Neuroethology, Sensory, Neural, and Behavioral Physiology* **189**:519–526. doi: 10.1007/s00359-003-0427-x.

Kumar BN, Taylor RW, Pask GM, Zwiebel LJ, Newcomb RD, Christie DL. 2013. A conserved aspartic acid is important for agonist (VUAA1) and odorant/tuning receptor-dependent activation of the insect odorant co-receptor (Orco). *PLOS ONE* **8**:e70218. doi: 10.1371/journal.pone.0070218.

Larsson MC, Domingos AI, Jones WD, Chiappe ME, Amrein H, Vosshall LB. 2004. Or83b encodes a broadly expressed odorant receptor essential for *Drosophila* olfaction. *Neuron* **43**:703–714. doi: 10.1016/j.neuron.2004.08.019.

Liu K, Linder CR, Warnow T. 2011. RAxML and FastTree: comparing two methods for large-scale maximum likelihood phylogeny estimation. *PLOS ONE* **6**:e27731. doi: 10.1371/journal.pone.0027731.

Lundin C, Käll L, Kreher SA, Kapp K, Sonnhammer EL, Carlson JR, Heijne Gv, Nilsson I. 2007. Membrane topology of the *Drosophila* OR83b odorant receptor. *FEBS Letters* **581**:5601–5604. doi: 10.1016/j.febslet.2007.11.007.

Missbach C, Harzsch S, Hansson BS. 2011. New insights into an ancient insect nose: the olfactory pathway of *Lepismachilis y-signata* (Archaeognatha: Machilidae). *Arthropod Structure & Development* **40**:317–333. doi: 10.1016/j.asd.2011.03.004.

Mombaerts P. 1999. Seven-transmembrane proteins as odorant and chemosensory receptors. *Science* **286**: 707–711. doi: 10.1126/science.286.5440.707.

Nakagawa T, Pellegrino M, Sato K, Vosshall LB, Touhara K. 2012. Amino acid residues contributing to function of the heteromeric insect olfactory receptor complex. *PLOS ONE* **7**:e32372. doi: 10.1371/journal.pone.0032372.

Peñalva-Arana DC, Lynch M, Robertson HM. 2009. The chemoreceptor genes of the waterflea *Daphnia pulex*: many Grs but no Ors. *Bmc Evolutionary Biology* **9**:79. doi: 10.1186/1471-2148-9-79.

Pitts RJ, Fox AN, Zwiebel LJ. 2004. A highly conserved candidate chemoreceptor expressed in both olfactory and gustatory tissues in the malaria vector *Anopheles gambiae*. *Proceedings of the National Academy of Sciences of the United States of America* **101**:5058–5063. doi: 10.1073/pnas.0308146101.

Price MN, Dehal PS, Arkin AP. 2009. FastTree: computing large minimum evolution trees with profiles instead of a distance matrix. *Molecular Biology and Evolution* **26**:1641–1650. doi: 10.1093/molbev/msp077.

Rebora M, Salerno G, Piersanti S, Dell'Otto A, Gaino E. 2012. Olfaction in dragonflies: electrophysiological evidence. *Journal of Insect Physiology* **58**:270–277. doi: 10.1016/j.jinsphys.2011.11.018.

Regier JC, Shultz JW, Zwick A, Hussey A, Ball B, Wetzer R, Martin JW, Cunningham CW. 2010. Arthropod relationships revealed by phylogenomic analysis of nuclear protein-coding sequences. *Nature* **463**:1079–1083. doi: 10.1038/nature08742.

Robertson HM. 2001. Taste: independent origins of chemoreception coding systems? *Current Biology* **11**: 560–562. doi: 10.1016/S0960-9822(01)00343-8.

Robertson HM, Warr CG, Carlson JR. 2003. Molecular evolution of the insect chemoreceptor gene superfamily in *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences of the United States of America* **100**:14537–14542. doi: 10.1073/pnas.2335847100.

Robertson HM, Thomas JH. 2006. The putative chemoreceptor families of *C. elegans* (January 06, 2006). In: The C. elegans Research Community, editor. WormBook. doi: 10.1895/wormbook.1.66.1. http://www.wormbook.org

Robertson HM, Wanner KW. 2006. The chemoreceptor superfamily in the honey bee *Apis mellifera*: expansions of the odorant, but not gustatory, receptor families. *Genome Research* **16**:1395–1403. doi: 10.1101/gr.5057506.

Rota-Stabelli O, Daley AC, Pisani D. 2013. Molecular timetrees reveal a Cambrian colonization of land and a new scenario for ecdysozoan evolution. *Current Biology* **23**:392–398. doi: 10.1016/j.cub.2013.01.026.

Sargsyan V, Getahun MN, Lavista Llanos S, Olsson SB, Hansson BS, Wicher D. 2011. Phosphorylation via PKC regulates the function of the *Drosophila* odorant co-receptor. *Frontiers in Cellular Neuroscience* **5**:5. doi: 10.3389/fncel.2011.00005.

Sato K, Pellegrino M, Nakagawa T, Nakagawa T, Vosshall LB, Touhara K. 2008. Insect olfactory receptors are heteromeric ligand-gated ion channels. *Nature* **452**:1002–1006. doi: 10.1038/nature06850.

Scott K, Brady R Jnr, Cravchik A, Morozov P, Rzhetsky A, Zuker C, Axel R. 2001. A chemosensory gene family encoding candidate gustatory and olfactory receptors in *Drosophila*. *Cell* **104**:661–673. doi: 10.1016/S0092-8674(01)00263-X.

Silbering AF, Rytz R, Grosjean Y, Abuin L, Ramdya P, Jefferis GS, Benton R. 2011. Complementary function and integrated wiring of the evolutionarily distinct *Drosophila* olfactory subsystems. *Journal of Neuroscience* **31**:13357–13375. doi: 10.1523/jneurosci.2360-11.2011.

Smadja C, Shi P, Butlin RK, Robertson HM. 2009. Large gene family expansions and adaptive evolution for odorant and gustatory receptors in the pea aphid, *Acyrthosiphon pisum*. *Molecular Biology and Evolution* **26**:2073–2086. doi: 10.1093/molbev/msp116.

Strausfeld NJ, Hansen L, Ll Y, Gomez RS, Ito K. 1998. Evolution, discovery, and interpretations of arthropod mushroom bodies. *Learning & Memory* **5**:11–37. doi: 10.1101/lm.5.1.11.

Tadesse T, Schmidt M, Walthall WW, Tai PC, Derby CD. 2011. Distribution and function of splash, an achaete-scute homolog in the adult olfactory organ of the Caribbean spiny lobster *Panulirus argus*. *Developmental Neurobiology* **71**:316–335. doi: 10.1002/dneu.20862.

Tauxe GM, MacWilliam D, Boyle SM, Guda T, Ray A. 2013. Targeting a dual detector of skin and CO2 to modify mosquito host seeking. *Cell* **155**:1365–1379. doi: 10.1016/j.cell.2013.11.013.

Trautwein MD, Wiegmann BM, Beutel R, Kjer KM, Yeates D. 2012. Advances in insect phylogeny at the dawn of the postgenomic era. *Annual Review of Entomology* **57**:499–468. doi: 10.1146/annurev-ento-120710-100538.

Vosshall LB, Amrein H, Morozov PS, Rzhetsky A, Axel R. 1999. A spatial map of olfactory receptor expression in the *Drosophila* antenna. *Cell* **96**:725–736. doi: 10.1016/s0092-8674(00)80582-6.

Vosshall LB, Hansson BS. 2011. A unified nomenclature system for the insect olfactory coreceptor. *Chemical Senses* **36**:497–498. doi: 10.1093/chemse/bjr022.

Wicher D, Schäfer R, Bauernfeind R, Stensmyr MC, Heller R, Heinemann SH, Hansson BS. 2008. *Drosophila* odorant receptors are both ligand-gated and cyclic-nucleotide-activated cation channels. *Nature* **452**:1007–1011. doi: 10.1038/nature06861.

Wiegmann BM, Trautwein MD, Kim JW, Cassel BK, Bertone MA, Winterton SL, Yeates DK. 2009. Single-copy nuclear genes resolve the phylogeny of holometabolous insects. *BMC Biology* **7**:1–16. doi: 10.1186/1741-7007-7-34.

Yang Y, Krieger J, Zhang L, Breer H. 2012. The olfactory co-receptor Orco from the migratory locust (*Locusta migratoria*) and the desert locust (*Schistocerca gregaria*): identification and expression pattern. *International Journal of Biological Sciences* **8**:159–170. doi: 10.7150/ijbs.8.159.

Zhou X, Slone JD, Rokas A, Berger SL, Liebig J, Ray A, Reinberg D, Zwiebel LJ. 2012. Phylogenetic and transcriptomic analysis of chemosensory receptors in a pair of divergent ant species reveals sex-specific signatures of odor coding. *PLOS Genetics* **8**:e1002930. doi: 10.1371/journal.pgen.1002930.

Figure Supplementes

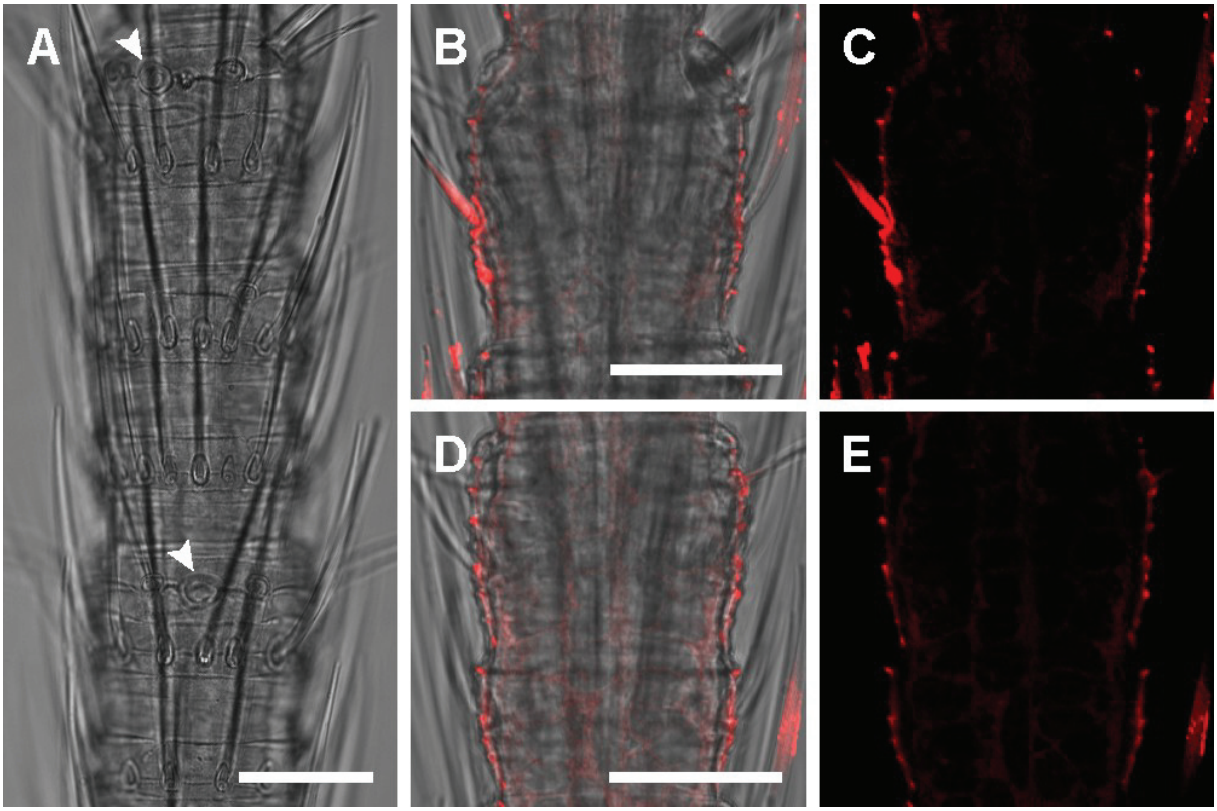


Figure 7-supplement 1: *In situ* hybridization on the antenna of *T. domestica* using sense probes directed against the TdomOrco1.

A: Transmitted light images taken with cLSM. The position of olfactory sensilla is indicated by arrowheads.

B, C, D, E: Projection section through the antennae. No Dig signals were obtained using the sense probes of TdomOrco1.

All scale bars 20 μm.

DOI: http://dx.doi.org/10.7554/eLife.02115.019

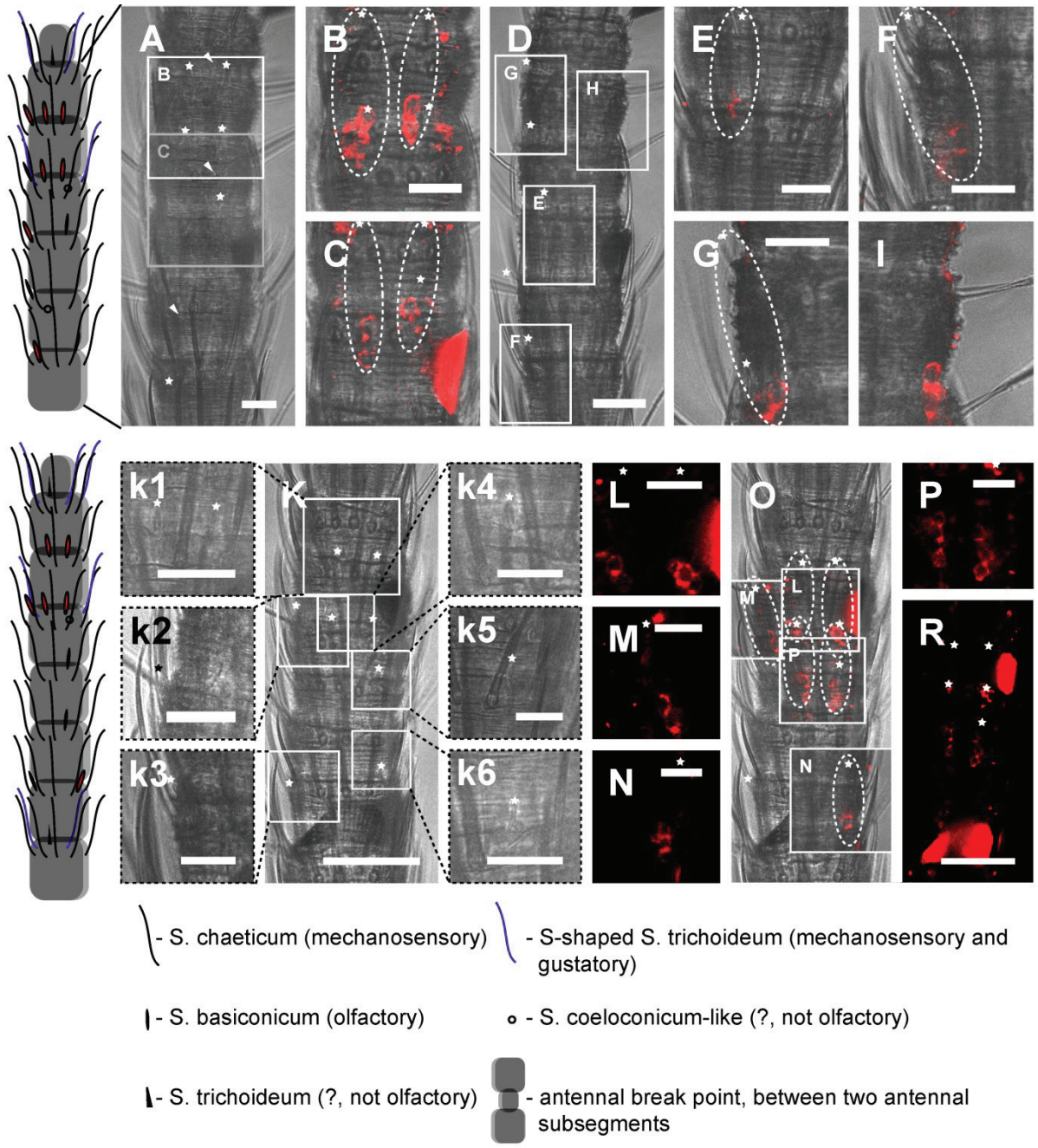


Figure 9-supplement 1: *In situ* hybridization on the antenna of *L. y-signata* using an antisense probe directed against the IR coreceptor IR8a.

On the left: Schematic drawings of the position of the different sensillum types on antennae. The legend for the sensillum types is given below the confocal images.

A-I: Labeling of somata in a subsegment of one antenna, with A and D showing the position of basiconic sensilla in the transmitted light channel. Images that are shown in B, C and E-I are indicated by white boxes and the corresponding letters. B, C, E-I: Overlaid transmitted

light and fluorescent images of single confocal planes through the dorsal side of the antenna. The signals are lineal to basiconic sensilla in a distance of about 20 to 25 µm to the base of the sensillum. Therefor we conclude that the labeled somata correspond to basiconic sensilla. For the somata in I we could not find a corresponding sensillum. It might be situated on the backside of the antenna. Red fluorescence on the sides is due to autofluorescence of the antennal cuticle.

K-R: Labeling of somata in a second antenna.

K,k1-k4: Transmitted light images of different focal planes, giving an overview about the position of basiconic sensilla on this antennal subsegment.

O: Merged image of transmitted light image and overlaid confocal and transmitted light image. Detailed images of the signals within the white boxes are displayed with corresponding letters around the overview image (L-N, P).

R: Single optical section through the antenna. Background fluorescence according to antennal cuticle and sensillum bases made whole antennal projections not possible. Very pronounced on this picture are the antennal scales on the right side of the antenna and the bottom of the picture.

Scale bars: A-I, k1-k6, L-N, P: 20 µm, K, R: 50 µm.

DOI: http://dx.doi.org/10.7554/eLife.02115.027

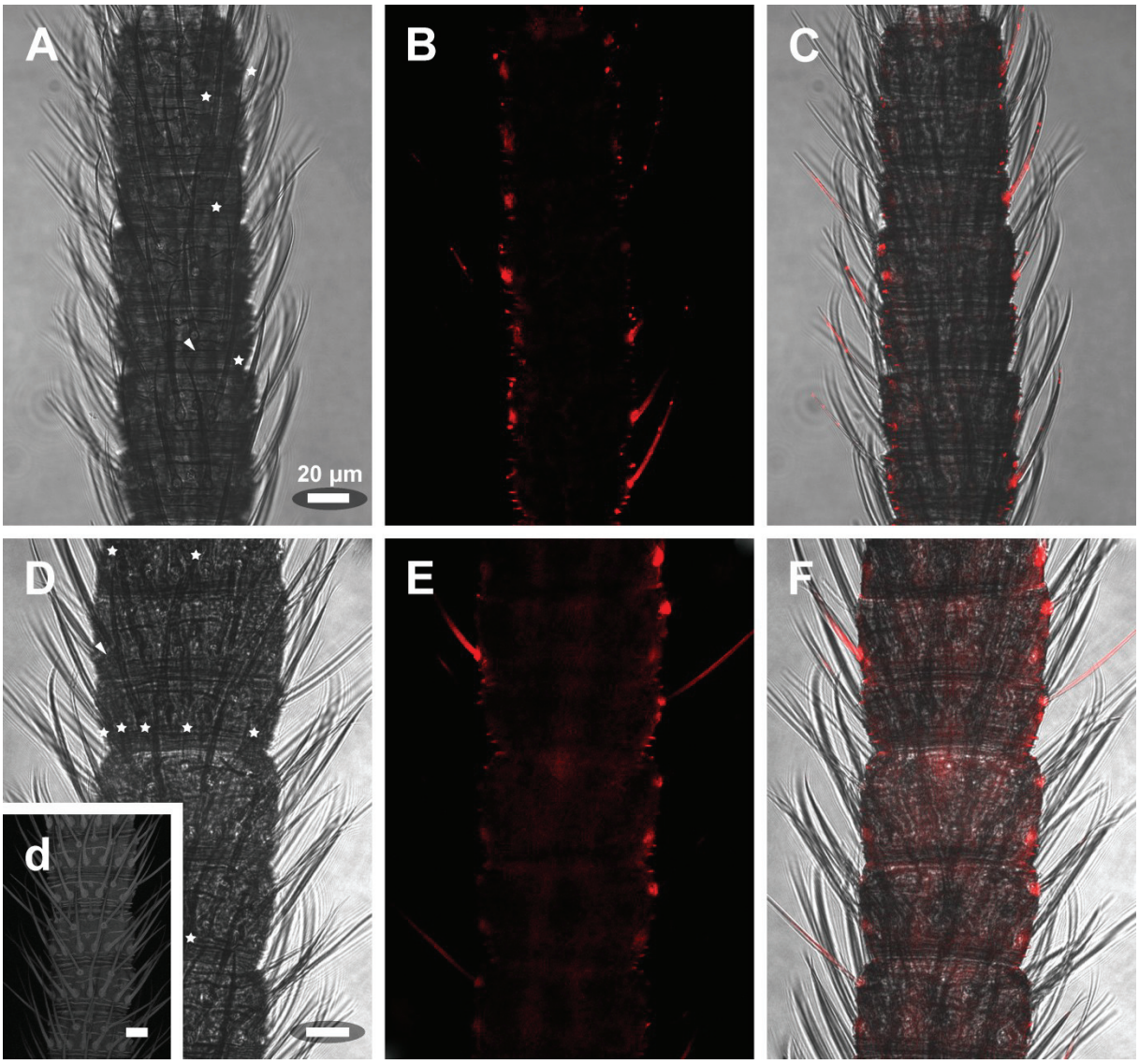


Figure 9-supplement 2: ***In situ*** **hybridization on the antenna of** ***L. y-signata*** **using sense probes directed against the IR coreceptors IR25a, IR8a.**

A, D, G: Transmitted light pictures of antennal excerpts. Asterisks mark positions of basiconic sensilla, arrowheads places were coeloconic-like sensilla are located. No signals were obtained in the sense controls.

A - C: IR25a sense probe.

D – F: IR8a sense probe.

All scale bars 20 µm.

DOI: http://dx.doi.org/10.7554/eLife.02115.028

Additional experiments

Experiment 1: Microarrays

Lepismachilis y-signata transcriptome sequence data of an initial assembly with a length above 400 nucleotides were used for the design of 4x 180k microarrays based on the eArray platform (Agilent Technologies; https: // earray.chem.agilent.com/ earray/), with a final number of 176030 non-control probe set and 4854 Agilent Technologies built in controls (structural and spike in). The cut-off of 400 nucleotides was set to maximize the precision of TMHMM profile prediction in the GO annotation for subsequent analysis. One-Color microarray hybridizations were performed on two SurePrint G3 Custom GE 4x180K (Agilent Technologies), using four independent pooled probes of thoracic musculature, representing non-antennal or non-sensory tissue. Total RNA was extracted as described in Chapter I, but double purified, using the RNeasy MinElute Cleanup Kit (Qiagen) following the manufacturer's protocol. RNA integrity and quantity was verified on an Agilent 2100 Bioanalyzer using the RNA Nano chips (Agilent Technologies). RNA quantity was determined on a Nanodrop ND-1000 spectrophotometer. Agilent Technologies spike-in RNA was added to 100 ng of total RNA and labelled using a combination of the Low Input QuickAmp Amplification kit (Agilent Technologies) and the Kreatech ULS Fluorescent Labeling Kit with cyanine 3-CTP dye following the manufacturer's instructions. Labelled amplified cRNA samples were purified using Qiagen RNeasy MinElute columns and analyzed on a Nanodrop ND-1000 spectrophotometer using the microarray function. Amplified cRNA samples were used for microarray hybridisation only if the specific activity is >6.0 pmol Cy3 per ug cRNA and 1600 ng of cyanine 3 labeled cRNA were used for each array. Hybridization was carried out at 65^{o}C for 17 hours and microarray slides were washed in GE Wash Buffers according to the manufacturer´s instructions (Agilent Technologies). Slides were treated in Stabilization and Drying Solution, scanned with the Agilent Microarray Scanner and data was extracted from the TIFF images with Agilent Feature Extraction software version 9.1.

Raw data output files (text files) from the feature extraction software were analyzed using the GeneSpring GX11 microarray analysis software. The data points were normalized between arrays to the median intensity and log base 2-transformation of the normalized data. Further analysis using Genespring GX 11 focused on the identification of absent/present scores of all probes, incorporating the Blast2GO results.

Cluster analysis

Contigs that were found to be present in at least three of the muscle samples were subtracted from the entire assembly. Remaining contigs were examined for interesting annotations. Contigs with GO-annotations like "protein" or without annotation, but putative transmembrane domains were blasted against each other using Geneious Pro 5.0.4 to find groups of contigs with high similarity. Additionally cluster analysis using the same contigs was performed in CLANS (Cluster ANalysis of Sequences, Frickey and Lupas, 2004, http://freelancingscience.com/2008/01/22/clans-java-tool-for-cluster-analysis-of-sequences/). Resulting clusters bigger than two contigs were analyzed in more detail using Geneious Pro.

Results

The fact that we were not able to identify any OR coding sequences using the techniques described in Chapter I could be an artifact due to the evolutionary distance of *L. y-signata* to all insect species with reported OR coding genes, as well as the diverse nature of these genes. To test this hypothesis we performed additional analyses using a microarray based on all contigs above 400 bases to create a secondary dataset, subtracting ESTs expressed in muscle tissue from the total sequence set. This procedure limits the total gene number and removes commonly expressed non-olfactory genes, reducing the number of contigs to assess to 3739 that are not active in muscular tissue. Of these 511 contigs were either not annotated or annotated only as "proteins" and could therefore not be excluded as candidates. Furthermore, they featured transmembrane domains predicted in Blast2GO, an expected characteristic of any receptor type. Cluster analysis was used to search for clusters of contigs that could represent a new group of olfactory receptors, or non-annotated OR genes. Sequences that formed clusters bigger than two sequences were analyzed in more detail. We found eight clusters (5x2 sequences, 2x3 sequences, 1x4 sequences). Sequences were translated in all frames and investigated for transmembrane domains. None of the sequences within clusters turned out to have TMDs or other motifs expected from a new receptor in their longest predicted ORF. The TMDs predicted in the course of BLAST2GO analysis belonged to translations in other frames and were interrupted by multiple stop codons, invalidating the prediction. Candidates of one triple cluster exhibited AAPA-tetrapeptide repeats in their hydrophobic region, similarly to chitin-binding proteins. Sequences of two double clusters related to other sequences within the transcriptome were excluded as putative receptors based on their translated amino acid sequence. All of these predicted amino acid sequences had a

very high glycine content and their overall identity was 23.4% (41.7% pairwise identity). By blasting the consensus sequence we got a hit against an *Ixodes* glycine-rich protein described as salivary gland peptides or cuticle proteins. A function in odor detection is therefore highly unlikely.

Experiment 2: Immunohistochemistry

Immunostaining of *L. y-signata* and *Thermobia domestica* was performed on whole mount antennae and on cryosections. *Sympetrum sanguimeum* (Odonata: Libellulidae)*, Phyllium siccifolium*, *Schistocerca gregaria* and *Manduca sexta* (Lepidoptera: Sphingidae) antennae were only treated as whole mount.

For whole mount immunostaining antennae were dissected in ice cold phosphate-buffered saline (PBS, 0.1 mol, pH 7.4) and fixed in 4% paraformaldehyde in 0.1M PBS overnight at 4°C. After fixation the antennae were washed several times with PBS for at least 2 h, followed by pre-incubation in 0.1M PBS containing 0.3% TritonX-100 (PBST) and 1% bovine serum albumin (Sigma-Aldrich) for 6 h at 4°C. Afterwards the antennae were incubated in primary antiserum (anti-R2 1:500 in PBST, kindly provided by Jürgen Krieger, University of Hohenheim) for 2 days at 4°C. After incubation in primary antiserum, antennae were washed several times with PBS for at least 2 h at RT and then incubated in secondary antiserum containing conjugated Alexa Fluor488 anti-rabbit (1:500, Invitrogen) for 2 days at 4°C. Then the antennae were washed several times with PBS for 4 h and mounted in MOWIOL (Calbiochem).

For cryosections antennae were dissected as described above, shortly dipped in PBS containing a little Triton for better surface coating in Tissue-Tek® OCT™ Compound (SAKURA). Antennae were cut in 12 µm sections (Cryo-Star HM560M, Microm) and mounted onto Superfrost* Ultra Plus Adhesion Slides (Thermo SCIENTIFIC). Sections were air-dried and fixed with PFA for 15 min. Staining procedure was performed as described above, but with shorter incubation times using 30 min for preincubation and blocking, overnight incubation with primary antibody solution and 2 h incubation with secondary antibody.

Preparations were analyzed using a Zeiss LSM510 Meta.

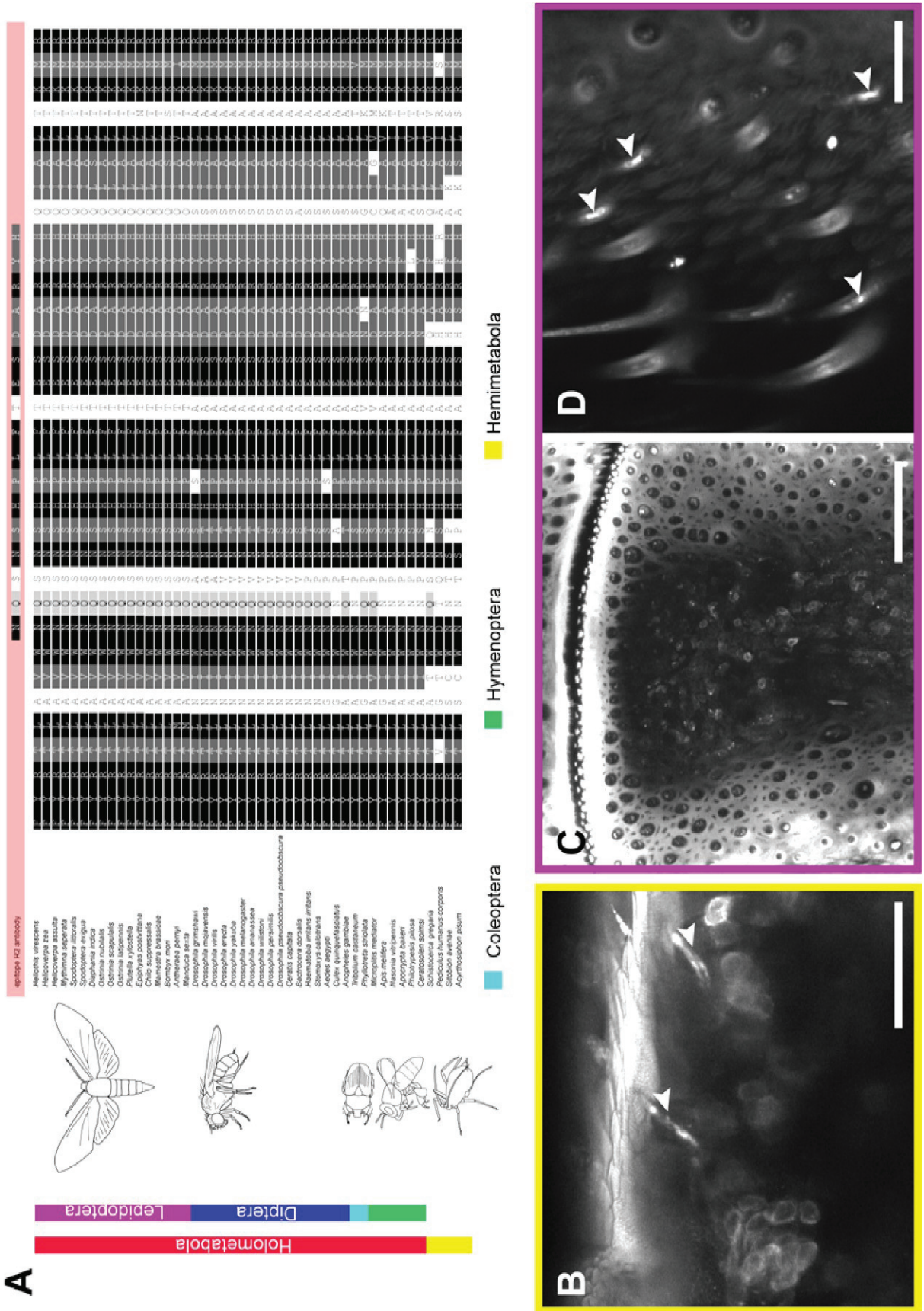


Figure 1. Labeling potential of the R2 antibody. A: Extract of a multiple sequence alignment of predicted Orco proteins of various insect species and the epitope used for R2 antibody production. The epitope is conserved enough to successfully label OSN dendrites (arrowheads in B and D) and cell bodies of *Schistocerca gregaria* (B) and *Manduca sexta* (C, D). Scale bars: B, C: 50 µm, D: 10 µm.

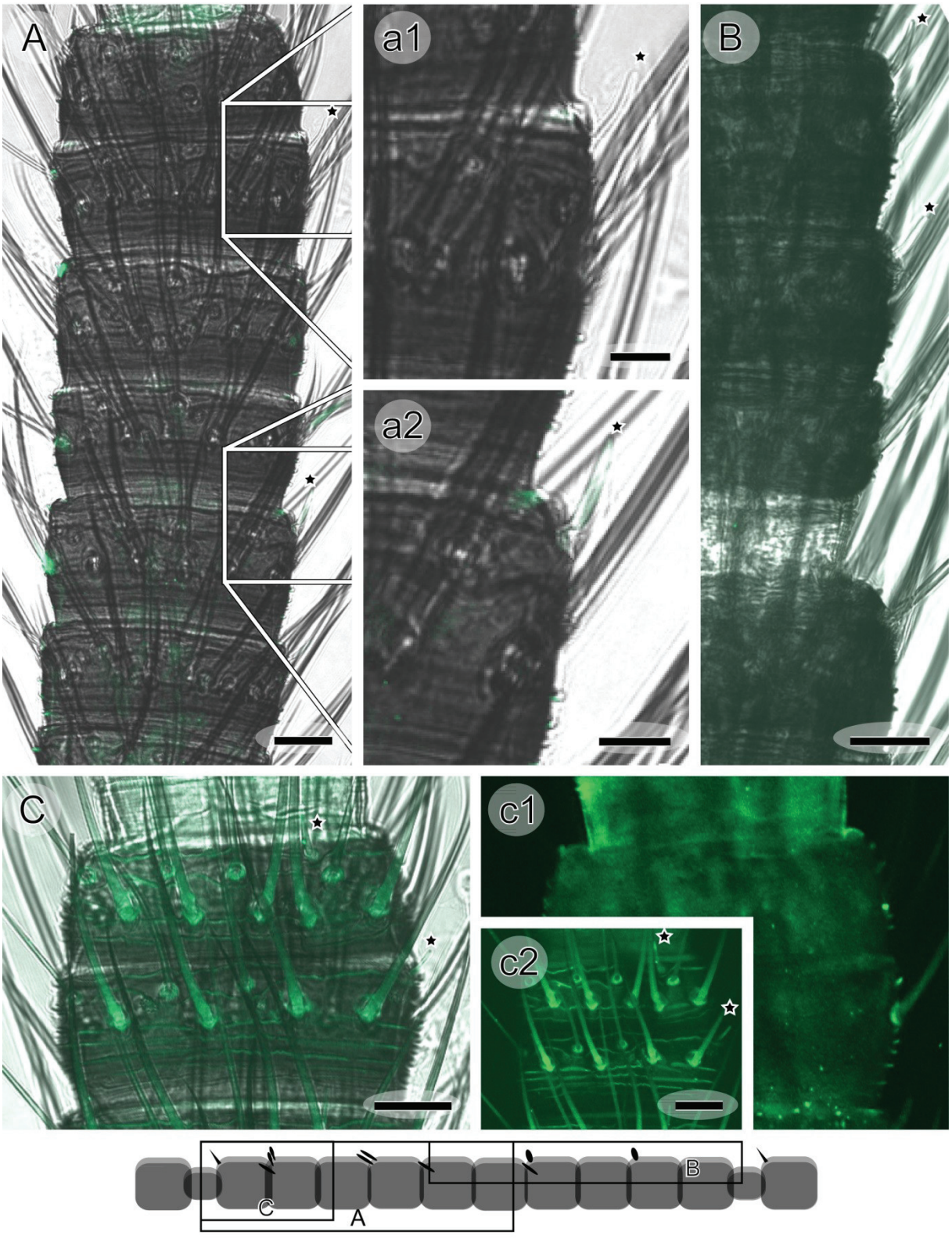


Figure 2. No Orco-immunoreactivity in the antenna of *Lepismachilis y-signata*
A-C: Details of one antennal compartment with special focus on olfactory basiconic sensilla. No signals were obtained from the dendrites, cell bodies and antennal nerve. Asterisks mark the position of olfactory sensilla. Below: Typical antennal segment of *L. y-signata* and distribution of basiconic sensilla. Black boxes show the position of the images displayed in A-C. Scale bars: A, B, C, c2: 20 µm; a1, a2: 10 µm.

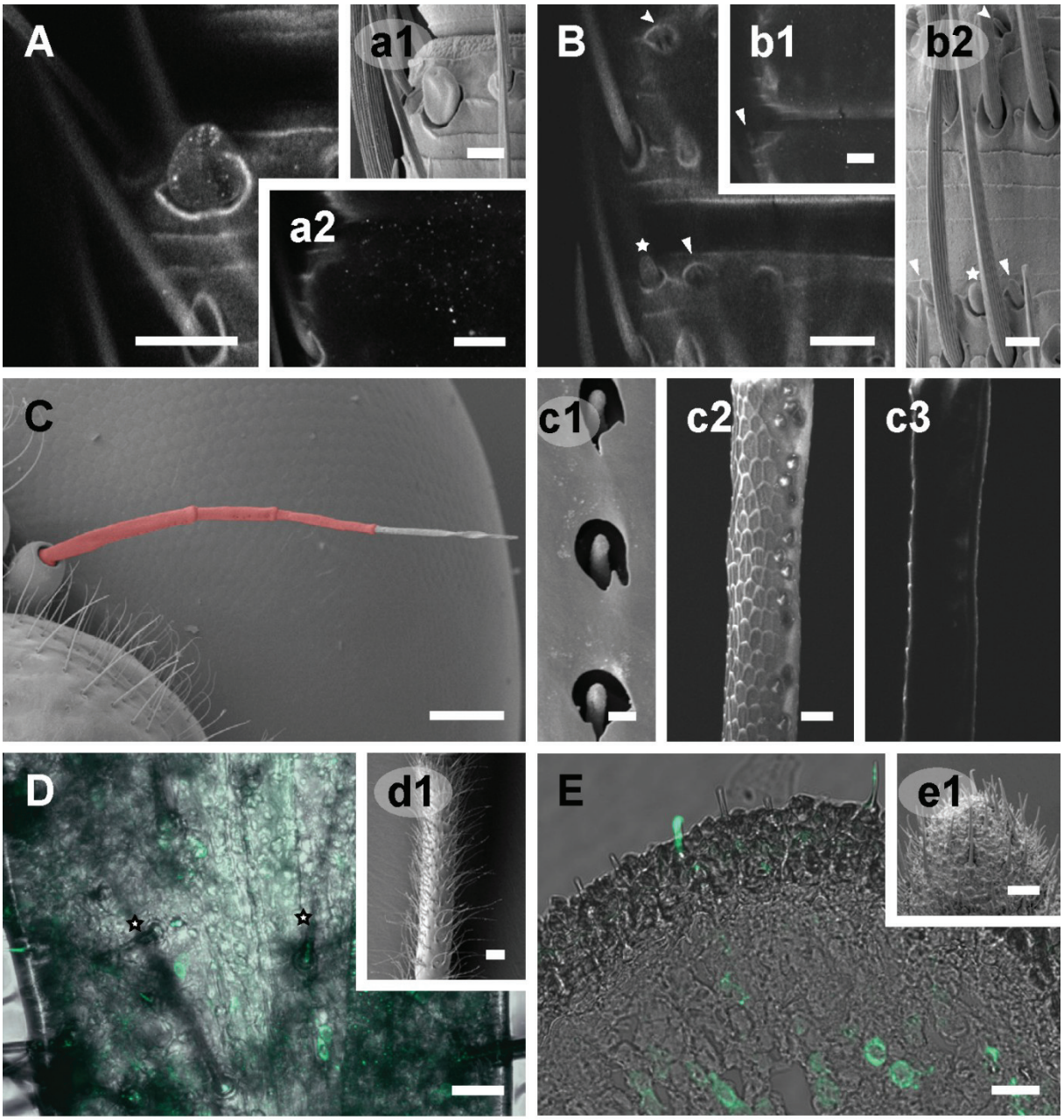


Figure 3. Localization of olfactory sensilla and Orco-immunoreactivity in antennae of *Thermobia domestica*, *Sympetrum sanguineum* and *Phyllium siccifolium*

A, B: Olfactory sensilla of *T. domestica*. Both sensilla with pores (A and asterisk in B) and grooved sensilla (arrowheads in B) were localized on the antennae. The pattern of sensilla is very sterotyped. SEM pictures (a1, b2) could be easily correlated with confocal images (A, a2, B, b1). No OSNs exhibiting Orco-ir were found in antennae of *T. domestica*.

C: Localization of olfactory sensilla on the antennae of *S. sanguineum* (the area were sensilla were located is labeled in red). c1: Sensilla with pores were localized in pits. c2: Projections of several cLSM slices. Sensilla were nicely visible inside the pits. c3: A single optical plane through the antenna. OSNs showed no Orco-ir.

D: Detail of a male antenna that is covered by trichoid sensilla (see also d1). These sensilla (asterisk) were innervated by Orco-ir exhibiting OSNs.

E: The antennal tip is covered by a high number of different sensilla, including porous and grooved sensilla (e1). Although Orco-ir was not obtained in all sensilla, it was not possible to assign the signals to either grooved or pores sensilla, because both sensillum types appeared quite similar in the sections.

Scale bars: A, Bb1, b2: 10 µm, c2, E. 20 µm D, e1: 50 µm, d1: 100.

Results

Since ORs are typically very diverse we focused on the considerably more conserved olfactory coreceptor Orco. We tried to verify Orco presence in *L. y-signata* using an Orco-antibody (kindly provided by J. Krieger, University of Hohenheim, Stuttgart, Germany). The antibody is directed against an epitope with 100% amino acid identity across lepidopteran Orco homologs. *M. sexta*, *S. gregaria and P. siccifolium* antennae were used as positive control to verify the labeling potential of this antibody. No signals whatsoever were obtained in *L. y-signata* and *T. domestica* using either whole antennae (Fig.2, 3) or cryosections (not shown), although clear signals were obtained in similar preparations from *S. gregaria, P. siccifolium* and *M. sexta* (Fig.1, 2). However, it is unclear if Orco is absent in *L .y-signata* or just too dissimilar to the original epitope the antibody is directed against, especially since we have shown the existence of multiple Orco variants in *T. domestica*, but did not obtain a signal using the R2 antibody.

Experiment 3: Orco cloning using degenerated primers

Two pairs of already published degenerated primer pairs (Krieger et al., 2003: sense: 5'-GYTNATHTTYGCNTGYGARC-3', antisense: 5'-GCYTTYTGRCAYTGYTGRCA-3' and Yang et al., 2012: sense: 5'-GCNATHAARTAYTGGGT-3', antisense: 5'-TTYTGRCAYTGYTGRCAYAC-3') as well as three additional custom made primer pairs (sense: 5'-TGGGTNGARMGNCAYAARCA-3', sense: 5'-AARTAYTGGGTNGARMGNCA-3', sense: 5'-GYTNATHTWYGCNTGYGARC-3', anti: 5'-GCNCCNARNACHGADRCRAA-3', anti: 5'-AYNKTRAARAAYTTNGCNCC-3', anti: 5'-TCYTCNGANCCRTCRTACCA-3') were used for amplification of Orco fragments from antennal cDNA template of *L. y-signata*, *T. domestica* and *P. siccifolium*. Antennal cDNA of *S. gregaria*, respectively *M. sexta* was used as positive control. Advantage® 2 Polymerase Mix (Clontech, USA), 1 µl of antennal cDNA and 100 pmol of each degenerated sense and antisense primer were used in a standard 25 µl PCR reaction. PCR conditions used were: 2 min at 95°C, then 40 cycles with 95°C for 30 s, 53°C for 40 s and 68°C for 1 min, followed by incubation for 7 min at 68°C. The conditions allowed for an amount of unspecific amplification even in the positive controls. PCR products were run on 1.2% agarose gels and visualized by ethidium bromide. We used all possible combinations of primers published by Yang et al., 2012 and our custom made primers. Resultant amplificates were cloned and sequenced.

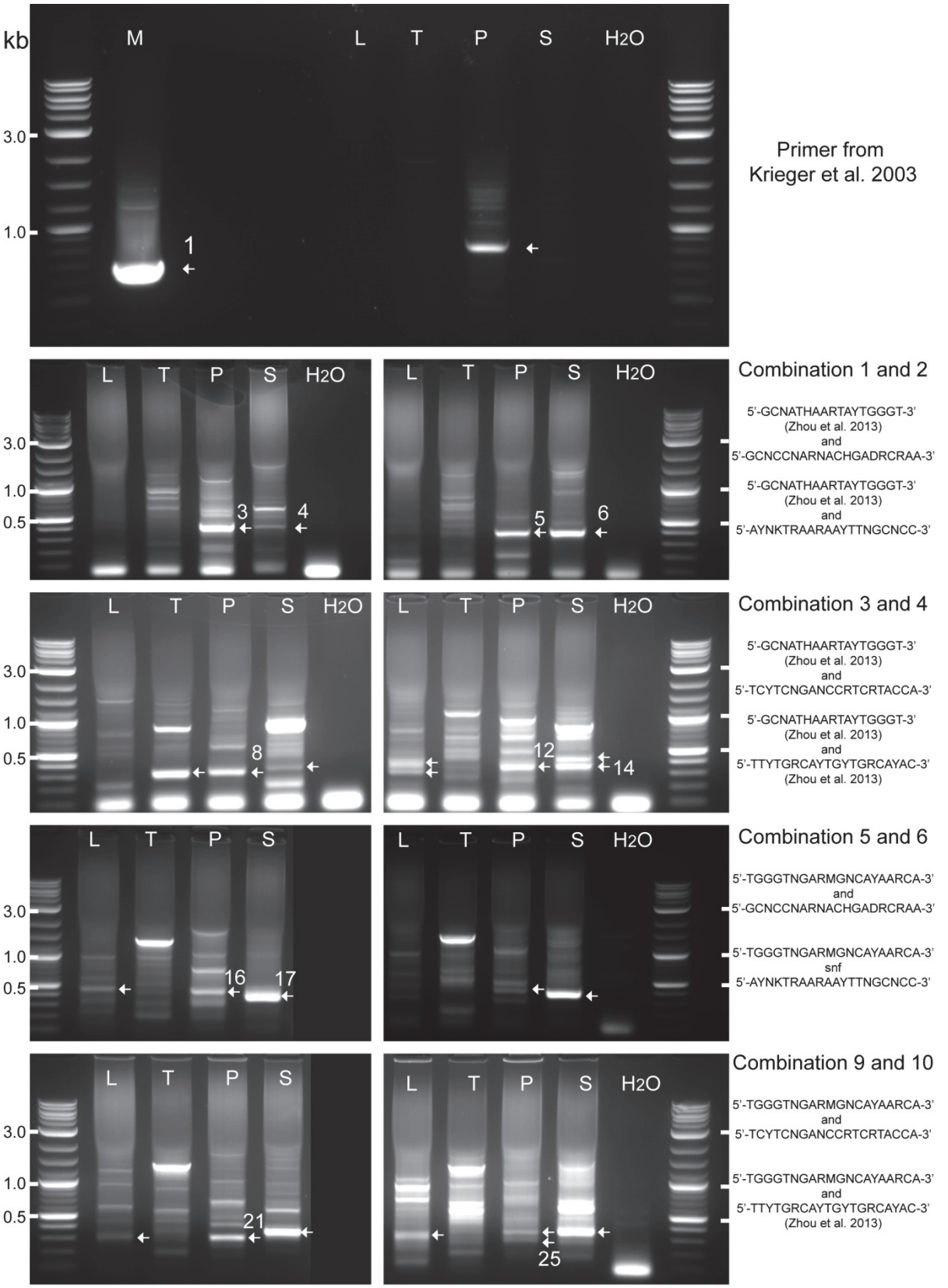


Figure 4. **PCR products of different primer combinations using antennal cDNA of *Lepismachilis y-signata*, T*hermobia domestica*, *Phyllium siccifolium* and *Schistocerca gregaria* as templates**
For primers from Krieger et al. 2003, *Manduca sexta* antennal cDNA was included as positive control. All fragments labeled with an arrow were cut, cloned and sequenced. Fragments coding for Orco were additionally labeled with a number.

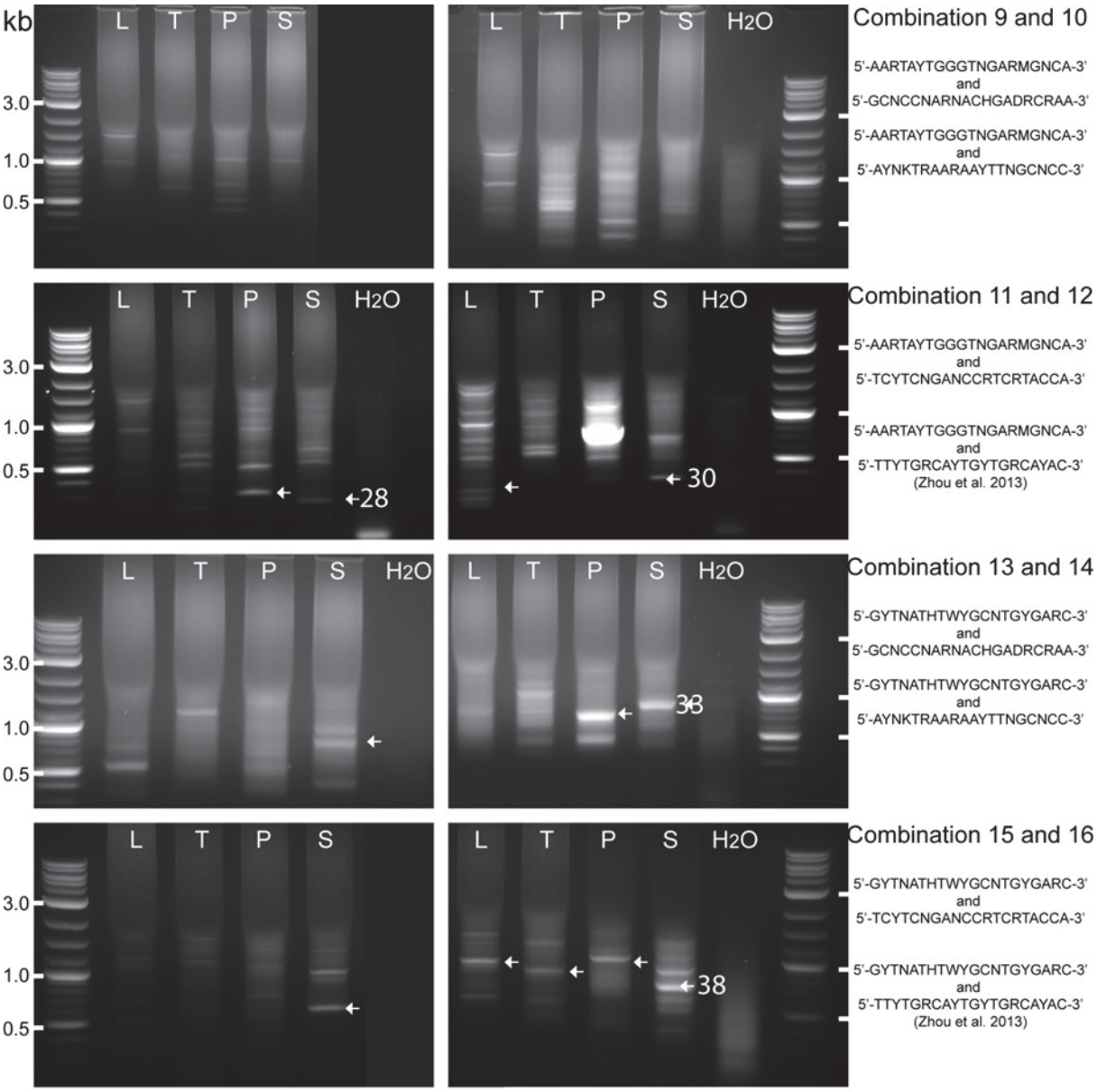


Figure 5. PCR products of different primer combinations using antennal cDNA of *Lepismachilis y-signata*, T*hermobia domestica*, *Phyllium siccifolium* and *Schistocerca gregaria* as templates
All fragments labeled with an arrow were cut, cloned and sequenced. Fragments coding for Orco were additionally labeled with a number.

Results

All possible combinations of primers (except the primers form Krieger et al. 2003) were used, deliberately choosing conditions that allowed for a limited amount of unspecific amplification even in the positive controls. All resultant amplificates of even remotely applicable size were cloned and sequenced. Using this approach Orco fragments were successfully amplified and identified for *S. gregaria* and *P. siccifolium*, but not for *T. domestica* and *L. y-signata.*

Chapter II

Identification of Odorant Binding Proteins in Antennal Transcriptomes of the jumping bristletail *Lepismachilis y-signata* and the firebrat *Thermobia domestica:* Evidence for an independent OBP-OR origin

Christine Missbach, Heiko Vogel, Bill S. Hansson and Ewald Grosse-Wilde

Manuscript

Identification of Binding Proteins in Antennal Transcriptomes of the jumping bristletail *Lepismachilis y-signata* and the firebrat *Thermobia domestica:* Evidence for an independent OBP-OR origin

Christine Missbach[1], Heiko Vogel[2], Bill S. Hansson[1]*, Ewald Grosse-Wilde[1]*

[1] Department of Evolutionary Neuroethology, Max Planck Institute for Chemical Ecology, 07745 Jena, Germany

[2] Department of Entomology, Max Planck Institute for Chemical Ecology, 07745 Jena, Germany

* these authors share senior authorship

Correspondence to be sent to: Ewald Grosse-Wilde, Department of Evolutionary Neuroethology, Max Planck Institute for Chemical Ecology, Jena, Germany. email: grosse-wilde@ice.mpg.de

Keywords: ***odorant binding proteins, chemosensory proteins, evolution***

Abstract

Chemosensory protein (CSPs) and gustatory receptor genes (GRs) have been identified in all major arthropod groups. However, odorant binding proteins (OBPs) and olfactory receptor genes (ORs) are insect specific, suggesting that both gene families originated after the Hexapoda–Crustacea split (~470 mya). The seemingly parallel diversification of OBPs and ORs has been suggested as coevolution between these genes after insect terrestrialization. Because OBPs have not been identified in pre-neopteran lineages (e.g. Odonata, Ephemeropotera, Thysanura, Archaeognatha) we used the recently published transcriptomes of the jumping bristletail *Lepismachilis y-signata* and the firebrat *Thermobia domestica* to search for putative OBP and CSP sequences and analyze their relationship to binding proteins of other insects and crustaceans. Our results suggest an evolution and expansion of OBPs as an adaptation to a terrestrial insect lifestyle, independently from the emergence of ORs.

Introduction

The sense of smell is crucial for insect behavior, such as location of food and oviposition sites as well as intraspecific communication (Hansson and Anton, 2000; Carlsson and Hansson, 2006). Insect olfaction is mediated by olfactory sensory neurons (OSNs). These OSNs are located in cuticular structures called sensilla, with their dendrites extending into the sensillum shaft. The shaft is filled with an aqueous solution, the sensillum lymph, which is secreted by support cells and contains a variety of proteins, including the odorant binding (OBP) and chemosensory (CSP) proteins (Vogt and Riddiford 1981; Steinbrecht 1998). Insect OBPs and CSPs are small (12e smkDa), water soluble proteins mainly containing α-helical domains, but are folded in 2 distinct patterns (Lartigue et al. 2002; Sandler et al. 2000; Tegoni et al, 2004). OBPs are generally divergent both between and within species, sometimes displaying as little as 8% amino acid conservation (Pelosi et al. 2005). According to their primary protein structure, mainly characterized by signature cysteines, OBPs have been divided into 4 groups: classical, Plus-C, Minus-C and Atypical OBPs (Hekmat-Scafe et al. 2002; Pelosi et al. 2006; Xu et al. 2003; Zhou et al. 2010a, Zhou et al. 2010b). CSPs are generally more conserved, with often >50% identical residues between members of even phylogenetically distant species. Their signature constitutes of 4 cysteines connected by disulphide bridges between adjacent residues (Pelosi et al. 2005). There is evidence that OBPs and CSPs represent 2 classes of proteins performing similar roles. Both protein classes reversibly bind small ligands with dissociation constants in the micromolar range (Pelosi 2005). OBPs are likely involved in chemosensory perception, participating in the solubilization and transfer of odorants through the sensillum lymph (Grosse-Wilde et al. 2006; Pelosi 1994; Pophof 2004; Prestwich et al. 1995, Tsuchihara

et al. 2005; Vogt et al. 1991). Additionally, they are supposed to contribute to the sensitivity of the olfactory system (Gomez-Diaz et al. 2013) and could protect odors from enzymatic degradation (Chertemps et al. 2012; Gomez-Diaz et al. 2013). Since different OBPs are present in a particular olfactory sensillum type, OBPs may also play a role in olfactory coding (Hekmat-Scafe et al. 1997). Depending on the species, OBPs and CSPs are expressed in gustatory as well as in olfactory sensilla (Angeli et al. 1999; Galindo and Smith 2001). They are, however, not restricted to chemosensory tissues and may thus also participate in other physiological functions (Celorio-Mancera et al. 2012; Furusawa et al. 2008; Graham et al. 2003; Iovinella et al. 2011; for a review, see Pelosi et al. 2006). Some CSPs have been proposed as participating in developmental processes (Maleszka et al. 2007), others mediate physiological and behavioral transitions, as it has been shown in the locust (Guo et al. 2011). CSPs as well as OBPs could act in these processes as carriers of hormones and other regulatory compounds (Iovinella et al. 2013).

Similar to insect olfactory receptors (ORs), OBPs were only found in Hexapoda, whereas CSP and gustatory receptor genes (GRs) have been identified in all major arthropod groups (Pelosi et al. 2006; Pewhere-Arana et al. 2009; Sanchez-Gracia et al. 2009; Smadja et al. 2009; Wanner and Robertson 2008; Wanner et al. 2007). This suggests that the OBP and OR gene families originated within the hexapodan lineage, whereas the CSP and GR families were already present in the ancestor of Hexapoda, Crustacea and Chelicerata (~700 Mya) (Hedges et al. 2006). The evolution of insect OBPs and ORs was suggested as coevolution after insects have colonized land and a new need for mediation and detection of volatile odors arose (Vieira and Rozas 2011).

Because OBPs so far have not been investigated in non-neopteran lineages (e.g. Odonata, Ephemeroptera, Thysanura, Archaeognatha) we used the recently published transcriptomes of the jumping bristletail *Lepismachilis y-signata* (Archaeognatha:

Machilidae) and the firebrat *Thermobia domestica* (Zygentoma: Lepismatidae) for identification of putative OBP and CSP sequences and analyzed their relationship to binding proteins of other arthropods.

Both Archaeognatha and Zygentoma are primary wingless insects. Their phylogenetic position within the insects with Archaeognatha as sister group of Zygentoma and Pterygota (winged insects, Bitsch and Bitsch 2004; von Reumont et al. 2009) and the putative age of these insect orders (Archaeognatha 390 mya; Labandeira 1988) make them excellent models to study evolution of various character systems. Missbach et al. previously showed that both the jumping bristletail *L. y-signata* and the firebrat *T. domestica* possess an acute but reduced olfactory system (Missbach et al. 2014), however, both differ in their genetic equipment from other insects. Neither ORs nor their coreceptor Orco have been identified in extensive antennal and maxillary palp transcriptomes of the jumping bristletail. Similar to *L. y-signata*, no OR-like sequences have been identified in the transcriptome of *T. domestica*. However, multiple Orco variants were found to be present (Missbach et al. 2014). Altogether this suggests that insect ORs evolved long after insects´ colonized land and that there seems to be a change in the olfactory system between the last common ancestor of Archaeognatha and Dicondylia (Zygentoma and Pterygota) and the ancestor of Dicondylia. Therefore, the investigation of the datasets with respect to presence or absence of OBPs and CSPs will shed light on the evolution of these chemosensory gene families, and can answer the question about a putative coevolution of insect ORs and OBPs.

Material and Methods

Bioinformatics

An antennal transcriptome of *T. domestica* and antennal and maxillary palp transcriptomes of *L. y-signata* (EMBL-EBI, study accession No.: PRJEB5093, sample accession No: ERS384175, ERS384176, ERS384177) were used for identification of odorant binding proteins (OBPs) and chemosensory proteins (CSPs). Generation of sequences and sequence databases is described in Missbach et al. 2014. Within these databases we searched for OBPs and CSPs using both text searches in BLAST2GO annotation and tBLASTn using Geneious Pro 5.0.4. Template for tBLASTn searches were published amino acid sequences of *Drosophila melanogaster*, *Bombyx mori*, *Pediculus humanus*, *Apis mellifera*, *Acyrthosiphon pisum* and *Daphnia pulex* OBPs and/or CSPs (sequences taken from Vieira and Rozas 2011 for OBPs, and Kulmuni and Havukainen 2013 for CSPs) as well as identified sequences of both *L. y-signata* and *T. domestica.*

Contigs with similarity to a member of these gene families were edited and subject to personal scrutiny of blast results, as well as further analysis. ORFs were identified and translated into amino acid sequence in Geneious Pro 5.0.4 (Biomatters). Alignments with other members of the respective gene families were carried out using MAFFT (E-INS-I parameter set; Katoh et al. 2005). Dendrograms were calculated using approximate maximum likelihood analysis with FastTree2 (Liu et al. 2011; Price et al. 2009) and displayed and edited with FigTree (http://tree.bio.ed.ac.uk/software/figtree). For OBPs the terminology of Hekmat-Scafe et al. 2002 for the C-terminal cysteines was used, because it reflected the position of the C6 cysteines in the overall sequence alignment as well as the position of disulfide bridges present in Plus-C OBPs (Lagarde

et al. 2011). Additionally the position of C1 of Plus-C OBPs is not consistent between different publications (e.c. Hekmat-Scafe et al. 2002: C1a-X13-C1-C1b-X11-13-C1c). Again the cysteine bridge information of AgamOBP47 was used as basis for terminology (Lagarde et al. 2011).

Signal peptides

Signal peptides were identified using the PrediSi program (Hiller et al. 2004).

Secondary structure

Secondary structures of full length candidates were predicted using the online platform psipred (Jones 1999) and compared to the results of secondary structure prediction in Geneious 5.0.4.

Ethics statement

Lepismachilis y-signata and *Thermobia domestica* are invertebrates that do not require *IACUC* approval. Additionally they are not categorized as endangered or protected in Germany.

Results

CSP candidates of *L. y-signata* and *T. domestica*

In total we identified 3 CSP encoding candidates in the transcriptome of *L. y-signata* (supplementary material 1, 2). All candidate sequences contained a predicted ORF, and the predicted protein product contained a signal peptide for secretion. ORFs encoded between 116 and 129 amino acids. *L. y-signata* CSPs displayed the cysteine pattern C1-X_6-C2-X_{18}-C3-X_2-C4 (Figure 1B), a pattern that is similar to CSP cysteine patterns described for other species (Figure 1C). Secondary structure prediction resulted in identification of 2 5-helical CSPs and 1 6-helical CSP, which was in agreement with the position of the *L. y-signata* CSPs within the dendrogram (Figure 1A). In a maximum likelihood derived tree, 2 CSP candidates grouped together with 5-helical CSPs of other insects and crustacean sequences. The third CSP grouped within the 6-helical CSPs (Figure 1A).

The antennal transcriptome of *T. domestica* contained 6 candidate CSP-coding sequences (supplementary material 1, 2). While only 3 candidates contained a complete coding region, all sequences covered dissimilar but overlapping regions in an amino acid sequence alignment, suggesting that they represent unigenes. Full-length candidates also contained a signal peptide in the translated amino acid sequence. Their predicted ORFs encoded 121 to 135 amino acids. The cysteine pattern of *T. domestica* CSPs was C1-X_6-C2-X_{18}-C3-X_2-C4 (Figure 1B), the same pattern that was identified for *L. y-signata* CSPs. Only CSP candidates spanning all key cysteines were included in further sequence analyses. In the dendrogram all *T. domestica* sequences grouped within

189 the 6-helical CSPs (Figure 1A). This is also consistent with the secondary structure
190 prediction obtained by Psipred.
191
192 **OBP candidates of *L. y-signata* and *T. domestica***
193
194 In total we identified 40 OBP transcripts in the *L. y-signata* transcriptomes and
195 32 in the transcriptome of *T. domestica* (supplementary material 3, 4). Most *L. y-signata*
196 OBP sequences were available as full-length coding sequences. Out 40 OBP candidates
197 31 sequences contained both a start and a stop codon and the predicted amino acid
198 sequence included a signal peptide (31 out of the 39) suggesting secretion of the protein.
199 The predicted ORFs of OBP sequences encoded between 127 and 247 amino acids.
200 OBP candidates displayed the respective conserved cysteine pattern, although most of
201 the candidates had 2 additional cysteines in a comparable position (C1b-X_{10-16}-C1-X_{25-49}-C2-X_3-C3-X_{41-52}-C4-X_{9-22}-C5-X_8-C6-X_8-C6a). The first cysteine (C1b) was located
202
203 in an N-terminal position to the C1, the second (C6a) 8 amino acids more C-terminal to
204 the C6 (alignment Figure 2). The C1b was found to be present in 34 OBPs, the C6a was
205 identified in 36 OBPs. Both cysteines can also be found in some *Drosophila* OBPs, for
206 example the conserved OBP59a and many Plus-C OBPs. However, the characteristic
207 C1a, C1c, C6b and C6c of the Plus-C OBPs were absent in the *L. y-signata* OBPs. In
208 most of the cases the proline that always follows the C6a of Plus-C OBPs was also
209 missing. For maximum likelihood analysis only the 37 sequences that contained all 6
210 core cysteines were included. In the tree no *L. y-signata* OBPs clustered within the
211 GOBPs, PBPs, Minus-C or Plus-C OBPs (Figure 3A), although many branches within
212 the tree are not very well supported (indicated by line width). The likely cause is the
213 high dissimilarity of OBPs in general. Many *L. y-signata* OBPs clustered close to the
214 conserved OBP59a and OBP73a subgroup and Plus-C OBPs, including 1 candidate

OBP most similar to the OBP59a subgroup, and 1 candidate closest to the OBP73a subgroup (Figure 3A). The putative *L. y-signata* OBP73a homologue (LsigOBP1) shared 24.3% of amino acids with the pea aphid homologue ApisOBP4 and 19 % with the DmelOBP73a. The similarity was comparable to homologues of other species (e.g. BmorOBP39 shared 26.8% of amino acids with DmelOBP73a and ApisOBP4 shared 22.6%). The putative *L. y-signata* OBP59a homologue (LsigOBP2) displayed a similarity of 23.9% to DmelOBP59a. When other putative orthologues of OBP59a were included in the analysis, LsigOBP2 sorted again into the OBP59a subgroup with LsigOBP2 grouping basal to the other members of this subgroup (Figure 3B). This clustering was found using different analysis techniques and including OBPs of different species. All the other *L. y-signata* OBPs form distinct clusters within the likelihood tree, sometimes together with OBPs of *T. domestica*.

Only 11 out of 32 predicted *T. domestica* OBP sequences contained an ORF with both start and stop codon and a putative signal peptide-coding sequence. The general cysteine pattern of *T. domestica* OBPs was C1-X_{20-71}-C2-X_3-C3-X_{37-48}-C4-X_{8-29}-C5-X_8-C6. Only 5 OBP sequences contained the C1b and C6a cysteines that were described above. 3 of those OBPs contained an additional second pair of cysteines, including one cysteine in a position next to C1b and 1 cysteine in a position C-terminal of C6a. Both cysteines were in a comparable position to the C1b and C6b of Plus-C OBPs. Furthermore, these OBPs also share the conserved proline of Plus-C OBPs next to C6a (Figure 2).

In contrast to the above-mentioned additional cysteines, we identified 6 *T. domestica* OBPs with 2 additional cysteines between the C3 and C4 (Figure 4B), a pattern that seemed to be specific for these *T. domestica* OBPs. As for *L. y-signata* only OBP sequences that are long enough to cover all core cysteines were included into the maximum likelihood tree calculation. In the resulting dendrograms most *T. domestica*

241 OBP candidate sequences were distributed across the Classic-OBPs, mostly close to
242 some OBPs of the head louse *P. humanus*, the pea aphid *A. pisum* or the bristletail
243 OBPs; however none of the sequences clustered within Plus-C, Minus-C or Dimer
244 OBPs. Furthermore, 2 *T. domestica* OBPs (TdomOBP20 and TdomOBP19) were
245 repeatedly grouped within the ABPII cluster next to sequences of *P. humanus* or *A.*
246 *pisum*.

247 Similar to *L. y-signata*, a putative homologue of OBP73a was detected. This
248 OBP, TdomOBP1, shared 20.5% of amino acids with DmelOBP73a. A putative
249 OBP59a candidate orthologue (TdomOBP2) had a pairwise identity of 31.9% to
250 DmelOBP59a, but as the 5´end of the sequence was missing, it was too short to include
251 in the analysis.

252

Discussion

CSP candidates of *L. y-signata* and *T. domestica* belong to 5- and 6-helical CSPs

CSPs can be classified mainly into 2 groups, the ancient 5-helical CSPs and the typical 6-helical insect CSPs (Kulmuni and Havukainen 2013). 2 out of 3 candidate CSPs of *L. y-signata* grouped within the 5-helical CSPs, very close to the sequences of the crustacean *Daphnia pulex*. 5-helical CSPs have been identified across Arthropoda including Myriapoda and Crustacea (Iovinella et al. 2013; Kulmuni and Havukainen 2013) with the exception of *Ixodes scapularis*, where the published CSP belong to the 6-helical CSPs. However, the sequence could not be found in the genome (Iovinella et al. 2013) and its existence needs to be validated. In all other species with a proposed complete set of CSPs at least 1 5-helical CSP has been described (Kulmuni and Havukainen 2013). For *T. domestica* we could not identify any 5-helical CSPs in our antennal dataset. Despite the lack of 5-helical CSPs in antennal tissue, expression elsewhere in the body is still possible, especially since some authors suggest that 5-helical CSPs do not function in chemosensation (Kulmuni and Havukainen 2013). For example in the honeybee *A. mellifera,* the 5-helical AmelCSP5 is only expressed in ovaries and eggs and has been identified as a regulator of embryonic development (Maleszka et al. 2007).

In more general terms, the number of CSPs is highly variable between species. In the locust *Locusta migratoria manilensis* 70 CSP genes have been identified (Zhou et al., 2012), whereas in *D. melanogaster* only 4 have been described (Wanner et al. 2004). In non-insect arthropods only a limited number of CSPs has been found in any given species, for example 3 in *D. pulex* and *Artemia franciscana*, and 4 in *Julida* sp

(Iovinella et al. 2013; Vieira and Rozas 2011). Similar numbers were also identified for the wingless insects *L. y-signata* and *T. domestica* in the present study, indicating that a rather small number of CSPs might be the ancestral state of insects.

Evolution of OBPs

In contrast to the relatively small number of CSPs, 40 OBPs were identified from the transcriptome of *L. y-signata* and 32 from that of the *T. domestica*. Many OBPs found in *L. y-signata* cluster close to DmelOBP59a and DmelOBP73a. These 2 OBPs have clear orthology relationships across insects (except in Hymenoptera, Zhou et al. 2010b), suggesting a critical and conserved role of these proteins. At least for DmelOBP59a a function in *Drosophila* olfaction has been suggested. Reduced expression of DmelOBP59a affects the detection of 1-hexanol, 2-heptanone and propanal (Swarup et al. 2011). It is conceivable that OBPs have a similar role in *L. y-signata* olfaction, where a broad response to an odor spectrum has been shown (Missbach et al. 2014). These odors have to travel through the sensillum lymph to be detected by the membrane-bound receptors, implying the presence and importance of carrier proteins. An alternative hypothesis regarding the evolution of OBPs came from Shanbhag et al. (2001). For OBP19d of *D. melanogaster* an expression in coeloconic and gustatory sensilla, but also in epidermal cells and subcuticular space of the funiculus and maxillary palp was reported (Shanbhag et al. 2001). One hypothesis for the presence of OBPs in epidermal cells was that these cells have to secrete apolar, water-insoluble substances into the cuticle, especially for the epicuticular layers (for review see Locke, 1998). These materials have to pass through the aqueous environment in the cells and in the inner cuticle, thus maybe necessitating the establishment of a transfer system based on OBP-like carrier proteins. Additionally, epidermal cells are the

precursors of sensillum cells (for review see Keil 1997) and OBPs could have evolved by specialization from those general carrier proteins (Shanbhag et al. 2001). Since both *L. y-signata* and *T.domestica* molt during their whole lifespan, a permanent turnover of cuticular material is very likely and maybe requires a high number of different carrier proteins like OBPs.

Another interesting aspect concerns the cysteine pattern of OBPs. The cysteine pattern that was identified in most of *L. y-signata* and some *T. domestica* OBPs, with 1 additional cysteine each at the C-and N-terminus, can also be found in all OBP59a and most of the OBP73a orthologues. The strong conservation of the additional cysteines between the *L. y-signata* OBP candidates suggests a critical role of these cysteines, maybe forming an additional disulfide bridge, as described for AgamOBP47 (Lagarde et al. 2011) where the 2 cysteines in a comparable position to the cysteins of *L. y-signata* OBPs form a disulfide bond. Furthermore, in *T. domestica* we found OBP candidates with another 2 additional cysteines, 1 C- and 1 N-terminal to the C1b and C6a in a very similar position to the C1c and the C6b of Plus-C OBPs. It seems that there is a successive addition or reduction of cysteines within the evolution of Plus-C OBPs. Plus-C OBPs evolved only once in insects (Vieira and Rozas 2011) with a secondary lost in Hymenoptera. The present scenario with 8 cysteines in most *L. y-signata* OBPs might have been reached either from a classic 6 cysteine OBP with an expansion of 8 cysteine OBPs in *L. y-signata* or from an ancient 8 cysteine pattern of OBPs to Plus-C OBPs and the common 6 cysteine pattern of classic OBPs.

Whether OBPs do have an impact on bristletail and firebrat olfactory functions or not, needs to be investigated. Nevertheless, the OBPs of *L. y-signata* and *T. domestica* are the oldest OBPs identified so far, suggesting the presence of OBPs in the last common ancestor of Insecta *sensu stricto*. A selective pressure leading to the diversification of an existing gene family to fill the function as mediator of airborne

molecules to their detectors, after terrestrialization can well be imagined (Vieira and Rozas 2011). The detectors of these airborne molecules are mainy ORs. However, ORs were found to be absent in *L. y-signata* and *T. domestica* (Missbach et al. 2014). Therefore olfaction seems to be based on the evolutionary older variant ionotropic glutamate receptors and GRs in these animals. This fact adds further to the idea of an independent origin of OBP and OR gene families and a cooption of OBPs in a OR/Orco mediated detection of odorants at a later point in insect evolution.

Funding

The work was supported by the Max Planck Society.

Acknowledgements

We gratefully thank Katrin Groh for constructive comments on the manuscript.

343 ## References

344

345 Angeli S, Ceron F, Scaloni A, Monti M, Monteforti G, Minnocci A, Petacchi R, Pelosi
346 P. 1999. Purification, structural characterization, cloning and
347 immunocytochemical localization of chemoreception proteins from *Schistocerca*
348 *gregaria*. Eur J Biochem. 262:745-754.

349 Bitsch C, Bitsch J 2004. Phylogenetic relationships of basal hexapods among the
350 mandibulate arthropods: a cladistic analysis based on comparative
351 morphological characters. Zool Scr. 33:511-550.

352 Celorio-Mancera M de la P., Sundmalm SM, Vogel H, Rutishauser D, Ytterberg J,
353 Zubarev RA, Janz N. 2012. Chemosensory proteins, major salivary factors in
354 caterpillar mandibular glands. Insect Biochem Molec. 42:796-805.

355 Chertemps T, Francois A, Durand N, Rosell G, Dekker T, Lucas P, Maïbèche-Coisne.
356 2012. A carboxylesterase, Esterase-6, modulates sensory physiological and
357 behavioral response dynamics to pheromone in *Drosophila*. BMC Biol. 10:56.

358 Furusawa T, Rakwal R, Nam HW, Hirano M, Shibato J, Kim YS, Ogawa Y, Yoshida Y,
359 Kramer KJ, Kouzuma Y et al. 2008. Systematic investigation of the hemolymph
360 proteome of *Manduca sexta* at the fifth instar larvae stage using one- and two-
361 dimensional proteomics platforms. J Proteom Res. 7:938.

362 Galindo K, Smith DP. 2001. A large family of divergent *Drosophila* odorant-binding
363 proteins expressed in gustatory and olfactory sensilla. Genetics. 159:1059–1072.

364 Gomez-Diaz C, Reina JH, Cambillau C, Benton R. 2013. Ligands for Pheromone-
365 Sensing Neurons Are Not Conformationally Activated Odorant Binding
366 Proteins. PLoS Biol. 114:e1001546.

Grosse-Wilde E, Svatos A, Krieger J. 2006. A pheromone binding protein mediates the bombykol-induced activation of a pheromone receptor in vitro. Chem Senses. 31:547-555.

Guo W, Wang X, Ma Z, Xue L, Han J, Yu D, Kang L. 2011. CSP and Takeout Genes Modulate the Switch between Attraction and Repulsion during Behavioral Phase Change in the Migratory Locust. PLoS Genet. 7:e1001291.

Hedges SB, Dudley J, Kumar S. 2006. TimeTree: a public knowledge-base of divergence times among organisms. Bioinformatics. 22:2971-2972.

Hekmat-Scafe DS, Steinbrech RA, Carlson JR. 1997. Coexpression of Two Odorant Binding Protein Homologs in *Drosophila*: Implications for Olfactory Coding. J Neurosci. 17:1616-1624.

Hekmat-Scafe DS, Scafe CR, McKinney AJ and Tanouye MA. 2002. Genome-wide analysis of the odorant-binding protein gene family in *Drosophila melanogaster*. Genome Res. 12:1357-1369.

Hiller K, Grote A, Scheer M, Münch R, Jahn D. 2004. PrediSi: prediction of signal peptides and their cleavage positions. Nucl Acids Res. 32 suppl 2:W375-W379.

Iovinella I, Dani FR, Niccolini A, Sagona S, Michelucci E, Gazzano A, Turillazzi S, Felicioli A, Pelosi P. 2011. Differential expression of odorant-binding proteins in the mandibular glands of the honey bee according to caste and age. J Proteom Res. 10:3439-3449.

Iovinella I, Bozza F, Caputo B, della Torre A, Pelosi P. 2013. Ligand-Binding Study of *Anopheles gambiae* Chemosensory Proteins. Chem Senses. 38:409-419.

Jones DT. 1999. Protein secondary structure prediction based on position-specific scoring matrices. J Mol Biol. 292:195-202.

Katoh K, Kuma K, Toh H, Miyata T. 2005. MAFFT version 5: improvement in accuracy of multiple sequence alignment. Nucleic Acids Res. 33:511-518.

393 Keil TA. 1997. Comparative morphogenesis of sensilla: a review. Int J Insect Morphol.
394 26:151-160.
395 Kulmuni J, Havukainen H. 2013. Insights into the Evolution of the CSP Gene Family
396 through the Integration of Evolutionary Analysis and Comparative Protein
397 Modeling. PLoS ONE. 8:e63688.
398 Labandeira CC, Beall BS, Hueber FM. 1988. Early insect diversification - Evidence
399 form a lower devonian bristletail from Quebec. Science. 242:913-916.
400 Lagarde A, Spinelli S, Qiao H, Tegoni M, Pelosi P, Cambillau C. Crystal structure of a
401 novel type of odorant-binding protein from *Anopheles gambiae*, belonging to the
402 C-plus class. J Biochem. 437:423-30.
403 Lartigue A, Campanacci, Roussel A, Larsson AM, Jones TA, Tegoni M, Cambillau C.
404 2002. X-ray structure and ligand binding study of a moth chemosensory protein.
405 J Biol Chem. 277:32094-32098.
406 Liu K, Linder CR, Warnow T. 2011. RAxML and FastTree: Comparing Two Methods
407 for Large-Scale Maximum Likelihood Phylogeny Estimation: Plos One. 6:
408 e27731.
409 Maleszka J, Foret S, Saint R, Maleszka R. 2007. RNAi-induced phenotypes suggest a
410 novel role for a chemosensory protein CSP5 in the development of embryonic
411 integument in the honeybee (*Apis mellifera*). Dev Genes and Evol. 217:189-196.
412 Matsuo T, Sugaya S, Yasukawa J, Aigaki T, Fuyama Y. 2007. Odorant-binding proteins
413 OBP57d and OBP57e affect taste perception and host-plant preference in
414 *Drosophila sechellia*. PLoS Biol. 5:e118.
415 Missbach C, Dweck HKM, Vogel H, Vilcinskas A, Stensmyr MC, Hansson BS, Grosse-
416 Wilde E. accepted. Evolution of insect olfactory receptors. ELife.

417 Nagnan-Le Meillour P, Cain AH, Jacquin-Joly E, François MC, Ramachandran S,
418 Maida R, Steinbrecht RA. 2000. Chemosensory proteins from the proboscis of
419 *Mamestra brassicae*. Chem Senses. 25:541-53.
420 Nomura A, Kawasaki K, Kubo T, Natori S. 1992. Purification and localization of p10, a
421 novel protein that increases in nymphal regenerating legs of *Periplaneta*
422 *americana* (American cockroach). Int J Dev Biol. 36:391- 398.
423 Pelosi P. 1994. Odorant-binding proteins. Crit Rev Biochem Mol Biol. 29:199-228.
424 Pelosi P, Calvello M, Ban L. 2005. Diversity of odorant-binding proteins and
425 chemosensory proteins in insects. Chem Senses. 30 suppl 1:i291-292.
426 Pelosi P, Zhou JJ, Ban LP, Calvello M. 2006. Soluble proteins in insect chemical
427 communication. Cell Mol Life Sci. 63:1658-1676.
428 Peñalva-Arana DC, Lynch M, Robertson HM. 2009. The chemoreceptor genes of the
429 waterflea *Daphnia pulex*: many GRs but no ORs. BMC Evol Biol. 9:79.
430 Pophof B. 2004. Pheromone-binding proteins contribute to the activation of olfactory
431 receptor neurons in the silkmoths *Antheraea polyphemus* and *Bombyx mori*.
432 Chem Senses. 29:117-125.
433 Prestwich GD, Du G, LaForest S. 1995. How is pheromone specificity encoded in
434 proteins? Chem. Senses. 20:461-469.
435 Price MN, Dehal PS, Arkin AP. 2009. FastTree: Computing Large Minimum Evolution
436 Trees with Profiles instead of a Distance Matrix. Mol Biol Evol. 26:1641-1650.
437 Sanchez-Gracia A, Vieira FG, Rozas J. 2009. Molecular evolution of the major
438 chemosensory gene families in insects. Heredity. 103:208-216.
439 Sandler BH, Nikonova L, Leal WS, Clardy J. 2000. Sexual attraction in the silkworm
440 moth: structure of the pheromone-binding-protein–bombykol complex. Chem
441 Biol. 7:143–151.

Shanbhag SR, Hekmat-Scafe D, Kim MS, Park SK, Carlson JR Pikielny C, Smith DP, Steinbrecht RA. 2001. Expression mosaic of odorant-binding proteins in *Drosophila* olfactory organs. Microsc Res Tech. 55:297-306.

Smadja C, Shi P, Butlin RK, Robertson HM. 2009. Large Gene Family Expansions and Adaptive Evolution for Odorant and Gustatory Receptors in the Pea Aphid, *Acyrthosiphon pisum*. Mol Biol Evol. 26:2073-2086.

Steinbrecht RA. 1998. Odorant-binding proteins: expression and function. Ann N Y Acad Sci. 855:323-332.

Swarup S, Williams IW, Anholt RRH. 2011. Functional Dissection of Odorant Binding Protein Genes in *Drosophila melanogaster*. Genes Brain Behav. 10:648-657.

Tegoni M, Campanacci V, Cambillau C. 2004. Structural aspects of sexual attraction and chemical communication in insects. Trends Biochem Sci. 29:257-264.

Tsuchihara K, Fujikawa K, Ishiguro M, Yamada T, Tada C, Ozaki K, Ozaki M. 2005. An Odorant-binding Protein Facilitates Odorant Transfer from Air to Hydrophilic Surroundings in the Blowfly. Chem Senses. 30:559-564.

Vieira FG, Rozas J. 2011. Comparative Genomics of the Odorant-Binding and Chemosensory Protein Gene Families across the Arthropoda: Origin and evolutionary history of the chemosensory system. Genome Biol Evol. 3:476-490.

Vogt RG, Riddiford LM. 1981. Pheromone binding and inactivation by moth antennae. Nature. 293:161-163.

Vogt RG, Prestwich GD, Lerner MR. 1991. Odorant-binding-protein subfamilies associate with distinct classes of olfactory receptor neurons in insects. J Neurobiol. 22:74-84.

von Reumont BM, Meusemann K, Szucsich NU, Dell'Ampio E, Gowri-Shankar V, Bartel D, Simon S, Letsch HO, Stocsits RR, Luan Y et al. 2009. Can

comprehensive background knowledge be incorporated into substitution models to improve phylogenetic analyses? A case study on major arthropod relationships. Bmc Evol Biol. 9:1-19.

Wanner KW, Willis LG, Theilmann DA, Isman MB, Feng Q, Plettner E. 2004. Analysis of the insect OS-D-like gene family. J Chem Ecol. 30:889-911.

Wanner, K.W., Anderson AR, Trowell SC, Theilmann DA, Robertson HM, Newcomb RD. 2007. Female-biased expression of odourant receptor genes in the adult antennae of the silkworm, *Bombyx mori*. Insect Mol Biol. 16:107-119.

Wanner KW, Robertson HM. 2008. The gustatory receptor family in the silkworm moth *Bombyx mori* is characterized by a large expansion of a single lineage of putative bitter receptors. Insect Mol Biol. 17:621-629.

Xu PX, Zwiebel LJ and Smith DP. 2003. Identification of a distinct family of genes encoding atypical odorant-binding proteins in the malaria vector mosquito, *Anopheles gambiae*. Insect Mol Biol. 12:549-560.

Xu P, Atkinson R, Jones DN, Smith DP. 2005. *Drosophila* OBP LUSH is required for activity of pheromone-sensitive neurons. Neuron. 45:193-200.

Zhou JJ, Kan Y, Antoniw J, Pickett JA, Field LM. 2006. Genome and EST analyses and expression of a gene family with putative functions in insect chemoreception. Chem Senses. 31:453-465.

Zhou JJ, Field LM and He XL. 2010a. Insect odorant-binding proteins: do they offer an alternative pest control strategy? Outlooks Pest Manag. 21:31-34.

Zhou, J.J., Vieira FG, He XL, Smadja C, Liu R, Rozas J, Field LM. 2010b. Genome annotation and comparative analyses of the odorant-binding proteins and chemosensory proteins in the pea aphid *Acyrthosiphon pisum*. Insect Mol Biol. 19 suppl 2: 113-122.

493 Zhou XH, Ban LP, Iovinella I, Zhao LJ, Gao Q, Felicioli A, Sagona S, Pieraccini G,
494 Pelosi P, Zhang L et al. 2012. Diversity, abundance and sex-specific expression
495 of chemosensory proteins in the reproductive organs of the locust *Locusta*
496 *migratoria manilensis*. Biol Chem. 394:43-54.
497

Figure capture

Fig1: Evolution of CSPs.

A: Approximate Maximum likelihood tree of arthropod CSPs. 5- and 6-helical CSPs form distinct branches within the CSP family. 2 *Lepismachilis y-signata* CSPs belong to the 5-helical group, whereas all *Thermobia domestica* CSPs sorted into the branch of 6-helical CSPs. The secondary structure is indicated next to the CSP sequences of *L. y-signata* and *T. domestica* (red box: α-helix; blue arrowhead: β-sheet). Branches are colored according to the different taxa (Crustacea - light green, Archaeognatha - dark red, Zygentoma - magenta, Hemiptera - cyan, Hymenoptera - yellow, Coleoptera - purple, Diptera - blue, Lepidoptera - green). Line width of branches reflects the likelihood based support values with thick branches having a high support value.

B: Sequence alignment of *L. y-signata* and *T. domestica* CSPs that are also represented in the tree. Conserved cysteines are highlighted by black boxes.

C: Cysteine pattern of *L. y-signata* and *T. domestica* CSPs in comparison with CSPs of other insects, emphasizing the highly conserved nature of the cysteine pattern. Data for other insect taxa were taken from Xu et. al 2009.

Fig2: Successive gain of cysteines leads to the insect Plus-C OBPs.

Sequence alignment of *Lepismachilis y-signata* OBPs, *Thermobia domestica* OBPs and Plus-OBPs displaying the additional C1c and C6a cysteines. Conserved cysteines are highlighted by red boxed. The description next to the red boxes is referring to the terminology used for cysteines in this paper. Names of Plus-C OBPs of other insects are highlighted in blue. Below the Plus-C OBPs 3 OBP sequences of *T. domestica* can be found. These sequences not only possess the additional C1c and C6b, but also have the conserved proline (blue box) next to C6a.

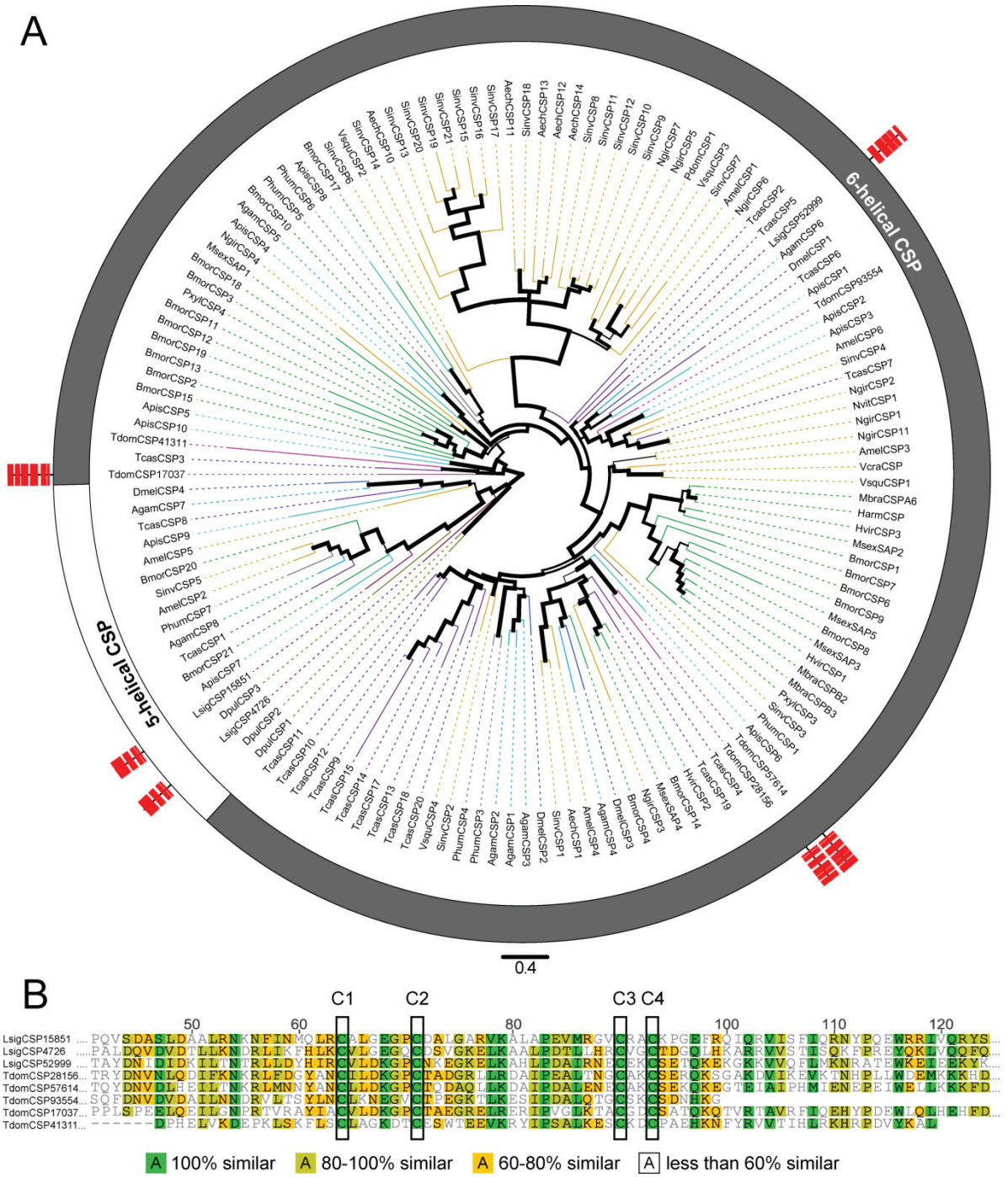
524 **Fig3: Evolution of insect OBPs.**

525 A: Approximate Maximum likelihood tree of insect OBPs. Branches leading to an OBP
526 of a certain species are labeled in different colors (*Lepismachilis y-signata* - dark red,
527 *Thermobia domestica* – magenta, *Acyrthosiphon pisum* – cyan, *Pediculus humanus* –
528 grey, *Drosophila* sp. –blue). The outer circle indicates the different OBP subfamilies
529 according to Vieira and Rozas 2011 (Classic OBPs - black, Minus-C OBPs - green,
530 Plus-C OBPs - blue, Dimers - red, ABPII - gray). The secondary structure information
531 of *L. y-signata* and *T. domestica* OBPs is given outside the circle next to the candidate
532 (red box: α-helix; blue arrowhead: β-sheet). Line width of branches reflects the
533 likelihood based support values.
534 B: Subtrees of the conserved OBP73a and OBP59a subgroups.
535

536 **Fig4: Evolution of OBPs in Lepismachilis *y-signata* and *Thermobia domestica.***

537 A: Unrooted approximate Maximum likelihood tree of *Lepismachilis y-signata* and
538 *Thermobia domestica* OBPs. Changes of cysteine patterns are marked by arrowheads
539 along the branches. Important groups are highlighted by different background colors. B:
540 Alignment of the 6 *T. domestica* OBPs that possess 2 additional cysteines between C3
541 and C4, named here C3a and C3b.
542 C: Comparison between cysteine pattern of different insect taxa. Data for other insect
543 taxa were taken from Xu et al. 2009.
544

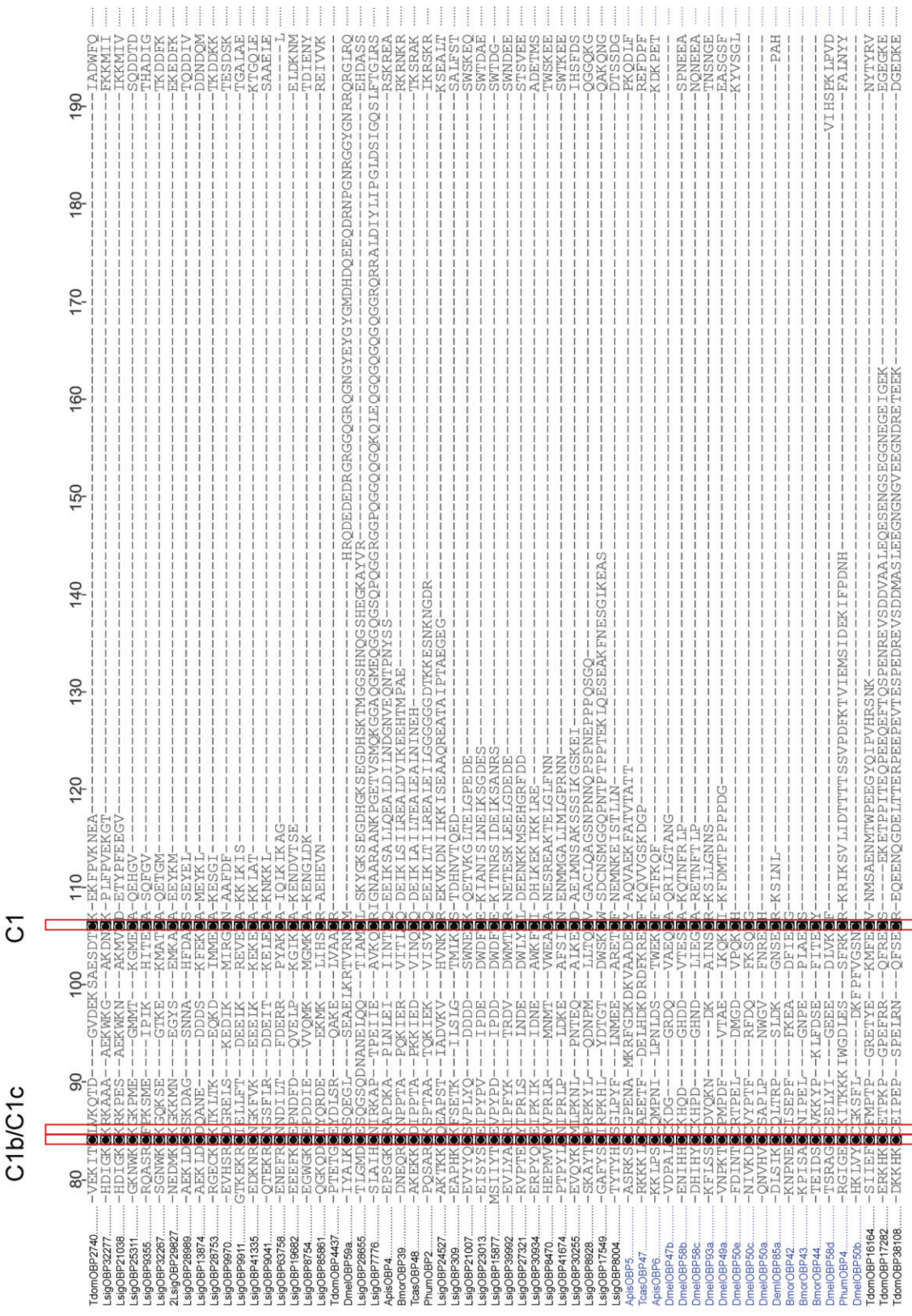
545



L.y-signata	3	C1-X6-C2-X18-C3-X2-C4
T.domestica	6	C1-X6-C2-X18-C3-X2-C4
Orthoptera		C1-X6-8-C2-X18-19-C3-X2-C4
Hemiptera		C1-X5-6-C2-X18-19-C3-X2-C4
Hymenoptera		C1-X6-8-C2-X18-19-C3-X2-C4
Coleoptera		C1-X6-8-C2-X18-C3-X2-C4
Diptera		C1-X6-8-C2-X18-C3-X2-C4
Lepidoptera		C1-X6-C2-X18-C3-X2-C4

546

547 **Figure 1: Evolution of CSPs.**


C1b/C1c
C1
C1
C1b/C1c

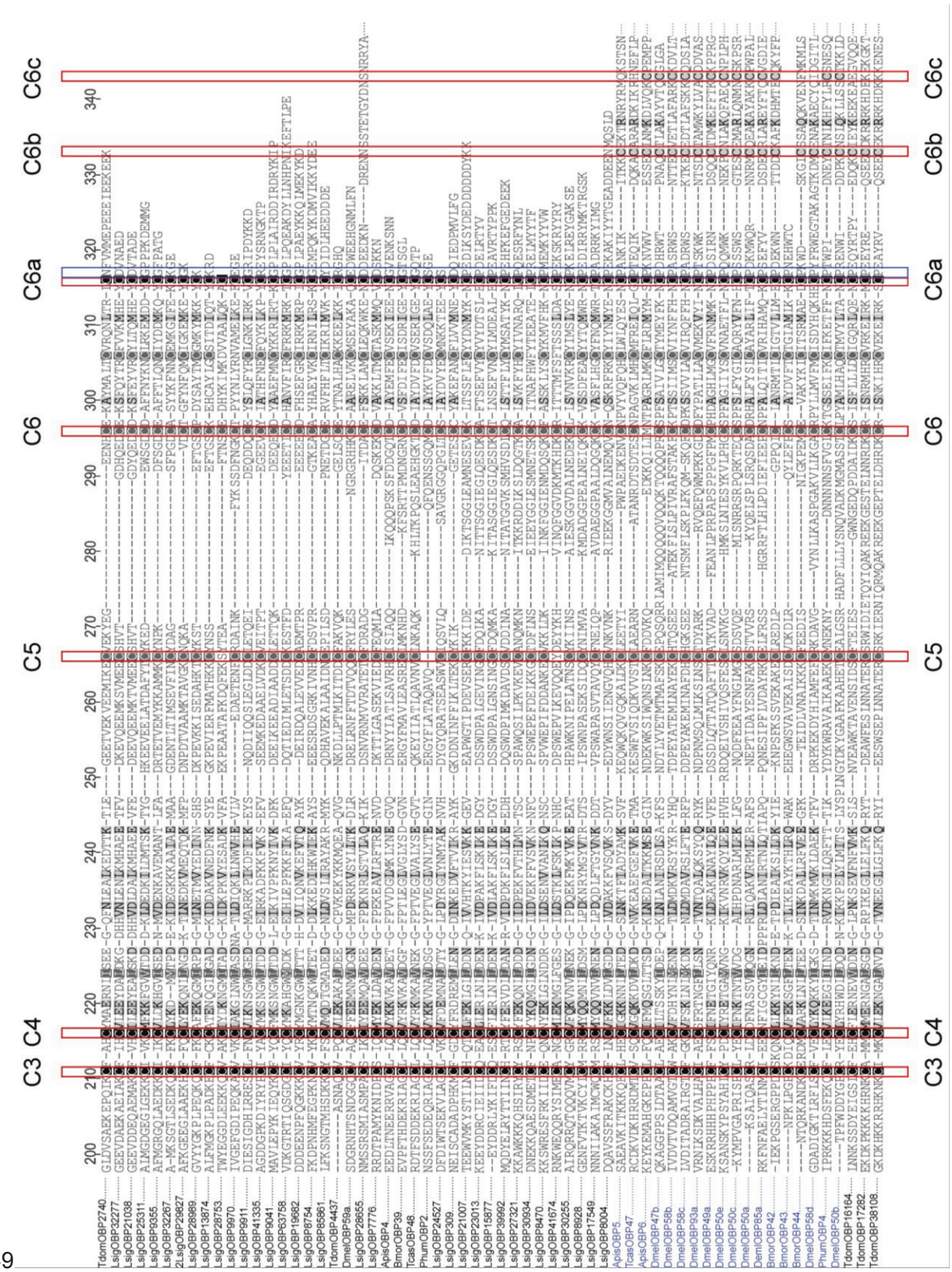


Figure 2: Successive gain of cysteine pairs leads to Plus-C OBPs.

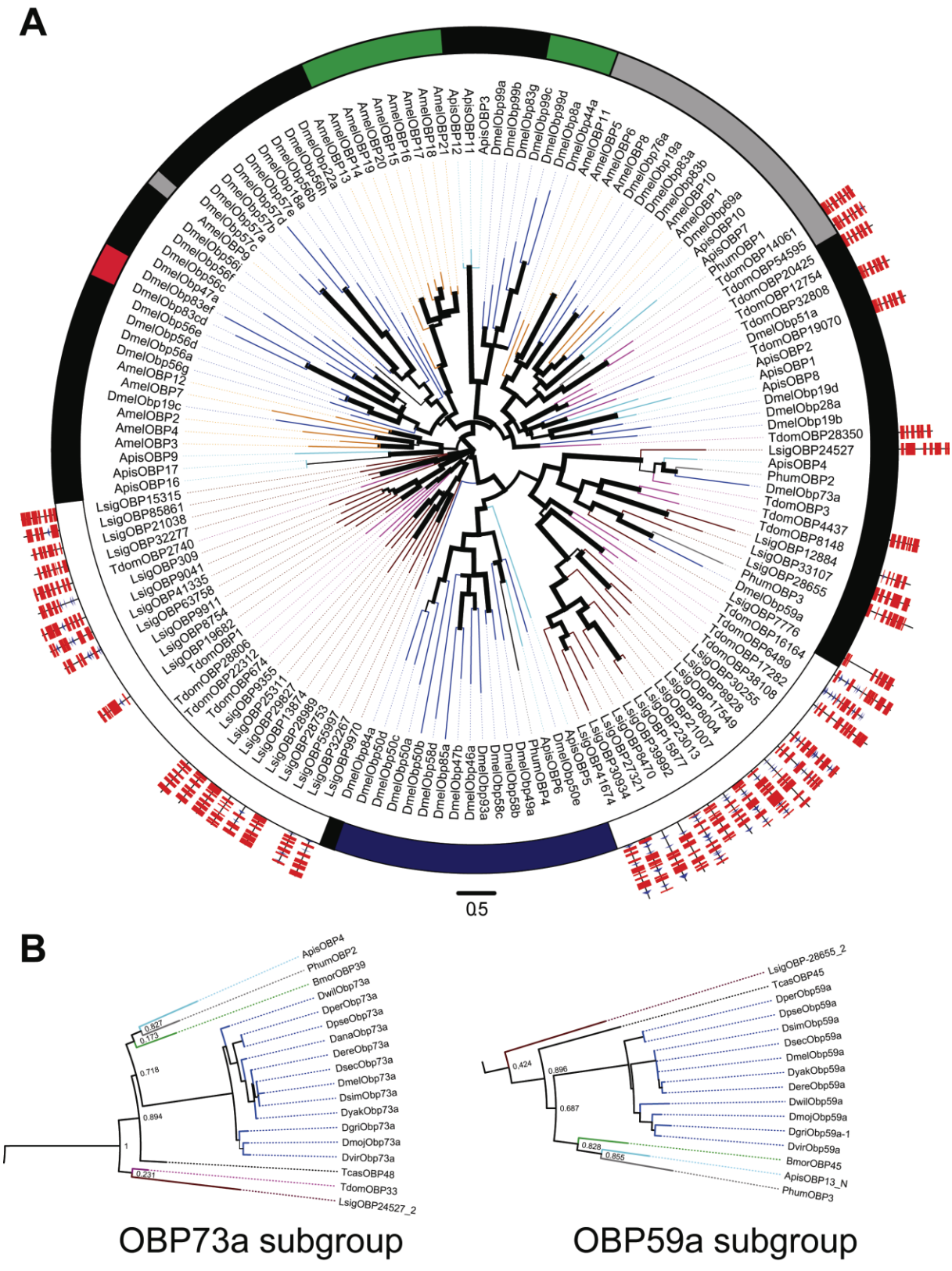
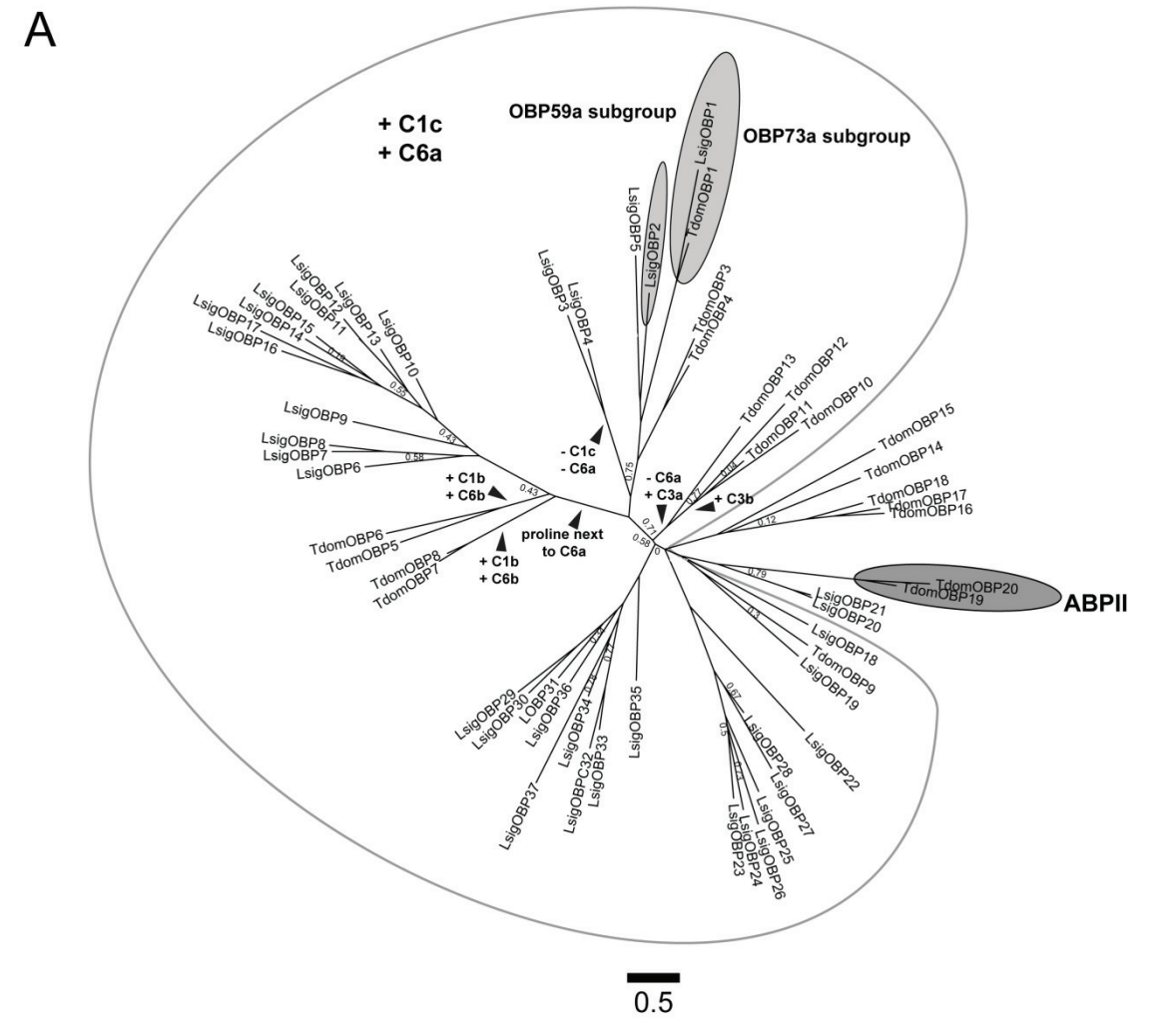


Figure 3: Evolution of insect OBPs.



L.y-signata	40	C1c-X10-24-C1-X25-49-C2-X3-C3-X41-52-C4-X9-22-C5-X8-C6-X8-C6a
T.domestica	32	C1-X20-71-C2-X3-C3-X37-48-C4-X8-29-C5-X8-C6
Orthoptera		C1-X26-27-C2-X3-C3-X35-41-C4-X8-12-C5-X8-C6
Hemiptera		C1-X22-32-C2-X3-C3-X36-46-C4-X8-14-C5-X8-C6
Hymenoptera		C1-X23-35-C2-X3-C3-X27-45-C4-X7-14-C5-X8-C6
Coleoptera		C1-X23-44-C2-X3-C3-X36-43-C4-X8-12-C5-X8-C6
		C1-X21-68-C2-X3-C3-X21-46-C4-X8-28-C5-X8-9-C6
Diptera		C1-X20-58-C2-X3-C3-X55-76-C4-X9-C5-X8-C6-X10-11-C7-X7-11-C8
Lepidoptera		C1-X25-30-C2-X3-C3-X36-42-C4-X8-14-C5-X8-C6

Figure 4: Evolution of OBPs in *L. y-signata* and *T. domestica.*

Chapter III

Variant Ionotropic Receptors Are Expressed in Olfactory Sensory Neurons of Coeloconic Sensilla on the Antenna of the Desert Locust (*Schistocerca gregaria*)

Mei Guo, Jürgen Krieger, Ewald Große-Wilde, Christine Mißbach, Long Zhang and Heinz Breer

Int. J. Biol. Sci. 2014, Vol. 10 1

IVYSPRING INTERNATIONAL PUBLISHER

International Journal of Biological Sciences

2014; 10(1):1-14. doi: 10.7150/ijbs.7624

Research Paper

Variant Ionotropic Receptors Are Expressed in Olfactory Sensory Neurons of Coeloconic Sensilla on the Antenna of the Desert Locust (*Schistocerca gregaria*)

Mei Guo[1], Jürgen Krieger[2], Ewald Große-Wilde[3], Christine Mißbach[3], Long Zhang[1] and Heinz Breer[2]✉

1. China Agricultural University, Key Lab for Biological Control of the Ministry of Agriculture, Beijing 100193, China
2. University of Hohenheim, Institute of Physiology, 70593 Stuttgart, Germany
3. Department of Evolutionary Neuroethology, Max Planck Institute for Chemical Ecology, Jena, Germany

✉ Corresponding authors: Prof. Dr. H. Breer, University of Hohenheim, Institute of Physiology, Garbenstr. 30, 70599 Stuttgart, Germany. e-mail: breer@uni-hohenheim.de. Prof. Long Zhang, Ph. D., China Agricultural University, Key Lab for Biological Control, Ministry of Agriculture, Beijing 100193, China, e-mail: locust@cau.edu.cn

Received: 2013.09.09; Accepted: 2013.10.31; Published: 2013.12.01

Abstract

The behaviour of the desert locust, *Schistocera gregaria*, is largely directed by volatile olfactory cues. The relevant odorants are detected by specialized antennal sensory neurons which project their sensory dendrites into hair-like structures, the sensilla. Generally, the responsiveness of the antennal chemosensory cells is determined by specific receptors which may be either odorant receptors (ORs) or variant ionotropic receptors (IRs). Previously, we demonstrated that in locust the co-receptor for ORs (ORco) is only expressed in cells of sensilla basiconica and sensilla trichodea, suggesting that cells in sensilla coeloconica may express different types of chemosensory receptors. In this study, we have identified the genes of *S. gregaria* which encode homologues of co-receptors for the variant ionotropic receptors, the subtypes IR8a and IR25a. It was found that both subtypes, SgreIR8a and SgreIR25a, are expressed in the antennae of all five nymphal stages and in adults. Attempts to assign the relevant cell types by means of *in situ* hybridization revealed that SgreIR8a and SgreIR25a are expressed in cells of sensilla coeloconica. Double fluorescence *in situ* hybridization experiments disclosed that the two IR-subtypes are co-expressed in some cells of this sensillum type. Expression of SgreIR25a was also found in some of the sensilla chaetica, however, neither SgreIR25a nor SgreIR8a was found to be expressed in sensilla basiconica and sensilla trichodea. This observation was substantiated by the results of double FISH experiments demonstrating that cells expressing SgreIR8a or SgreIR25a do not express ORco. These results support the notion that the antenna of the desert locust employs two different populations of OSNs to sense odors; cells which express IRs in sensilla coeloconica and cells which express ORs in sensilla basiconica and sensilla trichodea.

Key words: locust, olfaction, ionotropic receptors, *in situ* hybridization

Introduction

The desert locust, *Schistocerca gregaria*, is a dreaded pest in afro-asian regions, due to its potential to form huge swarms, which invade and crop complete agricultural areas thus causing tremendous economical damage. Many aspects of locust behavior, including aggregation, feeding, mating and oviposition are triggered and guided by volatile chemical cues emitted from con-specifics, food plants or oviposition sites [1]. Insects sense volatile chemicals by olfactory sensory neurons (OSNs) on antennae, which extend their dendrites into hair-like structures. On locust antennae three types of morphologically iden-

tifiable olfactory sensilla are distinguished housing different number of OSNs: sensilla basiconica (up to 50 OSNs), sensilla trichodea (1-3 OSNs) and sensilla coeloconica (1-4 OSNs) [2]. Single sensillum recordings from antennae of *Schistocerca gregaria* and the closely related *Locusta migratoria* have provided some first insight into the response spectrum of OSNs in the different sensilla types. It was found that basiconic OSNs responded to nymphal as well as to adult aggregation pheromones, while OSNs in s. trichodea responded to odorants from locust feces and to a putative sex pheromone [3, 4]. Finally, OSNs in s. coeloconica responded to organic acids, plant volatiles and nymphal odors; but were inhibited by putative aggregation pheromones [3].

In the past decades significant progress has been made to unravel the molecular mechanisms mediating the odorant-responses of insect OSNs [5-8]. Distinct receptor types residing in the dendritic membrane of OSNs are considered as key elements in odorant detection. Originally in *Drosophila* [9, 10] and subsequently in many species from various insect orders, large families of highly diverse olfactory receptors have been identified [11-13]. Interestingly, recent studies have provided evidence that two classes of chemosensory receptors may exist in the olfactory system of insects [14-17]. Members of the large and diverse family of odorant receptors (ORs) are expressed in OSNs housed in sensilla trichoidea and sensilla basiconica from flies [18, 19], mosquitoes [20] or moths [21, 21, 22]. These seven transmembrane domain receptor proteins confer ligand-specificity to the OSN [18, 23-25] and most probably heteromerize with a common OR-coreceptor (ORco) to form a receptor-complex which is activated by appropriate odorants leading to a depolarization of OSNs through ionotropic [26] and/or metabotropic mechanisms [27].

The second type of olfactory receptors, named "variant ionotropic receptors" (IRs) due to their sequence relation and structural similarity to ionotropic glutamate receptors (iGluRs) [8, 14] was found to be expressed in OSNs housed in sensilla coeloconica of *Drosophila*. In *Drosophila*, each coeloconic OSN appears to express combinations of several IRs from a repertoire of antennal IR genes. IRs are considered to mediate responsiveness of OSNs to organic acids, amines and alcohols [14, 28]. Generally, several variable IRs appear to be co-expressed with one or both IR-subtypes, IR8a and IR25a [29]. These two subtypes are phylogenetically highly conserved and are considered to function as co-receptors [30, 31], thus resembling the functional role of ORco protein in basiconic and trichoid OSNs.

In a previous study exploring the expression of ORco in the antenna of *S. gregaria* and *L. migratoria* [32] we could assign the expression of ORco to OSNs located in sensilla basiconica and sensilla trichodea, but found no expression in OSNs of sensilla coeloconica. In addition, for some ORs expression in ORco-positive sensilla types was demonstrated [33]. Together, these results imply that olfactory receptors of the OR-class are involved in odorant responses of basiconic and trichoid OSNs, while s. coeloconica likely express different receptor types. In this study, we set out to explore whether variant ionotropic receptors may be expressed in the antennae of the locust *S. gregaria.* Towards this goal attempts were made to identify the genes encoding the IR co-receptors IR8a and IR25a and to visualize their expression in the antenna.

Materials and Methods

Insect rearing and tissue collection

Locusts, *Schistocerca gregaria,* were obtained from local suppliers (Zoo&Co, Filderstadt, Germany). Body parts (antennae, mouth parts, tarsi and brains) of adult animals and antennae of different nymphal stages were dissected from cold anaesthetized insects. Tissues were collected in liquid N_2 and subsequently used to isolate total RNA. For *in situ* hybridization experiments antennae were directly embedded in Tissue-Tek O.C.T. compound (Sakura Finetek Europe, Zoeterwoude, The Netherlands) and stored at -70°C until sectioning.

Identification of IR sequences (SgreIRs) from the antennal transcriptome of *Schistocerca gregaria*

We used a collection of IR sequences reported in Croset et al, 2010 to generate a BLAST database in Geneious 6 (Biomatters, Auckland, New Zealand), and carried out tblastx queries with a cut off of 10^{-5} against this database using *S. gregaria* antennal transcriptome data, kindly provided by Heiko Vogel (Department for Entomology, MPI for Chemical Ecology Jena, Germany) and Andreas Vilcinskas (Institute of Phytopathology and Applied Zoology, Justus-Liebig-University of Giessen, Germany). Identified hits indicating candidate SgreIR sequences were used to re-tblastx the NCBI nr (non-redundant) database to verify identity. This identified several sequences annotated as ionotropic glutamate receptors or variant ionotropic receptors, which were used as queries to perform tblastx again with the *Schistocerca gregaria* transcriptome database. Finally, identified and extracted contig sequences were assembled to yield putative IR sequences of *S. gregaria* (SgreIRs).

Amplification of SgreIRs sequences

Total RNA was extracted from frozen male and

female antennae using Trizol reagent (Invitrogen, Germany) according to the supplier´s protocol. Poly A+ RNA was purified from 100 µg total RNA using oligo $(dT)_{25}$ magnetic dynabeads (Invitrogen) following recommended protocols. cDNAs were synthesized from 50 ng mRNA using the Smarter Race cDNA Amplication Kit (Takara, Japan). In order to amplify the 5′ terminal and 3′ terminal sequences of SgreIR8a and SgreIR25a coding sequence specific primers (Supplementary Material: Table S1) were used in PCR reaction with Fermentas High Fidelity Taq (Fisher Scientific, Germany). To overcome GC rich regions in the 5′ part of the SgreIR8a sequence a Taq(R) high GC enhancer (New England Biolabs, USA) was added to the standard PCR reaction. PCR conditions used in SgreIR8a 5′ part were: 95°C for 5 min, then 35 cycles with 94°C for 30 s, 68°C for 30 s and 72°C for 2 min, followed by incubation for 10 min at 72°C. PCR conditions used in SgreIR8a 3′ part were: 95°C for 5 min, then 20 cycles with 94°C for 30s, 70°C for 30 s and 72°C for 1 min 30 s, decreasing the annealing temperature by 0.5°C per cycle. Subsequently, 20 cycles with 60°C annealing temperature were performed followed by incubation for 10 min at 72°C. SgreIR25a sequences (5´ and 3´ parts) were amplified using the following conditions: 94°C for 1 min 40 s, then 20 cycles with 94°C for 30 s, 48°C for 30 s and 72°C for 1 min 30 s, with decreasing the annealing temperature by 0.5°C per cycle. This was followed by 20 further cycles with 38°C annealing temperature and a final incubation for 10 min at 72°C. PCR products were gel-purified using the Geneclean kit (MP Biomedicals, Germany) and adenine nucleotide overhangs were added by incubation with 10 mM dATP and 5U Taq polymerase (Gennaxxon, Germany) at 72°C for 20 min. The resulting A-tailed PCR products were cloned using the pGEM-T vector system (Promega, USA) and sequenced on an ABI310 automatic sequencer employing the BIG dye cycle sequencing kit (v3.1; Applied Biosystems, Foster City, Ca, USA) with vector and gene specific primers.

Tissue and stage-specific expression of IRs

Total RNA was extracted from different adult tissues and nymphal stages using Trizol reagent (Invitrogen) following recommended protocols. Male and female cDNAs were transcribed from 1 µg of total RNA using 4 µl first strand buffer (250 mM Tris pH 8.3, 375 mM KCl, 15 mM MgCl2), 1 µl 10 mM dNTP mix, 1 µl RNaseout, 2 µl DTT (0.1M), 1 µl oligo-dT18 primer (500 ng μl^{-1}) and 1 µl Superscript II reverse transcriptase (Invitrogen) in a total volume 20 µl. Synthesis of cDNA was performed at 50°C for 50 min followed by incubation for 15 min at 70 °C. Non-quantitative RT-PCR was performed using IR-specific sense and anti-sense primers (Supplementary Material: Table S1). PCR conditions used for SgreIR8a were: 94°C for 1 min 30 s, then 20 cycles with 94°C for 30s, 55°C for 30 s and 72°C for 1 min 30 s, with decreasing the annealing temperature by 0.5°C per cycle. Subsequently, 20 cycles at 45°C annealing temperature were performed followed by incubation for 10 min at 72°C. PCR conditions for SgreIR25a were: 94°C for 1 min 30 s, then 40 cycles with 94°C for 30 s, 45°C for 30 s and 72°C for 1 min, followed by incubation for 10 min at 72°C. Primers matching the actin gene of *S. gregaria* (Supplementary Material: Table S1) were used to verify the quality of the cDNA preparations. PCR conditions for actin were: 94°C for 1 min 30 s, then 40 cycles with 94°C for 30 s, 45°C for 30 s and 72°C for 1 min, followed by incubation for 10 min at 72°C. PCR products were run on 1.2% agarose gels and visualized by ethidium bromide staining.

Sequence analysis and comparison

Sequence alignments shown for IR8a and IR25a sequences, respectively, were conducted using ClustalW [34] and further arranged using the BioEdit pro-
pro-
gram (www.mbio.ncsu.edu/BioEdit/bioedit.html).
For SgreIR8a and SgreIR25a structure domain annotation was added according to the DmelIR8a definitions reported in [31]. An unrooted neighbour joining tree comparing the relationship of IR8a and IR25a amino acid sequences from various insect species was calculated based on a ClustalW alignment using the MEGA5 program [35].

In situ hybridization

Digoxigenin (Dig)-labeled or biotin-labeled anti-sense and sense probes were synthesized from linearized pGEM-T vectors containing partial cDNA of SgreIRs or the coding sequence of SgreORco [32] using the T7/Sp6 RNA transcription system (Roche, Germany) following the protocol recommended by the manufacturer. For SgreIR8a riboprobes were transcribed from two plasmids containing 1906 nucleotides of the 5′ part and 1283 nucleotides of the 3′ part, respectively. Accordingly, for SgreIR25a plasmids containing either the 5′ part (1438 nucleotides) or the 3′ part (1669 nucleotides) were used. In ISH experiments 1:1 mixtures of 5′ part and 3′ part riboprobes were used for both SgreIRs. Antennae (embedded in Tissue-Tek) of male and female locusts were used to make 12 µm sections with a Jung CM300 cryostat at -21°C. Sections were thaw mounted on Super Frost Plus slides (Menzel-Gläser, Braunschweig, Germany) and stored at -70°C until use. *In situ* hybridization was performed using the protocol described in detail previously [32] with few modifica-

tions. Briefly, sections were taken out from the -70°C freezer and immediately transferred to 4% PFA for 20 min at 4°C. This was followed by a wash in PBS for 1 min, incubation in 0.2 M HCl for 10 min and two washes in PBS for 1 min each. Then sections were incubated for 10 min in acetylation solution (25% acetic anhydride freshly added in 0.1 M triethanolamine) followed by three 3 min washes in PBS. Pre-hybridization was for 1 hour at 65°C for SgreIR8a and 60°C for SgreIR25a. Hybridization with labeled probes was performed at the same temperatures for 24 hours.

Visualization of Dig-labeled probe hybridizations using color substrate was performed as described earlier [32] using an anti-Dig alkaline phosphatase (AP) conjugated antibody (1:500, Roche) and NBT/BCIP substrate. To increase the signal intensity, polyvinyl alcohol (PVA, MW: 89-98K, Sigma) (1% for SgreIR8a; 2.5% for SgreIR25a) was added to the developing buffer containing NBT/BCIP substrate. Tissue sections were analyzed on a Zeiss Axioskope2 microscope (Zeiss, Oberkochen, Germany) equipped with Axiovision software.

Single and double fluorescent RNA *in situ* hybridization (FISH) with Dig- and/or biotin-labeled probes was conducted in the same way. Visualization of labeled probes was performed as described earlier [33, 36]. In short, Dig-labeled probes were visualized by the anti-Dig AP-conjugated antibody in combination with HNPP/Fast Red (Roche). For biotin-labeled probes the TSA kit (Perkin Elmer, MA, USA), including an anti-biotin strepavidin horse radish peroxidase-conjugate and fluorescein-tyramides as substrate was used. Sections were analysed for hybridization signals (epifluorescence) using a Zeiss LSM510 Meta laser scanning microscope (Zeiss, Oberkochen, Germany). Confocal image stacks were recorded from antennal segments in the red and green fluorescence channel as well as the transmitted-light channel. Pictures presented are projections of selected optical planes. The red and green fluorescence channels have been overlaid with the transmitted-light channel or are shown separately.

Results

Identification of IRs from the locust, *Schistocerca gregaria*

In order to identify olfactory ionotropic receptors from *S. gregaria* (SgreIRs) we have bioinformatically screened transcriptome sequence data from the antenna using a collection of reported IR sequences [29]. These approaches provided nine overlapping contigs with significant similarity to DmelIR8a or putative IR8a sequences of other insects. An assembly of the locust sequences resulted in a putative SgreIR8a sequence which comprises 3719 nucleotides and encodes a protein of 902 amino acids (Fig. 1). The correct assembly of the full-length SgreIR8a sequence was verified by RT-PCR amplification of 5′ and 3′ parts from locust antennal mRNA and sequencing of the PCR products. Similarly, three non-overlapping antennal transcriptome sequences were identified which showed high similarity to LmigIR25a or other candidate insect IR25a sequences. The gaps between stretches of partial sequences were closed by RT-PCR amplification employing gap-spanning primer pairs and sequencing of the PCR products. These efforts led to a SgreIR25a sequence of 2505 nucleotides which encoded a protein of 834 amino acids; sequence comparison suggested that part of the N-terminus is missing (Fig. 2).

To explore the similarity of candidate IR8a sequences from different insect orders we aligned the orthopteran SgreIR8a sequence to lepidopteran, dipteran, coleopteran and hymenopteran sequences (Fig. 1) and calculated the pair-wise identity. This revealed an overall sequence identity between 42.7 and 68.6 %; for certain protein domains a high degree of conservation is particularly evident (Fig. 1). The highest identity across species was found in the region between transmembrane (M) segments M1 – M3, in especially in M2 and the pore loop (P). Fewer identical amino acids are present in the binding domain loops S1 and S2; and very little similarity exists in the amino terminal domain (ATD) and the C-terminus (C) of the proteins. When comparing the sequence of SgreIR25a with the sequences of IR25a from other insects a similar pattern of sequence conservation emerged (Fig. 2). The pair-wise sequence identity ranged from 50.1% to 69.9% between species and the same domains were conserved as in the IR8a proteins, except for the amino terminal domain which was more conserved in the IR25a proteins (Fig. 2). Overall, SgreIR8a and Sgre25a share 29.2% of their amino acids. To further analyze the phylogenetic relationship of the locust IR8a and IR25a with representatives from other insects, a sequence similarity tree was calculated using the MEGA5 program [35]. The resulting neighbor joining tree (Fig. 3) shows that SgreIR8a and SgreIR25a cluster into clearly separated branches comprising insect IR8a and IR25a sequences, respectively. Within the IR8a branch as well as in the IR25a branch, the sequences cluster in an order-specific manner, reflecting that the highest similarity exists between sequences of insects belonging to the same orders.

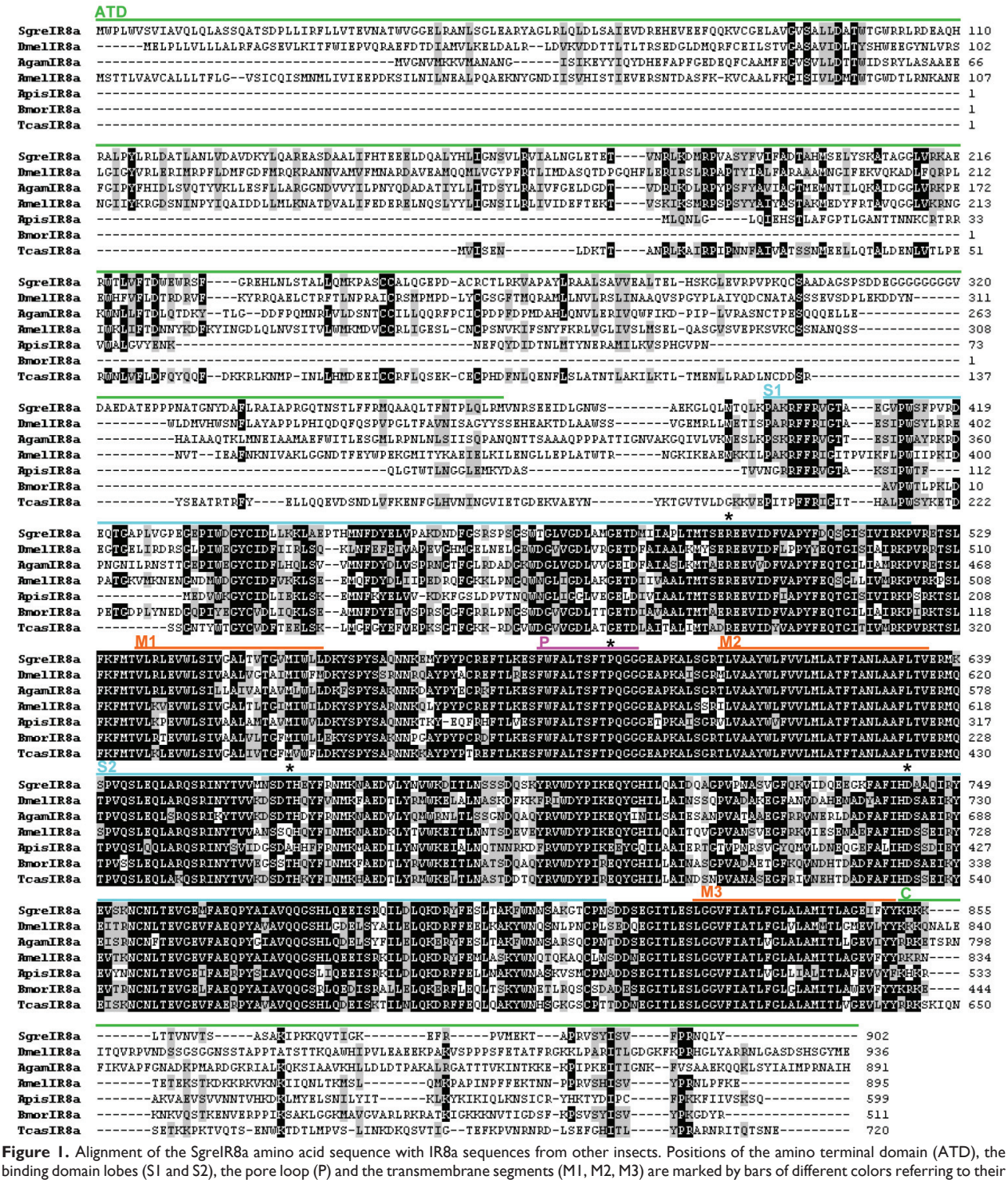


Figure 1. Alignment of the SgreIR8a amino acid sequence with IR8a sequences from other insects. Positions of the amino terminal domain (ATD), the binding domain lobes (S1 and S2), the pore loop (P) and the transmembrane segments (M1, M2, M3) are marked by bars of different colors referring to their position in DmelIR8a [14]. The positions of key ligand binding residues in iGluRs are marked by asterisks above the sequences. Numbers on the right refer to the number of the last amino acid in the line. Amino acids with at least 50% identity or similarity between sequences are shaded black and grey, respectively. The IR8a amino acid sequences from Agam = *Anopheles gambiae*, Amel = *Apis melifera*, Apis = *Acyrthosiphon pisum*, Bmor = *Bombyx mori*, Dmel = *Drosophila melanogaster*, and Tcas = *Tribolium castaneum* were taken from [29].

Figure 2. Alignment of the SgreIR25a amino acid sequence with IR25a sequences identified in other insects. Numbers on the right refer to the number of the last amino acid in the line. Black and grey shadings indicate amino acids which show at least 70% identity, respectively similarity, between sequences. Labeling of protein domains, abbreviations and origin of sequences are the same as indicated in figure 1. LmigIR25a = *Locusta migratoria* IR25a (GenBank: AFP33229.1)

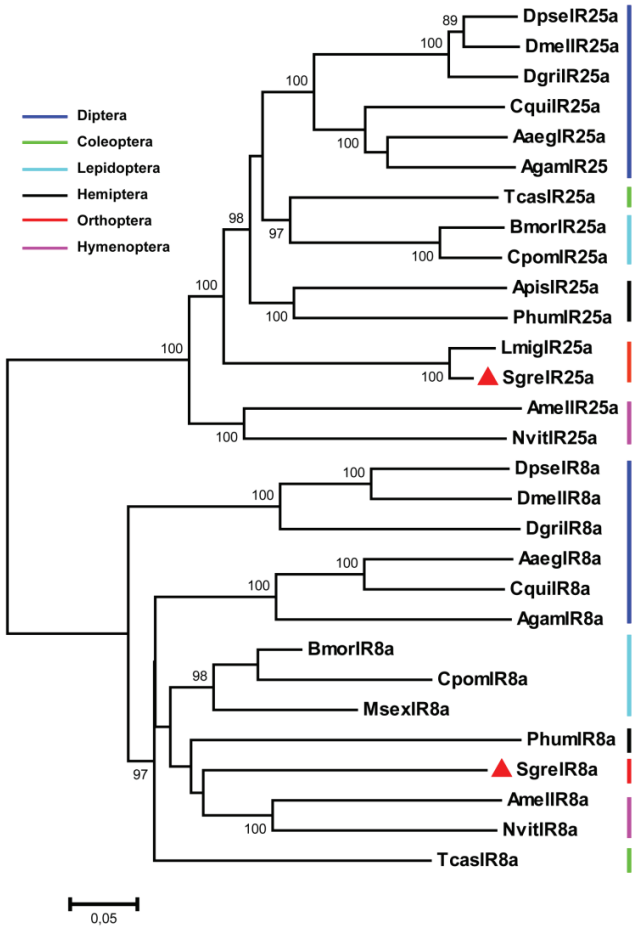


Figure 3. Phylogenetic relationship of IR8a and IR25a sequences from *S. gregaria* and species belonging to various insect orders. A neighbour-joining tree was constructed using MEGA5 [35] based on a ClustalW alignment of the IRs indicated in figures 1 and 2, as well as sequences from Aaeg = *Aedes aegypti*, Cpom = *Cydia pomonella*, Cqui = *Culex quinquefasciatus*, Dgri = *D. grimshawi*, Dpse= *D. pseudoobscura*, Msex = *Manduca sexta*, Nvit = *Nasonia vitripennis* and Phum = *Pedeculus humanus*, [16, 29, 43]. Bootstrap support values are based on 1000 replicates, only support values above 80% are shown. Branch lengths are proportional.

Spatiotemporal expression patterns of SgreIR8a and SgreIR25a

To determine the level of expression for SgreIR8a and SgreIR25a in male and female antenna and to assess if the two IRs may also be expressed in other parts of the body, RT-PCR experiments were performed using IR-specific primers and cDNA prepared from chemosensory (male and female antennae, mouthpart, tarsi) and non-chemosensory (brain) tissues of locust. With primer pairs specific for SgreIR8a and SgreIR25a, respectively, bands of the expected size were found in the antennae of both sexes, with no obvious differences in the expression level. In addition, transcripts for SgreIR25a were also found in cDNAs from mouthparts and at a low level from tarsi. This result indicates that SgreIR25a is expressed not only in the antennae. No PCR products were obtained with cDNA preparations of brain (Fig. 4A).

To explore the onset and time course of SgreIR8a and SgreIR25a expression during development different stages were compared. cDNAs prepared from antennae of different nymphal stages (1st to 5th instars) and adult animals were analysed with IR-specific primers (Fig. 4B). With templates from all stages, PCR products were obtained with slightly different intensities, especially for SgreIR8a. Together the results indicate that both IR-subtypes are expressed in antennae throughout development from the first instar stage to adult.

Identification of the IR-expressing cells on the antenna

Four morphological distinct sensilla types housing sensory cells have been identified on the antenna of the desert locusts: olfactory sensilla basiconica, sensilla trichodea and sensilla coeloconica, while the sensilla chaetica are supposed to serve a gustatory/mechanosensory function [2]. To visualize the cells which express SgreIR8a and SgreIR25a in antennae *in situ* hybridization (ISH) experiments were performed. Sections through the antennae were incubated with IR-specific anti-sense RNA probes and positive cells visualized employing colour substrates.

Experiments with a SgreIR8a specific Dig-labeled anti-sense RNA probe led to the labeling of several cells within an antennal segment (Fig. 5A and B). Control experiments with a corresponding sense RNA probe did not result in any labeled cells; thus confirming the specificity of the ISH signals (Fig. 5H). More detailed analysis revealed that within a section the SgreIR8a anti-sense RNA probe visualized either individual cells (Fig. 5C and F), two adjacent cells (Fig. 5D) and in some cases even clusters of three cells (Fig. 5E). SgreIR8a-positive cells could clearly be assigned to s. coeloconica (Fig. 5C - F), but were not found under any s. basiconica (Fig. 5C), s. trichodea (Fig. 5F) or s. chaetica (Fig. 5E). For comparison we performed ISH with a SgreORco-specific probe resulting in a complementary labeling pattern, thus confirming our previous results [32] that ORco is expressed in the 20-30 OSNs housed in s. basiconica (Fig. 5G) as well as in the 2-3 OSNs in the s. trichodea (not shown). Together, these results suggest that expression of SgreIR8a is restricted to s. coeloconica.

ISH-experiments with a SgreIR25a-specific anti-sense RNA probe resulted in a labeling pattern quite similar to SgreIR8a (Fig. 6), with either single cells or with two or three adjacent cells on a single section (Fig. 6A - C). In addition with the SgreIR25a probe occasionally clusters of four labeled cells were found (Fig. 6D). While no labeled cells were seen under s. trichodea (Fig. 6E) or s. basiconica (Fig. 6F), we

regularly found labeled cells under some of the s. chaetica (Fig. 6G and C). These results indicate that SgreIR25a is more broadly expressed; both in s. coeloconica as well as in a subpopulation of s. chaetica.

Since both IR-subtypes are predominantly expressed in the s. coeloconica, the possibility exists that they could be co-expressed in the same cell. To scrutinize this view, double FISH experiments were performed employing differentially labeled SgreIR8a- and SgreIR25a-specific probes. The results are depicted in (Fig. 7); cells containing IR transcripts were visualized by red or green fluorescence for, respectively, SgreIR25a and SgreIR8a. Although the experiments were hampered by the relatively low FISH signal intensities, we regularly visualized cells which were clearly co-labeled by both probes indicating co-expression of SgreIR8a and SgreIR25a (Fig. 7A - C). In addition, we found cells that appear to express only one of the two receptors (Fig. 7D - I). Together our results indicate heterogeneous expression of SgreIR8a and SgreIR25a in distinct but partly overlapping populations of OSNs.

To support the specific expression of SgreIR8a and SgreIR25a in OSNs of s. coeloconica but not in OSNs of s. basiconica or s. trichodea we performed double FISH with SgreIR- and SgreORco-specific probes. The results for the combination SgreIR8/SgreORco are shown in (Fig. 8). On longitudinal sections through the antenna cells labeled with SgreIR8a (green) and labeled with SgreORco (red) are clearly separated (Fig. 8C and G). The SgreIR8a probe labeled 1-3 cells under s. coeloconica (Fig. 8A and D), while SgreORco probe labeled a cluster of many cells (Fig. 8B and F) thus confirming the results obtained with single probes (Fig. 5). Analysis of more horizontal sections of the antenna revealed that the SgreIR8a-positive cells (Fig. 8E) are intermingled but clearly separated from the clusters of SgreORco-positive cells (Fig. 8G). This labeling pattern is reminiscent of the mixed topography described for s. coeloconica and s. basiconica on the locust antenna [2]. Two-color FISH experiments using the combination SgreIR25a/SgreORco probes gave a similar labeling pattern (Fig. 9). Areas labeled with a SgreIR25a-probe (Fig. 9A) or with a SgreORco-probe (Fig. 9B) were clearly separated (Fig. 9C), indicating that ORco is not expressed in SgreIR25a-positive cells of sensilla coeloconica. In accordance with the result obtained with the single probe ISH and a chromogenic visualization, double FISH experiments demonstrated that a SgreIR25a probe labeled cells also under some s. chaetica (Fig. 9D - F). Also, the SgreIR25a-positive cells of sensilla chaetica (Fig. 9D) did not co-express SgreORco (Fig. 9E) but were well separated from the ORco-expressing cells (Fig. 9F).

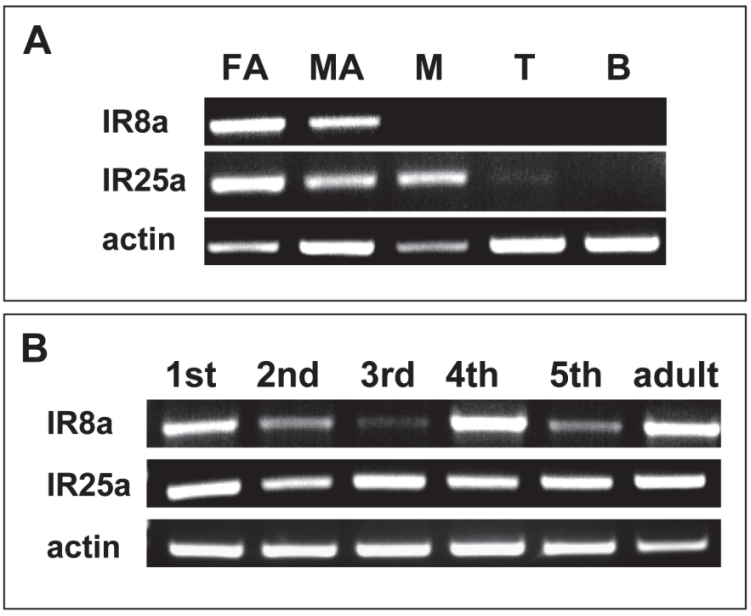


Figure 4. Tissue specificity and developmental expression of SgreIR8a and SgreIR25a. RT-PCR experiments were performed using cDNAs prepared from the tissues indicated and primer pairs specific for SgreIR8a and SgreIR25a, respectively. Primers to actin were used as control for the integrity of the cDNA preparations. **A**, Expression of the IRs in different locust tissues. FA, female antenna; MA, male antenna; M, mouthparts (maxillary and labial palps); B, brain; T, tarsi. **B**, Comparison of the IR expression in the antennae of different nympal stages (1st to 5th instar) and adults. Amplification products were analysed on agarose gels and visualized by UV illumination after ethidium bromide staining.

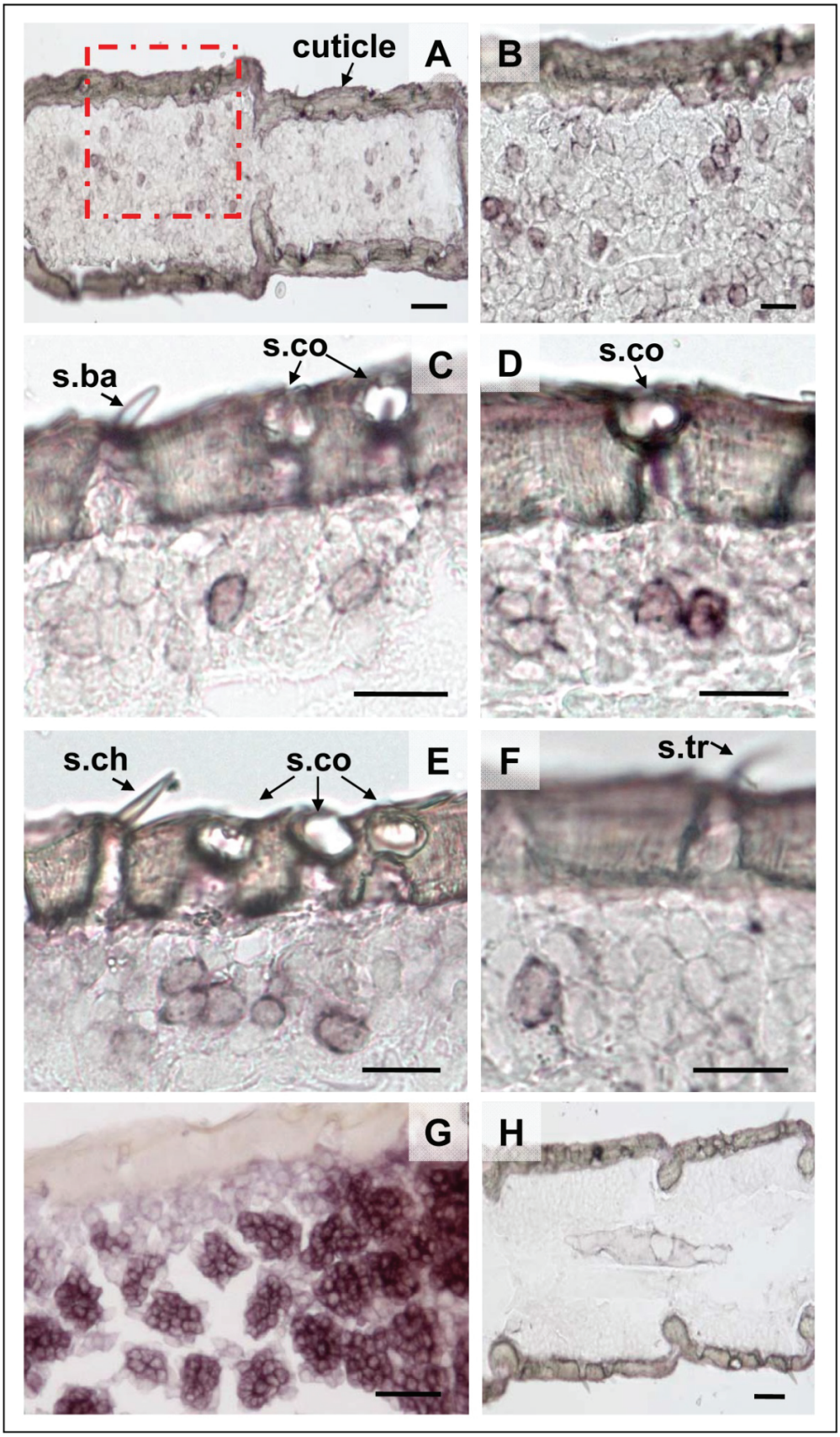


Figure 5. Topography of SgreIR8a gene expression in the antenna *S. gregaria*. *In situ* hybridization using SgreIR8a-specific Dig-labeled sense and anti-sense riboprobes and visualization with color substrates. **A**, Labeling of cells by the SgreIR8a anti-sense RNA probe in two antennal segments of the desert locust. **B**, Higher magnification of the area boxed in A. **C - F**, The SgreIR8a anti-sense RNA probe labeled one to three cells under sensilla coeloconica (s.co), but never cells under sensilla basiconica (s.ba, **C**) , sensilla chaetica (s.ch, **E**) or sensilla trichodea (s.tr, **F**). **G**, Labeling of cells by a Dig-labeled SgreORco-specific anti-sense RNA probe. **H**, No hybridization signals were observed with the SgreIR8a sense probe. A, B, D, E: female antennae; C, F, G, H: male antennae. Scale bars: 100 µm in A; 50 µm in B, G, H; 20 µm in C, D, E, F.

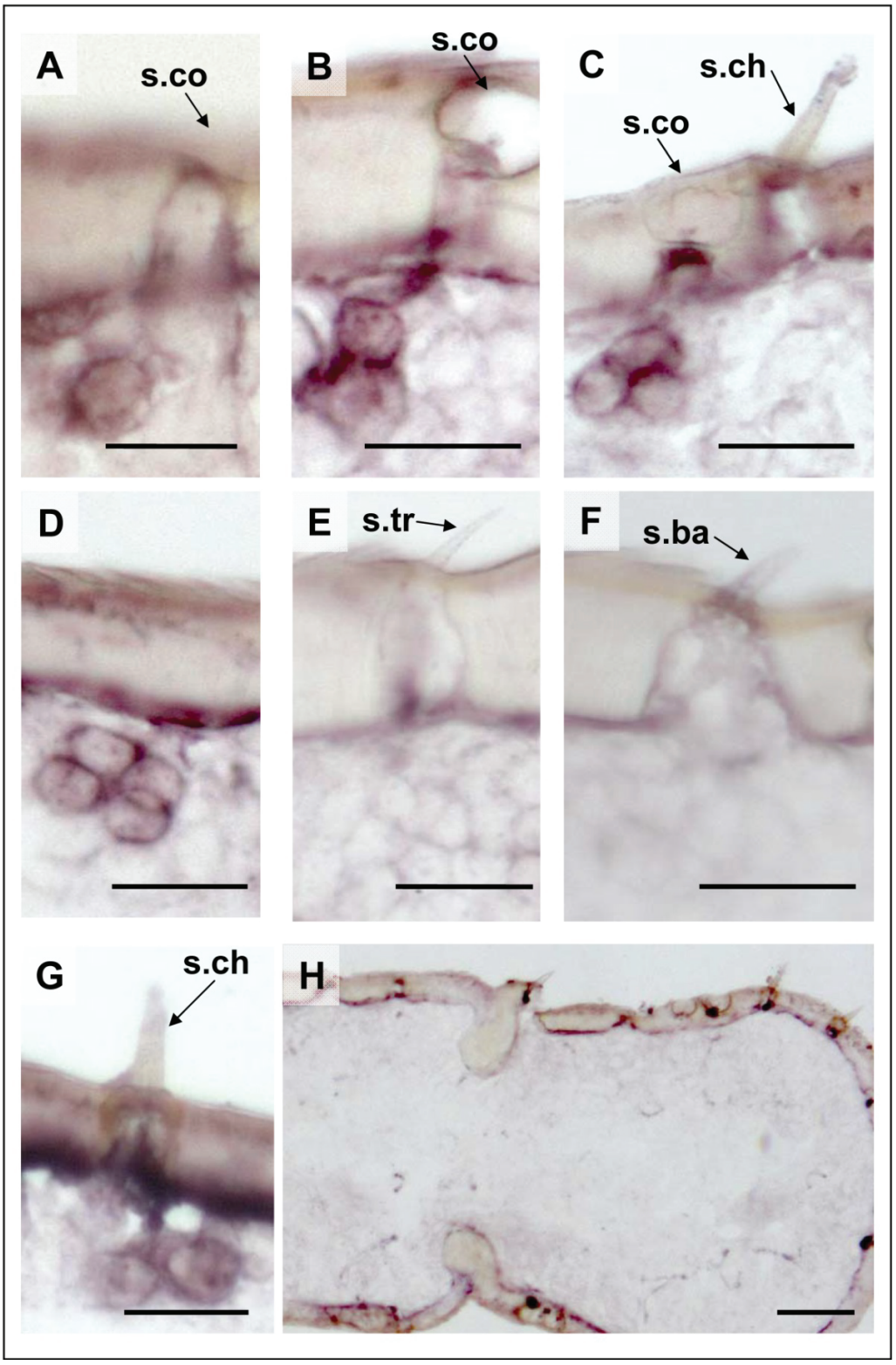


Figure 6. Localisation of SgreIR25a gene expression in the antenna of *S. gregaria*. *In situ* hybridization using SgreIR25a-specific Dig-labeled sense and anti-sense riboprobes and chromogenic visualization. **A - D**, The SgreIR25a anti-sense RNA probe labeled one (A), two (B), three (C) or four (D) cells under sensilla coeloconica (s.co). No cells under sensilla trichodea (s.tr, **E**) and sensilla basiconica (s.ba, **F**) were labeled. For sensilla chaetica (s.ch), cases of no labeled cells (C) and SgreIR25a-positive cells (G) were found. **H**, No labeling of cells were obtained with the SgreIR25a sense riboprobe. B, C, E, H: female antennae; A, D, G, F: male antennae. Scale bars: 20 μm in A - G; 50 μm in H.

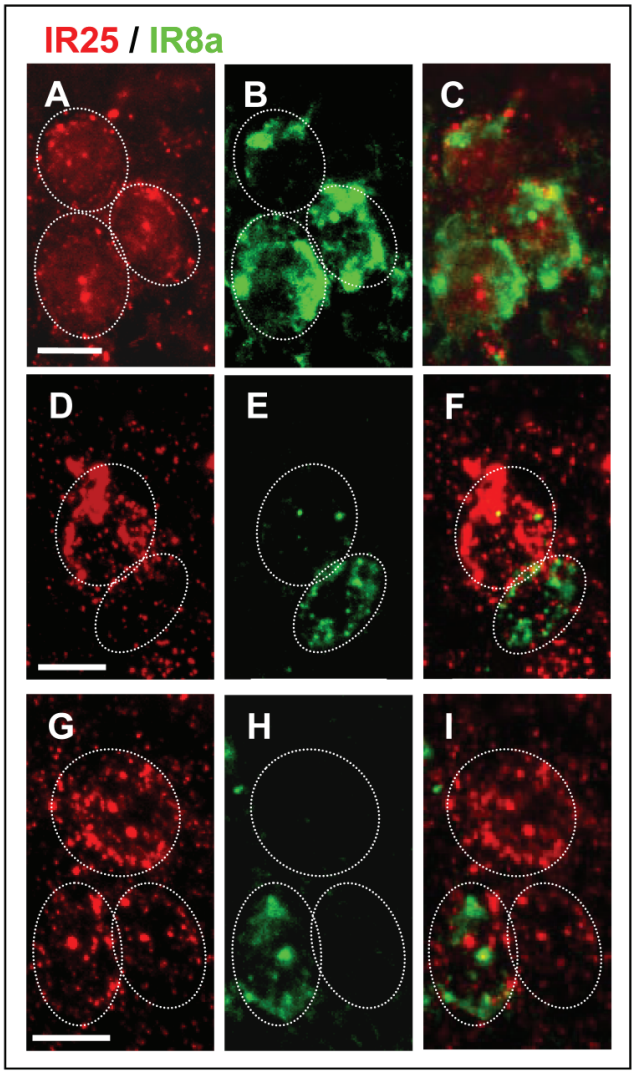


Figure 7. Partial overlap of SgreIR8a and SgreIR25a expression. Double FISH on antennal sections using Dig-labeled SgreIR25a and biotin-labeled SgreIR8a probes with visualization of FISH signals in red (SgreIR25a) and green (SgreIR8a). **A - C**, Cluster of three cells labeled by both (C), the SgreIR25 probe (red, A) and the SgreIR8a probe (green, B). **D - F**, Distinct cells that only express SgreIR25a (red, D) or SgreIR8a (green, E) without overlap (F). **G - I,** Cluster of three cells, with one cell co-expressing SgreIR8a and SgreIR25a (I), the other two cells express SgreIR25a (red, G) but not SgreIR8a (H, green). A - C, G - I: female antennae; D - F: male antennae. Scale bars: 20 µm.

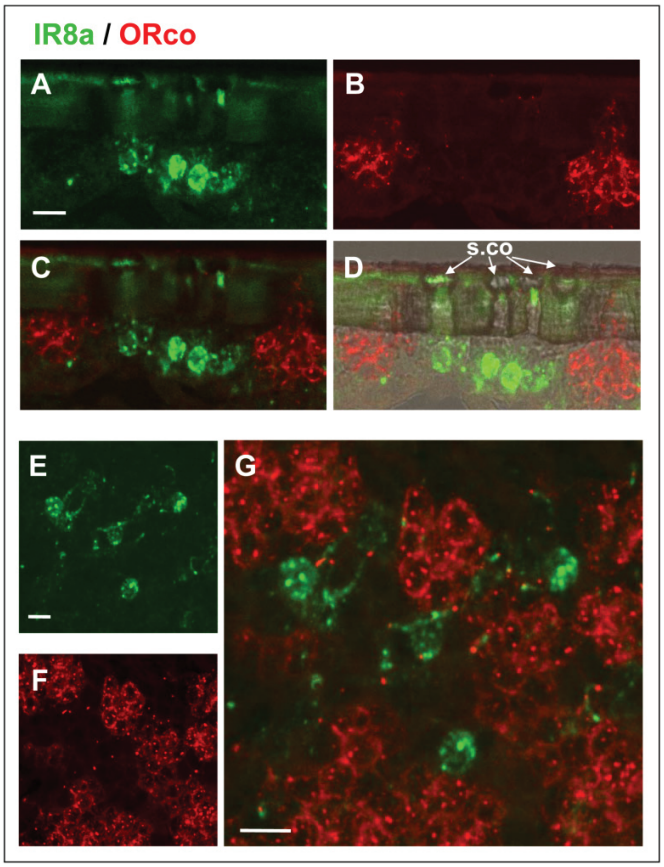


Figure 8. SgreIR8a is not expressed in cells expressing SgreORco. Two-color FISH on antennal sections of male locust using a biotin-labeled SgreIR8a and a Dig-labeled SgreORco probe and detection by green (SgreIR8a) and red (SgreORco) fluorescence. **A - D**, Hybridization signals on a sagital section of the antenna. The IR8a anti-sense RNA probe labeled cells under sensilla coeloconica which are not labeled by the ORco probe. Pictures show projections of confocal image stacks showing the separated (A, green; B, red) or overlaid (C) fluorescence channels. To better show the morphology of the section the transmitted light channel has been overlaid with the fluorescence channel in D. **E - G**, Two-color FISH on a more horizontal section of the antenna section confirming the expression of SgreIR8a (green) and SgreORco (red) in different cells. Clusters of ORco-positive cells are intermingled with SgreIR8a-positive cells. Pictures show the separated green (E) and red (F) fluorescence channels and the overlay at higher magnification in G. Scale bars: 20 µm.

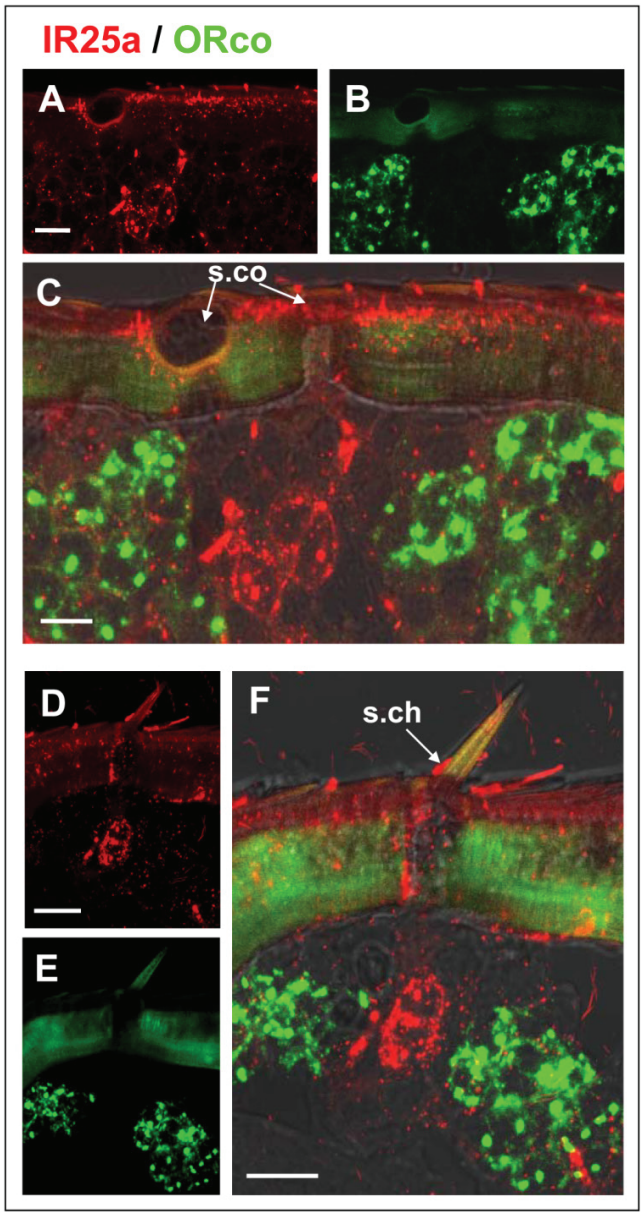


Figure 9. Expression of SgreIR25a and SgreORco locate in different cells. Double FISH on female antennal sections was performed employing Dig-labeled SgreIR25a and biotin-labeled SgreORco probes. Positive cell were visualized by red (SgreIR25a) and green (SgreORco) fluorescence. **A - C**, Hybridization signals on a longitudinal section of the antenna. The IR25a anti-sense RNA probe labeled cells under sensilla coeloconica which are not labeled by the ORco probe. **D - F,** The SgreIR25a anti-sense RNA probe labeled cells under sensilla cheatica which do not express SgreORco. Pictures are projections of confocal image stacks showing the separated (A, D, red; B, E, green) or overlaid fluorescence channels (C, F with transmitted light channel). Scale bars: 20 µm.

Discussion

In this study we identified two genes which encode putative co-receptors of variant ionotropic receptors, the subtypes, IR8a and IR25a, of the locust *Schistocera gregaria*. The identification is based on the pronounced sequence similarity of SgreIR8a and SgreIR25a with the respective sequences from other insect species. Originally the IR8a and IR25a receptors were identified in the fruitfly *Drososophila melanogaster* as members of a novel type of olfactory receptor family [14]. By means of bioinformatic genome screenings and transcriptome sequence analyses orthologs of IR8a and IR25a have been identified in a number of insect species as well as in other arthropods, such as crustaceans [16, 17, 29, 37]. Most remarkably, IR25a homologs were also found in the nematode *Caenorabditis elegans*, the mollusc *Aplysia californica* and the annelid *Capitella capitata*. Thus, these receptor types seem to be under high selective pressure to maintain the primary structure of the protein [29] suggesting an important functional role of the proteins.

The *in situ* hybridization experiments have shown that both receptor types, IR8a and IR25a, are mainly expressed in sensory neurons located in sensilla coeloconica. They are not expressed in OSNs of the sensilla basiconica and sensilla trichodea. This notion was substantiated by the results of double FISH experiments, demonstrating that both SgreIRs are not co-expressed with ORco (Figs. 8 and 9), which is selectively expressed in OSNs of s. basiconica and s. trichodea of locust [32]. A similar distribution pattern of ORco was also found in other insects [38, 39]. Recent studies suggest IR8a and IR25a may act as co-receptors and may form heteromers with another variant ionotropic receptor [30, 31] thus resembling the role of ORco for the ligand-specific OR-subtypes. Consequently, the expression of ORco is considered as an indicator for the expression of other OR-types and similarly IR8a and IR25a may be indicators for the expression of IR-subtypes. This view would imply that the sensory cells housed in s. coeloconica of the locust antenna express the variant ionotropic receptor and they are only present in this sensilla type.

Previous electron microscopic studies have identified two morphological distinguishable types of sensilla coeloconica on the antenna *of S. gregaria* [2]. The double wall type is penetrated by radial pores and contains one to three unbranched sensory neurons (type I), while the non-porous wall type (type II) contains four sensory neurons [2]. The *in situ* hybridization experiments have shown that SgreIR8a and SgreIR25a are apparently expressed in both types of s. coeloconica; in most cases the number of labeled cells varied from 1 to 3 (Figs. 5 and 6). Clusters of four labeled cells were only obtained using the probes for SgreIR25a (Fig. 6D), suggesting that only SgreIR25a may be expressed in all neurons of type II sensilla. The results of double-labeling studies showed that SgreIR8a and SgreIR25a are co-expressed in a subpopulation of cells, but there are also cells which ex-

press only one of the two subtypes. This expression pattern is reminiscent of that in *Drosophila*, where immunohistochemical studies with specific antibodies have demonstrated that IR8a and IR25 are expressed in distinct but partially overlapping populations of neurons [31]. Although we cannot exclude that in some cases SgreIR co-expression was not detected due to transcript levels below the detection limit, the data indicate a heterogeneous expression pattern of SgreIR8a and SgreIR25a in the sensilla coeloconica of the locust.

SgreIR25a-positive cells were also found in some sensilla chaetica which are supposed to serve gustatory/mechanosensory functions [2]. The notion that IR25a may be present in gustatory chemosensory cells was supported by the result of RT-PCR experiments indicating expression of SgreIR25a in mouths parts, which carry hundreds of s. chaetica (labial palps) and peg-like sensilla (maxillary palps); these sensilla are supposed to have a primary gustatory function [40]. The concept that locust gustatory neurons may co-express SgreIR25a and other ligand binding IRs is in line with some recent studies demonstrating that in *Drosophila* IR25a is co-expressed with IR7a in gustatory cells on the labellum [29] and that IR76b is involved in the detection of salt [41].

The results of our *in situ* hybridization experiments that there are no obvious gender differences in the number of SgreIR8a- or SgreIR25a-expressing cells (Figs. 5 and 6) as well as in the levels of SgreIR8a and SgreIR25a transcripts in male and female antennae (Fig. 4) are in agreement with similar numbers of s. coeloconica on the antenna of male and female animals [2, 42]. Overall these data suggest that the two co-receptors are of similar importance in the male and female olfactory system. The presence of SgreIR8a and SgreIR25a transcripts in the antennae of all five nymphal stages is in accordance with the observation that s. coeloconica exist already in first instar stage and are maintened till the adult stage [2]. This may further underline the importance of the variant ionotropic receptor for chemoreception of *Schistocera gregaria* throughout the entire locust lifespan.

Supplementary Material

Table S1.
http://www.ijbs.com/v10p0001s1.pdf

Acknowledgements

We are grateful to Heidrun Froß for excellent technical assistance. Furthermore, we thank Heiko Vogel (Department for Entomology, MPI for Chemical Ecology Jena, Germany) and Andreas Vilcinskas (Institute of Phytopathology and Applied Zoology, Justus-Liebig-University of Giessen, Germany) for providing access to the transcriptome data.

Funding

This work was supported by the DFG (German Research Foundation), the NSFC (Natural Science Foundation of China) Project No. 30730012 and the China Scholarship council.

Data deposition

The IR sequences reported in this paper have been deposited in Genbank under accession numbers: KF528686 (SgreIR25a) and KF528687 (SgreIR8a).

Competing Interests

The authors have declared that no competing interest exists.

References

1. Hassanali A, Njagi PG, Bashir MO. Chemical ecology of locusts and related acridids. Annu Rev Entomol 2005; 50: 223-45.
2. Ochieng SA, Hallberg E, Hansson BS. Fine structure and distribution of antennal sensilla of the desert locust, *Schistocerca gregaria* (Orthoptera: Acrididae). Cell Tissue Res 1998; 291: 525-36.
3. Ochieng SA, Hansson BS. Responses of olfactory receptor neurones to behaviourally important odours in gregarious and solitarious desert locust, *Schistocerca gregaria*. Physiol Entomol 1999; 24: 28-36.
4. Cui X, Wu C, Zhang L. Electrophysiological response patterns of 16 olfactory neurons from the trichoid sensilla to odorant from fecal volatiles in the locust, *Locusta migratoria manilensis*. Arch Insect Biochem Physiol 2011; 77: 45-57.
5. Leal WS. Odorant reception in insects: roles of receptors, binding proteins, and degrading enzymes. Annu Rev Entomol 2013; 58: 373-91.
6. Su CY, Menuz K, Carlson JR. Olfactory perception: receptors, cells, and circuits. Cell 2009; 139: 45-59.
7. Nakagawa T, Vosshall LB. Controversy and consensus: noncanonical signaling mechanisms in the insect olfactory system. Curr Opin Neurobiol 2009; 19: 284-92.
8. Rytz R, Croset V, Benton R. Ionotropic Receptors (IRs): Chemosensory ionotropic glutamate receptors in *Drosophila* and beyond. Insect Biochem Mol Biol 2013.
9. Vosshall LB, Amrein H, Morozov PS, Rzhetsky A, Axel R. A spatial map of olfactory receptor expression in the *Drosophila* antenna. Cell 1999; 96: 725-36.
10. Clyne PJ, Warr CG, Freeman MR, Lessing D, Kim J, Carlson JR. A novel family of divergent seven-transmembrane proteins: candidate odorant receptors in *Drosophila*. Neuron 1999; 22: 327-38.
11. Hansson BS, Stensmyr MC. Evolution of insect olfaction. Neuron 2011; 72: 698-711.
12. Jacquin-Joly E, Merlin C. Insect olfactory receptors: contributions of molecular biology to chemical ecology. J Chem Ecol 2004; 30: 2359-97.
13. Sanchez-Gracia A, Vieira FG, Rozas J. Molecular evolution of the major chemosensory gene families in insects. Heredity 2009; 103: 208-16.
14. Benton R, Vannice KS, Gomez-Diaz C, Vosshall LB. Variant ionotropic glutamate receptors as chemosensory receptors in *Drosophila*. Cell 2009; 136: 149-62.
15. Liu C, Pitts RJ, Bohbot JD, Jones PL, Wang G, Zwiebel LJ. Distinct olfactory signaling mechanisms in the malaria vector mosquito *Anopheles gambiae*. PLoS Biol 2010; 8.
16. Bengtsson JM, Trona F, Montagne N, Anfora G, Ignell R, Witzgall P, Jacquin-Joly E. Putative Chemosensory Receptors of the Codling Moth, *Cydia pomonella*, Identified by Antennal Transcriptome Analysis. PLoS ONE 2012; 7: e31620.
17. Olivier V, Monsempes C, Francois MC, Poivet E, Jacquin-Joly E. Candidate chemosensory ionotropic receptors in a Lepidoptera. Insect Mol Biol 2011; 20: 189-99.
18. Hallem EA, Ho MG, Carlson JR. The molecular basis of odor coding in the *Drosophila* antenna. Cell 2004; 117: 965-79.
19. Ha TS, Smith DP. A pheromone receptor mediates 11-cis-vaccenyl acetate-induced responses in *Drosophila*. J Neurosci 2006; 26: 8727-33.
20. Schultze A, Pregitzer P, Walter MF, Woods DF, Marinotti O, Breer H, Krieger J. The Co-Expression Pattern of Odorant Binding Proteins and Olfactory Receptors Identify Distinct Trichoid Sensilla on the Antenna of the Malaria Mosquito *Anopheles gambiae*. PLoS ONE 2013; 8: e69412.
21. Gohl T, Krieger J. Immunolocalization of a candidate pheromone receptor in the antenna of the male moth, *Heliothis virescens*. Invert Neurosci 2006; 6: 13-21.
22. Sakurai T, Nakagawa T, Mitsuno H, Mori H, Endo Y, Tanoue S, Yasukochi Y, Touhara K, Nishioka T. Identification and functional characterization of a sex

pheromone receptor in the silkmoth *Bombyx mori*. Proc Natl Acad Sci U S A 2004; 101: 16653-8.
23. Wang G, Carey AF, Carlson JR, Zwiebel LJ. Molecular basis of odor coding in the malaria vector mosquito *Anopheles gambiae*. Proc Natl Acad Sci U S A 2010; 107: 4418-23.
24. Carey AF, Wang G, Su CY, Zwiebel LJ, Carlson JR. Odorant reception in the malaria mosquito *Anopheles gambiae*. Nature 2010; 464: 66-71.
25. Hallem EA, Carlson JR. Coding of odors by a receptor repertoire. Cell 2006; 125: 143-60.
26. Sato K, Pellegrino M, Nakagawa T, Nakagawa T, Vosshall LB, Touhara K. Insect olfactory receptors are heteromeric ligand-gated ion channels. Nature 2008; 452: 1002-6.
27. Wicher D, Schafer R, Bauernfeind R, Stensmyr MC, Heller R, Heinemann SH, Hansson BS. *Drosophila* odorant receptors are both ligand-gated and cyclic-nucleotide-activated cation channels. Nature 2008; 452: 1007-11.
28. Ai M, Min S, Grosjean Y, Leblanc C, Bell R, Benton R, Suh GS. Acid sensing by the *Drosophila* olfactory system. Nature 2010; 468: 691-5.
29. Croset V, Rytz R, Cummins SF, Budd A, Brawand D, Kaessmann H, Gibson TJ, Benton R. Ancient protostome origin of chemosensory ionotropic glutamate receptors and the evolution of insect taste and olfaction. PLoS Genet 2010; 6: e1001064.
30. Ai M, Blais S, Park JY, Min S, Neubert TA, Suh GS. Ionotropic Glutamate Receptors IR64a and IR8a Form a Functional Odorant Receptor Complex In Vivo in *Drosophila*. J Neurosci 2013; 33: 10741-9.
31. Abuin L, Bargeton B, Ulbrich MH, Isacoff EY, Kellenberger S, Benton R. Functional architecture of olfactory ionotropic glutamate receptors. Neuron 2011; 69: 44-60.
32. Yang Y, Krieger J, Zhang L, Breer H. The olfactory co-receptor Orco from the migratory locust (*Locusta migratoria*) and the desert locust (*Schistocerca gregaria*): identification and expression pattern. Int J Biol Sci 2012; 8: 159-70.
33. Xu H, Guo M, Yang Y, You Y, Zhang L. Differential expression of two novel odorant receptors in the locust (*Locusta migratoria*). BMC Neurosci 2013; 14: 50.
34. Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 1994; 22: 4673-80.
35. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 2011; 28: 2731-9.
36. Krieger J, Raming K, Dewer YME, Bette S, Conzelmann S, Breer H. A divergent gene family encoding candidate olfactory receptors of the moth *Heliothis virescens*. Eur J Neurosci 2002; 16: 619-28.
37. Corey EA, Bobkov Y, Ukhanov K, Ache BW. Ionotropic crustacean olfactory receptors. PLoS ONE 2013; 8: e60551.
38. Larsson MC, Domingos AI, Jones WD, Chiappe ME, Amrein H, Vosshall LB. Or83b encodes a broadly expressed odorant receptor essential for *Drosophila* olfaction. Neuron 2004; 43: 703-14.
39. Pitts RJ, Fox AN, Zwiebel LJ. A highly conserved candidate chemoreceptor expressed in both olfactory and gustatory tissues in the malaria vector *Anopheles gambiae*. Proc Natl Acad Sci U S A 2004; 101: 5058-63.
40. Blaney WM, Chapman RF. The fine structure of the terminal sensilla on the maxillary palps of *Schistocerca gregaria* (Forskal) (Orthoptera, Acrididae). Z Zellforsch Mikrosk Anat 1969; 99: 74-97.
41. Zhang YV, Ni J, Montell C. The molecular basis for attractive salt-taste coding in *Drosophila*. Science 2013; 340: 1334-8.
42. Greenwood M, Chapman RF. Differences in numbers of sensilla on the antennae of solitarious abd gregarious *Locusta migratoria L.* (Orthoptera: Acrididae). Int J Morphol and Embryol 1984; 13: 295-301.
43. Grosse-Wilde E, Kuebler LS, Bucks S, Vogel H, Wicher D, Hansson BS. Antennal transcriptome of *Manduca sexta*. Proc Natl Acad Sci U S A 2011; 108: 7449-54.

General discussion

The major aim of the present thesis was to shed light on the evolution of the insect olfactory gene families. We used deep antennal transcriptome sequencing as the basis for identification of olfactory gene families in both the jumping bristletail *Lepismachilis y-signata* (Archaeognatha) and the firebrat *Thermobia domestica* (Zygentoma). For both examined species we could demonstrate the existence of a functional olfactory system (Chapter I), however, the genetic basis of this sense of smell differs from other insects that have been previously studied (Chapter I). Although we could identify OBPs in both species (Chapter II), an OR/Orco based olfactory system was completely lacking in the jumping bristletail *L. y-signata* and only the coreceptor Orco was identified in the firebrat transcriptome (Chapter I). Moreover, in the firebrat we have identified multiple coreceptor variants, a situation not previously found in any other insect (Chapter I).

In both of the examined species, other receptors besides ORs seem to cover the animals´ olfactory abilities, such as the evolutionary older IRs that we successfully identified in the transcriptome datasets of both species (Chapter I). For the putative IR coreceptors of *L. y-signata* we have shown an expression in antennal cells that closely resemble sw-wp olfactory sensilla (Chapter I). In contrast, antennal IRs of *Schistocerca gregaria* are expressed in OSN populations housed in dw-wp coeloconic sensilla (Chapter III), whereas Orco expression is restricted to sw-wp trichoid and basiconic sensilla (Yang et al. 2012).

Which receptors are involved in odor detection in non-flying insects?

The insect olfactory system mainly relies on two different receptor families, the insect specific ORs and the evolutionarily older IRs. The conservation of IRs within protostomes and the restriction of ORs to insect genomes suggest that IRs were the ancient type of insect odorant receptor (Robertson et al. 2003; Croset et al. 2010, Silbering et al. 2011). This sequence of receptor evolution is reflected in developmental properties of the insect IR and OR olfactory subsystems, including the order of evolution of upstream developmental regulators and the developmental timing of IR and OR related circuits (Silbering et al. 2011). In *Drosophila melanogaster*, the peripheral antennal OR pathways differentiate after the IR pathways have been established during development (Silbering et al. 2011). In agreement to the hypothesis that IRs were the first insect olfactory receptors, we neither identified ORs nor their coreceptor Orco within the antennal and maxillary palp transcriptomes of *L. y-signata*,

while we found the evolutionarily older IRs to be present in this species (Chapter I). Further experiments, such as microarrays, immunohistochemistry and attempts using degenerated primers for Orco-cloning (Chapter I, Additional experiments), as well as deep antennal transcriptome sequencing, all failed to show any indication of an OR/Orco based olfactory system. Investigation of an additional whole-body transcriptome did not reveal the transcription of OR and Orco genes elsewhere in the body of *L. y-signata*. Since Orco is conserved across insects and it is normally expressed in a majority of OSNs, it is highly unlikely that we missed Orco-coding sequences due to dissimilarity. In addition, the considerable depth of the sequencing approach makes is seem unlikely that we missed Orco due to low expression levels. Therefore, Orco and ORs seem to be absent in this species, and the olfactory system of *L. y-signata* must rely on other receptor types, presumably IRs.

The IRs are also most likely the exclusive olfactory receptors in crustaceans, the closest relatives of insects (Peñalva-Arana et al. 2009, Croset et al. 2010, Corey et al. 2013). While IRs have been reported from crustacean antennal transcriptomes, ORs or GRs have never been found (Corey et al. 2013, Groh et al. 2013). Since ORs are thought to be an adaption to a terrestrial insect lifestyle, terrestrial crustaceans might show similar adaptations. However, the molecular makeup of the terrestrial hermit crab *Coenobita clypeatus* (Decapoda, Coenobitidae) did not change much in comparison to its aquatic relative *Pagurus bernhardus* (Decapoda, Coenobitidae). The terrestrial hermit crab did not evolve an additional novel class of receptors as detectors for volatile compounds but instead use the same subclass of IRs as its aquatic relative (Groh et al. 2013), which lends further strength to our analysis of the primarily flightless insect species. It will be interesting to investigate the molecular basis of olfaction in the Coconut crab *Birgus latro* (Decapoda, Coenobitidae) in the future. Compared to other terrestrial hermit crabs, like *C. clypeatus*, the olfactory system of *B. latro* appears much more similar to the insect olfactory system (Stensmyr et al. 2005, Hansson et al. 2011). While *C. clypeatus* mainly respond to short chain water-soluble molecules like acids and amine (Krång et al., 2012), *B. latro* has developed a higher capacity to detect airborne volatiles including odors that are insoluble or only slightly soluble in water (Stensmyr et al., 2005), something which is similar to what we observed in our study of *L. y-signata* (Chapter I).

It has been shown that IRs indeed have the capability to detect ligands other than amines and acids, as was the case for *D. melanogaster* IR64a. This IR is expressed in different subpopulations of sensilla in the third chamber of the sacculus, a three-chambered pit organ on the third antennal segment of *D. melanogaster* (Silbering et al. 2011). Corresponding

OSNs are activated either by free protons or organic acids, as well as many other odors, including esters, alcohols and ketones (Ai et al. 2010). Expression of this IR together with IR8a is both necessary and sufficient to introduce sensitivity towards organic acids and other odors, but probably requires a different, still unknown cofactor to mediate the specific response of these OSNs to inorganic acids and to CO_2 (Ai et al., 2010). However, ectopic expression of IR64a and IR8a in *Xenopus* oocytes did not completely recapitulate the whole spectrum measured in antennal lobe calcium imaging (Ai et al. 2013), which again suggests other cofactors.

But how can the response of the neurons expressing the same set of receptors be altered? One possibility might be due to the fact that many IR expressing OSNs do not only show excitation, but also demonstrate inhibition. Most agonists are amines or carboxylic acids. Antagonists, however, belong to various chemical classes, including amines, acids, but also alcohols, ketones and esters (Silbering et al. 2011). IR mediated excitatory and inhibitory olfactory signaling is already well known in lobster OSNs where both excitation and inhibition are mediated by G protein-activated secondary messenger pathways. Odor-evoked excitatory signaling in lobster ORNs involves activation of phosphoinositide-3-kinase and phospholipase C (Xu and McClintock 1999, Corey et al. 2010); however, odor-evoked inhibitory signaling requires activation of cyclic nucleotide signaling (Michel and Ache 1992). A similar signaling pathway was observed in the vertebrate supraoptic nucleus of the hypothalamus where GABA release is mediated by kainate iGluRs through an ionotropic mode of action, whereas its inhibition is mediated by a phospholipase C-dependent metabotropic pathway activated by the same receptor (Bondfardin et al. 2010). Interestingly, the switch between the two modes of action is induced by increased levels of the receptor agonist glutamate. The mechanism that allows the receptors to change between excitatory and inhibitory signaling is not well understood, but this might be a general feature of iGluRs and also IRs (Corey et al. 2013). Nevertheless, a switch between the two modes of activation could also alter the spectrum of agonists and antagonists of IRs dramatically, allowing the detection of a broader chemical range, as we have shown it for *L. y-signata* (Chapter I).

Potential role of GRs

Even though ORs are only distantly related to GRs, one should also consider those as possible receptors for volatile substances. In a recent paper by Tauxe et al. (2013) it has been shown that CO_2-sensitive, GR-expressing cpA olfactory neurons on the maxillary palps of the mosquitoes *Anopheles gambiae* (Diptera: Culicidae) and *Aedes aegypti* (Diptera: Culicidae)

are also sensitive to human skin odors. CpA neurons are housed in capitate peg (cp) sensilla which are found on the maxillary palps and express three conserved members of the GR gene family. Beside CO_2 these neurons responded to 3-hexanol, 3-methyl -2-buten -1-ol, 2,5-dimethylpyrazine, 4-methyl -3-penten -2-one, 1-pentanol, 1-butanol, butanone, 3-methyl -1-butanol and many more (Tauxe et al. 2013). The GR-encoded receptors are required for detecting odorant ligands as well as CO_2, a likely evolutionarily conserved function since a similar response spectrum was shown for *D. melanogaster* CO_2 neurons (Tauxe et al. 2013). The receptors themselves are conserved across dipterans, lepidopterans and coleopterans (reviewed in Robertson and Kent 2009).

In accordance with previous studies that suggest the lineage of CO_2 receptors to be absent in crustaceans and more basal insect orders (Robertson and Kent 2009), we did not identify the orthologue CO_2 receptors in the transcriptomes of *L. y–signata* or *T. domestica* (Chapter I). Most gustatory receptors we found were sorted into the sugar receptor or the fructose receptor groups. Both receptor groups are evolutionarily old and conserved across insects (Kent and Robertson 2009, Freeman et al. 2014). Both *L. y-signata* and *T. domestica* have gustatory sensilla on their antennae and at least for *T. domestica* a response of those gustatory sensilla towards sugars has been previously shown (Hansen-Delkeskamp 2001). Both detection of sugars and the detection of a proposed contact-pheromone (Fröhlich and Lu 2013) likely involve GRs. Because only a small set of GRs was identified in *L. y-signata* and *T. domestica* an involvement of the limited set of GRs beyond this scope is very unlikely (Chapter I).

Evolution of Orco

Although it does not bind ligands, Orco is an essential part of the insect OR/Orco complex. Orco is necessary for both, the localization of ORs to dendritic membranes (Larsson et al. 2004, Benton et al. 2006) and the odor induced ionotropic signalling (Sato et al. 2008, Wicher et al. 2008). In contrast to the bristletail *L. y-signata*, where no ORs and Orco were found, we have identified three Orco-like variants in the firebrat transcriptome (Chapter I). Previously, only one Orco orthologue had been identified in each insect studied (reviewed in Stengl and Funk 2013). The three *T. domestica* Orco variants we found are not splicing variants and differ in their actual sequences. All Orco-like variants of *T. domestica* were found to be expressed in the antennae, and for one Orco we could show its expression specifically in the antennal OSNs (Chapter I). While the antennal expression argues for a potential involvement in chemosensation, the existence of three Orco types found in the absence of ORs remains mysterious, since they were always found together in other insect studies. One possibility

could be that the proteins interact with other receptors like the more ancient GRs, building GR/Orco complexes. Thus far, no single Orco-like counterpart has been identified among GRs. Although some GRs are coexpressed inside the same gustatory neuron, for example the CO_2 receptors (Jones et al. 2007, Kwon et al. 2007), the sugar receptors (Jiao et al. 2008) and bitter receptors (Lee et al. 2009), the combined ectopic and mutant analysis suggested that each receptor contributes to detection of ligands and does not exclusively serve as a coreceptor (Freeman et al. 2014). Additionally, these receptor pairs often form individual receptor lineages in phylogenetic analyses, which indicate a common origin of those GRs, a situation that is comparable to what we found for the *T. domestica* Orcos. A second possible explanation could be that the *T. domestica* Orcos form a functional receptor, building heteromers with one or two of the other coreceptor-like variants, as the sugar or CO_2 receptors do. Alternatively, Orco homomers could form functional channels on their own (Jones et al. 2011), mediating a cation current gated by cyclic nucleotides (Wicher et al. 2008). Cyclic nucleotides are produced in secondary messenger pathways by adenylyl cyclases. In crustaceans an antennal specific adenylyl cyclase is a candidate for producing cAMP in the inhibitory olfactory transduction pathway (Doolin and Ache 2005), likely activated by IRs. The *T. domestica* Orco-like candidates therefore might serve as an ion channel in OR-independent processes.

Evolution of ORs

Although insect ORs are unrelated to vertebrate and nematode olfactory receptors they appear to have derived from the GR family (Scott et al. 2001, Robertson et al. 2003). GRs have been identified in insects as well as in the related aquatic crustaceans (Penãlva-Arana et al. 2009) and beyond (Vieira and Rozas 2011). The transition of the hexapodan ancestors from an aquatic to a terrestrial lifestyle was often seen as a driving force for the evolution of ORs (Robertson et al. 2003). The olfactory system had to adapt to terrestrial conditions and the detection of volatile, air-borne chemicals. However, our data suggest that ORs evolved in insect evolution after the emergence of Archaeognatha and Zygentoma, and therefore long after insects transitioned to a terrestrial lifestyle (Chapter I). At the time when pterygote insects appeared, the vegetation on earth was rapidly spreading and diversifying. This was connected to a fast extension of available chemical cues that could be used by the insects. Odor detection might also have needed to change during the evolution of insect flight, since a much higher temporal resolution and a higher sensitivity are necessary to navigate at higher speeds and further from the ground. It has been shown that IR-expressing OSNs are better in

close range odor detection where odor interaction time is not a limiting factor. This might be the case for non-flying insects that live on or in the ground. In contrast OR-expressing neurons are more sensitive and better at resolving brief stimuli, but show desensitization/adaptation at longer stimulus duration (Getahun et al. 2012). ORs therefore might not only increase the chemical detection ability of insects, but also allow the olfactory system to more rapidly assess airborne odors. We therefore hypothesize that insect ORs evolved after insects acquired flight.

Single-walled versus double-walled sensilla

Chemosensory sensilla are well known in the different arthropod groups, including Arachnida (Tichy and Barth 1992), Myriapoda (reviewed in Tichy and Barth 1992), Crustacea (Schmidt and Gnatzy 1984) and Hexapoda (reviewed in Hallberg and Hansson 1999). Hexapod olfactory sensilla are of two categories: sw-wp sensilla of various shapes and dw-wp sensilla (Altner 1977) with spoke channels. The oldest insect taxon where dw-wp sensilla were identified is Zygentoma, having both sw-wp and dw-wp sensilla as well as an intermediate sensillum type (Berg and Schmidt 1997). Meineke (1975) suggests that dw-wp sensilla are the ancient type of insect olfactory sensilla, which he assessed by comparing different sensillum types in lamellicornian beetles. Based on this view, Berg and Schmidt (1997) hypothesized that the intermediate sensillum type in Zygentoma might represent the precursor for sw-wp sensilla, but it might well be the other way around. Following Meinekes argument, the dw-wp sensilla must have been lost independently in Collembola, Protura and Archaeognatha, because as in Archaeognatha (Berg and Schmidt 1997) also in Collembola (Slifer and Sekhon 1978) and Protura (Dallai and Nosek 1981) only sw-wp basiconic sensilla were identified. The evidence that dw-wp sensilla evolved within the lineage of Dicondylia is more sparse.

So far OR and IR expression in insect antennae has only been characterized in a few species. In contrast to *L. y-signata* were we could show IR coreceptor expression close to sw-wp sensilla (Chapter I), both in the vinegar fly *D. melanogaster* (Benton et al. 2009) and in the desert locust *S. gregaria* (Chapter III) IRs and ORs are expressed in complementary sets of OSNs. OSNs in coeloconic sensilla (dw-wp sensilla) express one or both putative IR coreceptors (Benton et al. 2009; Chapter III) in combination with other antennal IRs (Benton et al. 2009), whereas OSNs of basiconic and trichoid sensilla (both sw-wp sensilla) express Orco and ORs (Yang et al. 2012, Benton et al. 2009). OR and Orco expression is reported for only one coeloconic OSN type in *D. melanogaster* (Benton et al. 2009) and is completely absent inside the coeloconic sensilla of *S. gregaria* (Yang et al. 2012, Chapter III).

The reasons for a tendency for certain receptor types to be expressed in one or the other type of insect sensillum, and the actual effect of the sensillum type on the neuronal response to odorants is still unknown. Different wall structures of the sensilla probably act as a filter for stimulating substances (Steinbrecht and Müller 1976, Altner 1977, Altner and Prillinger 1980, Schaller 1982, Zacharuk 1980, 1985, Keil and Steinbrecht 1984). In a tracer study by Hawke and Farly (1971) it was found that the tracer lanthanum did not penetrate the spoke channels of dw-wp sensilla unless chloroform had been used, whereas acetone as solvent was necessary for filling sw-wp sensilla. The authors concluded that the walls of the grooves are lined with a lipid material that is more rapidly dissolved by chloroform. Chloroform is a more polar molecule that dissolves phospho- and neutral lipids better than acetone, whereas acetone dissolves more hydrophobic layers. This might be a hint that hydrophilic odors can more easily penetrate into dw-wp sensilla whereas hydrophobic odors better diffuse into sw-wp sensilla (Steinbrecht 1997), what matches very well to the complementary ligand spectra of IRs and ORs in higher insects (Silbering et al. 2011).

In SSR-experiments we have found that OSNs of different sensillum types of the firebrat *T. domestica* showed a slightly different response spectrum when compared to the bristletail *L. y-signata*, where only sw-wp sensilla are present (Chapter I). In the latter species, many OSNs were rather broadly tuned and responded to acids, aldehydes and many other odors, while other OSNs within the same sensillum were specific to acids and aldehydes. In *T. domestica* a much better spatial separation between the detection of hydrophilic and hydrophobic odorants was seen. OSNs in sw-wp sensilla mostly responded to alcohols, ketones and esters, whereas OSNs in dw-wp sensilla mostly responded to aldehydes, amines and acids. However, some of the OSNs inside the grooved sensilla of *T. domestica* also responded to esters, ketones and alcohols.

At least one Orco-variant of *T. domestica* is expressed in a subpopulation of OSNs inside sw-wp sensilla, but not in OSNs of dw-wp sensilla (Chapter I). It therefore seems that the beginning evolution of a second receptor family, with the coreceptor Orco present in *T. domestica* (discussed above) co-occurred with the design of a second olfactory sensillum type.

Independent evolution of OBPs and ORs

There is a hypothesis that insect OBPs and ORs evolved in parallel after insects have colonized land, similar to the binding proteins and olfactory G protein-coupled receptors of vertebrates (Vieira and Rozas 2011). However, OBPs, but not ORs were identified in *L. y-*

signata and *T. domestica* (Chapter I and II). The *L. y-signata* and *T. domestica* OBPs are the oldest insect OBPs described so far. But what was the origin of these genes?

OBPs are not exclusivity expressed in olfactory tissue. Many OBPs were localized in gustatory pits (Angeli et al. 1999, Galindo and Smith 2001) and non-chemosensory tissue (e.g. Gong et al. 2009, Zhang et al. 2013). These proteins may thus participate in other physiological functions (reviewed in Pelosi et al. 2006), for example, as carriers of hormones and other regulatory compounds (Iovinella et al. 2013). One *D. melanogaster* OBP, DmelOBP19d (also Pbprb2), was found to be expressed in coeloconic sensilla. Within coeloconic sensilla the protein has only been detected in the outer, but not in the inner sensillum lymph and could therefore not be involved in the actual transfer of odors through the sensillum lymph (Shanbhag et al. 2001). Additionally, DmelOBP19d was located in gustatory sensilla and epidermal cells of the funiculus and the maxillary palp (Shanbhag et al. 2001). One hypothesis for the presence of OBPs in epidermal cells is that these cells secrete apolar, water-insoluble substances into the cuticle, especially for building the epicuticular layers (reviewed in Locke 1998, Shanbhag et al. 2001). These substances have to pass through the aqueous environment of the cells and the inner cuticle, maybe with the help of OBP-like carrier proteins. Epidermal cells are the precursors of sensillum cells (for review see Keil 1997) and OBPs could have evolved by specialization from those general carrier proteins (Shanbhag et al. 2001). After terrestrialization, there might be a selective pressure leading to the diversification of those carrier proteins to function as mediator of airborne molecules by transferring odor molecules to the receptors (Vieira and Rozas 2011).

The idea that at least some of the OBPs identified in *L. y-signata* and *T. domestica* play a significant role in olfaction is supported by the presence of putative homologues of the only two conserved insect OBPs, the OBP59a and OBP73a (not found in Hymenoptera, Zhou et al. 2010, Chapter II). At least for the *D. melanogaster* OBP59a, a function in olfaction has been proposed. A reduced expression of DmelOBP59a alters the behavioral response of the flies to 1-hexanol, 2-heptanone and propanal (Swarup et al. 2011). The strong conservation of both of these OBPs indicates a critical role for these proteins, especially since OBPs normally are highly divergent (Pelosi et al. 2005). Since OBPs are thought to mediate the transferring process of odorants through the lymph (Vogt et al. 1991, Pelosi 1994, Pophof 2004, Prestwich et al. 1995, Tsuchihara et al. 2005, Grosse-Wilde et al. 2006), they could also have an influence on the actual response spectrum of other receptors, such as the IRs of *L. y-signata*. Odorants that rarely reach the receptors by just diffusion could interact with receptor proteins in the presence of OBPs.

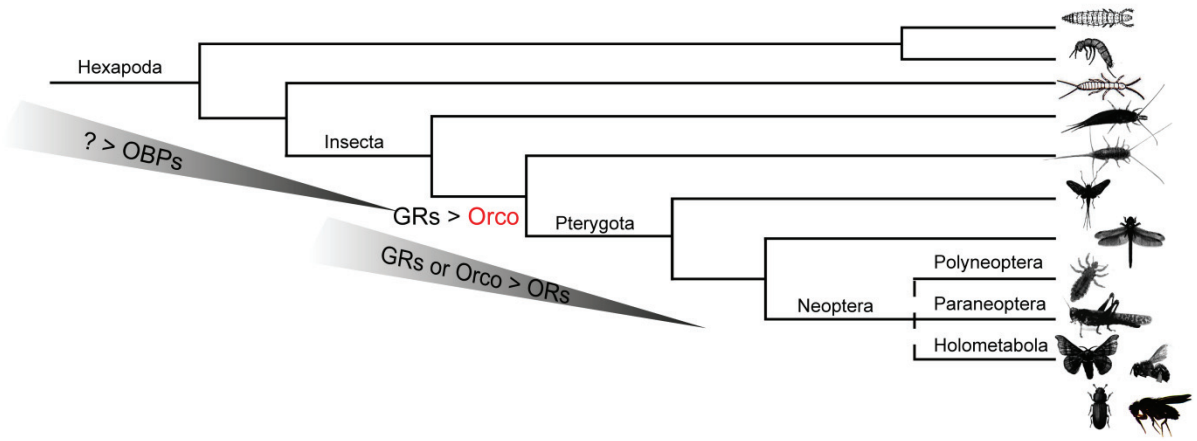


Figure 1. Hypothetical evolution of insect odorant binding proteins (OBPs) and insect olfactory receptors (ORs).
Both ionotropic receptors (IRs) and gustatory receptors (GRs) were found in other arthropod groups as chemoreceptors, however OBPs and ORs are specific to the insects. Both families evolved independently with OBPs present in the last common ancestor of Insecta *sensu stricto*. An insect OR/Orco based olfactory system evolved after the development of wings.

After the evolution of ORs and Orco, OBPs might have been secondarily coopted into an OR/Orco subunit based olfactory pathway and the diversification of ORs and OBPs that we see today in higher insects could be the result of a coevolution of both gene families.

Altogether this thesis can demonstrate that OBPs and ORs did not evolve together, but they rose independently, most likely OBPs first, followed by ORs, as supported by the presence of OBPs already found in the oldest insect taxon, the Archaeognatha. ORs occurred long time after insects colonized land with their coreceptor first (Figure 5). Lastly, this thesis supports the idea that the full OR/Orco complex could be an adaptation to insect flight and the demands on sensory speed and acuity raised thereby.

Future prospects

My thesis presents pioneering work in the field of insect olfactory receptor evolution and provides a very good starting point for future studies in this area. By investigating the two apterygote species we were able to identify a critical evolutionary step that is worthy of further analysis. Investigation of more antennal transcriptomes or genomes of both Archaeognatha and Zygentoma, as well as the first flying insect orders, the Ephemeroptera and Odonata, will help to understand more about the evolution of the OR gene family and provide possible ideas for the forces driving this evolution. Heterologous expression of the different *T. domestica* Orco variants will show if these receptors already possess the same properties as the Orco proteins of other insects and if they can take over the coreceptor

function by combining them with ORs of other insects such as *D. melanogaster*. Future expression of a single Orco or combinations of the different variants as well as GRs or IRs together with application of odors (e.g. ligands from Chapter I) could show if these proteins are sufficient to introduce odor responses in heterologous expression systems.

There also remains the question of OBP function in an OR independent olfactory system. OBP localization and binding assays could give hints about their possible function in *L. y-signata* and *T. domestica* olfaction or provide evidence for an ancient and not yet known function. Investigation of OBPs in Collembola, Protura and Diplura could add additional information about the origin of the OBP gene family.

Summary

Chemosensation is essential for the survival of organisms ranging from bacteria to animals, and the gene families involved are as divergent as the organisms themselves. In addition, our understanding of the biochemical basis of chemosensation is incomplete because only a limited number of species have been studied thus far. This also holds true for insects, which represent the most diverse and abundant animal group. Insects owe this success not least a highly elaborated sense of smell. Many insect behaviors are highly dependent on olfaction, such as inter- and intraspecific communication, as well as locating food and oviposition sites. Mainly two receptor families have been described as detectors of olfactory stimuli in insects, these include: Olfactory receptors (ORs) and ionotropic receptors (IRs). While IRs are expressed in chemosensory tissue across protostomes, ORs were only found to be present in insects. The ligands of ORs are mainly hydrophobic molecules that have to cross the aqueous lymph inside the olfactory sensory structures, the sensilla. Odorant binding proteins (OBPs) are thought to facilitate this transfer of these odorant molecules across the aqueous lymph to the necessary receptors. Both the OR and OBP gene families are thought to have evolved after insects colonized terrestrial habitats, and that they coevolved in order to assist in the detection of airborne chemicals.

Since no previous studies were conducted on insect taxa other than the modern wing-folding insects, namely the Neoptera, my research presented in this thesis was designed to fill this gap in our understanding of OR and OBP evolution through the study of the older, primarily wingless insect taxa.

Deep antennal transcriptome sequencing combined with electrophysiological investigations was able to show that an airborne or terrestrial sense of smell is already present in the oldest insect taxa, namely the Archaeognatha and Zygentoma (Chapter I). Both the jumping bristletail *Lepismachilis y-signata* (Archaeognatha: Machilidae) and the firebrat *Thermobia domestica* (Zygentoma: Lepismatidae) possess a functional olfactory system, but the genetic basis of olfaction is different in these taxa than that of the Neoptera. Neither ORs nor their coreceptor Orco were found in the different transcriptome datasets of *L. y-signata* and only different coreceptor variants were identified in *T. domestica*. Unlike ORs, the Orco sequence

is highly conserved across different insect odors, facilitating its identification. Various attempts to identify Orco in the *L. y-signata* failed (Chapter I, Additional experiments). Instead of ORs and Orco, the evolutionary older IRs were detected in transcriptomes of *L. y-signata*. In addition, the IR coreceptor coding RNA was located in sensory neurons close to single-walled basiconic sensilla (Chapter I). In flying insects such as the vinegar fly *Drosophila melanogaster* (Diptera: Drosophilidae) or the desert locust *Schistocerca gregaria* (Caelifera: Acrididae) (Chapter III) antennal IRs are mainly expressed in double-walled coeloconic sensilla, a sensillum type that is completely absent in *L. y-signata* and other archaeognathan species.

Similar to *L. y-signata*, not a single OR candidate was identified in *T. domestica*. However, we found three Orco-like sequences, a situation never before described in insects (Chapter I). Both in previous insect transcriptomes and in insect genomes, only one Orco has ever been detected. All Orco-like variants of *T. domestica* were amplified from antennal cDNA, but not from the leg, the head (without antennae and palps) nor from body samples. For one Orco variant of *T. domestica* we were able to show its transcription in OSNs of single-walled olfactory sensilla, which strongly suggests a function of this Orco in olfaction. However, the exact function of the three Orco-like candidates that were identified in this research need to be further investigated.

Although ORs were completely missing in both species that we examined, we could still successfully identify OBPs (Chapter II), including homologues of the only two conserved insect OBPs (the OBP59a and the OBP73a subgroup). For one orthologue, the *D. melanogaster* DmelOBP59a, a role in olfaction has been shown. Moreover, the strong conservation of this OBP across the insects suggests a critical and conserved role for the protein, especially since OBPs are typically highly divergent.

Altogether the results of my thesis suggest that the insect ORs evolved a long time after insects had already colonized land, and additionally that the coreceptor Orco evolved before ORs. The results also show an independent origin of insect ORs and OBPs, with OBPs already present in the last common ancestor of Insecta *sensu stricto*. In addition, the results of my thesis suggest that the full OR/Orco complex could be an evolutionary adaptation in conjunction with insect flight and a necessary adaptation to the increased demands for sensory speed and acuity at higher movement speeds.

Zusammenfassung

Die Wahrnehmung von chemischen Stoffen ist für die meisten Lebewesen lebensnotwendig. Nicht nur Tiere, sondern auch Bakterien, Pilze und Pflanzen detektieren chemische Stoffe in ihrer Umgebung. Die Detektoren (Chemorezeptoren) sind dabei so vielfältig, wie die Organismen selbst. Da bislang nur wenige Organismen auf das Vorkommen von Chemorezeptoren untersucht wurden, bestehen noch gravierende Lücken im Verständnis zur Evolution der zugrunde liegenden Genfamilien. Das gilt auch für die Insekten, die mit nahezu einer Millionen beschriebenen Arten die größte und erfolgreichste Tiergruppe darstellen. Diesen Erfolg verdanken Insekten nicht zuletzt ihrem extrem gut entwickelten Geruchssinn. Insekten nutzen ihren Geruchssinn für inner- und zwischenartliche Verständigung, sowie für das Auffinden von Futter und Eiablageplätzen. Hierbei spielen hauptsächlich zwei Familien von Geruchsrezeptoren ein Rolle: die Olfaktorischen Rezeptoren (ORs) und eine spezialisierte Gruppe innerhalb der ionotropen Glutamatrezeptoren (IRs). Während die Expression von IRs in chemosensorischen Geweben verschiedenster Protostomier (Altmünder), wie Krebsen, Spülwürmern und auch Schnecken gezeigt werden konnte, wurden ORs bislang nur bei Insekten gefunden. Dies trifft auch auf eine zweite Proteinfamilie zu, die sogenannten Odorant-Bindeproteine (OBPs). Es wird angenommen, dass diese Proteine dabei helfen die überwiegend wasserunlöslichen Geruchsmoleküle durch die wässrige Sensillumlymphe zu transportieren, in der die Dendriten der sensorischen Neurone und somit auch die Geruchsrezeptoren selber liegen. Es existiert die Hypothese das sowohl ORs, als auch OBPs im Zuge des Landgangs der Insekten entstanden sind, um die flüchtigen, überwiegend wasserunlöslichen Substanzen aus der Luft wahrnehmen zu können.

Diese Hypothese basiert aber ausschließlich auf Untersuchungen an geflügelten Insekten. Die evolutionär gesehen älteren, flügellosen Insektengruppen sind bislang nicht auf diese Hypothese hin untersucht worden. Die hier vorliegende Arbeit soll genau diese Lücke schließen.

Elektrophysiologische Untersuchungen im Rahmen der vorliegenden Arbeit konnten zeigen, dass sowohl der Felsenspringer *Lepismachilis y-signata* (Archaeognatha: Machilidae), als

auch das Ofenfischchen *Thermobia domestica* (Zygentoma: Lepismatidae) über einen guten Geruchssinn verfügen (Chapter I). Die bioinformatische Auswertung sehr umfangreicher Transkriptome brachte jedoch zum Vorschein, dass die genetische Basis ihres Geruchssinns sich von der der geflügelten Insektengruppen unterscheidet. Im Transkriptom des Felsenspringers konnten weder die oben angesprochenen ORs, noch ihr stark konservierter Korezeptor Orco gefunden werden. Jedoch waren die evolutionärgeschichtlich älteren IRs vorhanden. In der Antenne des Felsenspringer *L. y-signata* konnte die RNA der IR-Korezeptoren in enger räumlicher Nahe zu den entsprechenden olfaktorischen Sensillen gezeigt werde (Chapter I), was die Annahme unterstützt, dass diese Tiere andere Rezeptoren als die ORs zur Geruchsdetektion verwenden. IRs werden bei anderen Insekten wie der Fruchtfliege *Drosophila melanogaster* (Diptera: Drosophilidae) oder auch der Wüstenheuschrecke *Schistocerca gregaria* (Caelifera, Acrididae) (Chapter III), in den sensorischen Neuronen der coeloconischen Sensillen exprimiert, ein Sensillumtypus der jedoch bei *L. y-signata* und anderen Felsenspringerarten fehlt.

Ähnlich wie in den Datensätzen des Felsenspringers, wurden keinerlei Anzeichen für ORs im Transkriptom des Silberfischchens gefunden. Allerdings wurden mehrere Sequenzen identifiziert, die der des OR-Korezeptors sehr ähnlich sind. Alle drei Kandidaten konnten in der Antenne, jedoch nicht in Beinen, Köpfen (ohne Antennen und Maxillarpalpen) oder Körpern nachgewiesen werden. Die RNA eines der Orco-Kandidaten konnte mittels *in situ* Hybridisierung in einzelnen sensorischen Neuronen der einwandigen Geruchssensillen lokalisiert werden, was eine Funktion in der Geruchswahrnehmung vermuten lässt. Ihre genaue Funktion gilt es jedoch in der Zukunft zu erforschen.

Obwohl weder beim Felsenspringer, noch beim Ofenfischchen ORs identifiziert werden konnten, sind doch bei beiden Arten OBPs vorhanden (Chapter II). Darunter befanden sich Homologe der einzigen beiden innerhalb der Insekten konservierten OBPs (OBP59a und OBP73a). Für eines dieser Proteine konnte in vorangegangen Studien an *D. melanogaster* eine Funktion im Geruchsinn gezeigt (DmelOBP59a) werden. Da dieses Protein auf der Sequenzebene relativ stark konserviert sind, könnte dies auch auf seine Funktion zutreffen, vor allem da die meisten Insekten OBPs nichts als ihr konserviertes Cysteinmuster und ihre Sekundärstruktur gemein haben.

Zusammenfassend zeigen die Ergebnisse der hier vorliegenden Arbeit, dass die insektenspezifischen ORs lange Zeit nach dem Landgang der Insekten entstanden sind. Der vollständige Rezeptorkomplex, bestehend aus einem OR und dem Korezeptor könnte eine

Anpassung an das Fliegen darstellen, da während des Fliegens sowohl die Geschwindigkeit, also auch die Sensitivität viel höher sein müssen, um das gewünschte Ziel zu finden.
Zusätzlich konnte diese Arbeit zeigen, dass OBPs unabhängig von den ORs entstanden sind, denn OBPs dürften bereits beim letzten gemeinsamen Vorfahren der Insecta *sensu stricto* vorhanden gewesen sein.

Figure 2. ***Thermobia domestica*** (Photo: Sascha Bucks)

References

Abuin L, Bargeton B, Ulbrich MH, Isacoff EY, Kellenberger S, Benton R. 2011. Functional Architecture of Olfactory Ionotropic Glutamate Receptors. *Neuron* **69(1)**:44-60.

Adel T. 1984. Sensilla and sensilla pattern on the antenna of *Thermobia domestica* and *Lepisma saccharina* (Insecta: Zygentoma). *Braunschweiger naturkdl Schr.* **2**:191-218.

Ai M, Min S, Grosjean Y, Leblanc C, Bell R, Benton R, Suh GS. 2010. Acid sensing by the *Drosophila* olfactory system. *Nature* **468**(7324):691-695.

Ai M, Blais S, Park JY, Min S, Neubert TA, Suh GS. 2013. Ionotropic Glutamate Receptors IR64a and IR8a Form a Functional Odorant Receptor Complex In Vivo in *Drosophila*. *J Neurosci*. **33**(26):10741-10749.

Altner H. 1977. Insect sensillum specificity and structure: an approach to a new typology. In: Le Magnen J, MacLeod P, editors. *Olfaction and Taste VI.* London: Information Retrieval. p.295-303.

Altner H, Ernst KD. 1974. Structural peduliarities of antennal sensilla of soil inhabiting Collembola. *Pedobiologia* **14**(2-5):118-122.

Altner H, Prillinger L. 1980. Ultraststructure of invertebrate chemo-, thermo-, and hygroreceptors and its functional significance. *Int Rev Cytol*. **67**:69-139.

Angeli S, Ceron F, Scaloni A, Monti M, Monteforti G, Minnocci A, Petacchi R, Pelosi P. 1999. Purification, structural characterization, cloning and immunocytochemical localization of chemoreception proteins from *Schistocerca gregaria*. *Eur J Biochem.* **262**(3):745-754.

Anton S, Homberg U, 1999. Antennal lobe structure. In: Hansson BS, editor. *Insect Olfaction*. Berlin: Springer. p. 97-124.

Angerer LM, Angerer RC. 1992. In situ hybridization to cellular RNA with radiolabeled RNA probes. In: Wilkinson DG, editor. *In Situ Hybridization. A Practical Approach*. Oxford: IRL Press. p15-32.

Bargmann CI. 2006. Comparative chemosensation from receptors to ecology. *Nature* **444**(7117):295-301.

Bengtsson JM, Trona F, Montagne N, Anfora G, Ignell R, Witzgall P, Jacquin-Joly E. 2012. Putative Chemosensory Receptors of the Codling Moth, *Cydia pomonella*, Identified by Antennal Transcriptome Analysis. *PLoS ONE.* **7**:e31620.

Benton R, Sachse S, Michnick SW, Vosshall LB. 2006. Atypical membrane topology and heteromeric function of Drosophila odorant receptors in vivo. *PLoS Biol*. **4**(2):e20.

Benton R, Vannice KS, Gomez-Diaz C, Vosshall LB. 2009. Variant Ionotropic Glutamate Receptors as Chemosensory Receptors in *Drosophila*. *Cell.* **136**(1):149-162.

Berg J, Schmidt K. 1997. Comparative morphology and moulting of sensilla basiconica of *Lepisma saccharina* Linnaeus (Zygentoma: Lepismatidae) and *Machilis* spec. (Archaeognatha: Machilidae). *Int J Insect Morphol Embryol.* **26**(3):161-172.

Bitsch C, Bitsch J. 2004. Phylogenetic relationships of basal hexapods among the mandibulate arthropods: a cladistic analysis based on comparative morphological characters. *Zool Scr.* **3**(6):511-550.

Blaney WM, Chapman RF .1969. The fine structure of the terminal sensilla on the maxillary palps of *Schistocerca gregaria* (Forskal) (Orthoptera, Acrididae). *Z Zellforsch Mikrosk Anat.* **99**(1):74-97.

Bockhorst KG. 1988. Patterns of sensilla on the antennae of some Machilidae (Insecta, Archaeognatha). *Braunschweiger naturkdl Schr.* **3**:137-170.

Bonfardin VD, Fossat P, Theodosis DT, Oliet SH. 2010. Glia-dependent switch of kainate receptor presynaptic action. *J Neurosci.* **30**(3):985-995.

Boore JL, Lavrov DV, Brown WM. 1998. Gene translocation links insects and crustaceans. *Nature.* **392**(6677)*:*667-668.

Buck L., Axel R. 1991. A novel multigene family may encode odorant receptors: A molecular basis for odor recognition. *Cell.* **65**(1): 175-187.

Carapelli A, Nardi F, Dallai R, Frati F. 2006. A review of molecular data for the phylogeny of basal hexapods. *Pedobiologia*. **50**(2):191-204.

Carapelli A, Li ´o P, Nardi F, van der Wath E, Frati F. 2007. Phylogenetic analysis of mitochondrial protein coding genes confirms the reciprocal paraphyly of Hexapoda and Crustacea. *BMC Evol Biol.* **7**(Suppl. 2):S8.

Carey AF, Wang G, Su CY, Zwiebel LJ, Carlson JR. 2010. Odorant reception in the malaria mosquito *Anopheles gambiae*. *Nature*. **464**(7285):66-71.

Cascales-Miñana B; Cleal CJ. 2011. Plant fossil record and survival analyses. *Lethaia.* **45**(1):71-82.

Celorio-Mancera M de la P., Sundmalm SM, Vogel H, Rutishauser D, Ytterberg J, Zubarev RA, Janz N. 2012. Chemosensory proteins, major salivary factors in caterpillar mandibular glands. *Insect Biochem Molec.* **42**(10):796-805.

Chertemps T, Francois A, Durand N, Rosell G, Dekker T, Lucas P, Maïbèche-Coisne. 2012. A carboxylesterase, Esterase-6, modulates sensory physiological and behavioral response dynamics to pheromone in *Drosophila*. *BMC Biol.* **10**:56.

Clyne PJ, Warr CG, Freeman MR, Lessing D, Kim J Carlson JR**.** 1999. A novel family of divergent seven-transmembrane proteins: candidate odorant receptors in *Drosophila. Neuron*. **22**(2)*:*327-338.

Clyne PJ, Warr CG, Carlson JR. 2000. Candidate taste receptors in *Drosophila*. *Science.* **287**(5459):1830-1834.

Conesa A, Götz S, García-Gómez JM, Terol J, Talón M, Robles M. 2005. Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. *Bioinformatics.* **21**(18):3674-3676.

Corey EA, Bobkov Y, Pezier A, Ache BW. 2010. Phosphoinositide 3-kinase mediated signaling in lobster olfactory receptor neurons. *J Neurochem*. **113**(2):341-350.

Corey EA, Bobkov Y, Ukhanov K, Ache BW. 2013. Ionotropic crustacean olfactory receptors. *PLoS ONE.* **8**(4):e60551.

Croset V, Rytz R, Cummins SF, Budd A, Brawand D, Kaessmann H, Gibson TJ, et al. 2010. Ancient Protostome Origin of Chemosensory Ionotropic Glutamate Receptors and the Evolution of Insect Taste and Olfaction. *PLoS Genet.* **6**(8):e1001064.

Cui X, Wu C, Zhang L. 2011. Electrophysiological response patterns of 16 olfactory neurons from the trichoid sensilla to odorant from fecal volatiles in the locust, *Locusta migratoria manilensis*. *Arch Insect Biochem Physiol.* **77**(2):45-57.

Dallai R, Nosek J. 1981. Ultrastructure of sensillum t_1 on the foretarsus of *Acerentomon majus* Berlese (Protura: Acerentomidae). *Int J Insect Morphol Embryol*. **10**(4):321-330.

Danty E, Briand L, Michard-Vanhée C, Perez V, Arnold G, Gaudemer O, Huet D, Huet JC, Ouali C, Masson C, Pernollet JC. 1999. Cloning and expression of a queen pheromone-binding protein in the honeybee: an olfactory-specific, developmentally regulated protein. *J Neurosci.* **19**(17):7468-75.

Dobritsa AA, Goes van Naters W, Warr CG, Steinbrecht RA, Carlson JR. 2003. Integrating the molecular and cellular basis of odor coding in the *Drosophila* antenna. *Neuron.* **37**(5):827-841.

Eddy SR. 2011. Accelerated Profile HMM Searches. *PLoS Comput Biol.* **7**(10):e1002195.

Elmore T, Smith DP. 2001. Putative *Drosophila* odor receptor OR43b localizes to dendrites of olfactory neurons. *Insect Biochem Mol Biol.* **31**(8):791-798.

Elmore T, Ignell R, Carlson JR, Smith DP. 2003. Targeted mutation of a *Drosophila* odor receptor defines receptor requirement in a novel class of sensillum. *J Neurosci.* **23**(30):9906-9912.

Farris SM. 2005. Evolution of insect mushroom bodies: old clues, new insights. *Arthropod Struct Dev*. **34**(3):211-234.

Firestein S. 2001. How the olfactory system makes sense of scents**.** *Nature.* **413**(6852):211-218.

Freeman EG, Wisotsky Z, Danhanukar A. 2014. Detection of sweet tastants by a conserved group of insect gustatory. *Proc Natl Acad Sci U S A*. **111**(4):1598-1603.

Friedrich M, Tautz D. 2001. Arthropod rDNA phylogeny revisited: a consistency analysis using Monte Carlo Simulation. *Ann Soc Entomol Fr.* **37**(1-2):21-40.

Fröhlich A, Lu Z. 2013. The "rosette-like" structures in the cuticle of *Petrobius brevistylis* are the openings of epidermal glands. *Arthropod Struct Dev.* **42**(1):89-94.

Furusawa T, Rakwal R, Nam HW, Hirano M, Shibato J, Kim YS, Ogawa Y, Yoshida Y, Kramer KJ, Kouzuma Y, Agrawal GK, Yonekura M. 2008. Systematic investigation of the hemolymph proteome of *Manduca sexta* at the fifth instar larvae stage using one- and two-dimensional proteomics platforms. *J Proteom Res*. **7**(3):938-959.

Galindo K, Smith DP. 2001. A large family of divergent *Drosophila* odorant-binding proteins expressed in gustatory and olfactory sensilla. *Genetics*. **159**(3):1059-1072.

Galizia CG, Rössler W. 2010. Parallel olfactory systems in insects: anatomy and function. *Annu Rev Entomol*. **55**:399-420.

Gao Q, Chess A. 1999. Identification of candidate *Drosophila* olfactory receptors from genomic DNA sequence. *Genomics.* **60**(1):31-39.

Gereau, RW, Swanson GT. 2008. *The Glutamate Receptors*. Totowa, N.J.: Humana Press.

Getahun MN, Wicher D, Hansson BS, Olsson SB. 2012. Temporal response dynamics of *Drosophila* olfactory sensory neurons depends on receptor type and response polarity. *Front Cell Neurosci.* **6**:54.

Gohl T, Krieger J. 2006. Immunolocalization of a candidate pheromone receptor in the antenna of the male moth, *Heliothis virescens*. *Invert Neurosci.* **6**(1):13-21.

Gomez-Diaz C, Reina JH, Cambillau C, Benton R. 2013. Ligands for pheromone-sensing neurons are not conformationally activated odorant binding proteins. *PLoS Biol.* **14**(4):e1001546.

Gong DP, Zhang HJ, Zhao P, Qing-You X, Xiang ZH. 2009. The Odorant Binding Protein Gene Family from the Genome of the Silkmoth, *Bombyx mori. BMC Genomics*. **10**:332.

Greenwood M, Chapman RF. 1984. Differences in numbers of sensilla on the antennae of solitarious abd gregarious *Locusta migratoria L.* (Orthoptera: Acrididae). *Int J Morphol and Embryol.* **13**:295-301.

Grimaldi D. 2001. Insect evolutionary history from Handlirsch to Hennig, and beyond. *J Paleontol.* **75**:1152-1160.

Grimaldi D, Engel MS. 2005. *Evolution of the Insects*. Cambridge, UK: Cambridge Univ. Press.

Groh CK, Vogel H, Stensmyr MC, Grosse-Wilde E, Hansson BS. 2013. The hermit crab´s nose – antennal transcriptomics. *Front Neurosci*. **7**:266.

Grosse-Wilde E, Svatos A, Krieger J. 2006. A pheromone-binding protein mediates the bombykol-induced activation of a pheromone receptor in vitro. *Chem Senses*. **31**(6):547-555.

Grosse-Wilde E, Kuebler LS, Bucks S, Vogel H, Wicher D, Hansson BS. 2011. Antennal transcriptome of *Manduca sexta*. *Proc Natl Acad Sci U S A*. **108**(18):7449-54.

Guo M, Krieger J, Grosse-Wilde E, Mißbach C, Zhang L, Breer H. 2014. Variant Ionotropic Receptors Are Expressed in Olfactory Sensory Neurons of Coeloconic Sensilla on the Antenna of the Desert Locust (*Schistocerca gregaria*). *Int J Biol Sci.* **10**(1):1-14.

Guo W, Wang X, Ma Z, Xue L, Han J, Yu D, Kang L. 2011. CSP and Takeout Genes Modulate the Switch between Attraction and Repulsion during Behavioral Phase Change in the Migratory Locust. *PLoS Genet.* **7**(2):e1001291.

Ha TS, Smith DP. 2006. A pheromone receptor mediates 11-cis-vaccenyl acetate-induced responses in *Drosophila*. *J Neurosci*. **26**(34):8727-8733.

Hallberg E, Hansson BS. 1999. Arthropod Sensilla: Morphology and Phylogenetic Considerations. *Microsc Res Tech.* **47**(6):428-439.

Hallem EA, Ho MG, Carlson JR .2004. The molecular basis of odor coding in the *Drosophila* antenna. *Cell.* **117**(7):965-979.

Hallem EA, Carlson JR. 2006. Coding of odors by a receptor repertoire. *Cell* **125**(1):143-160.

Hansen-Delkeskamp E. 2001: Responsiveness of antennal taste hairs of the apterygotan insect, *Thermobia domestica* (Zygentoma); an electrophysiological investigation. *J Insect Physiol.* **47**(7):689-697.

Hansson BS, Anton S. 2000. Function and morphology of the antennal lobe: New developments. *Annu Review Entomol.* **45**:203-231.

Hansson BS, Stensmyr MC. 2011. Evolution of insect olfaction. *Neuron*. **72**(5):698-711.

Hansson BS, Harzsch S, Knaden M, Stensmyr M. 2011. The neural and behavioral basis of chemical communication in terrestrial crustaceans. In: Breithaupt T, Thiel M, editors. *Chemical Communication in Crustaceans*. New York: Springer Verlag. p. 149-173.

Hassanali A, Njagi PG, Bashir MO. 2005. Chemical ecology of locusts and related acridids. *Annu Rev Entomol.* **50**:223-245.

Hawke SD, Farley RD. 1971. The role of pore structures in the selective permeability of antennal sensilla of the desert burrowing cockroach, *Arenivaga* sp. *Tiss Cell*. **3**(4):665-674.

Hedges SB, Dudley J, Kumar S. 2006. TimeTree: a public knowledge-base of divergence times among organisms. *Bioinformatics.* **22**(23):2971-2972.

Heisenberg M. 2003. Mushroom body memoir: from maps to models. *Nat Revi Neurosci* **4**(4):266-275.

Hekmat-Scafe DS, Steinbrech RA, Carlson JR. 1997. Coexpression of Two Odorant Binding Protein Homologs in *Drosophila*: Implications for Olfactory Coding. *J Neurosci.* **17**(5):1616-1624.

Hekmat-Scafe DS, Scafe CR, McKinney AJ and Tanouye MA. 2002. Genome-wide analysis of the odorant-binding protein gene family in *Drosophila melanogaster*. *Genome Res.* **12**(9):1357-1369.

Hennig W. 1981. In: Pont A, editor. *Insect phylogeny*. New York: John Wiley and Sons.

Hildebrand JG, Shepherd GM. 1997. Mechanisms of olfactory discrimination: converging evidence for common principles across phyla. *Annu Rev Neurosci.* **20**:595-631.

Hill CA, Fox AN, Pitts RJ, Kent LB, Tan PL, Chrystal MA, Collins FH, Robertson HM, Zwiebel LJ. 2002. G protein coupled receptors in *Anopheles gambiae*. *Science.* **298**(5591):176-178.

Hiller K, Grote A, Scheer M, Münch R, Jahn D. 2004. PrediSi: prediction of signal peptides and their cleavage positions. *Nucl Acids Res.* **32** (suppl 2):W375-W379.

Hollins B, Hardin D, Gimelbrant AA, McClintock TS. 2003. Olfactory-enriched transcripts are cell-specific markers in the lobster olfactory organ. *J Comp Neurol.* **455**(1):125-138.

Homberg U. 1994. Distribution of neurotransmitters in the insect brain. In: *Progress in Zoology*, vol. 40. Stuttgart, Jena, New York: Gustav Fischer.

Homberg U. 2005. Multisensory processing in the insect brain. In: Christensen TA, editor. *Methods in Insect Sensory Neuroscience*. Boca Raton: CRC Press. p. 3-25.

Iovinella I, Dani FR, Niccolini A, Sagona S, Michelucci E, Gazzano A, Turillazzi S, Felicioli A, Pelosi P. 2011. Differential expression of odorant-binding proteins in the mandibular glands of the honey bee according to caste and age. *J Proteom Res.* **10**(8):3439-3449.

Iovinella I, Bozza F, Caputo B, della Torre A, Pelosi P. 2013. Ligand-Binding Study of *Anopheles gambiae* Chemosensory Proteins. *Chem Senses*. **38**(5):409-419.

Jacquin-Joly E, Merlin C. 2004. Insect olfactory receptors: contributions of molecular biology to chemical ecology. *J Chem Ecol.* **30**(12):2359-97.

Jiao Y, Moon SJ, Wang X, Ren Q, Montell C. 2008. Gr64f is required in combination with other gustatory receptors for sugar detection in *Drosophila*. *Curr Biol.* **18**(22):1797-1801.

Jones DT. 1999. Protein secondary structure prediction based on position-specific scoring matrices. *J Mol Biol.* **292**(2):195-202.

Jones WD, Cayirlioglu P, Kadow IG, Vosshall LB. 2007. Two chemosensory receptors together mediate carbon dioxide detection in *Drosophila*. *Nature*. **445**(7123):86-90.

Jones PL, Pask GM, Rinker DC, Zwiebel LJ. 2011. Functional agonism of insect odorant receptor ion channels. *Proc Natl Acad Sci U S A*. **108**(21):8821-8825.

Katoh K, Kuma K, Toh H, Miyata T. 2005. MAFFT version 5: improvement in accuracy of multiple sequence alignment. *Nucleic Acids Res.* **33**(2):511-518.

Kent LB, Robertson HM. 2009. Evolution of sugar receptors in insects. *BMC Evol Biol*. **9**:41.

Kirkness EF, Haas BJ, Sun W, Braig HR, Perotti MA, Clark JM, Lee SH, Robertson HM, Kennedy RC, Elhaik E et al. 2010. Genome sequences of the human body louse and its primary endosymbiont provide insights into the permanent parasitic lifestyle. *Proc Natl Acad Sci U S A.* **107**(27):12168-12173.

Keil TA. 1997. Comparative morphogenesis of sensilla: a review. *Int J Insect Morphol.* **26**(3-4):151-160.

Keil TA, Steinbrecht RA. 1984. Mechanosensitive and olfactory sensilla of insects. In: King RC, Akai H, editors. *Insect Ultrastructure*. vol. 2. New York: Plenum Press. p. 477-516.

Koch M.2001. Mandibular mechanism and the evolution of hexapods. *Ann Soc Entomol Fr*. **37**:129-174.

Krång A-S, Knaden M, Steck K, Hansson BS. 2012. Transition from sea to land: olfactory function and constraints in the terrestrial hermit crab *Coenobita clypeatus*. *P Roy Soc B-Biol Sci.* **279**(1742):3510-3519.

Kirkness EF, Haas BJ, Sun W, Braig HR, Perotti MA, Clark JM, et al., 2010. Genome sequences of the human body louse and its primary endosymbiont provide insights into the permanent parasitic lifestyle. *Proc Natl Acad Sci U S A.* **107** (27):12168-12173.

Krieger J, Raming K, Dewer YME, Bette S, Conzelmann S, Breer H. 2002. A divergent gene family encoding candidate olfactory receptors of the moth *Heliothis virescens*. *Eur J Neurosci.* **16**(4):619-28.

Krieger J, Klink O, Mohl C, Raming K, Breer H. 2003. A candidate olfactory receptor subtype highly conserved across different insect orders. *J Comp Physiol A.* **189**(7):519-526.

Kristensen NP. 1998. The groundplan and basal diversification of the hexapods. In: Fortey RA, Thomas RH, editors. *Arthropod Relationships, Systematic Association*. Vol. 55. London: Chapman and Hall. p. 281-293.

Kulmuni J, Havukainen H. 2013. Insights into the Evolution of the CSP Gene Family through the Integration of Evolutionary Analysis and Comparative Protein Modeling. *PLoS ONE.* **8**(5):e63688.

Kumar BN, Taylor RW, Pask GM, Zwiebel LJ, Newcomb RD, Christie DL. 2013. A Conserved Aspartic Acid Is Important for Agonist (VUAA1) and Odorant/Tuning Receptor-Dependent Activation of the Insect Odorant Co-Receptor (Orco). *PLoS ONE* **8**(7): e70218.

Kwon JY, Dahanukar A, Weiss LA, Carlson JR. 2007.The molecular basis of CO_2 reception in Drosophila. *Proc Natl Acad Sci U S A*. **104**(9):3574-3578.

Labandeira CC, Beall BS, Hueber FM. 1988. Early insect diversification: Evidence form a lower devonian bristletail from Quebec. *Science.* **242**(4880):913-916.

Lagarde A, Spinelli S, Qiao H, Tegoni M, Pelosi P, Cambillau C. 2011. Crystal structure of a novel type of odorant-binding protein from *Anopheles gambiae*, belonging to the C-plus class. *J Biochem*. **437**(3):423-430.

Larsson MC, Domingos AI, Jones WD, Chiappe ME, Amrein H, Vosshall LB. 2004. Or83b encodes a broadly expressed odorant receptor essential for *Drosophila* olfaction. *Neuron.* **43**(5):703-714.

Lartigue A, Campanacci, Roussel A, Larsson AM, Jones TA, Tegoni M, Cambillau C. 2002. X-ray structure and ligand binding study of a moth chemosensory protein. *J Biol Chem.* **277**(35):32094-32098.

Leal WS. 2013. Odorant reception in insects: roles of receptors, binding proteins, and degrading enzymes. *Annu Rev Entomol*. **58**: 373-91.

Lee Y, Moon SJ, Montell C. 2009. Multiple gustatory receptors required for the caffeine response in *Drosophila*. *Proc Natl Acad Sci USA.* **106**(11):4495-4500.

Liu C, Pitts RJ, Bohbot JD, Jones PL, Wang G, Zwiebel LJ. 2010. Distinct olfactory signaling mechanisms in the malaria vector mosquito *Anopheles gambiae*. *PLoS Biol*. **8**(8):e1000467.

Liu K, Linder CR, Warnow T. 2011. RAxML and FastTree: Comparing Two Methods for Large-Scale Maximum Likelihood Phylogeny Estimation. *Plos One.* **6**(11):e27731.

Lundin C, Käll L, Kreher SA, Kapp K, Sonnhammer EL, Carlson JR et al. 2007. Membrane topology of the *Drosophila* OR83b odorant receptor. *FEBS Lett.* **581**(29):5601–5604.

Mayer ML. 2006. Glutamate receptors at atomic resolution. *Nature*. **440**(7083):456-462.

Maleszka J, Foret S, Saint R, Maleszka R. 2007. RNAi-induced phenotypes suggest a novel role for a chemosensory protein CSP5 in the development of embryonic integument in the honeybee (*Apis mellifera*). *Dev Genes and Evol*. **217**(3):189-196.

Matsuo T, Sugaya S, Yasukawa J, Aigaki T, Fuyama Y. 2007. Odorant-binding proteins OBP57d and OBP57e affect taste perception and host-plant preference in *Drosophila sechellia*. *PLoS Biol.* **5**(5):e118.

Meinecke CC. 1975. Riechsensillen und die Systematik der Lamellicornia (Insecta, Coleoptera). *Zoomorphologie.* **82**:1-42.

Mendes L. 2002. Taxonomy of Zygentoma and Microcoryphia: historical overview, present status and goals for the new millenium. *Pedobiologia.* **46**(3-4):225-233.

Michel WC, Ache BW. 1992. Cyclic nucleotides mediate an odor-evoked potassium conductance in lobster olfactory receptor cells. *J Neurosci.* **12**(10):3979-3984.

Missbach C, Harzsch S, Hansson BS. 2011. New insights into an ancient insect nose: The olfactory pathway of *Lepismachilis y-signata* (Archaeognatha: Machilidae). *Arthropod Struct Dev.* **40**(4):317-333.

Mombaerts P. 1999. Seven-transmembrane proteins as odorant and chemosensory receptors. *Science.* **286**(5440):707-711.

Nagnan-Le Meillour P, Cain AH, Jacquin-Joly E, François MC, Ramachandran S, Maida R, Steinbrecht RA. 2000. Chemosensory proteins from the proboscis of *Mamestra brassicae*. *Chem Senses.* **25**(5):541-53.

Nakagawa T, Vosshall LB. 2009. Controversy and consensus: noncanonical signaling mechanisms in the insect olfactory system. *Curr Opin Neurobiol*. **19**(3):284-92.

Nakagawa T, Pellegrino M, Sato K, Vosshall LB, Touhara K. 2012. Amino Acid Residues Contributing to Function of the Heteromeric Insect Olfactory Receptor Complex. *PLoS ONE.* **7**(3): e32372.

Nomura A, Kawasaki K, Kubo T, Natori S. 1992. Purification and localization of p10, a novel protein that increases in nymphal regenerating legs of *Periplaneta americana* (American cockroach). *Int J Dev Biol.* **36**(3):391-398.

Ochieng SA, Hallberg E, Hansson BS. 1998. Fine structure and distribution of antennal sensilla of the desert locust, *Schistocerca gregaria* (Orthoptera: Acrididae). *Cell Tissue Res.* **291**(3):25-36.

Ochieng SA, Hansson BS. 1999. Responses of olfactory receptor neurones to behaviourally important odours in gregarious and solitarious desert locust, *Schistocerca gregaria*. *Physiol Entomol*. **24**:28-36.

Olivier V, Monsempes C, Francois MC, Poivet E, Jacquin-Joly E. 2011. Candidate chemosensory ionotropic receptors in a Lepidoptera. *Insect Mol Biol*. **20**(2):189-99.

Pace U, Hanski E, Salomon Y, Lancet D. Odorant-sensitive adenylate cyclase may mediate olfactory reception. *Nature.* **316**(6025):255-258.

Pelosi P. 1994. Odorant-binding proteins. *Crit Rev Biochem Mol Biol.* **29**(3):199-228.

Pelosi P, Calvello M, Ban L. 2005. Diversity of odorant-binding proteins and chemosensory proteins in insects. *Chem Senses*. **30**(suppl 1):i291-292.

Pelosi P, Zhou JJ, Ban LP, Calvello M. 2006. Soluble proteins in insect chemical communication. *Cell Mol Life Sci.* **63**(14):1658-1676.

Peñalva-Arana DC, Lynch M, Robertson HM. 2009. The chemoreceptor genes of the waterflea *Daphnia pulex*: many Grs but no Ors. *Bmc Evol Biol.* **9**:79.

Pitts RJ, Fox AN, Zwiebel LJ. 2004. A highly conserved candidate chemoreceptor expressed in both olfactory and gustatory tissues in the malaria vector *Anopheles gambiae*. *P Natl Acad Sci USA.* **101**(14):5058-5063.

Plettner E, Lazar J, Prestwich EG, Prestwich GD. 2000. Discrimination of pheromone enantiomers by two pheromone binding proteins from the gypsy moth *Lymantria dispar*. *Biochemistry.* **39**(30):8953-62.

Pophof B. 2002. Moth pheromone binding proteins contribute to the excitation of olfactory receptor cells. *Naturwissenschaften.* **89**(11):515-518.

Pophof B. 2004. Pheromone-binding proteins contribute to the activation of olfactory receptor neurons in the silkmoths *Antheraea polyphemus* and *Bombyx mori*. *Chem Senses*. **29**(2):117-125.

Prestwich GD, Du G, LaForest S. 1995. How is pheromone specificity encoded in proteins? *Chem Senses.* **20**(4):461-469.

Price MN, Dehal PS, Arkin AP. 2009. FastTree: Computing Large Minimum Evolution Trees with Profiles instead of a Distance Matrix. *Mol Biol Evol.* **26**(7):1641-1650.

Rebora M, Salerno G, Piersanti S, Dell'Otto A, Gaino E. 2012. Olfaction in dragonflies: Electrophysiological evidence. *J Insect Physiol.* **58**(2):270-277.

Regier JC, Shultz JW. 2001. Elongation factor-2: a useful gene for arthropod phylogenetics *Mol Phylogenet and Evol.* **20**(1):136-148.

Regier JC, Shultz JW, Kambic RE. 2005. Pancrustacean phylogeny: hexapods are terrestrial crustaceans and maxillopods are not monophyletic. *Proc R Soc B*. **272**(1561):395-401.

Regier JC, Shultz JW, Zwick A, Hussey A, Ball B, et al. 2010. Arthropod relationships evealed by phylogenomic analysis of nuclear protein-coding sequences. *Nature*. **463**(7284):1079-1083.

Robertson HM. 2001. Taste: Independent origins of chemoreception coding systems? *Curr Biol.* **11**(14):560-562.

Robertson HM, Kent LB. 2009. Evolution of the gene lineage encoding the carbon dioxide receptor in insects. *L Insect Sci*. **9**:19.

Robertson HM, Thomas JH. 2006. *The putative chemoreceptor families of C. elegans*. In: WormBook, editor, The C. elegans Research Community. http://www.wormbook.org.

Robertson HM. Wanner KW. 2006. The chemoreceptor superfamily in the honey bee *Apis mellifera*: expansions of the odorant, but not gustatory, receptor families. *Genome Res.* **16**(11):1395-403.

Robertson HM, Warr CG, Carlson JR. 2003. Molecular evolution of the insect chemoreceptor gene superfamily in *Drosophila melanogaster*. *Proc Natl Acad Sci USA*. **100**(suppl2):14537-14542.

Rota-Stabelli O, Daley AC, Pisani D. 2013. Molecular Timetrees reveal a Cambrian Colonization of Land and a New Scenario for Ecdysozoan Evolution. *Curr Biol.* **23**(5):392-398.

Rytz R, Croset V, Benton R. 2013. Ionotropic Receptors (IRs): Chemosensory ionotropic glutamate receptors in *Drosophila* and beyond. *Insect Biochem Mol Biol.* **43**(9):888-897.

Saghatelyan A, Carleton A, Lagier S, de Chevigny A, Lledo PM. 2003. Local neurons play key roles in the mammalian olfactory bulb. *J Physiol Paris.* **97**(4-6):517-528.

Sakurai T, Nakagawa T, Mitsuno H, Mori H, Endo Y, Tanoue S, Yasukochi Y, Touhara K, Nishioka T. 2004. Identification and functional characterization of a sex pheromone receptor in the silkmoth *Bombyx mori*. *Proc Natl Acad Sci U S A*. **101**(47):16653-16658.

Sanchez-Gracia A, Vieira FG, Rozas J. 2009. Molecular evolution of the major chemosensory gene families in insects. *Heredity*. **103**(3):208-216.

Sandler BH, Nikonova L, Leal WS, Clardy J. 2000. Sexual attraction in the silkworm moth: structure of the pheromone-binding-protein–bombykol complex. *Chem Biol.* **7**(2):143-151.

Sargsyan V, Getahun MN, LavistaLlanos S, Olsson SB, Hansson BS, Wicher D. 2011. Phosphorylation via PKC Regulates the Function of the *Drosophila* Odorant Co-Receptor**.** *Front Cell Neurosci* **5**:5.

Sato K, Pellegrino M, Nakagawa T, Nakagawa T, Vosshall LB, Touhara K. 2008. Insect olfactory receptors are heteromeric ligand-gated ion channels. *Nature.* **452**(7190):1002-1006.

Schachtner J, Schmidt M, Homberg U. 2005. Organization and evolutionary trends of primary olfactory brain centers in Tetraconata (Crustacea plus Hexapoda). *Arthropod Struct Dev.* **34**(3):257-299.

Schaller L. 1982. Structural and Functional Classification of Antennal Sensilla of the cockroach *Leucophaea maderae*. *Cell Tiss Res*. **225**(1):129-142.

Schmidt M, Gnatzy W. 1984. Are the funnel-canal organs the ”campaniform sensilla” of the shore crab, *Carcinus maenas* (Decapoda, Crustacea)? II Ultrastructure. *Cell Tissue Res.* **237**(1):81-93.

Schultze A, Pregitzer P, Walter MF, Woods DF, Marinotti O, Breer H, Krieger J. 2013. The Co-Expression Pattern of Odorant Binding Proteins and Olfactory Receptors Identify Distinct Trichoid Sensilla on the Antenna of the Malaria Mosquito *Anopheles gambiae*. *PLoS ONE*. **8**(7):e69412.

Scott K, Brady R Jr, Cravchik A, Morozov P, Rzhetsky A, Zuker, et al. 2001. A chemosensory gene family encoding candidate gustatory and olfactory receptors in *Drosophila*. *Cell.* **104**(5):661-673.

Shanbhag SR, Hekmat-Scafe D, Kim MS, Park SK, Carlson JR Pikielny C, Smith DP, Steinbrecht RA. 2001. Expression mosaic of odorant-binding proteins in *Drosophila* olfactory organs. *Microsc Res Tech*. **55**(5):297-306.

Silbering AF, Rytz R, Grosjean Y, Abuin L, Ramdya P, Jefferis GS, et al. 2011. Complementary Function and Integrated Wiring of the Evolutionarily Distinct *Drosophila* Olfactory Subsystems. *J Neurosci.* **31**(38):13357-13375.

Slifer EH, Sekhon SS. 1978. Sense organs on the antennae of two species of Collembola (Insecta). *J Morphol.* **157**(1):1-20.

Smadja C, Shi P, Butlin RK, Robertson HM. 2009. Large Gene Family Expansions and Adaptive Evolution for Odorant and Gustatory Receptors in the Pea Aphid, *Acyrthosiphon pisum*. *Mol Biol Evol.* **26**(9):2073-2086.

Steinbrecht RA. 1969. Comparative morphology of olfactory receptors. In: Pfaffmann C, editor. *Olfaction and taste III*. New York: Rockefeller University Press. p. 3-21.

Steinbrecht RA. 1973. Der Feinbau olfaktorischer Sensillen des Seidenspinners (Insecta, Lepidoptera): Rezeptorfortsätze und reizleitender Apparat. *Z Zellforsch mikrosk Anat*. **139**:533-565.

Steinbrecht RA. 1997. Pore structures in insect olfactory sensilla: a review of data and concepts. *Int J Insect Morphol Embryol.* **26**(3-4):229-245.

Steinbrecht RA. 1998. Odorant-binding proteins: expression and function. *Ann N Y Acad Sci.* **855**:323-332.

Steinbrecht RA, Müller B. 1976. Fine structure of antennal contact chemoreceptors after cryofixation. In: Gribakin FG, Wiese K, Popov AV, editors. *Sensory Systems and Communication in Arthropods*. Basel: Birkhäuser. p. 59-61.

Stengl M, Funk NW. 2013. The role of the coreceptor Orco in insect olfactory transduction. *J Comp Physiol A neuroethol Sens Neural Behav Physiol*. **199** (11):897-909.

Stensmry MC, Erland S, Hallberg E, Wallén R, Greenaway P, Hansson BS. 2005. Insect-like olfactory adaptation in the terrestrial giant robber crab. *Curr Biol.* **15**(2):116-121.

Strausfeld, NJ, Hansen L, Ll Y, Gomez RS, Ito K. 1998a. Evolution, discovery, and interpretations of Arthropod mushroom bodies. *Learn Mem.* **5**(1-2):11-37.

Strausfeld NJ, Sinakevitch I, Brown SM, Farris SM. 2009. Ground plan of the insect mushroom body: functional and evolutionary implications. *J Comp Neurol.* **513** (3):265-291.

Su CY, Menuz K, Carlson JR. 2009. Olfactory perception: receptors, cells, and circuits. *Cell.* **139**(1):45-59.

Swarup S, Williams IW, Anholt RRH. 2011. Functional Dissection of Odorant Binding Protein Genes in *Drosophila melanogaster*. *Genes Brain Behav*. **10**(6):648-657.

Tadesse T, Schmidt M, Walthall WW, Tai PC, Derby CD. 2011. Distribution and function of splash, an achaete-scute homolog in the adult olfactory organ of the Caribbean spiny lobster *Panulirus argus*. *Dev Neurobiol.* **71**(4):316-335.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol.* **28**(10):2731-2739.

Tauxe GM, MacWilliam D, Boyle SM, Guda T, Ray A. 2013. Targeting a Dual Detector of Skin and CO2 to Modify Mosquito Host Seeking. *Cell.* **155**(6):1365-1379.

Tegoni M, Campanacci V, Cambillau C. 2004. Structural aspects of sexual attraction and chemical communication in insects. *Trends Biochem Sci.* **29**(5):257-264.

Tichy H, Barth FG. 1992. Fine structure of olfactory sensilla in myriapods and arachnids. *Microsc Res Tech*. **22**(4):372-391.

Tikhonov DB, Magazanik LG. 2009. Origin and molecular evolution of ionotropic glutamate receptors. *Neurosci Behav Physiol.* **39**(8):763-773.

Thompson JD, Higgins DG, Gibson TJ. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **22**(22):4673-4680.

Trautwein MD, Wiegmann BM, Beutel R, Kjer KM, Yeates D. 2012. Advances in Insect Phylogeny at the Dawn of the Postgenomic Era. *Annu Rev Entomol.* **57**:499-468.

Troemel ER, Chou JH, Dwyer ND, Colbert HA, Bargmann CI. 1995. Divergent seven transmembrane receptors are candidate chemosensory receptors in *C. elegans*. *Cell*. **83**(2):207-218.

Tsuchihara K, Fujikawa K, Ishiguro M, Yamada T, Tada C, Ozaki K, Ozaki M. 2005. An Odorant-binding Protein Facilitates Odorant Transfer from Air to Hydrophilic Surroundings in the Blowfly. *Chem Senses*. **30**(7):559-564.

Vieira FG, Rozas J. 2011. Comparative Genomics of the Odorant-Binding and Chemosensory Protein Gene Families across the Arthropoda: Origin and evolutionary history of the chemosensory system. *Genome Biol Evol*. **3**:476-490.

Vogt RG, Riddiford LM. 1981. Pheromone binding and inactivation by moth antennae. *Nature.* **293**(5828):161-163.

Vogt RG, Prestwich GD, Lerner MR. 1991. Odorant-binding-protein subfamilies associate with distinct classes of olfactory receptor neurons in insects. *J Neurobiol.* **22**(1):74-84.

von Reumont BM, Meusemann K, Szucsich NU, Dell'Ampio E, Gowri-Shankar V, et al. 2009. Can comprehensive background knowledge be incorporated into substitution models to improve phylogenetic analyses? A case study on major arthropod relationships. *BMC Evol Biol*. **9**:119.

Vosshall LB, Amrein H, Morozov PS, Rzhetsky A, Axel R. 1999. A spatial map of olfactory receptor expression in the *Drosophila* antenna. *Cell.* **96**(5):725-736.

Vosshall LB, Stocker RE. 2007. Molecular architecture of smell and taste in *Drosophila*. *Annu Review Neurosci.* **30:**505-533.

Vosshall LB, Hansson BS. 2011. A Unified Nomenclature System for the Insect Olfactory Coreceptor. *Chem Senses.* **36**(6):497-498.

Wang G, Carey AF, Carlson JR, Zwiebel LJ. 2010. Molecular basis of odor coding in the malaria vector mosquito *Anopheles gambiae*. *Proc Natl Acad Sci U S A.* **107**(9):4418-4423.

Wanner KW, Willis LG, Theilmann DA, Isman MB, Feng Q, Plettner E. 2004. Analysis of the insect OS-D-like gene family. *J Chem Ecol.* **30**(5):889-911.

Wanner KW, Anderson AR, Trowell SC, Theilmann DA, Robertson HM, Newcomb RD. 2007. Female-biased expression of odourant receptor genes in the adult antennae of the silkworm, *Bombyx mori*. *Insect Mol Biol.* **16**(1):107-119.

Wanner KW, Robertson HM. 2008. The gustatory receptor family in the silkworm moth *Bombyx mori* is characterized by a large expansion of a single lineage of putative bitter receptors. *Insect Mol Biol.* **17**(6):621-629.

Wicher D, Schäfer R, Bauernfeind R, Stensmyr MC, Heller R, Heinemann, SH et al. 2008. *Drosophila* odorant receptors are both ligand-gated and cyclic-nucleotide-activated cation channels. *Nature.* **452**(7190):1007-1011.

Wiegmann BM, Trautwein MD, Kim J-W, Cassel BK, Bertone MA, Winterton SL, et al. 2009. Single-copy nuclear genes resolve the phylogeny of holometabolous insects. *BMC Biol*. **7**:34.

Xu F, McClintock TS. 1999. A lobster phospholipase C-beta that associates with G-proteins in response to odorants. *J Neurosci.* **19**(12):4881-4888.

Xu PX, Zwiebel LJ and Smith DP. 2003. Identification of a distinct family of genes encoding atypical odorant-binding proteins in the malaria vector mosquito, *Anopheles gambiae*. *Insect Mol Biol.* **12**(6):549-560.

Xu P, Atkinson R, Jones DN, Smith DP. 2005. *Drosophila* OBP LUSH is required for activity of pheromone-sensitive neurons. *Neuron.* **45**(2):193-200.

Xu H, Guo M, Yang Y, You Y, Zhang L. 2013. Differential expression of two novel odorant receptors in the locust (*Locusta migratoria*). *BMC Neurosci.* **14**:50.

Yang Y, Krieger J, Zhang L, Breer H. 2012. The Olfactory Co-receptor Orco from the Migratory Locust (*Locusta migratoria*) and the Desert Locust (*Schistocerca gregaria*): Identification and Expression pattern. *Int J Biol Sci.* **8**(2):159-170.

Zacharuk RY. 1980. Ultrastructure and function of insect chemosensilla. *Annu Rev Entomol.* **25**:27-47.

Zacharuk RY. 1985. Antennae and sensilla. In: Kerkut GA, Gilbert LI, editors. *Comprehensive insect physiology, biochemistry and pharmacology*. vol. 6. New York: Pergamon Press. p. 1-69.

Zhang X , Zhang X , Firestein S. 2007. Comparative genomics of odorant and pheromone receptor genes in rodents. *Genomics.* **89**(4):441-450.

Zhang YV, Ni J, Montell C. 2013. The molecular basis for attractive salt-taste coding in *Drosophila*. *Science.* **340**(6138):1334-1338.

Zhang YN, Jin JY, Jin R, Xia YH, Zhou JJ, Deng JY, Dong SL. 2013. Differential Expression Patterns in Chemosensory and Non-Chemosensory Tissues of Putative Chemosensory Genes Identified by Transcriptome Analysis of Insect Pest the Purple Stem Borer *Sesamia inferens* (Walker). *PLoS One.* **8**(7):e69715.

Zhou JJ, Zhang GA, Huang W, Birkett MA, Field LM, Pickett JA, Pelosi P. 2004. Revisiting the odorant-binding protein LUSH of *Drosophila melanogaster*: evidence for odour recognition and discrimination. *FEBS Lett.* **558**(1-3):23-6.

Zhou JJ, Kan Y, Antoniw J, Pickett JA, Field LM. 2006. Genome and EST analyses and expression of a gene family with putative functions in insect chemoreception. *Chem Senses.* **31**(5):453-465.

Zhou W, Chen D. 2009. Sociochemosensory and Emotional Functions: Behavioral Evidence for Shared Mechanisms. *Psychol Sci.* **20**(9):1118-1124.

Zhou JJ, Field LM and He XL. 2010a. Insect odorant-binding proteins: do they offer an alternative pest control strategy? *Outlooks Pest Manag.* **21**(1):31-34.

Zhou, J.J., Vieira FG, He XL, Smadja C, Liu R, Rozas J, Field LM. 2010b. Genome annotation and comparative analyses of the odorant-binding proteins and chemosensory proteins in the pea aphid *Acyrthosiphon pisum*. *Insect Mol Biol*. **19**(suppl 2):113-122.

Zhou X, Slone JD, Rokas A, Berger SL, Liebig J, Ray A, et al. 2012. Phylogenetic and Transcriptomic Analysis of Chemosensory Receptors in a Pair of Divergent Ant Species Reveals Sex-Specific Signatures of Odor Coding. *PLoS Genet.* **8**(8): e1002930.

Zhou XH, Ban LP, Iovinella I, Zhao LJ, Gao Q, Felicioli A, Sagona S, Pieraccini G, Pelosi P, Zhang L, Dani FR. 2012b. Diversity, abundance and sex-specific expression of chemosensory proteins in the reproductive organs of the locust *Locusta migratoria manilensis*. *Biol Chem.* **394**(1):43-54.

Declaration of Independent Assignment

I declare in accordance with the conferral of the degree of doctor from the School of Biology and Pharmacy of Friedrich-Schiller-University Jena that the submitted thesis was written only with the assistance and literature cited in the text.

People who assisted in experiments, data analysis and writing of the manuscripts are listed as co-authors of the respective manuscripts. I was not assisted by a consultant for doctorate theses.

The thesis has not been previously submitted whether to the Friedrich-Schiller-University, Jena or to any other university.

Jena, ……

Christine Mißbach

Acknowledgements

This thesis would not have been possible without contributions and support of many people that I want to thank here. My gratitude is owed to many more people than I could list here.

First, I am very thankful to Bill S. Hansson for giving me the opportunity to follow up my ideas after finishing my diploma thesis in his lab. Without his scientific and financial support I would have never had this fantastic and fruitful time in his amazing department.

Ich möchte mich sehr herzlich bei meinem Betreuer Ewald Grosse-Wilde bedanken. Von seinem extrem breiten Wissen habe ich sowohl wissenschaftlich, als auch persönlich sehr profitiert. Unzähligen Diskussionen und seinem Einsatz für das Projekt habe ich es zu verdanken, das es zu dem geworden ist, was es heute ist. Unvergessen sind auch seine kulinarischen Abende, die sehr zur Stärkung des Sozialgefüges der kleinen Gruppe „Olfactory Genes“ beigetragen haben. Danke Ewald!

Ganz besonderes Dankeschön geht an Katrin Groh, die mich sowohl fachlich als auch privat sehr unterstützt hat. Vor allem in Zeiten, in denen ich ein offenes Ohr gebraucht habe, warst du für mich da. Du hast mir die nötige Kraft gegeben wieder zu mir und meinen Zielen zurück zu finden.

Bei Sascha Bucks möchte ich mich für seine Unterstützung bei vielen molekularbiologischen Fragestellungen bedanken. Dank ihm, Regina und allen anderen Mitgliedern der Gruppe „Olfactory Genes“ habe ich die Angst vor Pipetten, Gelbanden und den vielen A´s, T´s, G´s und C´s verloren.

Ein ganz großer Dank gebührt auch den ehemaligen und jetzigen Mitgliedern der nach Greifswald gezogenen AG Harzsch, vor allem meinem ehemaligen Betreuer Steffen Harzsch, Verena Rieger und Andy Sombke. Eure Unterstützung während meiner Diplomarbeit und deren Ergebnisse haben maßgeblich den Weg für meine Doktorarbeit geebnet.

I want to thank Hany K.M. Dweck and Marcus C. Stensmyr. Without your strong driving force my PhD project would have been not become so big and extensive.

Sorry Shannon, I have not incorporated your fantastic words “tilth” and “turves” into my dissertation. I hope you will forgive me. Nevertheless, thank you for helping me with the electrophysiology and the helpful comments on my manuscripts.

Heiko Vogel danke ich für die Hilfe beim Auswerten der Microarrays und die vielen konstruktiven Kommentare zu meinen Manuskripten. Es hat mich sehr gefreut mit dir zusammen arbeiten zu dürfen.

Bei Jette und Domenica möchte ich mich für die Unterstützung bei den Microarray Experimenten und für die unzähligen Sequenzierungen bedanken.

My special thanks belong to the whole Hansson group for providing a supportive and productive environment. Many discussions and comments during group retreats, lab meetings and PhD meetings positively contribute to the work. I really enjoyed the time with all of you, the scientific and the social side.

Bei Swetlana Laubrich möchte ich mich für die Unterstützung bei organisatorischen Dingen wie Reisplanung und Vertragsverlängerungen bedanken. Wie wichtig und außerordentlich diese Unterstützung war haben wir alle in Zeiten deiner Abwesenheit gespürt.

Vielen Dank Daniel für die Ideen und Hilfestellung zum Durchführen von Verhaltensversuchen. Ich danke dir auch für deine erfrischende Art und deinem Enthusiasmus bei allen Fragestellungen, denn „Die Arbeit ist kein *Frosch* - Sie hüpft nicht davon“.

Ich danke der Max-Planck-Gesellschaft für das Stipendium, welches ich während der kompletten Doktorarbeitszeit erhalten habe und ohne welches die Arbeit nicht möglich gewesen wäre.

Vielen Dank Caro, Sven, Rosi, Frank, Nicole, Matthias, Nancy und alle anderen die ich vergessen habe, für die schönen und entspannten Stunden außerhalb des Instituts.

Der allergrößte Dank gebührt aber meiner Familie. Ich möchte mich bei meinem Lebensgefährten Frank Hünefeld und meinen Kindern Felix und Lena für ihre Geduld, das stundenlange Warten vor dem Institut und die vielen Exkursionen in die Natur zum Tiere sammeln bedanken. Aber vor allem ihr emotionaler Rückhalt und ihre Liebe während dieser Zeit die Kraft und die Ausdauer gegeben meine Arbeiten durchzuführen, auch wenn es mal nicht so lief, wie ich es mir vorgestellt habe.

Zu guter Letzt möchte mich bei meinen Eltern Regina und Gerd bedanken, die mich während meines Studiums und meiner Doktorarbeit immer unterstützt haben.

Curriculum Vitae

Persönliche Daten

Name:	Christine Mißbach
Anschrift:	Wacholderweg 4 07745 Jena
Geboren am:	21. April 1983 in Eisenach/Thür.
Familienstand:	ledig 2 Kinder, Felix Hünefeld (geboren am 10. August 2003) Lena Hünefeld (geboren am 20. August 2005)

Ausbildung

seit 10/2009	Promotion Max-Planck-Institut für Chemische Ökologie/Abt. Evolutionäre Neuroethologie, Jena
09/09	Abschluss als Diplom-Biologin (Note 1,2)
10/01– 09/09	Studium der Biologie Friedrich-Schiller-Universität Jena Hauptfach Spezielle Zoologie, Nebenfächer Genetik und Ökologie Thema der Diplomarbeit *"Die Gehirnarchitektur bei apterygoten Insekten und basalen Gruppen der Pterygota: Evolution des zentralen olfaktorischen Pfades der Insekten"*
09/93 – 06/01	Abitur (Note 1,8) Ernst-Abbe Gymnasium, Eisenach/Thür.

Praktische Erfahrungen

07/08 – 12/08	studentische Mitarbeit Max-Planck-Institut für Chemische Ökologie Immunhistochemische Markierungen an Strepsipteren Larven
03/08 – 06/08	studentische Mitarbeit Friedrich-Schiller-Universität Jena Arbeiten an der Sammlung des Phyletischen Museums
12/07	studentische Mitarbeit Friedrich-Schiller-Universität Jena
05/05 – 06/05	studentische Mitarbeit Friedrich-Schiller-Universität Jena
07/02	studentische Mitarbeit Friedrich-Schiller-Universität Jena

Kenntnisse

Sprachen:	Deutsch (Muttersprache), Englisch (fließend), Französisch (Grundkenntnisse)
Computer:	Microsoft Windows: Word, Powerpoint, Excel; Adobe Photoshop, Adobe Illustrator

Publikationen

Veröffentlichungen in Fachzeitschriften

2014 **Missbach C**, Dweck HKM, Vogel H, Vilcinskas A, Stensmyr MC, Hansson BS, Grosse-Wilde E. Evolution of insect olfactory receptors. eLIFE: doi:10.7554/eLife.02115.

Missbach C, Vogel H, Hansson BS, Grosse-Wilde. Identification of Odorant Binding Proteins in Antennal Transcriptomes of the jumping bristletail *Lepismachilis y-signata* and the firebrat *Thermobia domestica:* Evidence for an independent OBP-OR origin. Chemical Senses. Under Revision.

Guo M, Krieger J, Grosse-Wilde E, **Mißbach C**, Zhang L, Breer H. Variant ionotropic receptors are expressed in olfactory sensory neurons of coeloconic sensilla on the antenna of the desert locust (*Schistocerca gregaria*). International Journal of Biological Science. doi:10.7150/ijbs.7624.

2012 Bisch-Knaden S, Carlsson MA, Sugimoto Y, Schubert M, **Missbach C**, Sachse S, Hansson B (2012). Olfactory coding in five moth species from two families. The Journal of Experimental Biology. doi:10.1242/jeb.068064.

Hünefeld F, **Mißbach C**, Beutel RG. The morphology and evolution of the female postabdomen of Holometabola (Insecta). Arthropod Structure & Development 41, 361-371. doi: 10.1016/j.asd.2012.05.002.

Missbach C, Harzsch S, Hansson BS (2011). New insights into an ancient insect nose - the olfactory pathway of *Lepismachilis y-signata* (Archaeognatha: Machilidae). Arthropod Structure & Development. doi:10.1016/j.asd.2011.03.004.

Wissenschaftliche Vorträge

11/13 **Mißbach C**, Grosse-Wilde E. Evolution of olfactory genes in insects. Seminar, Zoologisches Forschungsmuseum Alexander Koenig, Bonn, DE (invited)

03/12 **Mißbach C**. Olfaction in the Jumping Bristletail *Lepismachilis y-signata* - A 390 Million-Year-Old Insect Nose -. Workshop „Molecular Tools to Study Olfaction in Arthropods", Universität Konstanz, Konstanz, DE

09/11 **Mißbach C**. Olfaction in the jumping bristletail Lepismachilis y-signata – A 390 million-year-old insect nose? 12th European Symposium for Insect Taste and Olfaction (ESITO), St. Petersburg, RU

02/11 **Mißbach C**. Olfaction in the basal insect *Lepismachilis y-signata* (Archaeognatha, Machilidae). 10th IMPRS Symposium, MPI for Chemical Ecology, Dornburg, DE

09/2010 **Mißbach C**. Evolution of insect olfaction. ICE Symposium, MPI for Chemical Ecology, Jena, DE

09/2010 **Mißbach C**. Evolution of insect olfactory receptors. Chemical Ecology 2010 Mini-Symposium, Jena, DE

11/09 **Mißbach C**. Der zentrale olfaktorische Pfad von *Lepismachilis y-signata* (Archaeognatha). Seminar, FSU, Institut für Spezielle Zoologie und Evolutionsbiologie, Jena, DE (invited)

09/09 **Mißbach C**. New insights into an ancient insect nose - the olfactory pathway of *Lepismachilis y-signata* (Archaeognatha). Evolution of the arthropod nervous system, Jena, DE (invited)

Poster

01/14 **Mißbach C**, Dweck H, Vogel H, Stensmyr MC, Hansson BS, Grosse-Wilde E. Evolution of olfactory genes in insects. International Plant & Animal Genome XXII (PAG XXII), San Diego, Ca, US

09/13 **Mißbach C**, Dweck H, Vogel H, Stensmyr MC, Hansson BS, Grosse-Wilde E. Evolution of olfactory genes in insects. 13th European Symposium for Insect Taste and Olfaction (ESITO), Villasimius, IT

04/13 **Mißbach C**. Evolution of insect olfactory receptors. 12th IMPRS Symposium, MPI for Chemical Ecology, Jena, DE

03/13 **Mißbach C**, Dweck H, Harzsch S, Stensmyr MC, Knaden M, Hansson BS, Grosse-Wilde E. Olfaction in the jumping bristletail *Lepismachilis y-signata* (Archaeognatha, Machilidae). 10th Göttingen Meeting of the German Neuroscience Society, Göttingen, DE

10/12 **Mißbach C**. Evolution of Insect Olfaction - A 390 mya old Insect Nose - . SAB Meeting 2012, MPI for Chemical Ecology, Jena, DE

06/12 Dweck H, **Mißbach C**, Stensmyr MC, Hansson BS. On the origin of insect olfaction. XVI International Symposium on Olfaction and Taste (ISOT), Stockholm, SE

06/12 **Mißbach C**, Dweck H, Vogel H, Stensmyr MC, Grosse-Wilde E, Hansson BS. Are insect olfactory receptors an adaption to a terrestrial lifestyle? XVI International Symposium on Olfaction and Taste (ISOT), Stockholm, SE

02/12 **Mißbach C**. Olfaction in the jumping bristletail *Lepismachilis y-signata* – A 390 million-year-old insect nose? 11th IMPRS Symposium, MPI for Chemical Ecology, Dornburg, DE

03/11 **Mißbach C**, Grosse-Wilde E, Hansson BS. Chemosensory receptors of *Lepismachilis* Ysignata (Insecta: Archaeognatha). 9th Göttingen Meeting of the German Neuroscience Society, Göttingen, DE

10/10 Bisch-Knaden S, Carlsson M, Sugimoto Y, Schubert M, **Mißbach C**. Odor coding in the brain of moths: Impact of phylogeny and life history . SAB Meeting 2010, MPI for Chemical Ecology, Jena, DE

02/10 **Mißbach C**. Evolution of coreceptors in insect olfaction. 9th IMPRS Symposium, MPI for Chemical Ecology, Dornburg, DE

03/09 **Mißbach C**, Harzsch S, Hansson BS. The antennal lobes in basal hexapods: characterizing the ancestral insect olfactory system. 8th Göttingen Meeting of the German Neuroscience Society, Göttingen, DE

Jena, ……

Christine Mißbach

Appendix

Chapter II

Supplementary material 1: amino acids sequences of putative CSPs

>LsigCSP1_putative chemosensory protein_translated_partial
MRSATLTLCITVTVISLTSLCCQARLPVKRPQVSDASLDAALRNKNFINMQLRCALGEGPCDALGARVK
ALAPEVMRGVCRACKPGEFRQIQRVISFIQRNYPQEWRRIVQRYSGF
>LsigCSP2_putative chemosensory protein_translated full ORF
MLSTQFVVALTLASVLAAAPTEAPSKPVSLLSRYPALDQVDVDTLLKNDRLIKFHLKCVLGEGQCDSV
GKELKAALPDTLLHRCVGCTDGQLHKARRVVSTISQKFPREYQKLVQQFQGVS
>LsigCSP3_putative chemosensory protein_translated full ORF
MKTVLILAALVAFTAAARIVREEAQYTTAYDNIDIDKILTNTRLLDYHIRCVLDKGPCNKEGKELKAHL
PDAIRNECEKCSETQKEKGKKVVQFLMKNRATEWKEIEEKYKNYHPSGTPPPNYEKYIKA
>TdomCSP1_putative chemosensory protein_translated_partial
mWTLFACACACALLAVGSAQPPLSPEELQEILGNPRTVRAYIACVLDKGPCTAEGRELRERIPVGLKTA
CGDCSATQKQTVRTAVRFIQEHYPDEWLQLHEHFDPSGEYVDSFQHFIDSDD
>TdomCSP2__putative chemosensory protein_translated_partial
VCGGVLPDPHELVKDEPKLSKFLSCLAGKDTCESWTEEVKRYIPSALKESCKDCPAEHKNFYRVVTIHL
RKHRPDVYKAL
>TdomCSP3_putative chemosensory protein_translated full ORF
MQSMRLLFVVLGLAVAAQAARLRREEKYSTQYDNVDLHEILTNKRLMNNYANCLLDKGPCTQDAQL
LKDAIPDALENECAKCSEKQKEGTEIAIPHMIENEPEIWELLKKKFDPSNKYGERYAQLLKKAQEKRRG
>TdomCSP4_putative chemosensory protein_translated_partial
MHACAVLLVAACALASSFAAEMYSTRYDNVNLQDIFKNKRLFDGYANCILDKGPCTADGRLLRDAIPE
ALTNGCAKCSERQKSGAKDVIKEVKTNHPLLWDEMKKKHDPTGLFEKKNSDLLDQLWH
>TdomCSP5__putative chemosensory protein_translated_partial
MRTTLALVALASLVALGAAQGKYTSQFDNVDVDAILNNDRVLTSYLNCLKNEGVCTPEGKTLKESIPD
ALQTGCSKCSDNHKG
>TdomCSP6_putative chemosensory protein_translated_partial
DEVFGRNLLLPSVGFGLHRGLQASKSRNL??????????KRLLPDALQTNCTKCTDKQKEIGRKTITFLRKN
RSEDWERLRSKYDPENKYEQFEEALTR

Supplementary material 2: partial mRNA sequences of putative CSPs

>LsigCSP1_putative chemosensory protein_partial mRNA
CCTCAGTGAAAGCATCTCTCGATATTAGTCTTACCGCACCACTGCTCCAGACACAAAATGCGCTCC
GCAACATTAACACTGTGCATTACAGTTACCGTAATATCTCTAACGTCATTATGCTGCCAAGCCCGAC
TTCCGGTCAAGAGACCTCAAGTATCAGATGCTTCATTAGATGCAGCCTTGAGAAACAAAAATTTCA
TTAATATGCAGTTACGTTGTGCTCTTGGGGAAGGCCCTTGTGATGCACTCGGAGCACGAGTTAAAG
CCCTTGCTCCAGAGGTAATGCGAGGCGTGTGCAGAGCGTGTAAACCAGGTGAGTTCCGACAGATCC
AGCGGGTGATATCTTTCATTCAGCGGAATTATCCCCAGGAATGGCGTCGTATTGTGCAGAGGTATT
CAGGCTTTTAGGAGAGTGCAACATTATTCACACATTATAGAGTAAGGACTGAACGAGTGCATCATA
GTGAATGAACATGAACTTGAATCACTTTCATTGTCTCGACGATTAATTATATTTAATGCAAAATGCT
TCTTGTTTCTATTTAAACTGTGCATGGTGTTACAATACATTATTTTACGAAATTGTCTGAAAATAAG
GTCCCATCCATACAGTAACTTTTCACCTGAGTTGGGTGAGGCTGTATTCAAGAAATATCACCCAATT
TATGTACACTGGCGGTCATAAATGCCCTTCTCGT
> LsigCSP2_putative chemosensory protein_partial mRNA
TCCGATTAAAACACTAATCCTGTTTCGGTGTTCCGAACGTCAAGAAAAGAGATCGAGCGAAATGAG
CCACTCTTTCGAAGCAAAACCTCGCGTCTTGCCTGTCAATTGTGGGTCATACGCTCGCTTCCGTTGA
GAGTGAAATCGCGTCCTATATAAACTGCTTGTGCTTCCGGTTCTCCGAGACTGAAATAGAAGATGT
TGTCTACTCAATTTGTCGTGGCACTTACGCTGGCCAGCGTACTTGCGGCTGCCCCAACAGAAGCGC
CAAGCAAACCTGTCAGCCTGCTTTCCAGATACCCAGCTTTGGATCAAGTGGACGTAGACACCTTAT
TGAAAAATGATCGTCTCATCAAGTTTCACCTCAAGTGTGTACTCGGAGAGGGCCAGTGTGACAGTG
TCGGAAAAGAGCTGAAGGCTGCTCTACCGGATACTTTGTTGCACCGCTGCGTCGGGTGTACTGATG
GACAATTACACAAGGCCAGGCGAGTTGTGAGTACAATCTCACAGAAATTCCCACGAGAGTATCAA
AAGCTGGTTCAGCAGTTTCAAGGAGTATCTTGATTTTCTTCGTGAAACTACAATTATCATGTATAAT

ATCCCAGTGAAATACACGGCTACTGTTTGAAATAACAATAAAAAAAAACAGTTATGTTTTTATAAG
ATGAAGTAGCAAGCCTAACCAAAGAACGAGTGTTGAAATATTGTTACCTTGACCCTCCTTTTTTGTG
TCTTCTCCTTTTTTATTATTCTTTTGTAATACCTCATTTCATATTCATTAACTTTTGCAACCGCTGGAA
AGTTTTATTAAGCTTATTAATAACATGACATGGCATGGCATACACCGTACTCATTTCAAAACACAC
ATTAGGGATTCATTTTAGCACTCATTTAGAGTTGGAATTAAGGATGACGTCAGCTCTTAAAGTGTTG
TGTTTCTGGTCCT
>LsigCSP3_putative chemosensory protein_partial mRNA
CCGGGACATATCACTGTCGTTCCGGGAAGTTCGTGAACTGGCAGACGTAAGAGTCATGTGCCTGGG
AAATTACCAGAGAGATGGCAGAATAAAAAAAGAAGTGGTTGAACAGCTTTGGACGCAAGGTCGTT
AATGGCTCCTTCTCTACCACTGTCTTGGCATTATTGACGTCGGTACTTGATTGGCCATCGCGCTATG
ACGTAAAAGGTAGTAGGAACCTTTAAGACTACTTAAACGAATGTGTTACAGCAGAAAATCAGAAG
ATTAATCGAACCAACGCTATGAAGACTGTACTGATTCTTGCAGCCCTTGTGGCTTTTACAGCCGCCG
CTCGAATTGTTCGTGAAGAAGCCCAATATACTACTGCCTACGATAATATTGATATTGATAAAATTTT
GACGAATACTCGTCTGCTGGATTACCATATCAGATGTGTTCTCGACAAAGGACCTTGCAACAAAGA
AGGCAAAGAATTGAAAGCTCATCTTCCCGATGCCATTAGAAACGAATGTGAAAAGTGCTCTGAAA
CTCAAAAGGAGAAGGGCAAAAAGGTGGTTCAGTTTTTGATGAAGAACCGTGCTACCGAATGGAAA
GAGATTGAGGAGAAATACAAGAACTACCATCCCAGCGGAACACCACCACCAAACTACGAGAAATA
CATCAAGGCTTAATTAACTAATAATCGACCAATCCGCCATTCAGTTGTTTCTTCAGTTTATGCACTC
ATGTCTGCTTTCTCTCACCTAATAGAATTGCGTCCAAGTGACGACATTTTATAAGTTAGTGAACAGG
CCTCAGTTAATTTTTTAAAATAAAATAATTGTTTTGTGTAAAATCGCATGATTTCATTTATTATCCTG
TATGAACCTAATCTAAAAAAAGAAACATACATTTTTAATAGAAGATCAAACAAATATTACATGCCA
ATGAATTAGAATATTTTAAGCTTTTAGTGCAAACAAATTATTTTGTCAATATTGACTTTCTAAACAT
TTCAATAAAAAAACGGTCCTCTTTTATAAAATTATAGTCCTATATTTTCTTATTTAAAGATCAGTGGA
TGACCTTGTTTTCCAAGCGAAAACTTTTTTTTTTAAACAGCACCTTGACTAATTTATGATCCTTTATT
CCCTAATCATTTTTATTTCCTTCATAAGGCAAACATAATTAATCGAC
>TdomCSP1_putative chemosensory protein_partial mRNA
TGTGGACACTGTTTGCATGTGCATGTGCGTGCGCGCTGCTCGCTGTTGGCTCTGCCCAGCCGCCGCT
GTCACCCGAGGAGCTGCAGGAGATCCTGGGGAACCCTCGAACTGTACGGGCCTACATCGCTTGCGT
CTTGGACAAGGGTCCATGCACGGCAGAAGGCCGCGAGCTGCGAGAGCGCATCCCTGTCGGTCTGA
AGACAGCCTGTGGCGACTGCAGCGCCACCCAGAAGCAGACCGTGCGCACAGCGGTTCGCTTCATCC
AGGAGCACTACCCTGACGAATGGCTCCAGCTACACGAGCACTTCGACCCGAGTGGCGAGTATGTA
GACTCCTTCCAGCACTTCATCGACTCTGACGACTGAGGCTCGGCGACACTCCTGCCGCGC
>TdomCSP2_putative chemosensory protein_partial mRNA
GGTCTGCGGCGGCGTCCTTCCGGACCCTCACGAGCTGGTCAAGGACGAGCCCAAGCTGAGCAAGTT
CCTCTCCTGCTTGGCCGGCAAGGACACGTGCGAGTCCTGGACGGAGGAGGTGAAACGTTACATTCC
GTCCGCTCTGAAGGAGAGCTGCAAGGACTGCCCCGCAGAGCACAAGAATTTTTACAGGGTCGTGA
CGATCCACCTCAGGAAACACAGGCCGGACGTCTACAAAGCGCTCCT
>TdomCSP3_putative chemosensory protein_partial mRNA
GACGAGAAGAACGGCAGAAGATGCAGTCCATGAGGTTGTTGTTCGTGGTGCTGGGTCTGGCGGTG
GCCGCTCAGGCGGCCAGGCTGCGGCGGGAGGAGAAGTACAGCACGCAGTATGACAACGTGGACCT
GCACGAGATCCTGACGAACAAGCGGCTGATGAATAACTACGCCAACTGCCTGCTGGACAAGGGAC
CCTGCACACAGGACGCGCAGCTGCTCAAAGACGCGATCCCCGACGCGCTGGAGAACGAGTGCGCC
AAGTGCAGCGAGAAGCAGAAGGAAGGCACCGAAATCGCCATCCCGCACATGATCGAGAACGAGC
CGGAGATCTGGGAATTGCTGAAGAAGAAGTTCGACCCGAGCAACAAGTACGGCGAGAGGTACGCG
CAGCTGCTGAAGAAAGCTCAGGAGAAGAGGCGAGGCTGAGCTGCGACCTGACCTGCTCGTGCACA
CGCTGTTAAACTGCTATTAAACTGCTTGTTACAAAC
>TdomCSP4_putative chemosensory protein_partial mRNA
CAGCACCTCTCAAGATGCACGCCTGCGCTGTCCTGCTTGTCGCCGCCTGCGCGCTGGCCTCGTCCTT
CGCCGCGGAGATGTACTCCACCAGGTATGACAACGTGAACCTCCAGGACATATTCAAGAACAAGC
GACTGTTCGATGGCTATGCGAACTGCATCCTGGACAAGGGCCCCTGCACTGCTGACGGTCGTCTGC
TCAGAGATGCGATCCCTGAAGCGCTGACCAACGGTTGTGCCAAGTGTAGCGAGCGGCAGAAGTCG
GGCGCGAAGGACGTCATCAAGGAGGTGAAGACGAACCACCCGCTGCTCTGGGATGAGATGAAGAA
GAAGCATGACCCTACCGGCTTGTTCGAGAAGAAGAACAGTGACCTGCTGGACCAGCTGTGGCACC
>TdomCSP5_putative chemosensory protein_partial mRNA
TAGCTCGACACGATGAGGACCACCCTCGCCTTGGTTGCGTTGGCCAGCCTGGTCGCTCTCGGCGCC
GCCCAGGGCAAGTACACCTCGCAGTTCGACAATGTCGACGTGGACGCCATCTTGAACAACGACCG
GGTTCTCACCAGCTACTTAAACTGTCTGAAGAATGAGGGCGTCTGCACACCGGAGGGGAAAACGCT
CAAAGAAAGTATCCCGGACGCACTGCAGACGGGCTGCTCCAAGTGCAGCGACAATCACAAAGGGG
>TdomCSP6_putative chemosensory protein_partial mRNA
CGTCAGTGGCTTGCATTGAATGGTAGCTTGAGACAATGTGTATATATATTTGGGAGACTTTCTCTGC
TCATACACACACACACAGTTTGACATTGAATAACAAATTTACTCTCTCTGCGTGTTCAAGATGAAGT

CTTTGGTCGTAATCTCCTGCTTCCTTCTGTTGGTTTTGGTCTCCACAGAGGCCTTCAGGCTTCGAAGA
GCAGAAACTTANNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNAAACGATTGTTGCCAGATGCTTT
ACAGACGAACTGCACCAAATGCACTGATAAGCAGAAGGAGATTGGACGAAAGACCATCACTTTCT
TGAGGAAGAACCGTTCCGAAGACTGGGAAAGACTCAGGAGCAAGTACGATCCTGAGAATAAGTAC
GAACAGTTTGAAGAAGCTCTTACTCGTTAAACAAACAAACAAAAACAAAGACTATATTTAAAATC
GAACTGCTTTACCTAAAATTTAAGACTGAGTGATCGCTGATCGATATGTGCATATGCGTATGTGTGT
TTAAAAAATATGAAAAGAAAGTTTTGTACTATGTGTGTGAACGTTTGATGTAAATTTTGAATGCTA
AAAATATAAAAGTTAAAAATTTTAAAAAAAAAA

Supplementary material 3: amino acids sequences of putative OBPs

>LsigOBP1_putative odorant binding protein_translated_partial
MHLLRFVLCCVVCVTANAFVASQSAKTKKCQEAPSTIADVKVHVNKCREKVKDNIIKKISEAAQREAT
AIPTAEGEGKSEALTDFDIWTSEKVLAGCLVKCVFDEMNALDTYGLPDYRGIYNMYAKNVHDYGYQR
ATSEASWVCIQSVLQSAVGRGGQPGLTCSIAYDVYECMNKKTEEYCS
>LsigOBP2_putative odorant binding protein_translated_full ORF
MVFLKLVLLTACGVAVTLGMDCESQGSQDNANELQQTIAMCLSKYGKSEGDHGKSEGDHSKTMGGS
HNQGSHEGKAYVREHDASSNMSSRSMIASMPACSLKCIYEEMQAVDEEGFPDKNRLSTVLAQKIKDSN
VRNMVVRATETCFDRADGITDACEFSKKLAMCLEQKWKENCDDEEDKNDRENNSSTETGYDNSNRRY
AYTQHNHK
>LsigOBP3_putative odorant binding protein_translated_full ORF
MKYFISIVLCLSAIFSAAYAEEFQGFNSLVDEETVTLLATCDKGPDENCIPKCYLETLDGLDETGHASKE
KMYDSIPNKILEPRREMVTRSLEECFRDEIKTADPCQSASTLLACMANSIQGAMLITE
>LsigOBP4_putative odorant binding protein_translated_full ORF
MKFFSFILLIAFFVQSHANNISGLEDLMESNVLEGLRSCEGPGADENCVYKCYLQQLDAVDSKGYADKT
KLDARVETKIKQDFRPKVRKSIQKCYKGPEETMKIDPCASAAVLVSCIAQELQQTMNQGR
>LsigOBP5_putative odorant binding protein_translated_full ORF
MHLLQALLLVLTVASSLAIHCNIRKAPTPEIIEAVKQCRIGNAARAANKPGETVSMQKGGAQGMEQGG
QGSQPQGGRGGPQGGQQGQKQLEQGQGQGQGQGGRQRRALDIYLIPGLDSIGQSLFTGLRSRRDTPAM
YKNIDPCSIQCMYEKLDAVDENGFPEKEAVLRFTRENVDDKTTLGASEKVIEDCFEQMLADQSKEKCD
VAKKLTMCTASKMMQVCDSKKN
>LsigOBP6_putative odorant binding protein_translated_full ORF
MKPVISFVTMLAGIIVYVFMGTALCAPPQTENGHEIPIGSEFKLALLQSQDPDTNGRRMFTLKSEENSEES
KEVQYKCMLPKNLPNTEQALKECDAELMNSAKSSSLKGSKEIIHSFDSAIRQRRQTQQQVMCYGRCVF
QKVNVLNEKGIPDQEKFMKYVKEEATHPAWKNIPELATNSCYKIINSAIESKGGVDALNEDEKCLLSVN
VKRCYIMSLYENCPKELREYGAKSE
>LsigOBP7_putative odorant binding protein_translated_full ORF
MSKAYTCPRPKYLQDNPMLLTQCVGACLQAGSNPNNQPSPNEPPPQSGQQGGQKGGENFVTKTVKCY
LCMRRCMYQQMNLLDSMGLPDKNRYMGYVTRDTSIPSWNPASEKSIDQCYNIMVAKMDADGGPEAL
NEIQKCKVSSDFEACYYTQMWRNCPEDIRRYMKTRGSK
>LsigOBP8_putative odorant binding protein_translated_full ORF
MKAFIGLTFLFGILLFVVAEEMAHLVDLSSDEVNPGPLILKNLNENDVEMISPLHNSRTPRLMKLNLSNK
GAFYSCIRPKHLYDTGTDWSKCWSDCNSMGGQPNTPTPPTEKLQESEAKFNESGLKEASQAKQNGNNN
ILAKAIMCWQCMRSCMYQQVNVLNENGLPDQDLFTGYVNKDDTVPSWAPASVTAVQYCFNEIQPAV
DAEGGPAALDQGQKCKVASFLHGCYFNQMWRTCPADRRKYIMG
>LsigOBP9_putative odorant binding protein_translated_full ORF
MKAVTSILLVVLATLCTHALAKHYGRGMNLNPKRLQKIGGRRTNGMQSHEEGSQQTYTYHCSGLPYF
LNMEEARETCFNEMNKEISTLLNDTSSDGDQAVSSFRAKCKHCFLNCVFKKLDVLGDDGLVDAKKFVS
QVKSDYVEDYWNSIIENGVQHCVNKVNKRIEEKGGMVALNEMQVCNQSKRFRKCYIYNMYENCPEK
AKIYYTGEADDEENMQSLD >LsigOBP10_putative odorant binding protein_translated_full ORF
MYTLKDEEESDNSTEEVYYQCSVPLYQDDDDSWNECKQETVKGLTELGPEDESWSKEQTEEWVMKYS
TILNCHQTCLFEKLGLLDDNQIVVHTKYIESVKKGEVEAPWGTIPDEVSERCFKKIDEDIKTSGGLEAMN
ESEVCKLTSSFLFCYYDNMYKNCPEDLKSYDEDDDDDDYKK
>LsigOBP11_putative odorant binding protein_translated_partial
TVRDVKFLAESEAHYVTTHLKVKDSSKTNAYTLKSEAEYGNTTEISYSCEIPYPVIPDEDWDFCEKIANI
SLNELKSGDESSWTDAEKEEYDDRCIEIIDCQEACLLERLNLLDENKIVDPAKFLSKLKEDGYDSSWDPA
LGEVINGCFDQLKANITTSGGIEGLQESDKCAFTSEFVYCYVYDTSLECPDELRTYV
>LsigOBP12_putative odorant binding protein_translated_full ORF
MSRQTDGHRKLAMSIIYTCEVPYPDIPDDDWDFCEKITNRSIDELKSANRSSWTDGEYDDRLYKIFDCQ
ESCFLERLNLLDENKIVDLAKFLSKLKEDGYDSSWDPALGNSINGCFDQMKAKITASGGIEGLQESDKC
TLNSEFVNCYLMDEALECPEAVRTYPPK

>LsigOBP13_putative odorant binding protein_translated_full ORF
MWLVIRTGHYNMRGVLIFIPFTVAVLSASPSTENIIQLWDDSEARSQSLRIIPYKSDGSNIYTLKNEEDTE
NTEEVLYACRIPFYKTRDVDWMTCRNETESKLEELGDEDESWNDEEMQDYELRYTTIINCHRTCYFER
VDLLDANRVIDPDKFLSLLKEEDHDQSWDPIMKDAVDSCVDDINANITATGGVKSMHVSDLCQLSTTF
LYCYMSATYKNCPEHFKEFGEDEEK
>LsigOBP14_putative odorant binding protein_translated_full ORF
MDLTGIVISLILFLGVSLCATTTTPTTTGNPVHHEGPYQAHGNHEQMNFMRGDKKFNITHEPMVCYVPR
LRMNMTVWEACANESREAKTELGLFNNTWSKEEKKSWRESFRKIIDCEQNCNYEKLGLNDDRGILDSE
NVVANLKQNSCSPVWEPIFDDANKECVKKLDKIINKFGGIENMDQSQKCFASSKLYSCYKMVFHKNCP
MEMKYYVW
>LsigOBP15_putative odorant binding protein_translated_full ORF
MKAVVSINVVIGVLLIIIATVNGEFSEDDVDSYQLSDDFNQMEILTEDEDEHSIFITRSKRQADDKKRVPT
ECYIPRLSLNDEDWLYCLDEENKKMSEHGRFDDSTSVEEKKAWKKKQHSIRYCYRSCYFEKQNLLDDD
GIIDPEKFVTHLMNTSCSPAWQSILPKVKEVCANQMKNITKKRDDLKSLDQGTKCALSVKFYHCYIVNA
RQLCPQESRFYNL
>LsigOBP16_putative odorant binding protein_translated_full ORF
MKLVASFVVLVAIPLGVASVRYDVYSVYDEETHFLKEHFREVQFMATGKDHDAKYSLMVKRQTDDK
EEDYERPYQCEIPKIKIDNEAWKFCIDHLKEKIKKLREADETMSDNEKKQAESDMETCFNPCYYKQMGL
IGDNGIIDVEKFFVSVKNNFCPPSWEPEFDELKKGCTDFLNSEIEEYGGLESMNETSKCSITNAFHWFYTE
EATELCPRELMYYTF >LsigOBP17_putative odorant binding protein_translated_full ORF
MKMLVQIVLLIGVLQCVIATESFGYNGDYVIGLNMQDNLRQAQLMAEGKDMHTAKMEHQEETKRPTP
YLCNIPRLPLDKEAFSICNENMMGALIMLGPRNNSWTKEERNKWEQQRYSIMECANGCMLEKMGLFG
ESGILDSTKLFSKLKPNHCDPSWEPVLKEVQQEYDEYYKHVINQFGGVDKMTKHDKCRITTTMFSFTSS
SLDASCPEKSKRYYRY >LsigOBP18_putative odorant binding protein_translated_partial
MQPPKWILLLSVFLAMQLYLGEAKKTASKKGTKAVLKHCMDAYNVTSDIFERLCIQAPESNEGNTKCL
MNCVLGGLNFLDDTGLLDMKKAFKHTSNEKRKIFREKCNNAAQAASLSTDDACVRTNRLVGCLMEK
NKKFCRAIKDFKQDEKEE
>LsigOBP19_putative odorant binding protein_translated_full ORF
MKTFLLLGICYLSLVCFAQGKQDCTYQRDEEKMKLIHSCRAEHEVNREIVVKLFKSNGTMHSDKKCYF
SCVMQDTGMADEDGNLDVSLIRAYAKRMYKQDHAVEKALAAIDNCEPILSDPNETDGCERVFHFLTCI
KRIMVKYCYDIDLHEEDDDE >LsigOBP20_putative odorant binding protein_translated_full ORF
MTTMNLRVLLVIGLVSIASASHDIGKCKRKPESAEKWKNAKMVCDETYPFEEGVIKKMIVGEEVDDEQ
AEMAKCFVHCILEEYEALSKDDHHVDLDALKMHAEEVFEDEEVQEEMKTVMEECSHVTGDYQEDCD
KSFEYVRCYLTQMHEYCDVTADE >LsigOBP21_putative odorant binding protein_translated_full ORF
MMMKTVSALLLIGLVSLVSASHDIGKCKRKAAAAEKWKGAKDNCKPLFPVEKGTFKKMIIGEEVDAE
KAEIAKCFIHCVLEEYDALDKGDHHVNLENLKMHAEETFVDKEVQEEMKSVMEECSHVTGDHQEDCE
KSFQYTRCFVVKMHEYCDVNAED
>LsigOBP22_putative odorant binding protein_translated_full ORF
MKLIYCIWFMIVVAVFCEEEDYDDDDEAPHKCKFSETKILSLGTMLKCSTDHNVTQEDSALFSTNEISCA
DADPHKMCFGDCYFRDREMLLENGDINKEDVFTVIKRAYKGKDDNINFFLKLTEKCYKIKGETESCCY
AKEFANCFLVVMNEYCDQIEDPMVLFG >LsigOBP23_putative odorant binding protein_translated_full
ORF
MYLKMGIVVLLLALPLAVLTQTTTTAKSMTTTTEKPDLAQTEKRCNGSFLRDDEITKELECAKNKKLSA
AELEMAVLEPYKIEQYKCFYQCFMKSNGWLTDDLEIKIVPFKNYIVKDFKDEELKEEADIAADDCLETT
QKDEEQECDYAAEFMNCYKKRIRTKCGPLPLAIRDDIRDRYKIP
>LsigOBP24_putative odorant binding protein_translated_full ORF
MLRKMEIVVLLFTMPLAVLMGEDKNRCNGKFVKEEELKKEKECAKKLATKTGQLEAGDDGPKDIYRY
PCIYACYMKSNGWLTDDGEIRKADFKKFVKSEFVSEEMKEDAAELVDKCVEITPTEGEEVCYIATHFNE
CFQYKLKPYCREYSRNGKTP >LsigOBP25_putative odorant binding protein_translated_full ORF
MRPTFILLMIIFVVFAEEHYPGENEFRCNNDILTFDERRPYAKCAIQLKIKAGLVDKGTRTIQSGDGCIYQ
CYMKAHGWMDDKGEIHLEPFKKFIKAEFQDQTIEDIMLETSDDCKESTFDYEEETICDHANVFIRCFRR
KMRKTCGPLPQEAKDYLLNHPNIKEFTLPE >LsigOBP26_putative odorant binding
protein_translated_partial
MESTTDSGEAGTKEKRCIELLFTDEELKREVECAKKLKISTGALAEDIESIGDHLRRESCLFNCVLKNSG
WLGEDGMARRKPIKDFIEKEYSNQDIIQQSLEGLDDCLETQEIDEQDDQCEYSLQFYRCLGNKIRKVCG
RIPDYKKD
>LsigOBP27_putative odorant binding protein_translated_full ORF
MKIYAIIFIAFAVSALAMKEDEKEGWGKCEPDDIEVVQMKMGMKCAKENGLDKTDIENYFKDPNHMF
EGPKNCVYKCYMTNQKWLTETDDLKKEDIHKWIEKAYSEEESRDSGRKIVNHCFDSVPRGTKEACAY
HAEYVKCIRNILRSKCGEMPQKYKDMVIKKYDEE >LsigOBP28_putative odorant binding
protein_translated_full ORF

MKTFTIICVTALIFFDFARAGDDEEEEFKCEPNDFDQVELPKGIKCAKENDVTSEELDKNMDDDEENPFQ
GKKKCVYRCYMGNKGWLTTTHDVIIQNVKEFVTQAYKDEIRDQALEVVEDCAEMTPREEEEECDFHS
EFGRCIRRKMRPICGPLPAEYKKQLMEKYKD >LsigOBP29_putative odorant binding
protein_translated_full ORF
MYPINRIFFLTALACLLATSIAMPEKRQASRCFPKSMEIPIKHITECASQFGVTHADIGAFMGRGQLAEDK
KCIIKCVLDKHGVISEDNMVDENKAVEMANTLFADRTETVEMYKKAMMKCYNPKDFSGDCEAFFTLQ
NCIYDDMKQYCGFPATG
>LsigOBP30_putative odorant binding protein_translated_full ORF
MKITFIILAVLVTVSLAEKKKEGGKNWKCKGKPMEGMMTKGMECAQEHGVSQDDTDALMGDGEGLG
EKKCIVKCMFKKFGVVTDDDKLDEDKILDMTSKTYGHKEEVEELATDAFYTCYKEDEWSGDCDAFFN
YKNCLRKEMDDYCGPPPKDEMMG >LsigOBP31_putative odorant binding protein_translated_full ORF
MKTTLFLLAVILALAVANEDMKCKGKKMNEGYSEMKACAEEYKMEKEDFKAFKGEEGLAAEKHCFF
QCLYEKQNLLNGDKTLNEDKVMEQTQKMFPDNPDTVAAMKTAVGKCNQKAVDCTGFYNFQMCIGK
EMKEYCGK
>LsigOBP32_putative odorant binding protein_translated_full ORF
MAVTFYLILTVIIAGAIAEKLDCDQANEDDDSKFEKCAMEYKLDDNDQMALFMGKPLPADKECFCKCA
TENQGILGADGKIDDAKVNEDFNKSYEGKPEVIERFMATHKKCYNSSEFTGSCKEHCAYLGCSITDIQT
YCKRD
>LsigOBP33_putative odorant binding protein_translated_full ORF
MAVTSYLILSILIARAVAEKLDCSSKDAGSNNAHFDACSSEYELTQDDIVGVYYGKPLPEQKQCFFKCA
FEKQGMIRPDGMLNETMVYEDINNSHSDKPEVKKISEDAHKTCYKSTEFTGSCPDYSAYTMCGMKYM
KKYCK
>LsigOBP34_putative odorant binding protein_translated_full ORF
MKLTLALLSVILALAAAGERGECKCKTKLTKEQKDIMMECAKESGITKDDKKTWYEGGDLEEKKQCV
AKCIYDKNGMITADGKIDKPKVYESADKVFAEKPEAATAFKDQFEKSYTEAFTNSCEDHYKIMKDVVA
ALQKAC
>LsigOBP35_putative odorant binding protein_partial
SEGFKEDSKSCAEKYGITKDDAHAMRKGTVEGEKQCFYDCFAKARGWADDNGKLDESKINESIIELSE
GDAYTKQSLEKSLEDCKAKTEWSNDCSKSLEISNCVVRTFMKLCKKDDE
>LsigOBP36_putative odorant binding protein_translated_full ORF
MKTAIVLFAVVIATAFAHPSGNWKCKGQKSEGTKEKMATCAQETGMTKDDFKAMKSGTLSEDKQCFF
KCLYKDMMTPDEKIDEGKKKAAIAEMAAGDENTLTIMSEVFINCYDAGSFPGDCASYYKFNNCEMKGI
FEKCKGE
>LsigOBP37_putative odorant binding protein_translated_full ORF
MRPYLLAIVVIIGTTVTEVHSRCDGRELSKEDIKMIRGCNAAFDFTESDSKIVGEFGDIPEQKACVVKCIA
KGLNWLASDNATLDIQKLLNWVHEVLVEDAETENFCRDAINKFYKSSDFNGKCTPYYNLYRNVAMEL
KEFCEGE
>LsigOBP38_putative odorant binding protein_translated_partial
KIHVSLVVLLWVSQYVMATKTFNSHDDYEKGLHIKGNLRQLMAEGKAVHTNAFSEDQEELEKHRSKT
PFRCDLPRLEVNEEAYMECDKETQAQMEEFDAYNSSWSRQERRDWRKKINVIEDCNVVCYFGKLKLL
DGSGILDSTKLFPKLKQNSCNPAWEPVLEEVRQSYDKNLIDFKKDVGENYKWSDPDNCRLIKNFYSWT
VETLYRKCPKQLKQHIY
>LsigOBP39_putative odorant binding protein_translated_partial
MRVVNIFIVVVVVLLGVESTEIGENPLKNGQSHLSHGGERSSGMYTLKSQNEPEDGEDVFKVTYTCVSP
RPKLNKEAWIFCEDETEQKLKEIPKPESGITGARKKEARIQLRQARWEINQCFLPCYFEQTGIMGENGIV
DIEKYFTSVKTNFCDPNWDPVLATLKEDSTIFVNTKIEEQGGLEAMNGTTKCSISN
>TdomOBP1_Assembly of contig 4374, 2770, 4782_putative odorant binding protein_translated_partial
MKSLLVVLSVLIALASASPTAKNKRCRDTPTASKNIQKVINECQDEIKLAILQEALQSIQEAVRSTRTRRD
LFNDEEKKIAGCLLQCVYRKVQAVDSYGYPERNGLVRLYTDGVQDSEYYEATAQAVYSCLSKTQQNLI
GRQNEPALACHVSYDVFEC
>TdomOBP2_putative odorant binding protein_translated_partial
MKRTRYEYSSSSSSSSLSSGSAAVQNVPPCIIHCVFRHMQVLDDSGMPEKSVVSRVMLQGISDREVRQFI
EDSVDECFESLDNNNETDNRKCQYAKKLAICLIEKGSNTCEDWGETMDEKRRSSNSRTTYSGRKYLRPS
SNG
>TdomOBP3_putative odorant binding protein_translated_full ORF
MKVFVVLATLLVAALAGPTETGCEYDLSRQAKELVAACRASNAQCVPQCVLEKAKALDEEGCPVKEK
YKKMQEAQVGNKDLLPTMLKITDDCFAKVQKGELSCCEYTNALHACKKEELKKICRGHQ
>TdomOBP4_putative odorant binding protein_translated_partial
NIINKCGNKNNAKCLVHCVMKELQALNDDDCPEQDLYEKMLEKEVGNEELRPKMIEITKQCFSSTPQG
DSDCCDYATNLWLCKKKKLNDICKYER
>TdomOBP5_putative odorant binding protein_translated_full ORF

MEKICAVVLVAAAAITLCYGLLGGLLSLRDDLEGYERMREQNMDEDSYEIIPVRVKRSEKNISENSRGP
DDSEDISIIEFCCFMPTPGRETYEKMFECVNMSAENMTWPEEGYQIPVHRSNKNYTYRVLNNKSSDYEI
GSLCFEHCFLERNEVVDDNGLPNKSEVFNFVMKSLSNVEAWKTAVENSIDSCFTEIESGWNGEDQPDD
AIDKCSISFLLLDCIGQRLQENCPEQYRTPYEDQKCIEYKEEKEAEGVQQEQNFQN
>TdomOBP6_putative odorant binding protein_translated_partial
MLVTCIGYYFLLHLTLVSGVSVHLYQGEGVVFSGSISIGLAQPEDKQPTMNNNTENFCCFIPASSIKTSQA
VAVCFDTSSDDQEQKSTGLEVPVTAEDQFRFEYEEYENMNLTEEERVYLCRQQCFLDKFRVLNKKRFPI
KDKMLTFMKEQMVDNDQLWFPAINSSVDACFEEMENEFKGKDLSKVDTCITASAMLYCV
> TdomOBP7_putative odorant binding protein_translated_full ORF
MKSIAILTLCSVTVAAVAGSILGLFGRDVSSDWGGSVNDDNPRFFRVRRDQQDERKHKCCETPKPGPEF
RRQFRECSEKETPPITEQPEEQEFTQSPENREVSDDVAALEQESENGSEGGNEGEIGEKEGEGKEEKDKP
KKKKHRNKCAMMCMMERNGALSGDGRPIKEGLLELFKQRYTDEAWFESLNNATERCSRWIDIETQYI
QAKREEKGESTELNNRDKCRISNRMHRCVRKEIRKMCPEEYREQSEECDKRRRKHDEKEKGKTEEDNE
SNNRENSEAQQE
>TdomOBP8_putative odorant binding protein_translated_full ORF
MKSISILTWCAVTIAAVAGGSLSSFNKDISSAWDAAADYANPILFRVRRGKKKEDKKHKCCEIPEPSPEL
RNQFSECREQEENQGDELTTERPEEPEVTESPEDREVSDDMASLEEGNGNGVEEGNDRETEEKDGEDKE
GKDKHKKRRHKNKCIMKCVLEKNGALNVDGTVNEEGLLGLFKQRYIEESWSEPLNNATERCSRKIERN
IQRMQAKREEKGEPTELDHRDKCRISNKIHRCVEKEIRKSCPEAYRVQSEECEKRRRKHDKKKENESQG
DSDSNNEEGDSQAEE
>TdomOBP9_putative odorant binding protein_translated_full ORF
MKKWLAFTLLCLYAAVRTEEVEKITCLVKQTDGVDEKSAESDTCKEKFPVKNEAIADWFQGLDVSAE
KEPQIKCFAHCYMAERNLISEEGQFNLEALKEDTTKTLEGEETVEKVEEMIKECVEKYEGEENHCEKAY
MALTCVRQNLTRLCNFVMMEPEEEIEEKEEK >TdomOBP10_Assembly of contig 7661, 582,
6888_putative odorant binding protein_translated_partial
LLALTAF?YAIVYVKAEGI?DEKWKAVSEECGKETGATKEMWEKWRE?HVAEEPLQCFFKCLIDKLEYS
DDEGNYLKDKGLNYCNTNWEESSDDDKDDKKRACLKAVETCGDMPVKECSEAWDFKECLVIAYFGE
LYNDKEEEEEDN
>TdomOBP11_putative odorant binding protein_translated_partial
CSEENGITKEDWERYKETKEATDNIKCFKKCFMEKLGFIENGSLNKEKAKKKCERKSQGDEEKKQNCF
NAVDECGKNTVETCEDAWNMLHCLWQWKNM >TdomOBP12_putative odorant binding
protein_translated_partial
DCWNSYTCFRNHFKKAIYHYKKTIKKCFSESGVTSEDYSEYMESGVPTESIQCYWKCFFEKAGYVKEN
KVNTPHLQELCKRYYESEEDTRNKCLAVVDKCKDILVSDCKNAWKFKECVVIETQTEER
>TdomOBP13_putative odorant binding protein_translated_full ORF
MKIQHFVLIALVFVAVNCDEDQGAGGFDEAMRECAQELQITQDEFMRFKESGQPDEKIKCHFKCVMEK
KDMIREDGTFDTEPMEQCNIMRKFRDQSEENEQKFQEAKKNCNGKPATTCQEAFEAYMCVQAIAQ
>TdomOBP14_putative odorant binding protein_translated_full ORF
MNKLLIYLLVSNCIIGSFQQFTKEFKEDLKKKSKICADQHETDSARVLALMNGEIDHYNSKCFLMCMM
KTYKLMDDSGNFNENNIQEAVERISREDWKKGFKEHYPSCKQDHSQVTDNCEKVFQISKCLMDKNKV
QQS
>TdomOBP15_putative odorant binding protein_translated_full ORF
MAPLRTLVILAAATAFSMAITEEDMEVIIHELATQCEKVYPISEEDAELLHHRQAPKGSNSVCFLTCMFE
KLELMENGVFNAAHAKETVQKYVEDQPDILSKMETLLDICAGEVGSGDGACGAGLELFNCFKNHAEK
VGFMPDA
>TdomOBP16_putative odorant binding protein_translated_full ORF
MKIMSTFLLILSLGVSWVMPRPNDDPVEATRVIVNKCSKENGVTEGEIMAMKNGDIPNKRSVKCFLNC
YMSSVQVMKDGRYNIPLAISLAEQIAPDEETLKILKNMFEVCGTKFGADDCETAYEVSKCEVAMDKEIN
KLYFP
>TdomOBP17_putative odorant binding protein_translated_partial
DLSWTMTQDEISAMVRAIVDNCSKETGITDGDRAQLRDGNIPDNNNVKCFILCYFTSIQIMKDGKYELD
VAKGFAANIAPNEEVKNGIMHIIETCGVKTGTDPCDTAYEILKCKVAMSKNIVKAFFP
>TdomOBP18_putative odorant binding protein_translated_full ORF
MAKIVLLSLLFALYFVLLSCRPQDDAETLRKSISDKCVSESGISQGDREALIAGEMPNTRNVQCFIGCYM
TSTKVMQDGKYVSSAARAVVEKAIADEATQEVVFEVLDSCGNKSSGADYCETAYQIISCVIGKYKNAN
AFFF
>TdomOBP19_putative odorant binding protein_translated_full ORF
MQTDSILRAIAVALLGLAHSALGMTGRALEKAKEVDAKCRSETGAGEGSFGKFVAGKIDVDDGRFKCF
IKCIMNELASLDHAGTFNLEEELLNVPPEIKEEGHRIVISCQHIQGTDPCDTAYKLHKCYHDANPELYNR
VLSVWDFNAGVS
>TdomOBP20_putative odorant binding protein_translated_full ORF

MNSLGKMLVFVLVLGVGAYAMELPEDLTGRAMERAKEVDSACRAETNAGEEVFMEFILGDETDEDH
VYKCYVKCVMMKLHAMDADGNFRFEEELLNVPPEIEVEGHALVNKCKGVEKRPDPCETAYKIHMCYL
RENRELFLHAIAMWFEKAAS
>TdomOBP21_putative odorant binding protein_translated_partial
EQGEGGGIFKQCAEENEVTKDDFQKFKESGEAEEKLKCHFKCVMDKRGMVREDGTFNTEPMEHCGK
MKKFRSQSDENEEKFQKIRTECEGMK
>TdomOBP22_putative odorant binding protein_translated_partial
MEHLSSFILLQLVATLSITGSIMGRPTTSTSVAEDYAIIKSCNQTSPVSLHAINVALVHRKLTEDTTYGFK
CFLHCLYTKYGWMDEDGGFELTTMRHVLEKQITRTDVLR
>TdomOBP23_putative odorant binding protein_translated_partial
EETNWEQLKRRVKEMRKRWNCFTECVFNSAGWATEDGQVVDNTVREDVSRQADGQWTEVVESTLN
ECLGKNYNHDRHEGEGECQPHCARTMFCMFFHMLLKCPENYRNMDTEKCQNFWRKVDEKNQE
>TdomOBP24_putative odorant binding protein_translated_partial
CFESCLMKESGAMTWKGRINETALRDITKKMELKEPQRSYVFHHMKKCANKVESTQFINHCEKAYVF
GACFREHLRKDLRSLAITWKLYLTRGNISSIEKIH
>TdomOBP25_putative odorant binding protein_translated_partial
TTSNYGNRNNRFNSSHHILSGNNIYNRRHNNSNWRNNRTWRNRDNKHNDSNSNENSTNYSKDNNRTT
NSWRKMRGFFKSFDVLAKSRVLRQVFGTEKYIPACTIQCIFSKVNTVDQTGYPNESLLIKLCENAIKNEE
ARTTAIKIIRKCFRRLGTDDQENTCTFSKQLALCMGRDMSKICQD
>TdomOBP26_putative odorant binding protein_translated_partial
TDFMRKFHEAAEQCNKTYPISKEADEYFNNNSKLEDETSENGRCYVACFAAKIGVLKDGEFDPDHIKTL
LEHMEKYRGKKHGHKH
>TdomOBP27_putative odorant binding protein_translated_partial
MKAIAVYAIFAVVFTVVYADDYKQLLKDSLKNCAAKFNVPKPSAVSGSDLNTYLKFAKDNPTLVSCFY
DCTFKGAGLLGDSGFATDKLKNDLKVGKSSAP
>TdomOBP28_putative odorant binding protein_translated_partial
ILFFVIAVVFLTYVQADGNKKEKFKQMKKECKDESGVTDEEFQQWKENKKNDAYEPSENVKCFKRCM
MQKMGFVH??????????????DKPVESCDNAWELWRCVKSHWKQQREGNGEGGEEGGEEGQD
>TdomOBP29_putative odorant binding protein_translated_partial
MQCSTLSIKMIVLLVFLFSILALGSAGNMSNTADMNSINMTDINKRCNETFKISNGQLEALNNTGKFQNE
SDTAAKCYLHCIFNNTG???????????????YA
>TdomOBP30_putative odorant binding protein_translated_partial
MNYLLKTVAEDCLGRSSNISEECKCYWSCFWKKFNFELENGTINKDLIIEFIGSYSNSPGEDISPRIEETVD
VCVEKSTVEGCGQVKEILERVGDNYKSAED
>TdomOBP31_putative odorant binding protein_translated_partial
AKDEDEMCYAKCVGEKLNFIKNGRVNWEFVDLLTNRMPEERRDNFERIMEYCDAKGDEGEGCKPGY
RVFKCIQETMIR
>Tdom32_putative odorant binding protein_translated_partial
MKIQLFVSVLITSIVMSKGRPQDNMAVAKAIIDKCIQEHELSKEIEGVKTGDIPNKENVKCFIRCFMISFQ
LMKDG

Supplementary material 4: partial mRNA sequences of putative OBPs

>LsigOBP1_putative odorant binding protein_partial mRNA
GTCGAACAATACTCTTCCGTTTTCTTGTTCATGCACTCGTACACATCGTAAGCAATGGAGCAAGTCA
GGCCAGGTTGACCTCCTCTGCCGACGGCACTTTGCAAGACCGACTGAATGCATACCCAAGAAGCTT
CCGACGTTGCTCTTTGATAGCCATAGTCGTGTACATTTTTGGCATACATATTGTAAATTCCTCTGTA
GTCAGGGAGTCCATAAGTATCAAGAGCATTCATTTCATCAAAGACACACTTCACAAGGCATCCTGC
TAAAACTTTCTCACTGGTCCAGATGTCGAAATCTGTAAGCGCTTCGGATTTTCCTTCCCCTTCAGCT
GTAGGTATGGCCGTGGCTTCTCTCTGAGCTGCTTCACTAATTTTTTTGATTATATTATCTTTGACCTT
TTCACGGCATTTATTCACATGAACTTTGACGTCAGCAATGGTGCTTGGAGCTTCTTGGCATTTTTTC
GTCTTTGCTGACTGGGATGCAACGAATGCATTGGCAGTAACACACACGACACAACAAAGAACGAA
ACGTAGGAGATGCATGATTGAGTGCCACAGACAAACTCCAAGCACC
>LsigOBP2_putative odorant binding protein_partial mRNA
ATCAAATCAAATAAGCGCCACTCTTCAAAATGGTCTTTCTCAAACTGGTTTTGTTAACAGCATGCGG
TGTCGCAGTAACGCTTGGTATGGACTGTGAATCACAGGGAAGTCAGGACAATGCCAATGAACTAC
AACAAACAATTGCAATGTGCTTATCAAAGTATGGGAAATCAGAAGGTGATCATGGAAAATCAGAA
GGCGATCATTCGAAAACAATGGGCGGTTCTCATAATCAAGGCTCCCATGAAGGCAAGGCGTACGT
AAGGGAACATGACGCGTCATCAAACATGTCATCACGGTCTATGATAGCATCAATGCCCGCATGTTC
CCTGAAATGCATCTATGAAGAAATGCAGGCCGTAGACGAAGAGGGCTTCCCAGACAAGAATCGAC
TCTCGACTGTTCTGGCTCAGAAGATTAAAGATTCAAATGTTCGTAATATGGTTGTTCGTGCGACAGA

AACTTGCTTCGACAGGGCAGATGGTATTACTGATGCTTGCGAGTTTTCTAAGAAACTGGCAATGTG
CTTGGAACAAAAGTGGAAAGAGAACTGTGATGATGAAGAAGATAAGAATGACAGAGAAAATAAC
AGCAGCACCGAAACTGGCTATGACAATTCAAACAGACGTTACGCTTACACACAGCACAATCACAA
ATGATGATCGTCAGTCTACTTGA
>LsigOBP3_putative odorant binding protein_partial mRNA
TTTGAATCGACATCATGAAGTACTTCATTTCAATTGTTCTGTGCCTAAGTGCCATCTTCAGCGCCGC
CTACGCGGAAGAATTCCAAGGCTTCAATAGCCTAGTGGATGAAGAAACCGTAACACTTCTAGCTAC
GTGCGATAAAGGACCTGATGAAAACTGCATACCAAAGTGCTACCTGGAGACACTTGATGGTCTGG
ACGAAACTGGCCACGCGAGCAAAGAGAAAATGTATGACTCCATCCCAAATAAGATTTTAGAACCC
CGTCGTGAAATGGTTACAAGAAGTCTTGAAGAATGTTTCAGAGATGAAATCAAAACCGCAGATCC
ATGTCAATCAGCTTCCACTCTGCTTGCGTGCATGGCCAATTCCATTCAAGGGGCAATGCTAATCACC
GAGTGAACTGCAACACGGCAAGATGACTAAGAAAGAAACAACTGAATTTAATTCAACAGCGTTTT
GTCTTTTAAACACTTCTAGTCTTCTGTACTGATTAAAGAATCTAAAATGAAAAGAAAATGAAAAAA
AAACAAGAAAATAAAATGAAAGAAAAATATAAGAGAAGAC
>LsigOBP4_putative odorant binding protein_partial mRNA
TTTTTTTATTTTTTCTAAATTTATTGATAGGAAATGAGATATCAAAACATTATTTAAAACTGTTTCCA
ACGTGTCCAACAAATTTTATTAAATGTTGGTATCTCATATTTTCATTCCTCCATGTGTATGTCTTCAG
CGGCCTTGATTCATTGTCTGTTGCAACTCTTGGGCAATGCACGACACCAAAACGGCTGCAGATGCA
CACGGGTCGATTTTCATTGTCTCCTCTGGTCCTTTGTAACATTTTTGTATGCTCTTACGGACTTTGGG
ACGAAAATCTTGTTTAATCTTTGTCTCAACTCTGGCGTCCAGCTTCGTTTTATCCGCATAGCCTTTGC
TGTCCACTGCATCCAATTGCTGTAAGTAGCATTTGTAAACGCAATTTTCGTCTGCACCTGGCCCTTC
GCAAGAACGTAAACCTTCAAGCACATTCGACTCCATCAGATCCTCCAATCCCGATATATTGTTGGC
ATGTGACTGCACAAAGAAAGCAATAAGGAGAATAAAACTGAAGAACTTCATGATTTGTGAACAAC
ACTTTGATTTCATCTAACGAGGAGGAAAAAATTCCGTTTACGATGAATGGTGATGTTCG
>LsigOBP5_putative odorant binding protein_partial mRNA
CGACACCTGAAATGGTCAGTGTGGATAATCATCAACATGCATCTTTTGCAAGCACTTCTTCTGGTTC
TCACCGTTGCATCGTCATTGGCAATTCACTGCAACATTCGAAAAGCACCGACACCTGAAATTATCG
AGGCTGTTAAACAATGTCGCATAGGAAATGCTGCAAGGGCTGCAAATAAGCCAGGTGAAACAGTA
TCTATGCAGAAGGGTGGAGCCCAAGGCATGGAACAGGGTGGACAAGGCAGTCAGCCTCAGGGTGG
CAGAGGAGGACCTCAAGGTGGCCAGCAGGGTCAAAAGCAACTAGAACAAGGTCAAGGCCAAGGT
CAGGGCCAAGGAGGACGCCAAAGAAGAGCTCTCGATATCTATCTCATACCAGGACTCGACAGTAT
TGGCCAAAGTCTGTTTACAGGTTTAAGAAGTCGACGAGATACGCCAGCCATGTACAAAAACATTGA
CCCGTGCTCGATTCAATGTATGTACGAGAAACTCGATGCGGTTGATGAAAATGGATTCCCCGAGAA
AGAGGCCGTTCTTCGATTCACCAGAGAAAATGTCGATGATAAGACCACGCTCGGCGCATCAGAAA
AGGTCATCGAGGATTGTTTCGAACAAATGTTAGCAGATCAAAGCAAAGAGAAATGCGATGTTGCC
AAGAAACTGACCATGTGCACGGCAAGCAAAATGATGCAGGTATGCGACAGTAAAAAGAATTAGAA
GAAATAATTGGACTTCACCTCACTCTGTCTTCAATTTTGATATTTTCAAAATGTATTCAGTAAATGA
ACGGAATCTATTTTAATGTTATACTATTTTTTCTTCATATTCAGGTAAGGAGATAAAACTTAAAACA
CGGTTTAAGAGTAGATTTTTTTATTTACAAACTAAATGTTACAAAATGTTTCATCATGGTATCTGTC
AATCACTGCTTTTGTTTTTGTACCTGCAAAATAAAGATTGAACTCAGCAAATATCTCAGCAATTTTA
TTTTCTCCACAAAACCATACAAAGAAATTTATAGTTTTAATTTTTCAGTTTATGAATTTCTTCACATC
GGATGTCCCCTAAACATGTCTCCCATATAGATAAAATACATAATCTTTGACTTGTTAAATATATTAA
TAAAACCGAATCACTTTATCAAGTCGAATTATTAAAATGAAAGTTGAATAATTTGTTAAAATTCAA
TACATTTCGGAAACGCAGGCTTTCTTAATTAGTGGATAAAAT
>LsigOBP6_putative odorant binding protein_partial mRNA
CACATTTTACATTATATCAAAGACCAAAACGAATTACAGTGAAACAGTTAAAATTGATATGATATT
AATTTGCTCATCCTTTAATTAGTCTTCCCTTCAACATCTTCTTTGTAGTATACTTACATGTTAACATA
CATTTATTAACAAACGTTACAGATGTTGTATTAGAGTTTGTTCTGTTTTAATATGAACTCAGTTTTA
AACTTGAAATCTCATGTTGTCTTTAAACATTGGACATAATGTACGTGTTCTTTATGTTTTATTCTGAC
TTCGCTCCATACTCTCTTAATTCCTTTGGGCAGTTCTCATACAGGCTCATAATGTAACAACGTTTCA
CATTGACTGACAGGAGACATTTTTCATCCTCATTCAACGCATCAACACCTCCTTTGGATTCGATTGC
GCTGTTGATAATTTTGTAACAACTATTTGTAGCTAATTCCGGAATATTTTTCCAAGCTGGATGTGTA
GCTTCTTCTTTTACATACTTCATGAATTTTTCTTGATCAGGAATTCCTTTTTCATTTAGCACGTTCACT
TTTTGAAATACACATCGTCCATAACACATTACTTGTTGCTGTGTCTGACGACGCTGACGTATCGCCG
AATCAAAAGAATGGATAATTTCTTTACTTCCCTTCAAAGAAGAGCTTTTTGCGGAGTTCATCAATTC
AGCATCACACTCCTTCAAGGCTTGTTCTGTATTCGGCAGATTCTTGGGTAGCATGCATTTATATTGC
ACCTCTTTCGACTCTTCCGAGTTCTCTTCACTTTTCAAGGTGAACATCCTCCTTCCGTTCGTATCAGG
ATCTTGGGATTGTAGCAAGGCTAACTTAAATTCTGAGCCAATTGGGATCTCGTGTCCATTCTCTGTT
TGCGGAGGTGCACATAGTGCAGTTCCCATAAACACATATACTATTATCCCTGCTAACATCGTGACG
AAACTAATCACTGGTTTCATAAAAT
>LsigOBP7_putative odorant binding protein_partial mRNA

ATGTACAATTTTATTTCGCATAACAACCCTGCCTGCTACCATGAGAGCAATCGTTATCGTCGCATTC
CTGCTTGAAGCACTAGTGCTTGTGCTTGCTGACAGTGTGTTCGACTTTGGGGGAGACCTAGGTTTTC
CTGGCACACTGAAATTAAGGAAAGAATATGGCGGCGTGGATATCCCGTCGCCTGCCGAAGAAGAA
AGAACATTTAAAACACTGAAAATGAGTAAAGCTTACACTTGCCCTAGACCAAAGTATTTACAAGAC
AACCCGATGCTCCTCACCCAATGCGTAGGGGCATGTTTACAAGCTGGGTCTAATCCAAATAACCAA
CCTTCTCCAAATGAACCTCCACCACAATCAGGTCAGCAAGGAGGTCAAAAAGGAGGTGAAAACTT
TGTTACTAAAACAGTGAAATGTTACCTGTGTATGCGAAGGTGCATGTATCAACAGATGAATCTTCT
TGATTCAATGGGGCTTCCAGATAAGAACCGTTACATGGGATATGTAACTAGAGACACGTCAATTCC
GTCTTGGAACCCAGCAAGTGAAAAATCGATTGACCAATGCTACAACATAATGGTTGCTAAAATGGA
CGCCGATGGAGGCCCAGAGGCCTTGAACGAAATCCAAAAATGTAAAGTTTCGTCAGATTTTGAAGC
ATGTTACTATACGCAGATGTGGAGGAATTGTCCAGAAGATATAAGGAGATATATGAAAACTCGTG
GCAGTAAGTGATCTGAAGCCTTCAATAATTGTAGAAACAATATTATGTAATGAATAAAGAGATTTC
AAC

>LsigOBP8_putative odorant binding protein_partial mRNA
GTGGAAAAGTAATTTATTAATGCCTTTATTTAGTTCAGTTACAGTCCAACTTGCAAATTCTGTGAAG
GAAGACAATTTCAGTGAGAGAATTGTGGAGGCTTGAAGTCCTTATAATGTCACTGAATTTAGCCCA
TTATATATTTTCTTCTATCGGCCGGACAGGTCCTCCACATTTGGTTAAAGTAGCAGCCATGGAGAAA
AGAAGCCACTTTGCATTTTTGTCCTTGATCCAAAGCTGCGGGGCCTCCTTCTGCGTCCACGGCAGGC
TGTATTTCATTAAAGCAGTACTGTACGGCAGTTACACTTGCGGGTGCCCACGAAGGCACAGTGTCG
TCCTTGTTAACATATCCTGTAAAAAGATCTTGATCTGGAAGTCCGTTTTCATTCAACACATTTACCT
GTTGATACATACACGATCGCATACACTGCCAGCACATTATAGCCTTTGCTAATATATTATTATTGCC
GTTTTGCTTTGCTTGACTTGCTTCCTTAAGGCCACTTTCATTAAATTTGGCTTCTGATTCCTGTAACT
TTTCTGTTGGAGGAGTCGGTGTATTAGGTTGGCCTCCCATAGAATTACAGTCGCTCCAGCATTTTGA
CCAATCAGTTCCAGTGTCGTACAAATGTTTTGGTCTGATGCATGAGTAGAACGCCCCCTTATTTGAA
AGATTTAATTTCATTAATCTTGGAGTCCTTGAGTTGTGCAAAGGGGATATCATTTCCACGTCATTTT
CGTTTAGATTCTTCAGTATCAGTGGTCCAGGATTCACTTCATCACTGCTTAGATCTACCAGATGTGC
CATTTCCTCAGCAACCACGAACAGTAAAATTCCAAAAAGGAAGGTTAAGCCAATAAATGCTTTCAT
GGTGATAGGCGCTGTGCGTATACTCTATAGAAAGCAATTCACGTGTATTTTTTC

>LsigOBP9_putative odorant binding protein_partial mRNA
TTTTTTTTTTTTTTTCAAATGTTCATATTCAATGTTTATTAGAATACATTTTGTTACTAGCGAGATTAC
ACCATCTTTTGTTTTATATCTGATTGACATTAACTCTGTAATCGTTTTTTGTTTCAAAATTTAGGTTA
TTCAGTCCAGACTTTGCATATTTTCTTCATCGTCGGCTTCCCCTGTATAATATATTTTTGCTTTTTCCG
GGCAGTTCTCATACATGTTGTAGATATAACATTTCCGAAATCTTTTGGATTGATTACAAACCTGCAT
CTCATTTAAAGCCACCATGCCTCCTTTTTCTTCAATGCGTTTGTTCACTTTGTTGACACAATGCTGTA
CACCATTTTCAATAATTGAATTCCAATAGTCTTCAACATAATCTGACTTCACTTGTGATACAAATTT
TTTAGCATCAACCAATCCATCATCACCAAGCACGTCTAATTTCTTAAACACGCAGTTTAGGAAACA
ATGTTTGCATTTAGCTCGAAAGGATGAAACCGCCTGATCACCATCAGAAGACGTGTCATTTAAGAG
CGTCGATATTTCTTTGTTCATCTCGTTGAAACACGTCTCCCTTGCCTCTTCCATGTTCAGAAAATAAG
GTAAACCGCTGCAGTGGTATGTGTAGGTCTGCTGACTTCCCTCTTCATGTGATTGCATTCCATTTGT
ACGCCTTCCTCCTATCTTCTGTAATCTTTTTGGATTGAGATTCATGCCTCTTCCATAATGTTTTGCTA
AAGCATGAGTACACAGTGTAGCTAAAACTACGAGAAGGATAGATGTAACTGCTTTCATGTTCACAA
TCGCTTTGCAAGTAATTGAAGGTATAATTCGGTACTGTGAATTTCCTTTACTGAGATTAATAAAACC
ATTGCTGAAATACTAC

>LsigOBP10_putative odorant binding protein_partial mRNA
GTTTTTTCACAAAAAAATATTTATTCTGCTAACATTTAAAAGGCTGTAACACAATTGGAACTTGATT
GACTGATACAATTTTAAAAAGAAAAACAAATAACTCGGAACTACAAAATAAAACGGAAGTTCTTA
AAGTTTTCGTTCTGAATGTTTCATTTTTTATAATCATCATCATCATCATCTTCATCATATGATTTTAG
ATCCTCTGGACAATTCTTGTACATATTGTCGTAGTAGCAAAACAGAAATGATGAGGTTAATTTGCA
TACTTCTGATTCGTTCATTGCTTCTAAACCACCTGAAGTCTTGATGTCCTCGTCAATCTTTTTGAAAC
AGCGTTCAGATACTTCATCAGGAATGGTACCCCAAGGCGCTTCAACTTCTCCCTTTTTCACACTTTC
TATGTACTTAGTATGAACAACTATTTGATTATCGTCGAGTAAACCCAATTTCTCAAAAAGGCACGTT
TGATGACAATTTAAGATTGTACTGTACTTCATCACCCATTCCTCTGTTTGTTCTTTTGACCAGGATTC
ATCTTCAGGTCCAAGCTCAGTTAAACCCTTAACAGTCTCCTGTTTGCATTCGTTCCATGAGTCATCA
TCATCTTGATAGAGAGGCACGGAACATTGATAGTAGACCTCTTCCGTGGAATTATCTGATTCCTCTT
CATCCTTCAAAGTGTACATGTTATTCTTGACAGCATCCCTCTTGCTCAGATGAAGTGATTTGTATTTT
GCTTCGTAGTCATCCAAAACTTTTTCATAACTTTTGGTTTGCGAAGTCATACACAGTGCAGTTCCAA
TTAAAAAAATGAGAAAATGAGAAAAATGAGCTTCCATTGCAAAAAGTGTTCCCGGG

>LsigOBP11_putative odorant binding protein_partial mRNA
ACTGTCCGTGATGTGAAGTTTTTAGCTGAATCTGAAGCTCATTACGTAACAACACATTTGAAGGTA
AAGGATTCTAGCAAAACAAATGCTTATACATTGAAGAGTGAAGCGGAATACGGAAATACAACAGA
GATATCCTATTCGTGTGAAATACCATATCCCGTCATCCCAGATGAAGACTGGGATTTTTGCGAAAA

AATTGCAAACATTTCATTAAACGAACTAAAAAGTGGAGATGAATCATCATGGACCGACGCTGAAA
AAGAAGAATATGATGACCGTTGTATTGAAATTATTGATTGTCAAGAAGCTTGCCTTTTAGAGAGAT
TAAATCTTCTCGATGAAAATAAAATAGTTGATCCTGCAAAGTTCCTATCCAAATTGAAGGAAGACG
GTTATGACTCTTCCTGGGATCCGGCTTTAGGAGAAGTTATAAATGGTTGCTTCGACCAGCTGAAAG
CAAATATTACGACTTCAGGAGGAATAGAAGGATTGCAAGAATCAGATAAATGTGCGTTCACTTCCG
AATTTGTGTACTGCTACGTGTACGACACTTCATTGGAATGCCCAGATGAATTAAGAACATATGTCT
AAATGTAAACACCAAATTGTAAGTTCAACTATAATTAAATGGCCGAGGGTCTTGAAGTTATGCAGT
TTAGATTTTGATTTGTTAAATTGGTAACTGTTATAAATACAAATAAATCAAATATTTTTTGAATAGA
AAAA

>LsigOBP12_putative odorant binding protein_partial mRNA

CATTACGGTCAAGAAAAGAGATAATTATAACAGTTGCCAATTTAACAAATCCAAATCTAAACTGCA
TAACTTCAAGACTTTGCGCCATTTAATTGTAGTTAAAATTACGTCTTGGTGTTTACTTTGGGGGATA
CGTTCTTACTGCTTCTGGGCATTCCAATGCCTCGTCCATCAGGTAGCAGTTCACAAATTCCGAGTTG
AGCGTACATTTATCTGATTCTTGCAATCCTTCTATTCCTCCTGAAGCCGTAATCTTTGCTTTCATCTG
GTCGAAGCAACCATTTATAGAGTTTCCTAAAGCCGGATCCCAGGAAGAGTCATAACCGTCTTCCTT
CAATTTGGATAGGAACTTTGCAAGATCAACTATTTTATTTTCATCGAGAAGATTTAATCTCTCTAAA
AAGCAGGATTCTTGACAATCAAAGATTTTATACAAACGGTCATCATATTCACCGTCGGTCCATGAC
GATCTATTTGCACTCTTTAGTTCGTCTATTGAACGGTTTGTAATTTTTTCGCAAAAATCCCAGTCATC
ATCTGGGATGTCAGGATATGGTACTTCACACGTATAGATTATCGACATAGCAAGTTTTCGATGTCC
GTCCGTCTGTCTGCTCATTCGTCTGTTCTTCTGTCGTATGTGCAAAGGCTGCAAAGACAAATTTTAA
ATTCAATGTTTTTAGAGCATTTCTCAGAGGTATTTTTAATTGTTTTGATCAATCCCTCAAATAAGGA
TATAGTTCAGTGATGTTTGAAAATCGAAAATTGGTATTATCTTCTAAACGTCTCTAACGATTTTCGT
TTTTCATATTTCACTCATGGATGTGTATTTAATGACCTTACAATATACCATCAATCATTGCCTCGAA
AAAACAAAACAAAAATATATATGGGACGTTTCAC

>LsigOBP13_putative odorant binding protein_partial mRNA

TTTTACTCAAAAAAGTATATATTGCTTCGGTTACAAACATAATTCCAGACAAGAATACAGACGAAT
GAAATTAATGTAATCATGTGTGGACTTTAAACATCTCTTTTGTTTCCGTTAATCATTGAATAGTTTTA
CTTCTCCTCGTCTTCTCCAAATTCTTTAAAATGTTCTGGGCAATTCTTGTAAGTAGCAGACATGTAA
CAATACAGGAAGGTTGTACTTAACTGGCAGAGATCTGATACATGCATCGATTTCACCCCTCCAGTA
GCCGTGATGTTTGCATTAATGTCATCAACACAACTATCTACTGCGTCTTTCATAATAGGATCCCATG
ACTGATCATGGTCTTCCTCTTTCAGTAATGATAGAAACTTGTCAGGATCAATTACACGATTTGCATC
TAGCAGATCTACTCGCTCAAAATAACATGTTCGGTGACAATTAATAATTGTTGTATAGCGTAGTTCA
TAGTCTTGCATTTCCTCATCGTTCCAAGATTCATCTTCATCACCAAGTTCTTCTAATTTGCTCTCTGT
TTCATTCCTGCATGTCATCCAGTCAACATCCCTTGTTTTATAGAATGGTATACGGCATGCATATAAG
ACCTCTTCAGTATTTTCGGTGTCCTCTTCGTTCTTCAGAGTATAAATGTTACTTCCATCAGATTTGTA
GGGTATTATTCTTAATGACTGGGACCTGGCTTCTGAGTCGTCCCATAACTGTATAATATTTTCAGTT
GATGGCGAAGCACTCAAAACTGCCACAGTAAAAGGTATGAATATTAAAACTCCTCTCATATTGTAA
TGTCCTGTTCGAATGACCAACCACATTCACAAACAACGAGAGGAGACTTTAAACACGAGATTGTTA
GATG

>LsigOBP14_putative odorant binding protein_partial mRNA

GTAACTTTTACAAGTATTTAAAATCTATAACTCATCGACAGTTTTGACAGTAAAATGTTAGGACTGG
ATAATTAAAAACTTGTATTCGTTCAGATCCTTCGGTTATAATGTTGTACATTTAATAAATAAAAACA
TACAAAACATGTATCCTTTGGTTCAGGAATTAATTCATAATTTGCATAAACATCATGTTTCCGTTTT
CCTTCATTCAATATTTTACCATACATAATACTTCATTTCCATTGGACAGTTCTTGTGAAAAACCATTT
TATAACAAGAATATAATTTTGAAGATGCAAAGCACTTTTGCGATTGGTCCATGTTTTCGATTCCTCC
GAACTTGTTGATGATTTTGTCCAATTTCTTCACACATTCTTTGTTTGCATCGTCAAAAATTGGTTCCC
AAACTGGAGAGCATGAATTTTGTTTTAAATTCGCTACGACGTTTTCCGAATCAAGTATCCCTCTGTC
ATCATTCAGTCCTAGTTTCTCATAGTTGCAATTTTGTTCACAGTCGATAATTTTTCTAAAACTCTCTC
TCCATGATTTCTTTTCTTCTTTGGACCACGTATTATTGAAGAGACCGAGTTCCGTCTTTGCTTCTCTC
GACTCGTTGGCACATGCCTCCCACACCGTCATATTCATCCGGAGACGTGGCACATAACATACCATA
GGTTCATGAGTTATGTTGAACTTTTTATCTCCACGCATGAAATTCATCTGTTCGTGATTGCCGTGTG
CTTGATAAGGTCCTTCATGATGTACGGGATTTCCAGTAGTTGTTGGTGTAGTAGTTGTTGCACACAG
TGAAACTCCTAAAAAGAGGATCAATGATATGACTATTCCGGTAAGGTCCATGATCACGAATAAAG
AAAATAACGTGTTAAAGCACCACTATATGAAGAACAGCAGAATTGAATACGGG

>LsigOBP15_putative odorant binding protein_partial mRNA

CTTCTTTAATTTATATTTCTTCGTACTCTATAATTTATGTATTACATTTGTCCTTATTATATAAAAATA
AAATAGTAACGAATAACTAAAAAAATAATAATATCAGCATATCTTTTATGACTATACTTTTCTTTAA
CAATATTTATATTAGATACGCAATTAACTAAAACGTTTAAGCGTTATTATTTATTTCCTATTAGTTTT
ATACTTTGAACAAAAGACACATAAATATTGTTAATCTATTTTATTTTGATTTTACAAATGTCCGGTT
ACAAATATACTAGATTATATATCGCAACTCTAATTTTGGTGCTCTCACAAATGGACGTTTGTAGTTG
TTGTAGTTGTGCGTGTTGACATTTTTCTCCCTCCCCAGTTATTCAAAGATTATAAAATCTTGATTCCT

GTGGACATAACTGTCTTGCATTAACGATATAACAGTGATAGAATTTAACAGATAATGCGCACTTTG
TTCCCTGGTCCAGTGATTTTAAATCATCTCTCTTCTTCGTTATATTTTTCATTTGGTTCGCACATACTT
CCTTTACTTTTGGAAGAATCGATTGCCAAGCTGGAGAGCAGGAAGTATTCATCAGATGTGTAACGA
ATTTTTCAGGATCAATTATTCCGTCATCATCGAGCAGATTTTGTTTTTCGAAATAGCATGAACGATA
ACAATAACGAATTGAGTGTTGTTTCTTTTTCCATGCCTTCTTCTCTTCAACTGATGTGGAATCATCAA
ACCGTCCATGTTCTGACATTTTTTTGTTTTCTTCATCTAGACAATACAACCAATCTTCGTCGTTCAAG
CTGAGACGCGGAATATAACATTCAGTAGGTACCCGTTTTTTGTCATCCGCTTGTCGTTTGCTCCTGG
TTATGAAAATACTATGTTCATCTTCATCCTCTGTCAATATTTCCATTTGGTTGAAATCATCAGATAA
CTGATAACTGTCTACGTCATCTTCTGAGAATTCACCATTTACCGTTGCTATAATGATCAGTAAAACT
CCTATGACGACGTTGATTGATACGACTGCCTTCATCGCGTGTACGATAAGTAATGCTTTTGATGAAT
T

>LsigOBP16_putative odorant binding protein_partial mRNA
CCGATCTTAACAGATTTAAAAGTTTTATTTTTTATTTTGAAATTTAATTTACATACATAAAGCATTTA
CTTGACACAATATTCGAAACACTGGTTTCGTTACGATGGGAAACAGATTTCGGATTACAAATTTAT
GTTTTATCTTCTTGGGGAACAGCTACAGTGCATTTCCTTTCTCAGTATTTGTTAGAAGGTATAATAC
ATTAATTCCCTCGGGCATAGCTCAGTTGCTTCTTCAGTGTAAAACCAATGGAACGCATTAGTTATTG
AGCATTTGCTGGTCTCGTTCATTGACTCCAAACCTCCGTATTCCTCAATTTCTGAATTTAAGAAGTC
CGTACAACCTTTCTTTAATTCATCAAATTCTGGTTCCCAGCTTGGAGGGCAAAAATTGTTTTTCACG
CTTACAAAGAACTTTTCCACGTCAATTATTCCATTGTCCCCAATCAATCCCATCTGCTTGTAATAAC
ACGGATTAAAACATGTCTCCATGTCACTCTCGGCTTGTTTCTTCTCATTATCTGACATGGTTTCATCG
GCTTCACGAAGTTTTTTAATTTTCTCCTTCAAATGGTCAATGCAAAATTTCCAGGCTTCGTTATCAAT
CTTGATCTTAGGTATTTCACATTGATAGGGTCGCTCGTAATCCTCTTCCTTGTCATCTGTTTGTCGTT
TGACCATGAGAGAGTATTTAGCATCATGATCTTTGCCTGTCGCCATAAATTGTACTTCTCTGAAGTG
CTCCTTTAAAAAATGGGTTTCTTCATCATATACAGAGTATACGTCATATCGCACTGATGCTACACCC
AGAGGTATGGCTACTAATACTACGAAAGATGCAACTAGCTTCATGGTAACAAAAGTCACAAGTGG
TAAGCTCGTATGAAATTCACTTTTTTATACAAATATGATATCACAAAACATAATATCAAGTGTTCG

>LsigOBP17_putative odorant binding protein_partial mRNA
TCCAGAGCTATTAACAGGAATTTCAAGAAATATCTTATAGAATTTGAGGTGTTTAAAAATAAGGCA
CGACAACTTTCATCAGAATTTTAATTGTTACATTTGGTCACGTTCTACATAACAAAGTATGTCGAAA
CAACAAAAGTACACAACAAATACAATCGGTACATGAATTAAGATTTTTTTAATTTGTAGGGGAGGA
ATAATCTTCTTTTCAAATCTAATAACGGTAATATCGTTTCGATTTTTCCGGACAGCTCGCATCTAAA
CTAGACGAAGTAAAAGAAAACATGGTCGTGGTTATTCTGCATTTGTCGTGTTTGGTCATCTTGTCCA
CGCCTCCAAATTGGTTGATAACGTGTTTATAATATTCGTCATATTCTTGTTGCACTTCTTTCAGAACA
GGTTCCCAGGATGGATCACAATGATTCGGTTTCAATTTGGAAAATAATTTTGTCGAATCAAGTATTC
CGCTTTCACCAAACAGTCCCATTTTTTCTAACATGCAGCCATTGGCGCATTCCATAATTGAGTACCG
TTGCTGCTCCCATTTATTTCGTTCTTCTTTCGTCCAAGAATTATTCCTGGGTCCGAGCATTATAAGCG
CTCCCATCATATTTTCGTTACATATAGAAAATGCTTCTTTATCTAAAGGAAGGCGAGGTATGTTACA
TAGATAAGGTGTAGGTCTTTTAGTCTCTTCTTGATGTTCCATCTTTGCAGTGTGCATATCTTTTCCTT
CTGCCATTAACTGCGCTTGTCTTAGATTGTCTTGCATGTTTAACCCTATTACGTAATCGCCATTATAT
CCGAAACTTTCAGTTGCAATTACACACTGTAAAACCCCTATTAAGAGGACTATTTGCACAAGCATC
TTCATACTGTCC

>LsigOBP18_putative odorant binding protein_partial mRNA
GTTTGTGGCAGTTACACATTAGTGAGTGATCTTTAGTGAAGAAGTGAAGAGACAGACACCATGCAG
CCTCCGAAATGGATTCTTCTCTTGTCGGTATTTTTAGCGATGCAGTTGTATTTGGGTGAAGCAAAGA
AGACCGCTTCCAAAAAAGGAACAAAGGCTGTTCTAAAACACTGCATGGATGCATACAACGTAACA
TCAGATATATTTGAAAGACTTTGCATCCAGGCACCTGAAAGTAACGAAGGCAATACAAAGTGTCTA
ATGAACTGTGTACTCGGTGGATTGAACTTTTTGGATGATACAGGTTTGCTGGATATGAAAAAGGCC
TTCAAACATACCAGTAATGAGAAGAGGAAGATTTTCCGTGAAAAGTGCAATAACGCAGCGCAGGC
AGCCAGTTTAAGTACTGATGACGCCTGTGTCAGGACAAATAGACTTGTTGGATGTTTGATGGAAAA
AAATAAGAAGTTTTGTCGGGCCATCAAAGACTTTAAACAGGATGAGAAAGAAGAAGT

>LsigOBP19_putative odorant binding protein_partial mRNA
TGTCATCCGGCCTGTCGACAAGTTCCACAATGAAAACGTTTTTGCTTCTAGGAATATGCTATTTGAG
TTTGGTGTGCTTTGCACAGGGAAAACAGGACTGCACTTACCAGAGAGATGAAGAAAAAATGAAAC
TTATTCATAGCTGCCGAGCAGAACATGAGGTTAACAGAGAAATTGTTGTGAAACTCTTCAAATCAA
ATGGCACTATGCATTCTGATAAGAAGTGTTACTTTTCGTGCGTCATGCAAGACACTGGCATGGCTG
ATGAAGATGGCAATTTGGATGTTAGTTTGATTCGTGCCTATGCAAAACGGATGTACAAACAGGACC
ACGCTGTTGAAAAAGCCCTGGCAGCTATAGACAACTGTGAACCAATTCTTTCAGATCCAAATGAAA
CTGATGGATGCGAACGTGTTTTTCACTTCCTGACATGCATCAAAAGAATAATGGTCAAATACTGCT
ACGACATTGATCTACACGAAGAAGATGACGATGAATAGACTGAATATGCCTACCAGTTTCCAATAC
CATTTGTTGTTTCTAGTTTGTAATTGATTTATTAGTACAATATTAACTTCTGATAAGTAATTAAGCTC
TTTCACTCTAACAAAACAGTACTATGTTCTTAAAGGTATAGTTAGTACTCGTACATATATGTATATT

ATTAATAATGATTTCTTTGCGTATAAATAAATTGTATTTCTTATGTAGAAGTATTTCAGTGTGTTCAT
TAAATATGAATCACATTTTTTTAATTTAGAAATAATTTTGGATGTTTCGGGTTTGAATCTGCAATAC
GTGACATATCCCTAGCCAACAAAGAGCATATGCATTTCCACTACACCACTCCGTACTATTTACTAGT
ACGCAAAGTAATCATCATTAATATTATTATGTTTCGATATTTCTATGATCAATAAGACATATTGTTC
GCTTCGAAGAAGAGTGCTGCGAAACGAAATTTTTATTTGAGTTTTTTTTTTTACAAGAATAAGCCCC
AAATCATCTACATTTTTATAGGAAACAACAAATTTGAACTAATATTACAGTGTAATATAAGATGTA
TTTCTTTGGATCTGGTGGGGTTTGAACCCCACCAGATCCAAAATTATTCCAAATTATTTCTAAATTA
ATATATGTTTCATATTTAATGAACGTATTTAAAACTT

>LsigOBP20_putative odorant binding protein_partial mRNA
CGAGACCGGTACACGAGTCAAATAGATAAGATGACGACGATGAATCTTCGTGTTCTGCTTGTAATT
GGATTGGTGTCTATTGCATCAGCTAGTCACGATATTGGCAAATGTAAAAGGAAACCTGAGTCAGCC
GAGAAGTGGAAGAATGCAAAGATGGTGTGTGACGAGACCTATCCCTTTGAAGAGGGTGTTATCAA
AAAGATGATAGTTGGCGAAGAAGTAGATGACGAACAAGCCGAAATGGCAAAGTGTTTCGTGCATT
GCATTTTGGAAGAATACGAAGCTTTGAGTAAAGACGACCATCATGTCGATCTGGACGCCTTGAAGA
TGCACGCTGAAGAAGTATTCGAAGACGAGGAAGTGCAGGAAGAAATGAAGACAGTTATGGAAGA
ATGTAGTCACGTGACAGGTGATTACCAGGAAGACTGCGACAAATCGTTTGAATATGTCAGATGTTA
TCTTACTCAGATGCATGAGTACTGTGACGTCACTGCAGATGAATAAACAGCCAGCAATATTTGAAA
CAACCATCTTTTATCAAGTGCACATGTAATTAAATAATACCAATGCAATGTTTACACATTAATGTGT
TTGTTATCAAAAAATATATTAAATAAATATTTAAAAACTATTATAAATATATTTTATTGTGTCTATA
TATTAATAAAAC

>LsigOBP21_putative odorant binding protein_partial mRNA
AGAAAAGTGGGCTATTCTCGTGCAAAGAAGTCAACAAGATACGATCATCATGATGATGAAGACTG
TTTCCGCCCTTCTTCTAATTGGATTGGTGTCTTTGGTATCAGCTAGCCATGACATCGGGAAATGCAA
GAGGAAGGCCGCGGCTGCAGAAAAGTGGAAAGGCGCAAAGGACAATTGCAAACCATTGTTTCCGG
TTGAAAAAGGTACTTTTAAAAAGATGATTATTGGCGAGGAAGTGGATGCTGAAAAAGCTGAAATT
GCAAAGTGTTTCATCCACTGCGTCTTGGAAGAGTATGATGCGCTGGACAAAGGAGATCACCACGTT
AATCTGGAAAACTTGAAGATGCACGCCGAAGAAACATTCGTCGATAAAGAAGTACAAGAGGAAAT
GAAAAGCGTTATGGAGGAATGCAGTCATGTTACAGGAGACCACCAAGAAGATTGCGAGAAGTCTT
TCCAATACACCAGATGCTTCGTCGTCAAAATGCACGAGTACTGTGACGTCAATGCAGAAGATTAAG
CTGACACTTGGGGAGGAAGTGGTTAGCCTCAGACTCTCTGCAAAAGAGTTCAACGCGGCGTGTAAA
CAACAAAATTAAATTCATTTAACAAACTTTGTAATCAACGGTGTATGCTTGTTGACAAATTCTCAAA
ATAAAGATATTTAGTTGTAACACTTCTTTAAAAAAAAAAAAAAG

>LsigOBP22_putative odorant binding protein_partial mRNA
TTTTTTTATATTTTTTTACTTTTATTAGAGAATCGTATAACATTTTCGCACGTATGCTGCATGGGTAG
CTTAGCTGGTGTAATACACTGAAATTAAACTACAGTCTCTTCGGTATTAATATTCTTTATTTCTGAA
ATAGCTGGCATCTATACGAGATATTAATGCGTTCTTATCATCCAAATAGAACCATCGGATCTTCAAT
TTGATCACAGTACTCATTCATAACCACTAAGAAGCAGTTGGCAAATTCCTTAGCATAGCAACATGA
TTCCGTCTCGCCTTTGATTTTGTAACATTTTTCGGTTAGTTTTAAGAAGAAGTTTATGTTATCATCCT
TGCCTTTGTATGCCCTTTTAATGACAGTGAATACGTCCTCTTTATTTATGTCACCATTTTCAAGCAGC
ATTTCCCTGTCTCTGAAGTAGCAGTCACCAAAGCACATTTTATGAGGATCTGCATCGGCACAGGAT
ATTTCATTGGTGGAAAATAAAGCCGAGTCCTCCTGGGTGACGTTATGGTCCGTAGAGCATTTTAAC
ATTGTCCCTAAGCTGAGAATTTTTGTCTCCGAGAACTTGCATTTGTGTGGAGCCTCATCGTCATCGT
CGTAATCCTCCTCCTCACAAAAAACCGCCACTACAATCATAAACCAGATGCAATAGATTAGCTTCA
TCCTGACAGGTTTCCAGACACAGTATGCAACTAATTAAC

>LsigOBP23_putative odorant binding protein_partial mRNA
GTAGCAGTTGAACAATGTCAGGCAGGGAACGCACGTGGGGACTTTGGACGTCCGCTGCAATTGCG
ATGCGGAAGAGATGGTGCTATGTACACGTAGCTAAAGAGCAAGTCATCGCTGCGATACCGGAGTA
CGTGTACATCCAATGCCTCGCTAAGCCGCAACTCTGGCACCTCAATTGCGGCGGGCGTCCATTGTC
CGCATGTGTGCACAGGGACTTGTATTCACAAATTTGCATATACCATTTTACTTTCTCGCTACCATAG
TAGTAGGGAAAGTTTTGTCACTGCTAAAAAAATCGAATTCGACGGATTTGGGCTTTCATAGTAACT
TTTCACTTCTTTTTACTATCCGTAGGATCCTTTAAACCCTATGTATTTTTACATATAGCTATTTCATTT
TCTGAATGTCGCCACCTCAAAATACAACTATGTAAACTCCAAAACACATTTGGGTTTAGTGAAAAA
ATACGTTCGTAGGTTTCTTCTCTACAATATATTGGACAAAACTACTCATCTTTGCATCGTTTCCGTAT
GAGGAATTAAAAATCATACGCTTTTAAAAAAATGTTATTTGAAGAATCGTCTGCACATATAATTTA
TTCAACTATTTGTTTGTGTTACTTGCACTCTGGTGTACAGAATAAAATAAAAGTTTTTTTTTATTACT
TTCGAAAAATGCTCTGTTTATTGTTATTTTTTTTTAGCTTGTAACAGACAAATACAAATTATGCAATT
AAAAGTAAATCCTATGGTAATCTTCATCATAAACTCAGAGGGTGATAAAGGGAAAACTATTGCGTC
TCTGAGTGGCGCTAAACTTTGCAAAATATAGTTTGAGAACCTAAACACTAACAATCTACCCCAAAA
ATCACTTCAACAGTGCTTTGCTTAAAATTAATACGTAAATTTATTCTTATTTTTATTCAGTGATTTAA
AAAAGTGACTATAACCATTAGTAAAGGTCACAGGATTCTGGGAATGGGTCAATATGATTACACAAC
CTTACACCACAAAGATGTGAATTGCAGAAATTAGTTAATATTAAAAACACATATGACTGTAGTTCT

GCAAACACAAATAAAAACACACCTTTCCATTAAAAGTAATAATTGTATTATAACACTGTGAGATAC
AAATTAAAAATCCACTTAAGAAATTTCTCAATGTTTCAAAAATATATTTAAAACTGTGCATAAAGG
TGTGATAGCTACTTATAAAAATATATTACTGCTACTCCTTTAAAACCAAATCTTCGTTGACTTAGGG
TATTTTATATCTGTCTCTAATATCATCTCTGATAGCCAAGGGGAGAGGTCCGCATTTCGTTCGTATC
CTCTTTTTATAACAGTTCATAAATTCAGCTGCGTAATCGCATTCTTGTTCTTCATCTTTTTGTGTCGT
TTCAAGGCAGTCATCAGCGGCAATATCAGCTTCTTCTTTTAATTCTTCGTCCTTAAAATCCTTCACG
ATATAATTCTTGAATGGCACTATTTTAATTTCAAGGTCGTCAGTTAACCATCCATTTGACTTCATGA
AGCATTGATAGAAGCATTTGTATTGCTCAATCTTATATGGTTCAAGAACGGCCATCTCTAGTTCCGC
TGCTGATAGTTTCTTATTTTTTGCACATTCTAATTCTTTAGTAATTTCATCATCTCGTAAAAATGATC
CATTACAACGTTTCTCCGTCTGTGCTAAATCTGGCTTCTCAGTAGTAGTAGTCATGGACTTTGCCGT
AGTAGTTGTTTGCGTCAACACAGCGAGTGGCAACGCCAACAGCAAAACGACAATCCCCATTTTCAA
ATACATTTTGGAATCTCAAAATAATTGAACCTCAGATTTTGTGTGAACTTCGATGACGACAGCAGTT
TGTTATTTACTTCGAAGAAAAATG

>LsigOBP24_putative odorant binding protein_partial mRNA
GTGTGGTTTGCTTGCCTTTTTTTTTTACCACTTTTGTGACTTTAAAAATGTACAAATTAATGAAAACA
CCTTTTCGAAATTAAAATCAGGCCATTTATTCGTGCATTTTGAGTTACAAAATAGTTTCTAATAAAG
TCCATTTTTTAAACAATTTTCTTGAGATAAAGTAAAATTAGGTTGACCAATTACCTAAACTTCTAAT
ATACATGGCGTATATTCATTTCCATTTCTGCTCACTTGTTGACTTTTAAGGTGTCTTTCCATTTCTAC
TGTATTCTCTACAATATGGTTTTAACTTATATTGGAAACACTCGTTGAAGTGAGTTGCTATATAACA
TACTTCTTCGCCTTCTGTGGGTGTGATTTCCACGCATTTGTCCACTAATTCAGCAGCGTCTTCTTTCA
TTTCTTCACTAACAAATTCTGATTTCACAAATTTCTTGAAATCCGCTTTACGAATTTCACCGTCGTCA
GTTAACCATCCATTTGATTTCATATAACATGCATAGATGCAGGGGTAACGGTAAATATCTTTTGGTC
CATCATCACCTGCCTCCAGTTGGCCAGTTTTTGTTGCCAATTTTTTGGCACATTCTTTTTCTTTTTTAA
GTTCTTCCTCTTTAACAAACTTTCCATTACAGCGATTCTTATCTTCTCCCATTAACACAGCAAGTGGC
ATCGTGAACAGCAGAACCACAATTTCCATTTTCCGTAACATTTGATTCTCGGAATAATTGAACCTCA
GTTTTAGTCTGGATATTGATGTCGACAACAGTTTGATATTTACTTCAAAGAAAT

>LsigOBP25_putative odorant binding protein_partial mRNA
GTTTTAAAAAATTATGAATCTACAGAAAATATACATTTTCAATTTTAGATTAAAAAAAATTATTCAG
GAAGTGTAAATTCTTTGATATTTGGGTGATTTAAAAGATAGTCCTTGGCTTCTTGCGGTAGCGGTCC
ACATGTTTTTCTCATCTTCCTACGAAAACATCGGATAAATACGTTTGCGTGATCACAAATTGTTTCT
TCTTCATAATCAAAGGTTGATTCCTTACAGTCGTCAGATGTCTCAAGCATAATATCCTCTATGGTTT
GATCTTGAAATTCTGCCTTAATAAATTTCTTAAAAGGTTCTAAGTGGATTTCTCCCTTGTCGTCCATC
CATCCATGAGCTTTCATGTAGCATTGATAGATGCATCCGTCACCCGATTGTATAGTTCGAGTACCTT
TATCAACCAGTCCTGCTTTTATTTTAAGCTGTATGGCACACTTTGCGTACGGCCTCCGCTCATCAAA
AGTAAGGATGTCATTGTTACATCGGAATTCATTTTCTCCCGGATAATGCTCCTCAGCAAAAACGAC
GAAAATTATCATCAAGAGTATGAAAGTTGGCCTCATT

>LsigOBP26_putative odorant binding protein_partial mRNA
CTGATGATGCCTTTCGTTGTGTTCATGGAGTCAACTACTGATTCCGGTGAAGCGGGTACAAAAGAA
AAACGTTGTATCGAATTGCTTTTCACCGATGAGGAGCTTAAAAGGGAAGTTGAATGTGCAAAGAAA
CTCAAAATCTCAACAGGTGCGTTGGCTGAGGATATAGAGTCTATTGGTGACCATTTAAGGAGGGAA
TCGTGTCTCTTCAACTGCGTCTTAAAGAATAGTGGTTGGTTAGGTGAAGATGGGATGGCCCGTCGT
AAACCGATCAAGGATTTTATTGAAAAAGAATATTCCAACCAAGATATAATACAACAATCACTCGAA
GGTTTAGATGATTGTCTTGAAACACAGGAAATTGATGAGCAAGATGATCAATGCGAATACTCGTTA
CAGTTTTACAGATGTCTTGGAAATAAAATAAGAAAAGTGTGCGGACGCATTCCTGATTATAAAAAA
GAC

>LsigOBP27_putative odorant binding protein_partial mRNA
CGGAGGACGAAAAAGAAGGATGGGGTAAATGTGAACCGGAGATCCATTAGTTGAATAGTAGCATC
ATGAAGATCTATGCAATTATTTTCATCGCCTTTGCGGTCTCGGCGTTAGCCATGAAGGAGGACGAA
AAAGAAGGTTGGGGTAAATGTGAACCGGACGACATAGAAGTGGTCCAAATGAAGATGGGAATGA
AATGTGCAAAGGAGAACGGCCTTGACAAGACGGACATTGAAAATTACTTTAAAGATCCTAACCAC
ATGTTTGAAGGTCCTAAAAATTGTGTGTACAAATGCTACATGACCAATCAAAAATGGTTAACTGAA
ACTGACGACCTCAAGAAGGAAGATATTCACAAGTGGATCGAAAAAGCTTACTCCGAAGAAGAATC
CAGAGACAGTGGCAGAAAGATTGTAAACCATTGTTTCGATAGTGTTCCGAGAGGAACAAAGGAAG
CATGTGCCTACCACGCAGAATATGTCAAGTGTATTAGAAATATTCTGAGAAGTAAATGCGGTGAAA
TGCCTCAAAAATACAAAGACATGGTCATCAAGAAATATGATGAAGAATAAGCTGCCATCTAGTTCA
GGATCGAGAACTACATTATATGTAATTTTTGGTAATACTATTGAAATTTAATAAAAAACAATAATT
GCATTATGGAAAAAAAAAAAAAAAAAACTGTCAAAGAAAAAAAGAAACAAG

>LsigOBP28_putative odorant binding protein_partial mRNA
CGGTGATGATGAAGAAGAGGAATTCAAATGTGAGCACGGAAGAATTAAAAATCTGGTTCAGAAGC
GTCATTCGATAATAATCCAGCATCATGAAGACCTTTACTATCATCTGTGTTACAGCTCTCATATTTT
TTGACTTTGCCAGGGCCGGTGATGATGAAGAAGAGGAATTCAAATGTGAGCCTAACGATTTCGATC

AAGTGGAGCTGCCTAAGGGAATAAAATGTGCAAAGGAAAATGACGTCACGTCAGAGGAGCTTGAT
AAAAACATGGACGATGACGAAGAAAACCCATTTCAAGGCAAGAAAAAGTGTGTCTACAGATGTTA
CATGGGCAACAAGGGATGGCTTACAACCACTCATGATGTTATTATTCAAAATGTGAAGGAATTCGT
CACCCAAGCTTACAAAGATGAAATTAGAGATCAAGCTTTGGAAGTTGTTGAAGACTGCGCCGAAAT
GACACCCCGAGAAGAAGAGGAAGAATGCGACTTCCATTCTGAATTTGGAAGATGTATTAGACGAA
AAATGAGACCAATCTGTGGACCTCTTCCAGCAGAATACAAGAAGCAGCTGATGGAGAAATACAAA
GATTAAGGATTATGTTATCAGCCTGTCCAGAAGTTTTTCCTGCAAGATTTGTTCATGCTTTCTCAAC
AATAAATACTCTCTGAAAATGACTATATTTTTGTGTACTGAAACATCACATTATTTTTAGTTTTATTC
TTCAAAACAATTGATACATTACACTTAATTCTTTTTCGTAACTGTATGGCACATGTGTAATGACTTA
TAATTCATGGTGCACAATTAAAATTATCATTGGAAGTTGTATTCATTTTTCATGAGCAAATAAAAAC
AAAGTAGCAATAGTAAATCACTATTTTTTCCAAACTATTGTGCAACGCACTATCTAGTTGTGCCCAG
CTAAGTACGGATGCTGTGGCAGTGTTGGAAAATGCTTTTATGTGAGAAGGAGAAACAAAATTAAAT
ACGATTCACTTTCAGTAAGTACAGATTCTAATTTTAAATGTGATGTAAAAACTTTACATAAGAAACT
CTTATAACCACATGTTGAAGGTTAAAAGTCTAGATTTGATTAAAATGAGGACACACCACTCAAAAT
TAGTAATTGACAAAAAATATGAGAGGCCAATGAATAAAATTAATTTGAAATTTAAAAAAAA

>LsigOBP29_putative odorant binding protein_partial mRNA
GCGCCAAGCATCAAGATGGTGCATATACCTATTGGATTTATCGAGTGATACTCAAAGACATCTCAA
TGTATCCAATCAACAGAATATTCTTTCTGACAGCACTTGCTTGTCTGTTGGCGACAAGTATTGCGAT
GCCAGAAAAGCGCCAAGCATCAAGATGTTTTCCTAAAAGTATGGAGATTCCAATAAAACACATTAC
AGAATGCGCTTCTCAATTTGGAGTCACACACGCGGATATTGGAGCATTTATGGGAAGAGGCCAACT
TGCAGAAGATAAAAAGTGCATCATTAAATGTGTTTTGGACAAACATGGAGTGATCTCAGAAGATA
ATATGGTGGACGAGAATAAAGCCGTTGAAATGGCGAACACATTATTTGCAGACCGCACGGAGACT
GTTGAAATGTACAAGAAAGCAATGATGAAATGTTACAATCCTAAAGACTTTTCTGGTGATTGCGAA
GCCTTTTTCACGTTGCAAAACTGCATTTACGATGATATGAAACAGTATTGTGGATTTCCTGCTACTG
GCTAAGATGACGACAGTTGAACGTACAAGTGGTTCATATATTGAAGATATTATCCAGCGTATTCAC
ATTGTAAAAAAATAAATACTTATTGTAACATAATAACAAATCATTTCGTTTTTAAATGTTTAATGAA
AAACAAATTGTACTTAAACAACAAAATTTGTGTAAATGTTCTAAATTATTACCTATTTGAATTTTAC
TGTACTTCAAAGATGAAAATAAAAATGGTTGAAC

>LsigOBP30_putative odorant binding protein_partial mRNA
ATGAAAATTACATTCATTATCTTGGCTGTTCTTGTTACAGTGTCGTTAGCAGAAAAGAAAAAAGAA
GGCGGCAAGAACTGGAAATGCAAAGGAAAGCCTATGGAAGGAATGATGACGAAAGGCATGGAAT
GTGCTCAAGAACATGGAGTCTCGCAAGATGACACAGATGCCCTCATGGGTGACGGTGAAGGCTTG
GGCGAGAAGAAGTGCATTGTCAAGTGCATGTTCAAGAAGTTTGGTGTGGTGACAGACGATGACAA
ATTGGATGAAGATAAAATATTGGACATGACCAGCAAAACCTACGGACATAAAGAAGAAGTCGAAG
AATTAGCAACTGATGCTTTTTACACGTGCTACAAAGAGGATGAATGGAGTGGCGATTGTGACGCAT
TCTTCAACTATAAAAATTGTTTGAGGAAAGAAATGGATGACTACTGCGGACCACCACCAAAAGATG
AAATGATGGGGTAAATGG

>LsigOBP31_putative odorant binding protein_partial mRNA
TCTCTATGGGCAGTCGTTTACCTGTCGTAAATTGGAGTACAGCAGTCTCTACATTACATCTCACCAA
AATGAAAACTACATTATTTCTATTGGCCGTCATTTTGGCTTTGGCTGTCGCAAATGAAGATATGAAG
TGTAAAGGAAAGAAAATGAATGAAGGATATTCCGAAATGAAAGCATGTGCTGAGGAATACAAAAT
GGAAAAAGAGGACTTCAAGGCATTCAAAGGAGAAGAAGGTTTAGCCGCTGAAAAACATTGTTTCT
TCCAATGCTTGTACGAAAAGCAAAACTTGTTGAATGGTGATAAAACGCTTAACGAAGACAAGGTG
ATGGAGCAAACACAAAAAATGTTCCCTGACAACCCAGACACTGTAGCTGCCATGAAAACAGCAGT
TGGAAAATGCAATCAGAAAGCAGTTGATTGCACCGGCTTCTACAACTTCCAAATGTGTATCGGAAA
AGAAATGAAAGAGTACTGTGGCAAGGAATAATGGAAAAAAACAGCACACAAGAGAACGGCATGT
GATTCAATGTTGGCGATGATACATTGTTACAAAAATATAAATTAAAAATAATTATAAATTTAATTC
CTACTTTTGTAATTGTTACATCTTGAGATGTGTTAAGTAAACCAGAATAAATTATTTAAACACACAA
AAAAAAAAAAAAAAAAAAAAAAAAA

>LsigOBP32_putative odorant binding protein_partial mRNA
GTTGGAACACGCTGCTAACCGACGCAGTCGTGAAGTTTCAATAACAACCACAAGATGGCAGTTACG
TTCTATCTTATACTGACGGTAATAATTGCCGGGGCTATCGCTGAAAAACTGGACTGTGATCAAGCA
AATGAAGACGATGATTCAAAATTCGAAAAATGTGCAATGGAATACAAACTGGACGATAACGACCA
AATGGCTCTGTTTATGGGAAAACCCTTACCAGCAGATAAAGAGTGCTTCTGCAAATGCGCCACTGA
AAACCAAGGAATACTCGGTGCTGATGGAAAGATCGATGACGCCAAGGTGAATGAAGATTTTAACA
AGTCGTATGAAGGAAAACCGGAAGTTATAGAGCGATTCATGGCTACACACAAAAAATGTTACAAT
TCTTCAGAGTTTACCGGAAGTTGCAAAGAGCATTGCGCTTATCTTGGATGCTCTATAACAGATATCC
AAACGTACTGCAAGAGGGATTGACCTTATTATGGAGATGGATTGACCTTATTATGGAGATGGATTG
ACCTTATTATGGAGATGGAACTATAATTAATAACAATTAAATAAACCTTTTTTGTTATTTTGTAATT
CGTTGTGCAAAAAAAGTGGCCCAATAAAGATTTAACCATT

>LsigOBP33_putative odorant binding protein_partial mRNA

AAAATAAGATACGACGTCACTGACAGTCGTGTGGTGTCAACAACAATCACAAGATGGCAGTGACG
TCGTATCTTATTTTGTCGATATTAATTGCCAGGGCTGTCGCTGAAAAACTGGATTGCAGTTCAAAGG
ATGCGGGTAGTAATAACGCACATTTCGATGCATGTTCATCGGAATATGAACTAACCCAAGATGACA
TCGTAGGTGTTTATTATGGAAAACCCTTACCAGAACAGAAACAGTGCTTCTTCAAGTGTGCGTTTG
AAAAGCAAGGAATGATTAGACCTGATGGAATGCTTAATGAAACCATGGTGTATGAAGACATTAAT
AACTCACATTCTGACAAACCAGAAGTTAAGAAGATTAGCGAGGATGCACACAAAACGTGTTACAA
ATCTACAGAGTTTACTGGTAGTTGCCCAGATTATTCCGCTTACACCATGTGTGGTATGAAATATATG
AAAAAGTACTGCAAGTAATGCCGATTTGATTGTGAAGAAAATAAATATAATTTTAAATAAATTAAT
GAAATTATTAGACTGACGCATTTGTAAATCCATTGTTCCAAATATAGTGACTCAATAAAAAAAATA
TTCATTGAAAATATGTTTTACTAATAGTTACTAAACCGTATTCTTTAAGT
>LsigOBP34_putative odorant binding protein_partial mRNA
TTACAGTCGAATACGCTGTCTGTCGTTGAACCTACTTTACTGTAACAACCTCACCACATCTCACCAA
CATGAAACTGACTCTCGCTTTATTATCTGTTATTCTTGCCTTGGCTGCCGCTGGCGAACGAGGCGAG
TGCAAGTGTAAGACGAAACTAACAAAGGAGCAAAAGGACATAATGATGGAATGCGCGAAGGAAA
GTGGCATTACAAAAGATGATAAGAAAACGTGGTACGAAGGAGGCGATCTAGAAGAAAAGAAACA
GTGCGTTGCAAAATGTATTTATGATAAAAATGGAATGATTACCGCAGATGGCAAAATTGATAAACC
GAAGGTGTATGAATCAGCGGACAAAGTGTTTGCAGAAAAGCCAGAAGCTGCAACTGCTTTCAAAG
ATCAATTTGAGAAGAGCTATACTGAAGCATTTACCAACTCTTGCGAAGATCACTACAAAATCATGA
AGGACGTTGTTGCAGCTCTGCAAAAGGCGTGTTGAGGTCCAGTTCAGTATAACGGATAACAATTTT
TGCATTGCAAAAGTTGTTGGAAACTTCTTAATTTTCAAATAAGTATTTCACTATAGCAAGGTATACA
TTGTAAACGTTTCTTGATTTTTCGTCACATCATCAGTACATATGACTGCATATGGCTGATGATATGA
TC
>LsigOBP35_putative odorant binding protein_partial mRNA
TCTGAAGGCTTCAAAGAAGATTCGAAGAGTTGTGCAGAAAAATATGGTATCACAAAAGACGACGC
CCATGCCATGAGGAAGGGAACCGTGGAAGGTGAAAAGCAGTGTTTCTATGACTGCTTCGCAAAGG
CAAGAGGATGGGCCGACGATAATGGCAAACTTGACGAAAGTAAGATCAATGAATCCATAATCGAA
CTCTCTGAAGGAGACGCCTACACAAAGCAATCTCTGGAAAAGTCGTTAGAAGATTGCAAAGCAAA
AACCGAATGGAGCAATGACTGTTCAAAATCTCTGGAGATAAGTAACTGTGTGGTGAGAACATTTAT
GAAGCTATGCAAAAAGGACGACGAATGAAAACATCACGGAAACACAAATCAGAAAGAAAATATT
CACGCAAACTGAATATACTCTTTTTAAATTGTGCACTTATTTAGAACTATTTTCACGTAAATATATG
TGGACTACTAAATTAAAGCTATGTAAAATGATAATAAAAACATTACGAACATAA
>LsigOBP36_putative odorant binding protein_partial mRNA
TTTTTTTTTTTTTTTATTTTCACATGTCTTTATTTTGTATTTCGACAGTCTGTACAAATATTTACGAGA
AATTTTACACAATCAGTGCACATGATTGTGTAAAATTGTTTTTAGCTTGAGATGTTTTAGCTTGAGT
ACTAGTCGTTTTCCAGATAATTCATTATTCTCCCTTGCATTTCTCAAATATTCCCTTCATTTCACAGT
TGTTGAACTTGTAGTATGATGCGCAGTCACCTGGAAAAGAGCCAGCGTCATAGCAATTGATGAAGA
CCTCTGACATGATTGTCAGGGTGTTTTCGTCACCTGCTGCCATCTCAGCTATGGCCGCCTTCTTTTTG
CCTTCATCAATTTTCTCATCGGGTGTCATCATGTCTTTGTACAAACATTTGAAGAAACACTGCTTAT
CTTCAGACAGAGTGCCGCTCTTCATGGCCTTGAAATCGTCTTTCGTCATTCCAGTTTCTTGTGCGCA
TGTTGCCATCTTTTCTTTTGTGCCCTCTGACTTTTGTCCCTTGCATTTCCAATTTCCGCTAGGGTGAG
CAAAAGCCGTGGCAATTACTACAGCGAATAAAACTATTGCGGTTTTCATGTTGGAAGTTGATTTTG
TTGATGTAGCTAAACGATCTAGTTAACGACAGATTACAGACTGTTTTATTCGCTGTAGTAATTGCCA
CGGC
>LsigOBP37_putative odorant binding protein_partial mRNA
TACGTGACTTGCGACCCATCACTAATACTAGGTAATGGTTATGAAGAAATATATATGTATTAATTA
ACAATTTTACGAATTTTTATTGTAATTGTTATATCAATGAAACACAAATCAATATTCAATGTTGAAA
TTTCAAAAGTAGTCACTTTCATTCGCCTTCACAAAACTCCTTTAATTCCATTGCAACATTGCGATAC
AGATTGTAATATGGTGTACATTTGCCATTAAAATCAGAACTTTTATAAAATTTGTTGATTGCGTCTC
TGCAAAAGTTTTCGGTTTCCGCATCTTCGACTAACACCTCATGGACCCAGTTCAATAATTTCTGTAT
ATCAAGGGTTGCGTTATCTGAAGCTAGCCAATTCAACCCTTTAGCGATGCATTTAACAACGCACGC
CTTCTGCTCCGGTATATCTCCAAACTCACCTACAATCTTGGAATCACTTTCCGTAAAATCAAAAGCT
GCATTACAACCGCGTATCATCTTGATGTCTTCTTTTGATAATTCTCGTCCATCACATCTACTATGTAC
TTCAGTGACAGTAGTCCCGATAATAACAACAATTGCAAGTAAGTAAGGTCTCATGATGGACAAGA
GTGTTAAACCCAGTGTACACTTGAATCACCAGCA
>LsigOBP38_putative odorant binding protein_partial mRNA
AAGATACACGTATCTTTGGTCGTTTTACTATGGGTTTCACAGTATGTAATGGCAACTAAAACTTTCA
ACTCTCATGATGATTATGAAAAAGGGTTACATATAAAAGGCAATCTCAGACAACTCATGGCAGAA
GGCAAAGCTGTGCACACCAACGCTTTCAGTGAAGATCAGGAAGAGCTTGAGAAGCATCGTTCCAA
GACACCCTTTCGATGTGACTTACCTCGCCTCGAGGTAAATGAAGAAGCTTATATGGAGTGTGACAA
AGAAACACAGGCACAGATGGAAGAATTCGATGCTTATAACTCTTCATGGTCGAGGCAAGAACGTC
GTGATTGGAGGAAGAAAATCAATGTAATTGAAGATTGCAACGTTGTATGCTACTTCGGAAAACTAA

AATTGCTTGATGGAAGCGGAATACTTGATTCGACTAAACTCTTTCCTAAATTGAAACAAAACTCTT
GCAATCCAGCCTGGGAACCTGTTCTGGAAGAAGTTAGACAATCATACGACAAAAATTTAATCGACT
TTAAGAAAGACGTTGGTGAAAATTACAAGTGGAGCGACCCTGATAACTGTCGCCTAATTAAGAACT
TTTATTCTTGGACAGTGGAGACTTTATACAGGAAATGCCCAAAGCAATTGAAACAACATATCTATT
AAAAGTATTGGAGAGAA
>LsigOBP39_putative odorant binding protein_partial mRNA
GTTGGATATTGAGCATTTTGTCGTCCCGTTCATCGCCTCCAAACCCCCTTGCTCCTCGATTTTAGTAT
TTACGAATATCGTAGAGTCTTCTTTGAGTGTTGCAAGAACAGGATCCCAATTTGGATCGCAAAAAT
TGGTTTTCACACTTGTGAAGTATTTTTCAATATCAACTATCCCATTTTCTCCCATAATTCCTGTCTGC
TCGAAATAGCATGGTAGAAAACATTGATTGATTTCCCATCTGGCCTGGCGTAACTGTATCCTGGCTT
CCTTTTTTCTAGCCCCAGTAATCCCACTCTCTGGTTTAGGAATTTCTTTTAACTTTTGTTCTGTTTCAT
CTTCACAAAATATCCAGGCTTCCTTGTTTAATTTTGGACGAGGTGAAACACAGGTATAGGTTACCTT
AAACACGTCTTCACCGTCCTCTGGTTCATTTTGGGACTTGAGTGTGTACATTCCACTACTCCTTTCGC
CACCGTGAGAAAGATGGCTTTGTCCATTTTTCAAAGGATTTTCACCTATTTCTGTTGATTCCACGCC
CAGTAAAACCACTACTACGACGATGAAAATGTTGACTACCCTCATGGCTGATCTAG
>LsigOBP40_putative odorant binding protein_partial mRNA
TTTCATTATACTAAATACAGTATATTCCAAATTTTATTTCAACGACGTACTCAAATCAAGAGTTTAC
AGGATAATTAGCAGCTATGGCATGGATCAATTTAATTCCAACATTTAGCTGAATTTTTTCAGTAGCT
CTTCCTTGTACTTTTCCGGCAAAGGNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNAC
AATAATCAATCCAGCCTCCATAACTTCCAAATTGGATCCGTACGCTGTTTTAATGAATTGCTTTACA
TTTTGAATGATTATGTCTTGTCCACCTTGCAGCCAACCCTTGTCCTTCATGTAGCNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNAAGGTCAT
CCTTTGTAATTCCAGCTTGCTGGGCGCAGTTGATTGCCTTGTAAACTTCCACACTTGAAAAGTCGTT
CGGTTTACATTTGTAGTCTTCGTCGTCATCATCCGCCATTTCAGCGCCTGGCATTCCACCACCCGGC
GTACC
>TdomOBP1_Assembly of contig 4374, 2770, 4782_putative odorant binding protein_partial mRNA
AAAGCTCCAGTCTTCAATTCAGTCAGTGTGTCTGCAACCGGTTCACTATGAAGTCACTCCTTGTGGT
GCTCTCCGTTCTCATAGCGCTCGCATCGGCTTCACCTACCGCCAAGAATAAACGATGCCGAGATAC
ACCAACAGCCTCAAAAAATATACAGAAAGTAATCAACGAATGTCAAGATGAAATCAAATTGGCCA
TTTTACAAGAGGCCCTGCAAAGTATCCAGGAAGCTGTGAGGTCTACGAGGACGCGAAGAGATTTAT
TCAACGAYGAAGAGAAGAAAATTGCYGGGTGTYTGCTGCARTGTGTATACAGAAAAGTTCAAGCT
GTGGACTCTTACGGMTATCCTGAACGAAATGGCCTTGTGAGGTTGTACACAGATGGMGTYCAGGA
CAGCGAGTATTAYGAAGCCACTGCCCAAGCTGTTTATTCGTGTTTGTCAAAAACTCAACAAAATTT
GATAGGRCGGCAAAAYGAACCAGCGTTAGCATGCCATGTRTCATATGATGTTTTCGAATGCG
>TdomOBP2_putative odorant binding protein_partial mRNA
TGCAGTCTACCCATTGCTGCTGGGTCTGAGATATTTGCGACCGCTGTAAGTTGTTCTGCTGTTGCTG
GATCTGCGTTTTTCATCCATAGTCTCTCCCCAATCCTCGCAAGTGTTGCTTCCTTTCTCTATTAAGCA
AATAGCCAGTTTCTTCGCATATTGACACTTCCTGTTGTCAGTCTCGTTATTATTGTCCAGGGATTCA
AAACATTCGTCCACAGAATCTTCAATAAACTGTCTGACTTCTCTGTCACTGATTCCTTGCAGCATTA
CACGGGAGACCACAGATTTTTCTGGCATTCCACTGTCATCTAACACTTGCATATGTCGAAAAACAC
AGTGGATGATACACGGTGGAACATTCTGAACAGCCGCTGATCCAGATGACAATGATGAGGACGAC
GATGAAGAAGAATATTCATAGCGTGTTCTTTTCATACGATTTC
>TdomOBP3_putative odorant binding protein_partial mRNA
GTTGGACATAATTTTGAAATAAAGTTTATTTATTACAATACATACATACATATAATAGATAATAATT
AGATAATAACATTAAGAATGAAGTGATGCATTGTATTTTTGATGGATAATAAACTTATCAAAACAA
ACTTGAAGCGTATGATGAAATGCGGGTGCATTATAATGTTTTTTATAGATGAAGGCGTCGGTTTTGT
CCTCTGTATTTTACTGGTGTCCTCTGCAAATCTTCTTCAATTCTTCCTTCTTGCAAGCATGAAGAGCA
TTGGTATATTCGCAGCAAGAAAGCTCTCCTTTCTGGACCTTTGCGAAGCAGTCGTCAGTGATCTTCA
ACATGGTTGGAAGAAGGTCCTTGTTTCCAACTTGAGCTTCCTGCATCTTCTTGTATTTTTCTTTGACG
GGGCAGCCTTCTTCATCCAAAGCTTTTGCCTTTTCAAGAACACATTGTGGGACACATTGTGCATTGG
AAGCTCTGCAAGCAGCTACAAGTTCTTTGGCTTGCCGAGAGAGGTCATATTCACATCCAGTTTCGG
TGGGACCAGCAAGAGCTGCTACCAAGAGAGTTGCCAATACAACAAAGACTTTCATGTTGAAGAGG
ATTGTCGTAAGTGACTATT
>TdomOBP4_putative odorant binding protein_partial mRNA
GCTAGGTCTGCTTTTCTTCTCCATCCGACCAGCTAACGCTCGTATTTGCAAATATCATTTAATTTCTT
TTTCTTGCAAAGCCATAGATTTGTAGCGTAGTCACAGCAATCACTGTCACCTTGTGGAGTAGAACT
AAAGCACTGTTTCGTAATTTCAATCATTTTGGGTCGTAACTCTTCATTGCCAACTTCCTTTTCTAGCA
TCTTCTCGTAAAGATCTTGTTCAGGACAGTCATCATCGTTTAGCGCTTGCAATTCTTTCATCACGCA
ATGCACCAAACATTTTGCATTATTTTTGTTACCACATTTGTTGATTATATTCT
>TdomOBP5_putative odorant binding protein_partial mRNA

AATCTTTATTGATCTATATTTAATTTACAGAATGCCACTAAAATATCCAGAAAAATGTTAAGCTTGT
AAATAATTAGTTCTGAAAGTTTTGTTCTTGTTGTACTCCTTCGGCTTCTTTCTCCTCTTTGTACTCGA
TGCATTTTTGATCTTCATAAGGAGTTCTGTACTGCTCTGGGCAGTTCTCTTGAAGTCTTTGTCCAATA
CAATCCAATAGCAAAAATGAAATGCTACACTTGTCTATAGCGTCATCAGGTTGATCTTCGCCATTCC
AGCCACTCTCAATTTCTGTGAAGCAACTATCAATAGAATTTTCAACAGCTGTTTTCCATGCTTCCAC
ATTGCTTAAAGATTTCATGACGAAATTAAATACCTCGGACTTGTTTGGAAGACCATTATCATCTACC
ACCTCATTTCTTTCTAGGAAGCAATGTTCGAAACACAAGGAACCAATTTCATAATCCGAGGATTTG
TTATTCAACACTCGATATGTGTAATTCTTGTTACTTCTATGAACTGGAATTTGGTAGCCCTCCTCGG
GCCATGTCATATTTTCTGCTGACATATTCACACATTCGAACATTTTCTCGTAAGTTTCTCGTCCAGGT
GTTGGCATGAAACAGCAAAACTCTATTATAGAAATATCTTCGGAATCATCCGGCCCTCTACTATTTT
CACTAATATTTTTTTCACTTCTTTTCACTCTGACAGGTATTATTTCATATGAATCTTCATCCATATTCT
GTTCTCTCATACGTTCATAGCCTTCAAGATCATCCCGCAGGCTTAAAAGTCCTCCTAACAAACCATA
GCATAACGTAATTGCTGCTGCAGCTACTAGAACGACTGCACAAATTTTCTCCATATTTGAAAAAGA
ATATTATTATATTCAAAAGACTACAACTAAAAATATTTTATAGTTTTTCAACTGTGGATATTATAAT
GTTGCAACCACACCAACGACAAACTTCAAACATCGGAAGATGCCAT

>TdomOBP6_putative odorant binding protein_partial mRNA
CCTCGAACGTTGCCAGTATAGAAAATCATGTTAGTTACATGTATAGGATATTACTTTCTGCTGCATT
TAACACTGGTTTCTGGTGTCAGTGTTCATCTATATCAAGGAGAAGGAGTGGTATTTTCTGGAAGCAT
TTCTATTGGACTTGCGCAGCCAGAGGACAAACAACCAACAATGAATAATAATACTGAGAATTTCTG
TTGCTTCATTCCTGCCTCCTCGATCAAAACCAGTCAAGCAGTAGCTGTGTGCTTTGACACTTCCTCT
GATGATCAAGAGCAGAAATCCACTGGCCTAGAAGTGCCAGTGACGGCGGAAGATCAGTTCAGATT
TGAATATGAAGAATATGAAAATATGAATTTAACTGAAGAAGAAAGAGTCTATCTGTGTCGGCAGC
AATGTTTCTTAGATAAGTTTCGTGTGTTAAACAAGAAGAGATTTCCAATAAAGGACAAGATGCTAA
CTTTCATGAAGGAGCAAATGGTTGACAATGACCAATTATGGTTTCCAGCCATCAATTCTTCAGTAG
ATGCCTGTTTCGAAGAAATGGAAAACGAATTCAAAGGAAAAGATTTATCAAAAGTTGATACCTGTA
TAACTGCATCTGCAATGTTGTATTGCGTAGT

>TdomOBP7_putative odorant binding protein_partial mRNA
GCAGCTTATCACATATATTTATTTCGAAAGGACTAATTTGCATTTCTTAAAACATAGGGTGTCGTAT
TTCCTTCACAATCTTCGAGTGATGAAATTATTCTTGTTGAGCTTCTGAATTCTCTCTATTGTTGGATT
CATTATCCTCTTCTGTTTTTCCTTTTTCCTTTTCGTCGTGCTTCCTTCTTCTTTTATCACATTCTTCTGA
TTGCTCTCTGTATTCTTCGGGACACATCTTTCTTATTTCCTTTCGAACGCATCTGTGCATCCTGTTAC
TTATTCTACACTTGTCACGGTTATTAAGTTCAGTGGACTCTCCTTTCTCTTCACGCTTCGCTTGTATA
TACTGTGTCTCTATATCAATCCATCTGGAACATCTTTCAGTAGCATTATTCAGAGATTCAAACCACG
CTTCATCTGTATACCTCTGCTTAAAGAGTTCTAAAAGGCCTTCCTTAATTGGCCTTCCATCCCCACT
AAGCGCACCATTTCTCTCCATCATACACATCATGGCGCACTTATTTCTGTGTTTCTTCTTCTTAGGCT
TATCTTTTTCTTCCTTTCCTTCTCCCTCTTTTTCTCCAATTTCTCCTTCGTTTCCTCCTTCAGATCCATT
TTCACTTTCTTGTTCTAATGCTGCTACATCATCTGACACCTCTCTATTCTCCGGAGATTGGGTAAATT
CCTGTTCTTCAGGTTGTTCAGTGATAGGTGGCGTTTCCTTTTCGCTGCATTCTCTGAATTGTCTTCTG
AACTCTGGACCTGGTTTAGGAGTTTCACAACATTTATGTTTTCTTTCATCCTGTTGGTCTCGTCTCAC
TCTGAAAAATCTTGGGTTGTCATCGTTCACACTACCGCCCCAATCACTAGAAACATCTCTGCCAAAT
AAGCCTAAGATGCTACCAGCGACTGCTGCAACTGTCACAGAACACAAAGTCAAAATGGCGATACT
CTTCATAACAATCCGTGTTTGTTCTTTACCAATGTAAAGTTGAAATCAAGAATAATTTTA

>TdomOBP8_putative odorant binding protein_partial mRNA
TTCGCATTTCTTTCGTAATCTTCTAGTGAAAAAATTATTATTCTTCCGCTTGTGAATCTCCCTCCTCA
TTGTTGGAGTCAGAATCCCCTTGTGATTCGTTTTCTTTCTTTTTGTCGTGCTTCCTCCGCCTCTTCTCA
CATTCCTCTGATTGCACCCTGTATGCTTCGGGACACGACTTTCTTATTTCCTTTTCAACGCATCTGTG
TATCTTATTACTTATTCTACACTTGTCACGATGATCAAGTTCAGTAGGCTCTCCTTTTTCTTCACGCT
TCGCTTGCATACGTTGTATATTTCTTTCAATCTTTCTGGAACATCTTTCAGTAGCATTATTTAGAGGT
TCAGACCAAGATTCTTCAATGTATCTCTGCTTAAACAATCCTAAAAGGCCTTCTTCGTTTACCGTTC
CATCAACATTGAGCGCGCCATTTTTCTCCAACACACACTTCATAATACACTTATTTTTGTGTCTTCTC
TTCTTATGTTTATCTTTTCCTTCCTTGTCTTCTCCATCTTTTTCTTCAGTTTCTCTATCGTTTCCTTCTT
CAACTCCATTTCCATTTCCTTCTTCTAATGATGCCATATCATCCGACACCTCCCTATCCTCTGGAGAC
TCAGTGACTTCTGGTTCTTCTGGTCGTTCAGTTGTAAGTTCATCACCTTGGTTTTCTTCTTGTTCTCG
GCATTCACTGAATTGATTTCTTAACTCTGGACTTGGCTCAGGAATTTCACAGCATTTGTGTTTTTTGT
CTTCTTTCTTTTTTCCACGTCTCACTCTAAAAAGTATCGGGTTGGCATAATCTGCTGCAGCATCCCA
AGCACTAGAAATATCTTTATTAAATGAACTTAAGCTGCCACCTGCTACTGCTGCAATTGTAACAGC
ACACCAAGTGAGAATGGAGATACTCTTCATGACTAATACTGAATCACACGCTAAACAACTG

>TdomOBP9_putative odorant binding protein_partial mRNA
CTTTGCTTACGTTAGAAACTTGTCTTTAGTTTCCACTGATACGAATATGAAGAAATGGTTAGCTTTT
ACCTTATTGTGCTTGTATGCTGCAGTGCGAACAGAAGAAGTTGAAAAAATTACCTGCCTTGTAAAG
CAAACAGATGGTGTAGACGAAAAATCAGCAGAATCAGACACCTGCAAAGAAAAATTTCCAGTAAA

AAATGAGGCCATAGCGGATTGGTTCCAAGGTTTGGATGTTTCTGCAGAGAAGGAGCCTCAAATTAA
GTGTTTCGCACATTGTTACATGGCTGAACGAAATTTGATCAGTGAGGAGGGACAGTTCAACCTGGA
AGCTTTAAAAGAAGATACAACAAAAACATTAGAGGGTGAAGAAACAGTAGAGAAAGTTGAGGAA
ATGATTAAAGAATGTGTAGAGAAATATGAAGGTGAAGAAAATCATTGTGAGAAGGCATACATGGC
TTTGACTTGTGTTCGGCAGAATCTTACGCGGCTTTGTAACTTCGTGATGATGGAACCCGAAGAAGA
AATTGAGGAAAAGGAGGAGAAATAACGAAGATCAAATAGATGGTTGGAATGCTTGCTTAATTACT
TCTTTTTCACTGTTTGCACAGCAAAAAATTTAAAAAAGATTTTTAAAAAAACATCAACGCATTTAAT
TTTGTAATACACACATGCACATTAATTATAATTCATATGTTATGAATTAATAAAATATATTTTCAAG
>TdomOBP10_Assembly of contig 7661, 582, 6888_putative odorant binding protein_partial mRNA
TATACTAGAAAAATTTCCTGCATACTGTTACTGTTGCCCCTGCTGTGAATTTCATGCTTATCGGGGA
TATTTAAGGCATTCAATTCAATTATCTTCTTCTTCTTCTTTGTCATTGTATAACTCTCCAAAATA
TGCAATGACAAGACATTCTTTGAAGTCCCAAGCCTCTGAACACTCTTTCACAGGCATGTCACCACA
CGTCTCCACTGCCTTAAGGCACGCGCGTTTTTTGTCATCCTTGTCGTCGTCACTTGACTCTTCCCAAT
TGGTGTTGCAATAGTTTAATCCCTTGTCCTTCAAATAATTGCCCTCATCATCGCTATATTCCAGCTTG
TCTATCAGGCACTTGAAAAAACACTGAAGTGGTTCTTCTGCCACATGWTCTTCRCGCCATTTYTCCC
ACATTTCTTTCGTGGCACCWGTTTCTTTTCCACACTCTTCTGATACTGCCTTCCATTTTTCATCATYW
ATGCCTTCAGCCTTAACATATACTATAGCATAGRCAAACGCTGTTAAAGCCAGTAA
>TdomOBP11_putative odorant binding protein_partial mRNA
AACAAGTTCTTAACTCTTTATTATTCATAAAACCAATTACAGTTACAATATTGTGAACATTGTTAAA
TTTTCATCAACTCGTGCAATGATCAGCGGTATCAACAAAATTCTTTATTAGATGATTTCTTTGTTGA
ATTCAACTGGATGATGTTCTACATGTTCTTCCATTGCCATAAGCAATGGAGCATATTCCACGCATCT
TCACATGTTTCAACTGTATTTTTACCACATTCATCAACAGCATTAAAGCAGTTTTGCTTCTTCTCTTC
ATCTCCCTGACTCTTACGTTCGCATTTCTTCTTTGCTTTTTCCTTATTCAACGATCCATTTTCAATAAA
TCCAAGTTTCTCCATGAAGCACTTCTTGAAGCACTTAATATTGTCTGTAGCTTCTTTAGTTTCTTTGT
ATCTCTCCCAATCCTCTTTGGTAATACCATTTTCTTCAGAACAAC
>TdomOBP12_putative odorant binding protein_partial mRNA
AAGATTGTTGGAATTCATACACATGCTTCAGGAATCATTTCAAAAAGGCAATTTACCACTACAAGA
AAACAATAAAAAAATGTTTTAGTGAGTCTGGAGTTACATCAGAGGACTACTCCGAATATATGGAAT
CAGGGGTCCCTACAGAAAGTATTCAATGCTATTGGAAATGTTTCTTCGAGAAAGCAGGATATGTCA
AAGAAAATAAAGTTAACACTCCACATCTTCAGGAACTATGCAAACGCTATTACGAAAGTGAGGAA
GATACCAGGAACAAGTGTTTAGCTGTTGTTGACAAATGCAAAGACATACTAGTTTCAGACTGCAAG
AATGCATGGAAATTCAAGGAATGTGTCGTTATAGAGACACAGACAGAAGAACGT
>TdomOBP13__(reversed) _putative odorant binding protein_partial mRNA
TCCGATCAAACGTCTTAAGCACACGAAAGTATACATTGTAGTAACAAATTCAATTATGAAGATTCA
ACATTTCGTTCTTATTGCACTAGTCTTTGTAGCAGTCAACTGCGATGAAGATCAAGGAGCAGGAGG
CTTTGATGAAGCTATGAGAGAGTGTGCACAGGAACTTCAAATAACACAAGACGAGTTTATGAGGTT
CAAGGAAAGTGGCCAACCAGATGAAAAAATAAAGTGCCACTTTAAATGTGTGATGGAGAAGAAAG
ACATGATTAGAGAAGATGGAACATTCGACACTGAACCTATGGAGCAGTGTAATATTATGAGGAAA
TTCCGAGATCAAAGTGAGGAAAACGAACAAAAATTCCAGGAAGCAAAGAAAAATTGCAATGGAA
AACCTGCTACGACTTGCCAAGAAGCTTTCGAAGCGTACATGTGCGTACAAGCAATAGCTCAATGAA
TCACTCATTTCCAAAGTGCAGCTGCAAAACAACCTCGATAATTTGTACACATCCGAAGTGGAGAAT
ATTTCAATATTGTGAACGAGAAGACTTAAAATGTGTAATAGAAAGAAATGTTATCATTCTAAACAA
CATTTTATTAAATATTTGATTTATATATGAAAGGAGTCTTTCCGTTTATATAATTTAGG
>TdomOBP14_putative odorant binding protein_partial mRNA
GGCTTCCTTATCTTTTACATGAAACAAATCAAAGATACAGTACATTTACATTAATGTAGAGAAAAC
TCGTTCCAGCCAAGGTTTCTACAAATCGTAATCCGCCATCTTGTATCCTCAAAATACATCAGCTTTG
TTGTACTTTATTTTTATCCATTAGGCACTTAGATATTTGGAAAACTTTCTCGCAGTTATCAGTTACTT
GTGAATGATCTTGTTTACATGAGGGATAGTGTTCCTTGAACCCTTTCTTCCAATCTTCTCTTGAAATC
CTTTCGACAGCTTCTTGAATATTATTTTCGTTAAAATTCCCACTATCATCCATTAACTTATATGTCTT
CATCATGCACATAAGGAAGCATTTACTGTTATAGTGGTCTATTTCTCCATTCATAAGCGCAAGAACT
CTTGCACTGTCCGTTTCATGCTGGTCTGCGCATATTTTGCTTTTCTTTTTCAGGTCCTCTTTAAACTCC
TTGGTGAATTGCTGAAACGAACCAATAATACAATTGCTCACCAATAAATAAATCAATAATTTATTC
ATGGTAGACAATAAAGATTCGCAGTAAATCAAGAATACATCCGAATACCAGAGTTACGAAAAATT
ACGGACAAAAAATAGAAAAAACAAAAACAAATAATTTTCATAGTTCGCATGTTGT
>TdomOBP15_putative odorant binding protein_partial mRNA
GTGCAAGAAAATCGTAATTACATGACATTACAGCTGATTTTCTCTTCAAAATGTACTCTGAATTATC
TTAACAACTTTGGTGCAATGAAGAGTCAGGAATGTGAAATGCCACTATGCTCAAACAAAATGTTCT
GCAGTGAAATAAAGTATTATAAAGTGAGTACTGAAATAGTCTCCGTTGTGTGCTCATTTTGTTGCGT
TGGTATCAACGTCTTCGTTTGTAAATCTTCTATGCGTCTGGCATGAAACCAACCTTTTCAGCGTGGT
TCTTGAAACAGTTAAACAGTTCCAGTCCAGCTCCACATGCCCCATCGCCACTACCCACTTCTCCTGC
GCATATGTCCAGAAGTGTTTCCATCTTGCTCAGTATGTCAGGCTGGTCTTCGACGTACTTCTGCACC

GTTTCTTTCGCATGCGCTGCGTTGAATACGCCATTTTCCATCAACTCTAGTTTTTCGAACATGCAGG
TGAGGAAACACACTGAATTCGAACCTTTTGGGGCCTGGCGGTGGTGCAAGAGCTCGGCATCCTCTT
CTGAGATAGGGTAAACCTTTTCGCACTGCGTAGCCAATTCGTGGATGATCACTTCCATGTCTTCTTC
AGTTATCGCCATGGAGAAGGCGGTGGCAGCAGCCAAGATGACGAGTGTCCTCAGCGGCGCCATCG
TCCCGTGGATC
>TdomOBP16_putative odorant binding protein_partial mRNA
CATCGTCGTACCGCAGAAGTCACTAATTTCCTGCTTCTGAGTACTAGAAAATAGACAGCACAAGAA
CAGTAACATTATGTAGAGAAACAAGAACATAATTCAAGACTAGAAAGAACATAAAACATGGAGAG
TTACAATTGTGAAACCCATTTTAGCGAAGATTTCAAACTTTAAAACAACTATATCCTTAAAGTAAC
ATTTTGGATTTAGTTACTTCTGCCAGAATGACGACAATATTATATAGAAAATAAACACAGGAATTT
GGTTTTTTATAGACATTTATTAGCTGGTTATTTGAGTTGAACTGTTGGAGACCAGTTCGTTTGTAGTT
GCATGCTGATAAATGACTCCATTCACGGGAAATATAATTTATTTATTTCTTTGTCCATGGCCACCTC
ACACTTAGAAACTTCATACGCTGTTTCACAATCATCTGCACCAAATTTAGTGCCACAAACTTCAAAC
ATATTCTTAAGAATCTTCAGAGTTTCCTCATCAGGAGCGATTTGTTCTGCCAGACTAATCGCAAGAG
GTATATTGTATCTCCCATCTTTCATCACTTGTACACTGGACATATAACAATTCAGGAAGCACTTCAC
ACTTCTTTTATTTGGAATATCTCCATTCTTCATGGCCATTATTTCTCCTTCTGTTACTCCATTTTCTTT
GGAGCATTTATTAACAATCACCCTCGTAGCTTCCACTGGGTCATCATTCGGACGCGGCATTACCCA
GGAAACGCCTAAAGACAGTATCAGAAGGAAAGTTGACATTATTTTCATCTTCATAAAGAAGAAATT
AATAATTCTCTTGTGAATTCTTCTATCAAATCTAGA
>TdomOBP17_putative odorant binding protein_partial mRNA
TTGTCTAACTATAAAATAGTAACATTTTCTGTAGTATATATTATCTATTAAATTGAAAACTTAACAT
TATGGACATTTAGATCATACTTAGCATAGTAAATGAAAAATTTATTCAGTCAAAATCCTGAAAATT
GTCCATATCTTCATAATAGTCAAAATCTTCAATCCCGAAATCCATCAGTTAAAATCCGTTGTTGCCA
TAAGCAATGTTGTCTATTTAAGGGAAGAATGCTTTAACAATATTCTTGCTCATTGCCACCTTACACT
TGAGGATTTCGTATGCCGTATCACATGGGTCAGTGCCAGTTTTTACGCCACATGTTTCGATAATATG
CATGATGCCATTCTTGACCTCTTCGTTCGGTGCAATATTCGCTGCAAATCCTTTTGCAACATCTAATT
CGTATTTTCCATCTTTCATTATTTGGATACTCGTGAAGTAGCAAAGTATGAAACACTTAACATTGTT
ATTATCTGGAATATTTCCGTCTCTCAGTTGTGCCCTGTCCCCGTCAGTTATCCCAGTCTCTTTAGAGC
AATTATCAACAATTGCTCTGACCATTGCTGATATTTCGTCTTGTGTCATGGTCCAACTTAAATCTA
>TdomOBP18_putative odorant binding protein_partial mRNA
CCCCCTAGCCAATCATATTTTTGTTCTTGATTGCTGCATAATATGAGGCCATATTTCAAAAGAAGAA
GGCATTTGCATTTTTATACTTTCCAATTACACAACTGATGATCTGGTATGCTGTTTCACAATAATCA
GCACCACTACTTTTATTTCCGCAAGAGTCAAGAACTTCAAAAACAACTTCTTGGGTTGCTTCATCTG
CTATCGCTTTCTCCACCACAGCTCTAGCTGCTGAACTCACGTATTTTCCATCCTGCATCACTTTTGTA
GATGTCATATAGCAGCCAATGAAACACTGGACATTTCTTGTGTTTGGCATCTCTCCAGCAATCAGA
GCTTCTCTGTCTCCTTGGGATATCCCGCTTTCTGAGACGCATTTGTCACTTATAGATTTCCTCAAGGT
TTCCGCATCATCTTGTGGACGACACGATAATAGAACAAAATACAATGCAAAGAGCAAGGATAGTA
AAACAATCTTTGCCAT
>TdomOBP19_putative odorant binding protein_partial mRNA
GGGGGAATATTTACAGCGACCGAAATCCTGAGGTGGGCGTAGTCATCATCATTGAAGCATTCGTTT
TTCTGACTAAGATGGAATCTATGAGACTCCGGCGTTGAAGTCCCAGACGGACAGCACGCGGTTGTA
CAGCTCAGGGTTGGCGTCGTGGTAGCATTTGTGCAGCTTGTAGGCGGTGTCGCAAGGATCGGTGCC
CTGGATGTGCTGGCAGGAGATGACGATGCGGTGGCCCTCCTCCTTGATCTCCGGCGGCACGTTCAG
CAGCTCCTCCTCCAGGTTGAACGTACCGGCGTGGTCCAGCGAGGCCAGCTCGTTCATGATGCACTT
GATAAAGCACTTGAAACGTCCGTCGTCCACGTCGATCTTGCCCGCCACGAACTTGCCGAAGCTGCC
CTCCCCTGCGCCCGTCTCGGACCGGCACTTGGCGTCCACCTCCTTGGCTTTCTCCAAGGCGCGCCCC
GTCATACCCAGCGCGCTGTGCGCGAGCCCCAGCAGGGCCACGGCGATCGCCCGCAGGATGCTGTC
GGTCTGCATTGTCGTCGTC
>TdomOBP20_putative odorant binding protein_partial mRNA
GGAAGTTCATTTTACACAGAAGAAAAAGTTGTTAAAATATATTTCGATTTCTGCTTGGTCCAGACGT
CAGACAAGGCTGTTTCGGGCAATATATCTGCACGAAGTAGGTAGGAAGCATTTAGCTCGCAGCCTT
TTCAAACCACATTGCTATGGCGTGGAGGAACAGCTCTCTGTTCTCTCGCAGGTAGCACATGTGTATC
TTGTAGGCTGTTTCGCAAGGATCCGGTCTTTTCTCCACGCCCTTGCATTTGTTCACGAGCGCGTGGC
CTTCCACTTCGATTTCCGGCGGCACGTTTAGCAGCTCCTCTTCAAACCTGAAGTTGCCATCTGCGTC
CATCGCATGCAGTTTCATCATGACGCATTTGACGTAACACTTGTACACGTGGTCTTCGTCGGTCTCG
TCGCCCAGGATGAACTCCATGAACACCTCCTCGCCAGCGTTGGTCTCCGCTCGGCACGCAGAGTCC
ACTTCCTTGGCGCGCTCCATGGCTCGTCCCGTCAGGTCCTCCGGAAGTTCCATAGCATATGCACCTA
CTCCCAGCACCAGGACGAATACCAACATCTTCCCAAGACTGTTCATTTCGGAAGCAC
>TdomOBP21_putative odorant binding protein_partial mRNA
CTTTCATTCCCTCACATTCTGTTCTTATCTTCTGGAATTTCTCTTCATTCTCGTCACTTTGTGATCGGA
ATTTCTTCATCTTTCCACAGTGCTCCATAGGTTCTGTATTAAAAGTCCCGTCTTCTCTAACCATGCCC

CTCTTATCCATTACGCATTTGAAATGGCACTTCAGCTTTTCTTCTGCCTCACCAGACTCCTTAAATTT
CTGGAAATCATCTTTCGTTACTTCATTCTCTTCGGCACATTGCTTGAAAATCCCACCTCCTTCTCCTT
GTTCA
>TdomOBP22_putative odorant binding protein_partial mRNA
TCGAAGAACATCAGTGCGGGTGATTTGTTTTTCTAACACATGTCTCATAGTAGTCAGTTCAAAACCA
CCATCTTCATCCATCCATCCGTACTTCGTGTATAAACAATGAAGAAAACACTTGAATCCATACGTTG
TATCTTCAGTCAGCTTCCTGTGAACTAACGCTACATTGATGGCATGAAGGCTAACTGGTGATGTTTG
GTTGCAGCTCTTGATGATGGCATAATCCTCGGCAACACTGGTTGAAGTAGTGGGTCTGCCCATAAT
ACTTCCGGTGATTGAAAGAGTAGCAACTAACTGCAACAAAATAAACGAAGATAAGTGTTCCATGA
TATCTGGACCGTAAAACGTGTGTGACCTGTAGAGCCCTTTGTCAATTGCAGAAGAGGAGCACTGGC
AGCTGTACCACTGGAAGAGATGCAGAAGAGGGAAATACTGAAGAGGACCTAATTCTCTAACTCTT
AATAAGT
>TdomOBP23_putative odorant binding protein_partial mRNA
CACTACATTAAATCTTTTTTAATAGTTATTTTCATTTTGGTTCTTTAGTACATATTTATATAGTAGAT
TACATATTTATTTTATAGTATTTAGTATTTATAATCAGTGGGATTAGTAGTTTGAAATTTCCCTTGAT
CAATAACTGAATAATTTGAACTAAACAAGATCAATTATGATACTAAAATATAAATCGCCGCAAGGT
CGTAACCCTGGGACGCTATTCTTGATTCTTCTCGTCCACTTTCCTCCAAAAGTTTTGACATTTTTCCG
TGTCCATATTTCTGTAGTTTTCAGGACATTTCAATAACATATGGAAGAACATACAGAACATTGTTCT
CGCACAATGGGGTTGACATTCACCCTCACCTTCATGTCGGTCGTGATTATAATTTTTTCCCAAACAT
TCATTCAAAGTTGATTCAACAACTTCAGTCCATTGCCCATCTGCCTGACGTGACACATCTTCCCGAA
CAGTATTGTCCACTACCTGACCATCCTCTGTTGCCCATCCCGCTGAATTGAACACACATTCGGTGAA
GCAATTCCATCTTTTTCTCATCTCCTTAACACGTCTTTTCAGTTGTTCCCAATTAGTTTCTTC
>TdomOBP24_putative odorant binding protein_partial mRNA
TCATGGATCTTTTCTATGGAAGAAATATTTCCTCGAGTTAGGTATAGTTTCCAAGTTATCGCAAGAG
ATCTCAAATCCTTTCTAAGATGTTCTCGGAAACAAGCTCCAAACACGTAAGCTTTCTCGCAGTGGTT
GATGAATTGCGTTGACTCAACTTTATTGGCACATTTCTTCATGTGATGAAATACGTAAGATCTTTGA
GGTTCCTTGAGTTCCATTTTCTTTGTAATATCCCTTAGAGCAGTTTCATTAATACGTCCTTTCCATGT
CATAGCTCCAGATTCTTTCATCAGACAGCTCTCAAAACA
>TdomOBP25_putative odorant binding protein_partial mRNA
ACAACATCAAACTATGGAAATCGTAACAACAGGTTTAATTCCAGTCACCACATTTTAAGCGGAAAC
AACATCTACAACCGGAGACATAACAATTCAAACTGGAGAAACAACCGCACCTGGAGAAACAGGGA
CAATAAACATAATGACAGTAATAGCAACGAGAATTCTACCAATTATTCAAAGGATAACAACAGGA
CAACCAATTCCTGGAGAAAGATGAGAGGGTTTTTCAAGTCCTTTGATGTCCTTGCCAAGTCTCGAG
TTCTAAGACAAGTCTTTGGTACTGAGAAATATATTCCTGCGTGTACAATCCAATGTATTTTTTCAAA
AGTTAATACGGTGGATCAAACTGGATATCCGAATGAGTCGTTACTAATAAAATTATGTGAAAACGC
CATTAAAAATGAAGAAGCTCGAACGACTGCTATTAAGATAATTAGAAAATGTTTCCGAAGACTTGG
AACAGACGATCAAGAGAACACATGCACCTTCTCCAAACAATTGGCTCTTTGCATGGGAAGGGATAT
GAGCAAGATTTGTCAAGACTAACGGACCATCTGGAAATATAAGAAAAGGTCGCTGTAGTTCCTATT
CCTGGTAGACTGGCTAAAAATAT
>TdomOBP26_putative odorant binding protein_partial mRNA
ATGCTTATGGCCATGCTTCTTGCCGCGATATTTTTCCATATGTTCTAACAGTGTCTTGATGTGGTCAG
GATCGAATTCCCCATCTTTTAACACTCCAATTTTGGCTGCGAAACACGCCACATAGCATCTGCCGTT
TTCACTGGTTTCATCCTCAAGTTTAGAATTGTTGTTGAAGTATTCGTCAGCCTCTTTGGAGATTGGG
TATGTTTTATTGCATTGCTCTGCTGCCTCGTGAAATTTCCTCATGAAGTCCGT
>TdomOBP27_putative odorant binding protein_partial mRNA
CCGGCGCGCTGCTCTTGCCGACCTTCAGGTCGTTCTTCAGCTTGTCCGTGGCGAACCCGGAGTCGCC
CAGCAAGCCCGCGCCCTTGAACGTGCAGTCGTAGAAGCACGAAACCAGGGTGGGGTTGTCTTTGGC
GAATTTCAGGTAGGTATTCAGGTCCGAACCGCTGACGGCACTGGGTTTCGGCACGTTGAACTTCGC
GGCACAATTCTTCAGAGAGTCCTTCAGCAACTGCTTGTAGTCGTCGGCGTAGACCACAGTAAATAC
TACGGCGAAGATGGCGTAAACAGCTATGGCCTTCATTGTTGGCGGTTTTGGATCAATC
>TdomOBP28_putative odorant binding protein_partial mRNA
TTGAAATAAAGATATACATAACGCTGATCTCTGCACATAATTTACACTTGTCATATTAATTTCTGAG
ATTCCTTCAACAATGCTGGTAACTTTTGGTTTCTTGAATTCAGCCTGTTGTCTTTAATAGTCAAATAA
CTTAATCCTGTCCTTCTTCGCCACCTTCTTCGCCACCTTCTCCATTTCCTTCACGTTGTTGCTTCCAAT
GAGACTTAACACACCTCCATAGTTCCCAAGCATTGTCACAAGACTCAACAGGTTTATCNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNTCATGAACAAATCCCATTTTCTGCATCATGCA
ACGCTTGAAGCACTTAACATTTTCTGATGGTTCGTAAGCATCGTTTTTCTTGTTCTCTTTCCATTGTT
GAAATTCCTCATCGGTCACTCCAGATTCATCTTTACATTCTTTTTTCATCTGCTTAAATTTTTCCTTCT
TATTACCATCAGCCTGAACATACGTTAGGAAAACGACAGCAATGACAAAAAATAGTATGG
>TdomOBP29_putative odorant binding protein_partial mRNA

AGTGTTCTTCATCCCCACATAAAAAGTCTTCGTGCTGCAGATTTTTGCTTTTGTGCTGAGGGTCTTA
ATTTCTTCATTGTTGGCTTTTCGTACAAGCATGTGACAAACTTATAAGCCCTCTCACATTTATCTGA
AACACTTTGTTTGCTACAGGTATCCAACGCTTTGCTTACATCATCGTTAAGCGTACNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNCCAGTATTGTTGAAAATGCAATGAAGATA
ACACTTTGCAGCAGTGTCAGATTCATTTTGAAACTTTCCTGTGTTGTTCAGGGCTTCTAATTGACCG
TTAGAAATTTTGAATGTCTCATTACATCTTTTATTAATATCTGTCATATTGATGCTGTTCATATCAGC
AGTATTGCTCATGTTCCCAGCTGATCCTAGAGCCAAGATAGAAAATAAAAATACTAGAAGGACAAT
CATCTTGATGGACAATGTTGAGCACTGCATTCTGTCTAGGATTTCCTTATTGGGTTAAACGCCTTAT
AT

>TdomOBP30_putative odorant binding protein_partial mRNA
AATGAACTATTTGTTAAAAACTGTTGCAGAGGATTGTCTCGGGAGGAGTTCTAACATCAGCGAGGA
ATGCAAGTGCTACTGGTCTTGTTTCTGGAAGAAGTTCAATTTTGAACTGGAAAACGGAACGATCAA
TAAGGATTTGATCATAGAATTCATAGGCAGCTACTCGAACTCCCCTGGTGAAGACATAAGCCCACG
CATAGAAGAGACCGTAGATGTCTGCGTAGAAAAATCGACGGTTGAAGGCTGCGGCCAGGTGAAGG
AGATCCTGGAGCGCGTCGGCGACAACTACAAGAGCGCGGAAGACG

>TdomOBP31_putative odorant binding protein_partial mRNA
GACGTATCATAGTCTCCTGAATACATTTAAACACTCGGTACCCGGGCTTACAACCTTCCCCTTCATC
TCCTTTTGCGTCACAGTACTCCATTATTCTTTCAAAATTATCTCTCCTCTCTTCTGGCATCCGATTTG
TAAGCAAGTCTACGAATTCCCAGTTCACTCTTCCATTCTTAATGAAATTCAGTTTCTCTCCAACACA
TTTGGCGTAACACATTTCGTCCTCATCTTTCGC

>TdomOBP32_putative odorant binding protein_partial mRNA
TTCCCATCCTTCATGAGCTGGAAACTTATCATGAAACAGCGTATAAAACACTTGACATTTTCTTTAT
TTGGAATATCGCCAGTTTTCACTCCTTCTATTTCTTTCGATAATTCATGTTCCTGTATGCATTTATCT
ATAATTGCTTTTGCAACAGCCATGTTGTCCTGGGGTCTTCCCTTAGACATCACTATGCTAGTTATTA
AAACAGATACAAATAATTGTATCTTCATTGTGATGTAAATGCTGAAGCAGCAAAGTTATC