

Insights Into the Evolution of Chemoreceptor Genes Superfamily in *Tyrophagus putrescentiae* (Acari: Acaridae)

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Abstract

All living organisms, including animals, plants, fungi, and bacteria, use the olfactory system to recognize chemicals or pheromone from their environment. Insects detect a volatile substance using odorant receptors (ORs) or gustatory receptors (GRs) and ionotropic receptors (IRs). The gene families of the olfactory system in Acari are still not clear. In this study, we identified seven ORs, one GR, and five IRs from the transcriptome of the storage mite, *Tyrophagus putrescentiae* Schrank. No olfactory coreceptor was found in this transcriptome. Phylogenetic analysis of these gene families with other Arthropoda species revealed the conservation of carbon dioxide receptors in all tested flying insects and *T. putrescentiae*. Most of these ORs and GRs were unique to three mosquitoes (*Anopheles gambiae* Giles, *Culex quinquefasciatus* Say, and *Aedes aegypti* L.), *Ixodes scapularis* Say and *Pediculus humanus* L., indicating their involvement in specific aspects of both gustatory and olfactory perception. Some clades contained receptors obtained from all tested insect vector species, indicating a degree of conservation among some vector-dependent OR lineages. IRs family was a highly dynamic and independent original of the chemoreceptor genes subfamily. Our findings would make it possible for future research on the chemosensory recognition mechanism in Acari.

Key words: gustatory receptor, OR, ionotropic receptor, olfactory system

The storage mite (stored product mite or cheese mite), *Tyrophagus putrescentiae* Schrank, occurs throughout the world in a wide range of habitats, including stored products, crop seeds, mushrooms, feed of domestic animals and pets, and house dust (Zhang and Jin 1960, Hughes 1976). It also causes human allergic dermatitis, pulmonary acariasis, and intestinal acariasis (Li et al. 2003, Jeong et al. 2007). *Tyrophagus putrescentiae* is known as the third most common mite following *Dermatophagoides pteronyssinus* Trouessart and *Dermatophagoides farina* Hughes in Korea, and it is an important allergen in allergic diseases, especially for children (Choi 2010). The previous study demonstrated that around 38% of patients suffering from allergic symptoms had sera containing IgE against *T. putrescentiae* crude extract (Liao et al. 2013). Practitioners in the edible fungi industry are >20 million in China. Owing to allergies and the destruction of edible fungi, *T. putrescentiae* is an important pest in edible fungi cultivation (Duck et al. 2001, Kheradmand et al. 2007, Qu et al. 2015a). However, olfactory receptors have been given limited attention in Acari species, many of which have major economic importance in health, agriculture, and animal husbandry.

Animals use the olfactory system to recognize various volatile chemicals in the surrounding environment and to detect proper hosts, mates, oviposition, predators, and other important behavioral

responses (Asahina et al. 2008, De Bruyne and Baker 2008). In this process, several important chemosensory gene families are involved in chemosensory, including odorant-binding proteins (OBPs), chemosensory proteins (CSPs), pheromone-binding protein (PBP), odorant-degrading enzymes (ODEs), and olfactory receptors, such as odorant receptors (ORs), ionotropic receptors (IRs), and gustatory receptors (GRs). The research on the molecular mechanism of chemosensory showed that volatile chemicals activate a single class of sensory neurons that target the glomerulus and connect to projection neurons that respond exclusively to the chemicals (Stensmyr et al. 2012).

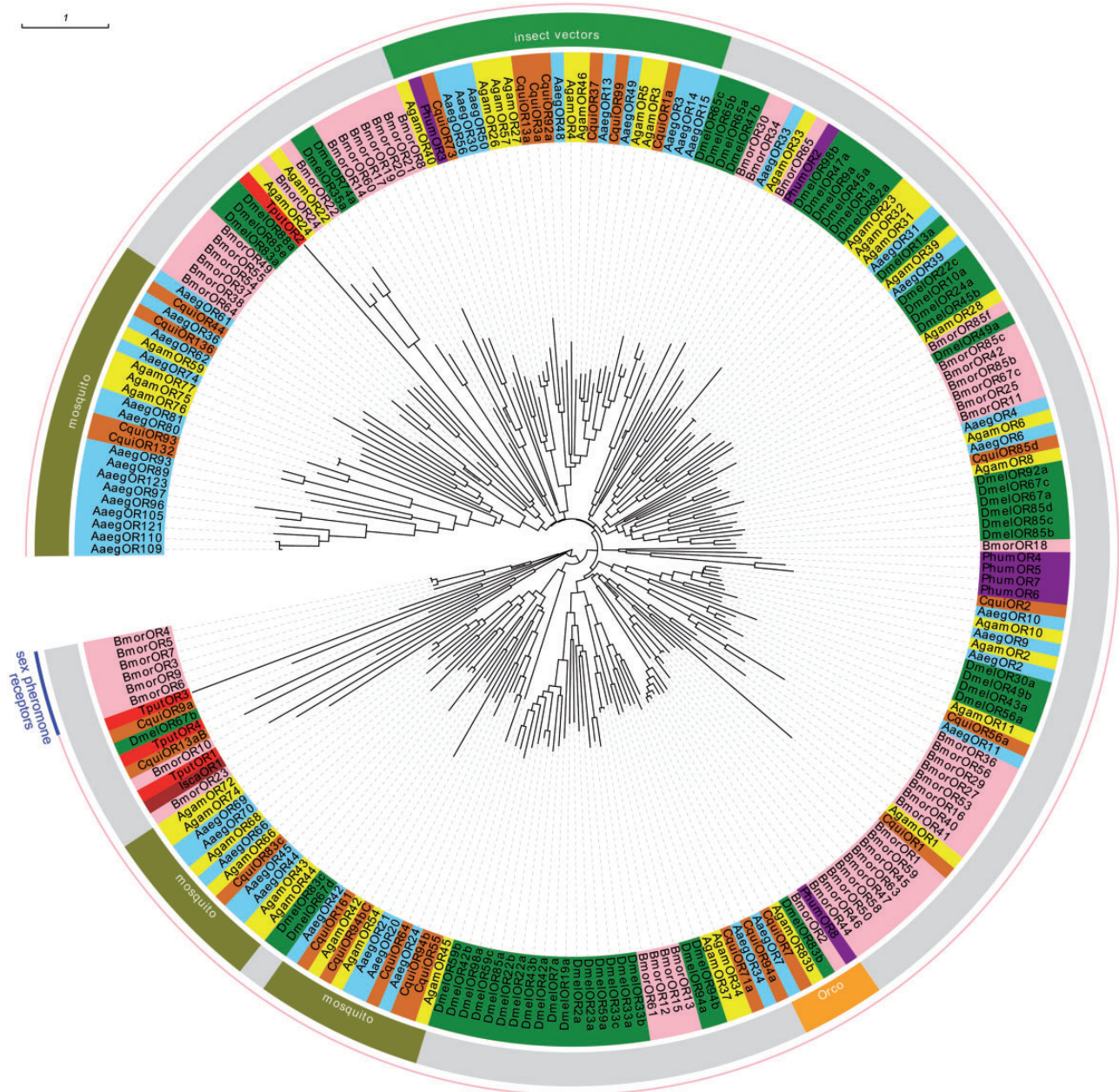
OBPs are found to be the initial molecular interactions for volatile chemicals, combined with the initial chemical signals and transported to the respective ORs triggering the mechanism of olfaction (Vogt and Riddiford 1981, Vogt et al. 1999). From the first odorant receptors that were discovered in the fruit fly, *Drosophila melanogaster* Meigen, in 1999, at present, many olfactory genes of parasites and vectors have been discovered in olfactory sensory neurons (OSNs), also known as olfactory receptor neurons or ORNs, such as *Anopheles gambiae* Giles, *Culex quinquefasciatus* Say, *Aedes aegypti* L., and *Pediculus humanus* L. (Melo et al. 2004, Pitts et al. 2004, Xia and Zwiebel 2006, Latorre-Estivalis et al. 2015). ORs are seven-transmembrane domain proteins expressed in the olfactory

receptor neurons (Clyne et al. 1999). Most olfactory neurons express two types of OR genes, one type called the OR83b family receptors, which are atypical odorant receptors involved in the process of odor recognition. A striking difference to G protein-coupled receptors (GPCRs) in mammals, insect OR/OR83b complexes form ligand-gated ion channels (Sato et al. 2008). The number of ORs varies from 10 in *P. humanus* to 350 in ants (Kirkness et al. 2010, Zhou et al. 2012).

GRs are recognized as insect-specific chemosensory receptor families, expressed in gustatory receptor neurons (GRNs). Most GRs are classic seven-transmembrane GPCRs, those involved in sweet and bitter taste and in the detection of nonvolatile pheromones. However, four members of the GRs family are expressed in ORNs, including two highly related GRs (GR21a and GR63a) that

function in the detection of CO₂ (Scott et al. 2001, Kwon et al. 2007). The GR5a gene is confirmed to taste the sugar trehalose; the GR genes with GR5a high homology, such as GR61a, GR64a, and GR64b, might also bind sugars (Robertson et al. 2003).

A new receptor family found in *D. melanogaster*, the ionotropic glutamate receptors (IRs), was involved in insect chemosensation. In *Drosophila*, IRs are expressed in a combinatorial manner in sensory neurons that respond to many distinct odors but do not express either insect ORs or GRs (Benton et al. 2009). IRs contain a predicted extracellular N-terminus, a bipartite ligand-binding domain (LBD) with two lobes (S1 and S2) separated by an ion channel domain (P), individual odor-specific receptors, and one or two of the broadly expressed coreceptors (Abuin et al. 2011). Multiple IRs can be expressed per neuron, which generally follows a one neuron–one



receptor rule. The odor responses of IR-expressing OSNs may be defined by expression of a particular set of IRs rather than a single IR gene, so a given IR may label axons projecting to multiple glomeruli. For example, a combination of three distinct IRs (IR25a, IR76a, and IR76b) assemble into functional odor-sensing complexes, in which IR25a is likely to have IR76b-independent roles as a coreceptor for sacculus and arista odor-specific (Benton et al. 2009). Further research suggests that IR8a has the same gene-targeting strategy used for IR25a as coreceptors acting in conjunction with more divergent odor-specific IRs (Croset et al. 2010, Abuin et al. 2011).

In our previous study, chemosensory genes (CSPs) in *T. putrescentiae* had played very important roles in host recognition. Based on a docking study, we found three ligands of host volatile substances, (–)-alloaromadendrene, 2-methylnaphthalene, and cyclopentadecane had high binding affinities for TputCSP1 (Qu et al. 2015b). In this study, olfactory receptor families had been identified for the first time in *T. putrescentiae* by transcriptome sequencing, which may further provide some molecular targets for control and insight into mite olfaction research, thus providing an essential foundation for the development of efficient, simple, and sustainable pest control strategies.

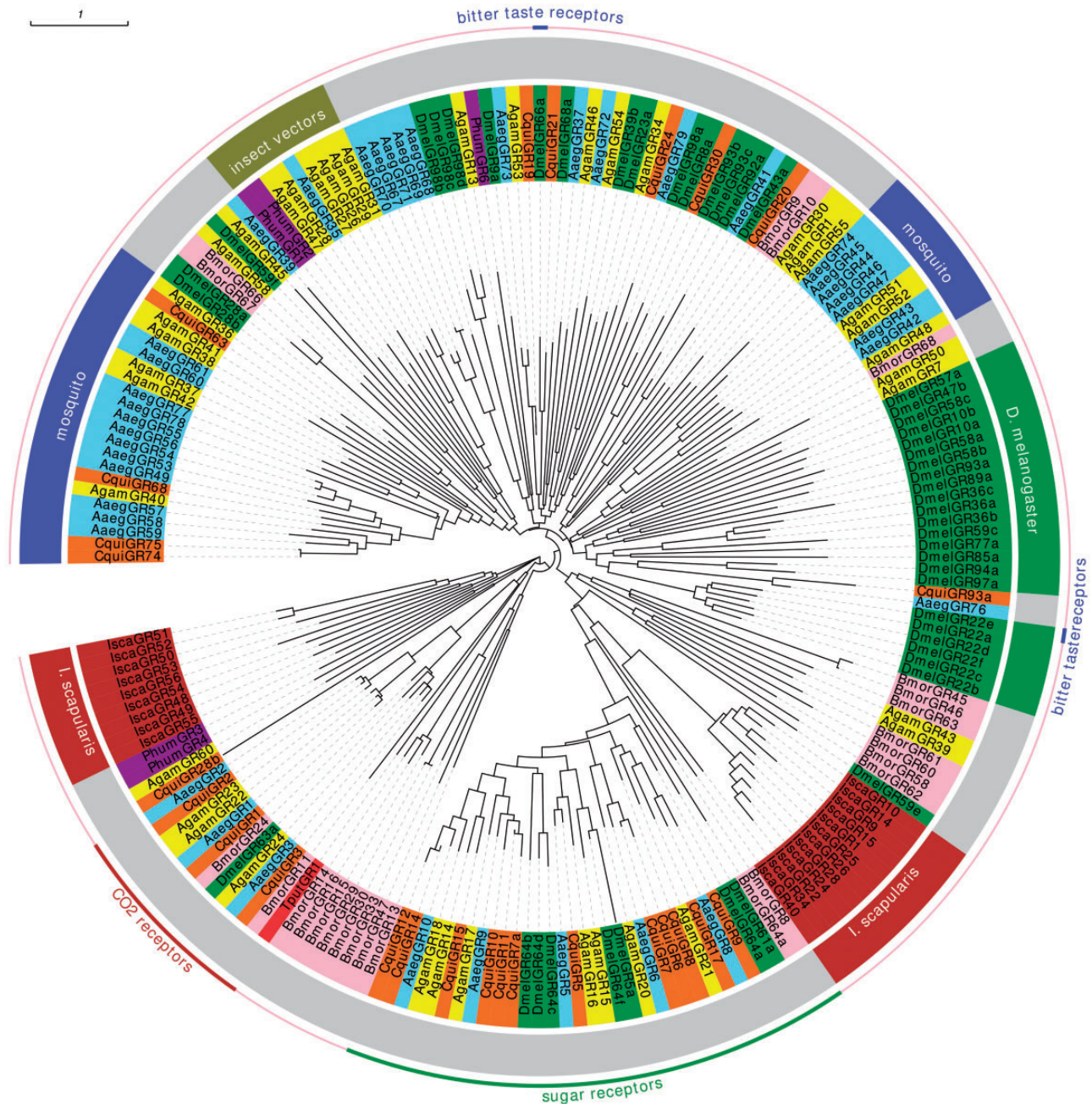


Fig. 2. Unrooted phylogenetic tree of GR protein sequences from *T. putrescentiae* (red branches), *D. melanogaster* (green branches), *An. gambiae* (yellow branches), *B. mori* (pink branches), *Ae. aegypti* (skyblue branches), *Cx. quinquefasciatus* (chocolate branches), *I. scapularis* (brown branches), and *P. humanus* (purple branches). Outer ring indicates the different groups and protein functions.

Materials and Methods

Mites

The strain of *Tyrophagus putrescentiae* Schrank originates from a population collected in 2012 on wood ear mushroom *Auricularia polytricha* Montagne in Feng County (Jiangsu, China). The mites were bred by the methods reported previously (Qu et al. 2015b).

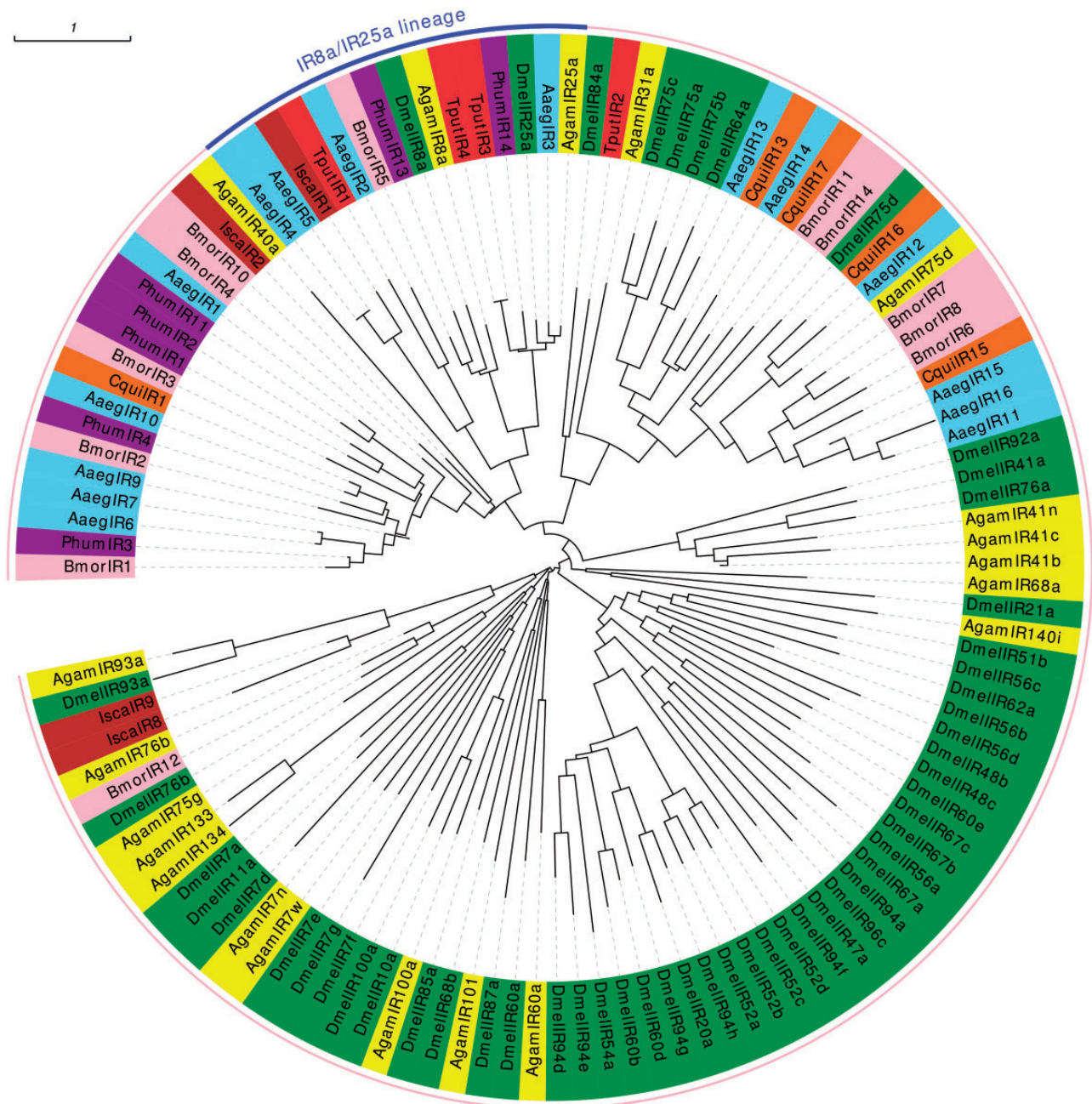
Transcriptome Sequencing

Total RNAs were extracted using SV Total RNA Isolation System (Promega, Madison, WI) from the adults (about 300 males and females mixed) according to the manufacturer's protocol. The cDNA

library construction and the Illumina HiSeq2000 sequencing were conducted at Novogene Company (Beijing, China).

Identification of Chemoreceptor Genes Superfamily From *T. putrescentiae* Transcriptome

Transcriptome (without a reference genome) assembly was accomplished based on the left.fq and right.fq using Trinity with min_kmer_cov set to 2 by default and all other parameters set default. Gene function was annotated based on the following databases: Nr (NCBI nonredundant protein sequences), Nt (NCBI nonredundant nucleotide sequences), Pfam (Protein family), KOG/COG (Clusters of Orthologous Groups of proteins), Swiss-Prot (a manually



annotated and reviewed protein sequence database), KO (KEGG Ortholog database), and GO (Gene Ontology). Within these databases, chemoreceptor genes superfamily was identified by manually searching for ORs with keywords “odorant receptors” or “olfactory receptors,” or “G protein-coupled receptors,” GRs with keyword “gustatory receptors,” and IRs with keywords “ionotropic receptors” or “ionotropic gustatory receptors.” The translated protein sequences of target genes were edited and subjected to BLAST online servers as well as further identification and analysis. TMHMM 2.0 (<http://www.cbs.dtu.dk/services/TMHMM/>) was used to predict transmembrane domains of candidate ORs, IRs, and GRs.

Sequence Analysis and Phylogenetic Tree Construction

The chemoreceptor genes superfamily from *T. putrescentiae* identified in this study was aligned with the respective gene families from other arthropod species, *Drosophila melanogaster* Meigen, *Anopheles gambiae* Giles, *Bombyx mori* L., *Aedes aegypti* L., *Culex quinquefasciatus* Say, *Ixodes scapularis* Say, and *Pediculus humanus* L. (Supp Table 1 [online only]). Genome sequence data and gene annotations were downloaded from public data repositories: *D. melanogaster* and *B. mori* from National Center for Biotechnology Information (<ftp://ftp.ncbi.nih.gov/genomes/>), and *An. gambiae*, *Ae. aegypti*, *Cx. quinquefasciatus*, *P. humanus*, and *I. scapularis* from VectorBase (<https://www.vectorbase.org/>). ORFs were identified and translated into amino acid sequence using ExPASy software (<http://web.expasy.org/translate/>). The putative N-terminal signal peptides of all predicted olfactory genes were predicted by SignalP 4.0 server version (<http://www.cbs.dtu.dk/services/SignalP/>) with >70 amino acids before conducting the analyses (Petersen et al. 2011). All sequence alignments were performed using ClustalX2.1 (Thompson et al. 1997). Phylogenetic trees were constructed by the neighbor-joining method with Jones–Taylor–Thornton amino acid substitution model as implemented in MEGA6.05 software and then edited in the iTOL web server (<http://itol.embl.de/>; Letunic and Bork 2007). Bootstrap support of tree branches was assessed by resampling amino acid positions 1,000 times.

Results

Identification of Chemoreceptor Genes Superfamily From *T. putrescentiae* Transcriptome

The unigenes related to the chemoreceptor genes superfamily were identified by keyword search of the seven databases’ annotation. We identified distinct unigenes encoding putative seven ORs, one unigene encoding GR, and five unigenes encoding IRs. We named TputOR1-7, TputGR1, and TputIR1-5 for these gene families as following the nomenclature established for *T. putrescentiae* (Zhou et al. 2010). Three of five predicted sequences were full-length IR genes, and four of seven predicted sequences were full-length OR genes with seven transmembrane helices encoding proteins with >428 amino acids (Supp Table 2 [online only]). All predicted ORs were found to belong to the large family of GPCRs. No ORs and Coreceptor (Orco) in *T. putrescentiae* and *I. scapularis* were identified using the keywords “odorant receptors” or “olfactory receptors” in this study. At the same time, we identified the genes of chemoreceptor superfamily in some species (1 OR gene, 15 IR genes, and 23 GR genes in *I. scapularis*, and 9 OR genes, 6 IR genes, and 4 GR genes in *P. humanus*) from their genome (Supp Table 1 [online only]). It was possible that a number of incompletely annotated genes may be the chemoreceptor superfamily and were not

identified. Presumably, this was due in some cases to lack of sequence conservation among the insect chemoreceptor superfamily.

Sequence Analysis and Phylogenetic Tree Construction

Phylogenetic analysis showed that the evolution of the chemoreceptor genes superfamily was highly dynamic, exhibiting a number of taxa-specific subfamilies. We found that the genetic distances between many chemoreceptor genes of these eight species were too low to be clustered in MEGA6.05 software. So, the number of chemoreceptor genes superfamily used in our study was lower than those reported by previous studies. Unlike the IRs family, ORs and GRs families have several branch-specific expansions, mosquito and insect vectors expansions, and almost no groups of orthologous genes shared across Arthropoda in our study (Figs. 1 and 2). Furthermore, short branch lengths were observed between many ORs within one species (Fig. 1).

Two-hundred and thirty sequences of ORs were clustered into different groups, insect vectors, and many are specific to the mosquitoes’ lineages. AegOR7, DmelOR83b, AgamOR83b (also named as AgamOR7), CquiOR7, BmorOR2, and PhumOR8 displayed high primary amino acid conservation with others; these sequences have been reported to be OR83b family receptors, also known as Oroc proteins (Meijerink et al. 2001, Melo et al. 2004, Xia and Zwiebel 2006, Wanner et al. 2007; Fig. 3). Six ORs of *B. mori*, CquiOR9a, and TputOR3 were grouped together in a single lineage; these ORs, for example, BmorOR3–7 and BmorOR9, were reported to be sex pheromone receptors (Wanner et al. 2007). Two ORs of *T. putrescentiae*, TputOR1 and TputOR4, were grouped with IscaOR1, BmorOR10, 23, and CquiOR13aB in a single lineage. TputOR2 formed a lineage with other four ORs (AgamOR22, AgamOR24, BmorOR22, and BmorOR24; Fig. 1). AegOR4 were most closely related to AegOR6 and AgamOR6, which had been found to recognize a component of human body odor (McBride et al. 2014).

Only one GR of *T. putrescentiae*, TputGR1, had a low homology with other GRs in other species and clustered into the subgroup of CO₂ receptors (including BmorGR11, BmorGR24, AgamGR22, AgamGR23, AgamGR24, DmelGR63a, AegGR1–3, and CquiGR1–3; Kwon et al. 2007, Wanner and Robertson 2008). Moreover, the GRs from other species are 33 sugar receptors in flying insects (DmelGR5a, 61a, 64a–d, and 64f; BmorGR8 and 64a; AgamGR14–18 and 20–21; AegGR5–6 and 8–10; and CquiGR7a, 5–15, and 17; Kwon et al. 2007, Kent et al. 2008). No orthologs were discovered for 21 GRs of *I. scapularis* with others, they clustered themselves together into two subgroups (Fig. 2). The expansion and diversification of *I. scapularis* GRs reflected the different evolutionary history in tick chemosensory receptors.

Within the IRs family, four TputIRs were clustered into two subgroups, TputIR1, 3, and 4 were grouped with IscaIR1 into IR8a/25a lineage (including DmelIR8a, 25a; AgamIR8a, 25a; AegIR2–5; PhumIR14, 15; and BmorIR5), and TputIR2 was grouped with DmelIR84a, which was accompanied by a significant odor-specific IR (Fig. 3; Abuin et al. 2011). We observed that some subgroups were present across almost tested Arthropoda species, including Diptera, Phthiraptera, and Acari (Fig. 3).

Discussion

In this study, we have identified a data set of seven ORs, one GR, and five IRs from the transcriptome sequencing of *T. putrescentiae*. Our approach has been proved to be successful in identifying low-expressing olfactory genes in a nonmodel species without genome

sequences. Previous research suggests that several olfactory gene families are identified in the tick *I. scapularis*, 1 OR, 1 GR, and 10 IRs (Van Zee et al. 2007, Vieira and Rozas 2011), while we identified other 21 GRs and 4 IRs. We found that the ORs of *T. putrescentiae* were characterized into the seven-transmembrane domain GPCRs superfamily. Moreover, no ORs and Orco (OR83b family receptors) were identified in this mite and *I. scapularis*. One proposed hypothesis has been that Orco and ORs of the insect type adapt to this terrestrial lifestyle (Robertson et al. 2003, Krång et al. 2012). From the evolution of ORs in different ancient insect orders, including Archaeognatha (jumping bristletails), Zygentoma (silverfishes and firebrats), and Phasmatodea (leaf and stick insects), Missbach et al. believed 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 (Missbach et al. 2014). In contrast to other receptor repertoires, IRs represent a highly divergent subfamily that is present across the Arthropoda, which was in agreement with the report of Croset et al. (2010). IRs are expressed in chemosensory neurons that do not express known insect chemosensory receptors, the ORs and GRs. Moreover, the IRs may reveal important evolutionary links in chemical detection mechanisms in diverse types of cells across prokaryotes and eukaryotes (Benton et al. 2009).

We present a characterization of the chemoreceptor genes superfamily, a superfamily of ligand-binding domains in *T. putrescentiae*. These findings will help to reverse chemical ecology-based approaches aimed at the development of better attractants by using chemosensory receptors as molecular targets.

Supplementary Data

Supplementary data are available at *Journal of Medical Entomology* online.

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