Comparative morphological and transcriptomic analyses reveal chemosensory genes in the poultry red mite, *Dermanyssus gallinae*

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Supplementary Materials-1

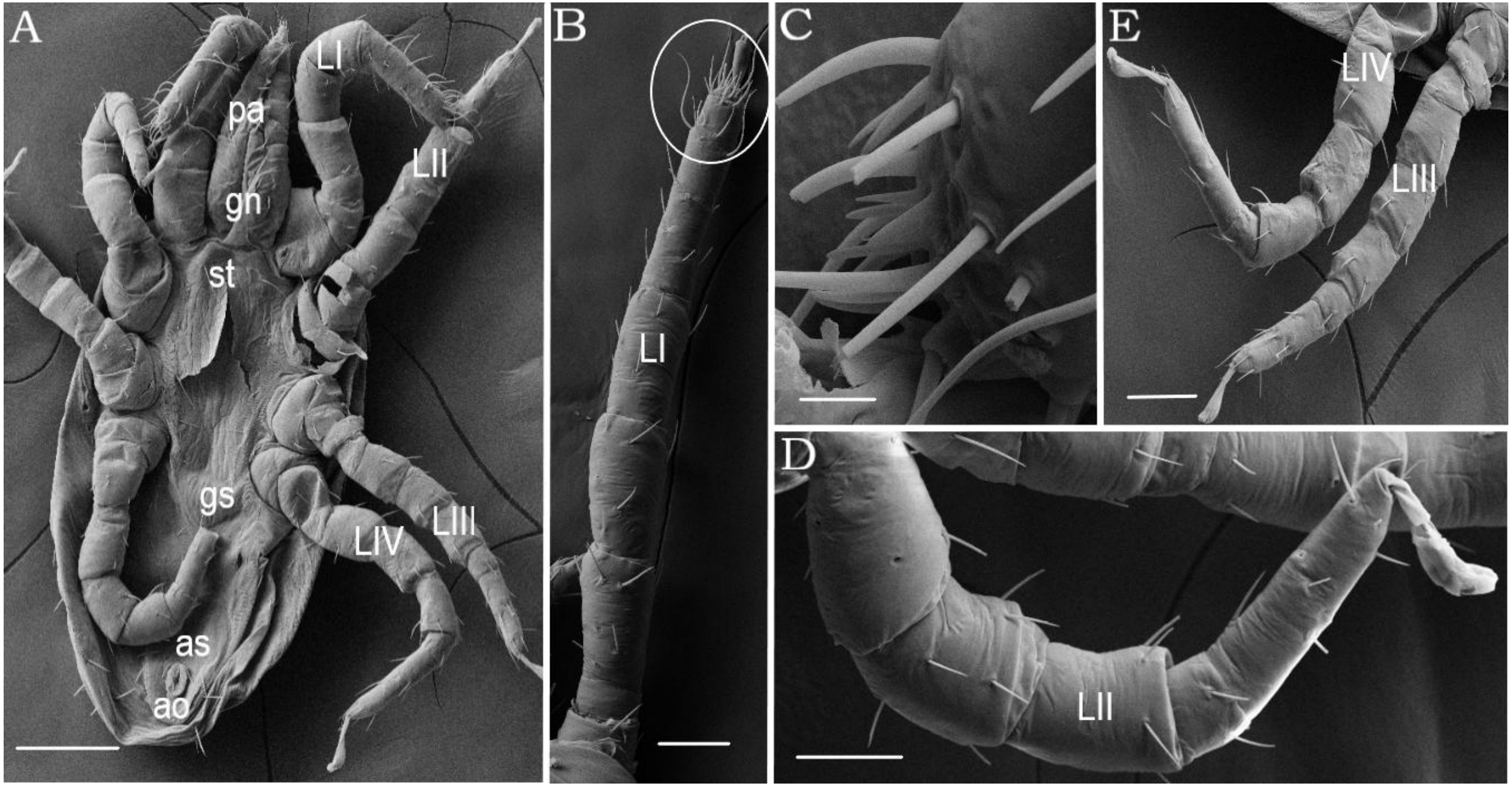

A
LI
pa
LII
gn
st
gs
LIV
LIII
as
ao
B
LI
C
E
LIV
LIII
D
LII

Figure S1. Scanning electron micrographs of PRM. A Ventral view of an adult PRM. B The first leg (LI), indicating the distal sensilla-rich structure (circle). C Different types of sensilla on the distal-most tarsomere (DT-I) of LI. D The second leg (LII). E The third leg (LIII) and fourth leg (LIV). Different types of sensilla are not discriminated in this figure because of low magnifications. *Scale-bars*: A, 100 µm B, D, E, F, 20 µm, C, 2 µm. *Abbreviations*: st, sternal plate; gn, gnathostoma; as, anal shield; ao, anal opening; ch, chelicera; Pa, pedipalp; (LI–IV), leg; gs, genitoventral shield

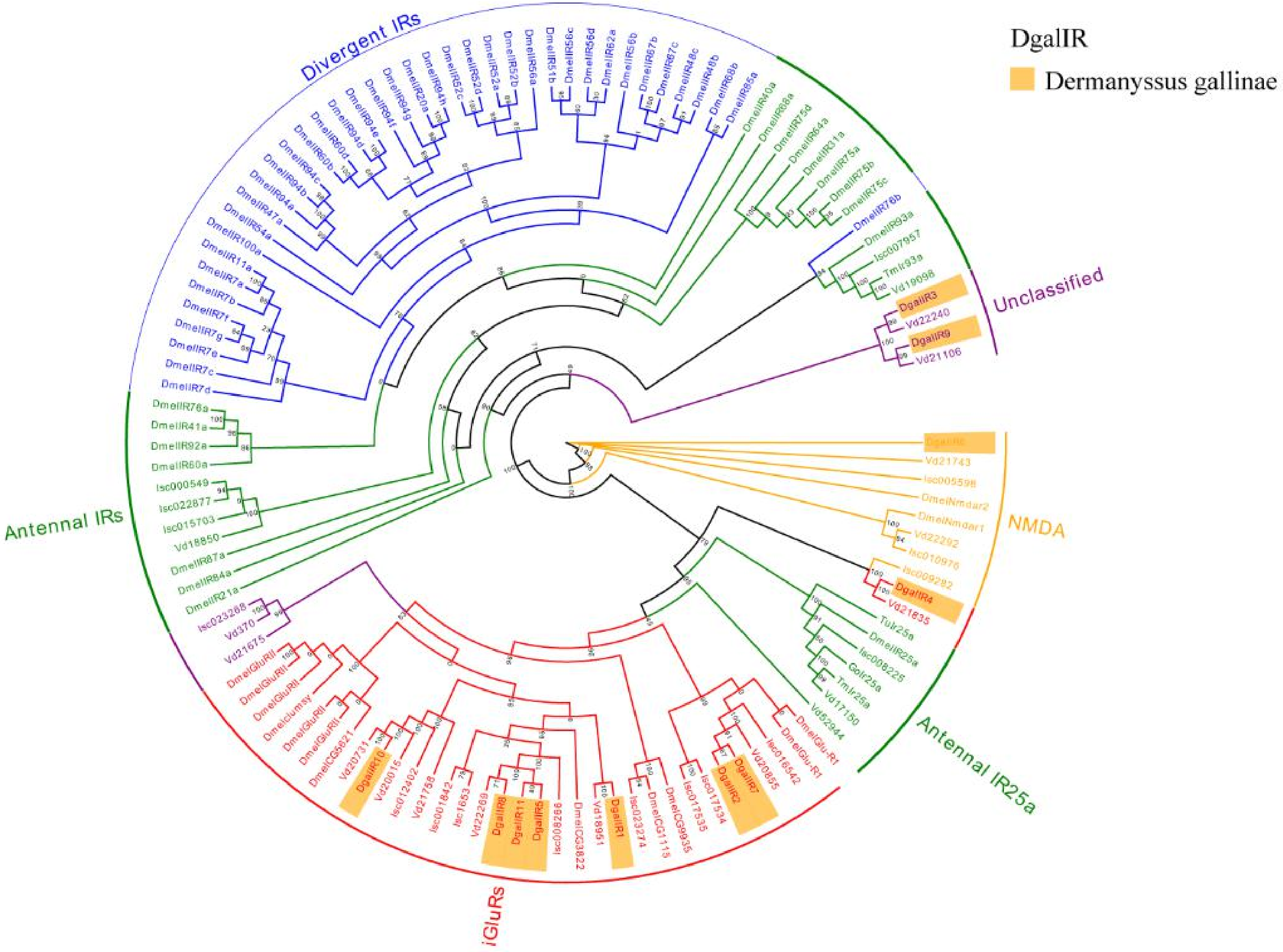

DgalIR
🟧 Dermanyssus gallinae
Divergent IRs
Unclassified
NMDA
Antennal IR25a
iGluRs
Antennal IRs
DmelIR7d
DmelIR7c
DmelIR7e
DmelIR7g
DmelIR7f
DmelIR7b
DmelIR7a
DmelIR11a
DmelIR100a
DmelIR54a
DmelIR47a
DmelIR94a
DmelIR94b
DmelIR94c
DmelIR60b
DmelIR60d
DmelIR94d
DmelIR94e
DmelIR94f
DmelIR94g
DmelIR20a
DmelIR94h
DmelIR52c
DmelIR52d
DmelIR52a
DmelIR52b
DmelIR56a
DmelIR51b
DmelIR56c
DmelIR56d
DmelIR62a
DmelIR56b
DmelIR67b
DmelIR67c
DmelIR48c
DmelIR48b
DmelIR68b
DmelIR85a
DmelIR40a
DmelIR68a
DmelIR75d
DmelIR64a
DmelIR31a
DmelIR75a
DmelIR75b
DmelIR75c
DmelIR76b
DmelIR93a
Isc007957
TmIr93a
Vd19098
DgalIR3
Vd22240
DgalIR9
Vd21106
DgalIR6
Vd21743
Isc005598
DmelNmdar2
DmelNmdar1
Vd22292
Isc010976
Isc009282
DgalIR4
Vd21835
TuIr25a
DmelIR25a
Isc008225
GoIr25a
TmIr25a
Vd17150
Vd52944
DmelGlu-RI
DmelGlu-R1
Isc016542
Vd20855
DgalIR7
DgalIR2
Isc017534
Isc017535
DmelCG9935
DmelCG11155
Isc023274
DgalIR1
Vd18951
DmelCG3822
Isc008286
DgalIR5
DgalIR11
DgalIR8
Vd22269
Isc1653
Isc001842
Vd21758
Isc012402
Vd26015
DgalIR10
Vd20731
DmelCG5621
DmelGluRII
DmelGluRII
Dmelclumsy
DmelGluRII
DmelGluRII
Vd21675
Vd370
Isc023268
DmelIR21a
DmelIR84a
DmelIR87a
Vd18850
Isc015703
Isc022877
Isc000549
DmelIR60a
DmelIR92a
DmelIR41a
DmelIR76a

Figure S2. Phylogenetic tree of putative *D. gallinae* iGluRs and IRs along with insect and non-insect arthropods iGluRs and IRs. To further distinguish putative IR gene families from the transcriptome of *D. gallinae*, all of the IRs in our transcriptomes were aligned with IRs from *Varroa destructor*, *Tetranychus urticae*, *Galendromus occidentalis*, *Ixodes scapularis*, *Tropilaelaps mercedesae* and *Drosophila melanogaster* by MAFFT. The *D. gallinae* genes are shown in light orange. The amino acid sequences and a comprehensive list of acronyms are listed in Supplementary file 2, Table S2.

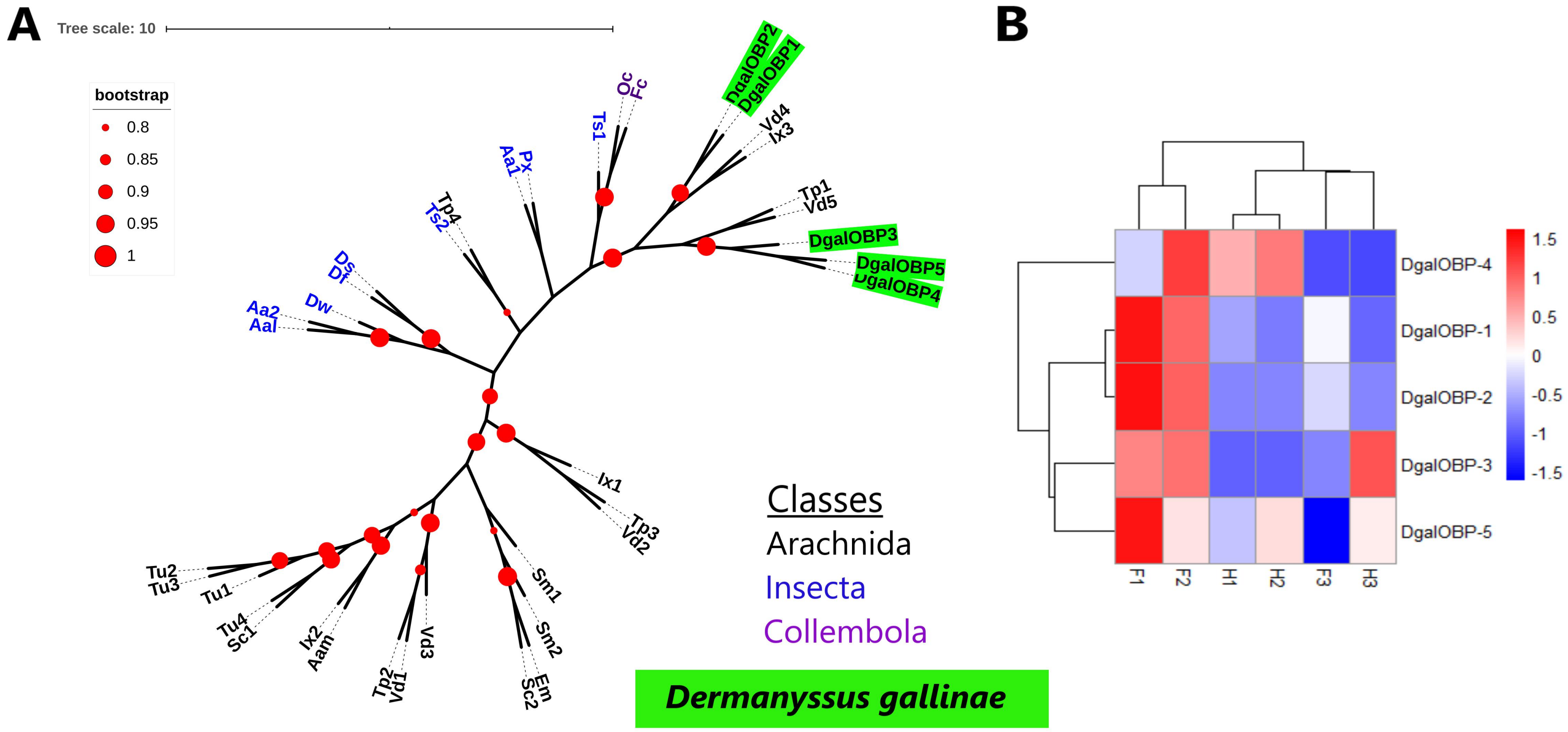

A
Tree scale: 10
bootstrap
0.8
0.85
0.9
0.95
1
Oc
Fc
Ts1
Px
Aa1
Tp4
Ts2
Ds
Df
Dw
Aa2
Aal
DgalOBP2
DgalOBP1
Vd4
Ix3
Tp1
Vd5
DgalOBP3
DgalOBP5
DgalOBP4
Ix1
Tp3
Vd2
Sm1
Sm2
Em
Sc2
Vd3
Tp2
Vd1
Ix2
Aam
Tu4
Sc1
Tu1
Tu2
Tu3
Classes
Arachnida
Insecta
Collembola
Dermanyssus gallinae
B
DgalOBP-4
DgalOBP-1
DgalOBP-2
DgalOBP-3
DgalOBP-5
F1
F2
H1
H2
F3
H3
1.5
1
0.5
0
-0.5
-1
-1.5

Figure S3 Phylogenetic unrooted tree of odorant-binding protein (OBPs) and hierarchical clustering analysis of the differentially expressed genes (DEGs). (A) Phylogenetic unrooted tree of odorant-binding protein (OBPs). Included are OBPs from *D. gallinae* (Dg: 5), *V. destructor* (Vd: 5), *I. scapularis* (Ix: 3), *A. americanum* (Aam: 1), *T. mercedesae* (Tp: 4), *Stegodyphus mimosarum* (Sm: 2), *Tetranychus urticae* (Tu: 4), *Sarcoptes scabiei* (Sc: 2), *Trichomalopsis sarcophagae* (Ts: 2), *Aedes albopictus* (Aal: 1), *Aedes aegypti* (Aa: 2), *Papilio xuthus* (Px: 1), *Folsomia candida* (Fc: 1), *D. sechellia* (Ds: 1), *D. ficusphila* (Df: 1), *Euroglyphus maynei* (Em: 1), *Orchesella cincta* (Oc: 1) and *D. willistoni* (Dw: 1). Leaves are coloured according to the organisms classes: arachnida (black), collembola (purple) and insecta (blue). *Dermanyssus* transcripts are highlighted in green.The amino acid sequences and a comprehensive list of acronyms are listed in S3. (B) Hierarchical clustering analysis of the differentially expressed genes (DEGs). Blue to red colors represent gene expression levels (i.e., FPKM values from −1.5 to 1.5). To clearly describe the DEGs of each olfactory-related family, we performed cluster analysis using a pheatmap package in R software based on FPKM-values, represented as a heatmap.

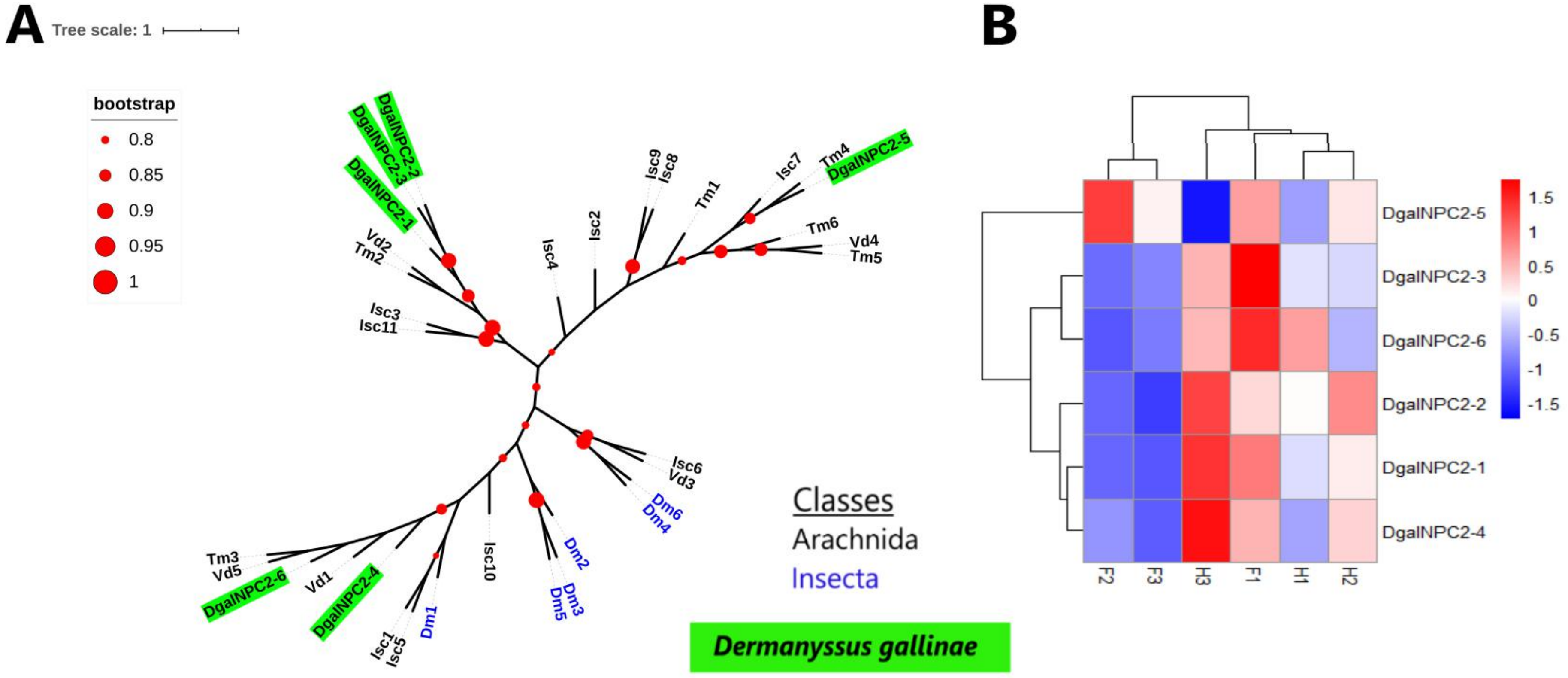
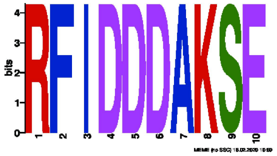


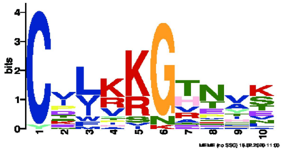
Figure S4. An unrooted phylogenetic tree of NPC2 proteins and hierarchical clustering analysis of the differentially expressed genes (DEGs). (A) An unrooted phylogenetic tree of NPC2 proteins. The evolutionary tree is constructed by aligning the NPC2 sequences of *V. destructor* (Vd:

6), *Dermanyssus gallinae* (Dg: 6), *Drosophila melanogaster* (Dm: 6), *Ixodes scapularis* (Isc: 11) and *Tropilaelaps mercedesae* (Tm: 6). Leaves are colored according to the organisms classes: Insecta (blue) and Arachnida (black). *Dermanyssus* transcripts are highlighted in green. The amino acid sequences and a comprehensive list of acronyms are listed in S5. (B) Hierarchical clustering analysis of the differentially expressed genes (DEGs). Blue to red colors represent gene expression levels (i.e., FPKM values from −1.5 to 1.5). To clearly describe the DEGs of each olfactory-related family, we performed cluster analysis using a pheatmap package in R software based on FPKM-values, represented as a heatmap.

motif 1 1.0e-017

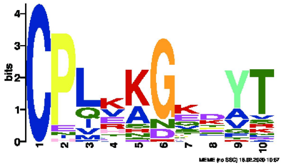


motif 2 1.5e-017

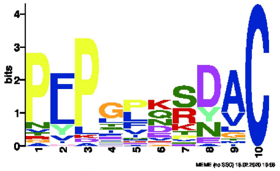


motif 3 2.3e-017

motif 4 3.0e-009



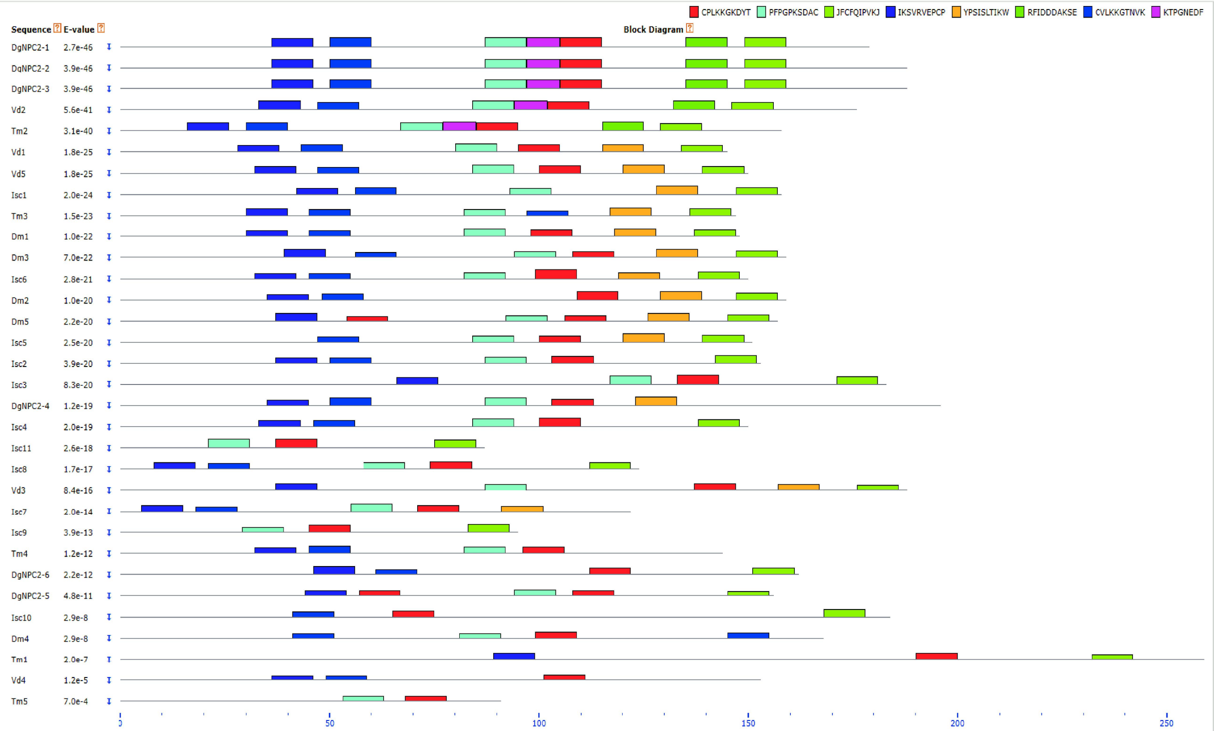
motif 5 5.8e-068



motif 6 7.9e-039

motif 7 3.8e-030

motif 8 1.2e-021



Sequence	E-value
DgNPC2-1	2.7e-46
DgNPC2-2	3.9e-46
DgNPC2-3	3.9e-46
Vd2	5.6e-41
Tm2	3.1e-40
Vd1	1.8e-25
Vd5	1.8e-25
Isc1	2.0e-24
Tm3	1.5e-23
Dm1	1.0e-22
Dm3	7.0e-22
Isc6	2.8e-21
Dm2	1.0e-20
Dm5	2.2e-20
Isc5	2.5e-20
Isc2	3.9e-20
Isc3	8.3e-20
DgNPC2-4	1.2e-19
Isc4	2.0e-19
Isc11	2.6e-18
Isc8	1.7e-17
Vd3	8.4e-16
Isc7	2.0e-14
Isc9	3.9e-13
Tm4	1.2e-12
DgNPC2-6	2.2e-12
DgNPC2-5	4.8e-11
Isc10	2.9e-8
Dm4	2.9e-8
Tm1	2.0e-7
Vd4	1.2e-5
Tm5	7.0e-4

Figure S5. Motif analysis of NPC2 protein from *D. gallinae* and other classes including insecta and arachnida. The parameters setting in this study for motif predictions were: minimum width of motif = 6, maximum width = 10 and maximum number of motifs to find = 8. Motif-based sequence analyses were predicted by using web-based version 5.1.0 of the MEME server. Eight amino acid motifs with various widths and corresponding *e*-values were identified, and the lower part indicates approximate locations of each motif on the protein sequence. The results showed that motif 1 (CPLKKGKDYT) and motif 2 (PFPGPKSDAC) were present in all the NPC2 orthologs except for *I. scapularis* (Isc1), *Tropilaelaps mercedesae* (Tm3,1), *D. melanogaster* (Dm2,4), *D. gallinae* (DgNPC2) and *V. destructor* (Vd4). In addition, the homologous NPC2 from different acari species had similar motif patterns; for example, *D. gallinae* (DgNPC2-1, 2 and 3), *V. destructor* (vd2) and *T. mercedesae* (Tm2) had same motif-pattern as 4-7-2-8-1-6-3. Different motifs are shown by different colored boxes, where small number indicate high conservation. Protein sequences of NPC2 used in this analysis are listed in Supplementary file 2, Table S6.

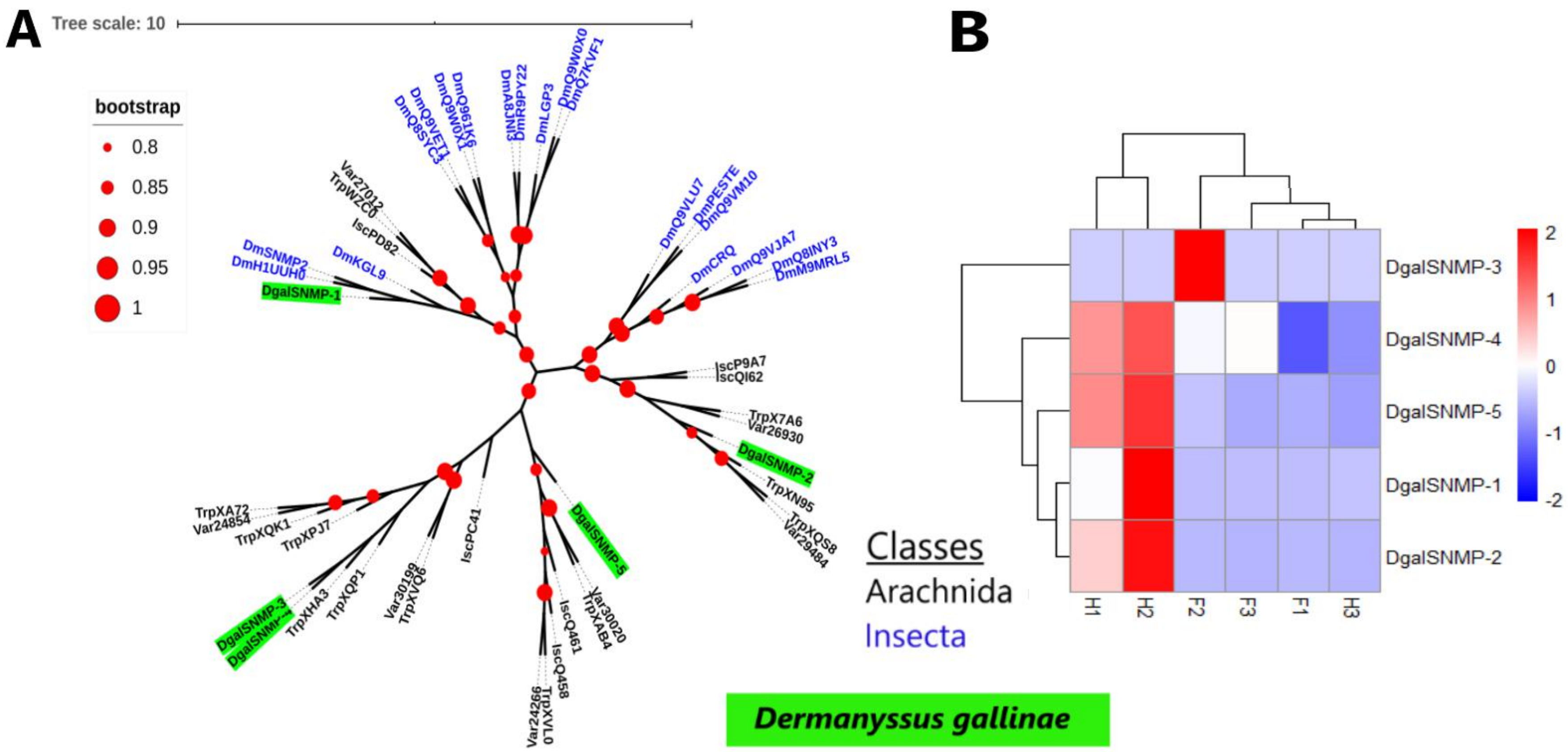


Figure S6. An unrooted phylogenetic tree of SNMP proteins and hierarchical clustering analysis of the differentially expressed genes (DEGs). (a) An unrooted phylogenetic tree of SNMP proteins. The tree is constructed by aligning the SNMP sequences of *V. destructor* (Var: 7), *D. gallinae* (Dg: 5) *T. mercedesae* (Trp: 12), *Ixodes scapularis* (Isc: 6) and *Drosophila melanogaster* (Dm: 19). Leaves are coloured according to the organisms classes: Insecta (blue) and Arachnida (black). *Dermanyssus* transcripts are highlighted in green. (b) Hierarchical clustering analysis of the differentially expressed genes (DEGs). Blue to red colors represent gene expression levels (i.e., FPKM values from −2 to 2). To clearly describe the DEGs of each olfactory-related family, we performed cluster analysis using a pheatmap package in R software based on FPKM-values, represented as a heatmap.

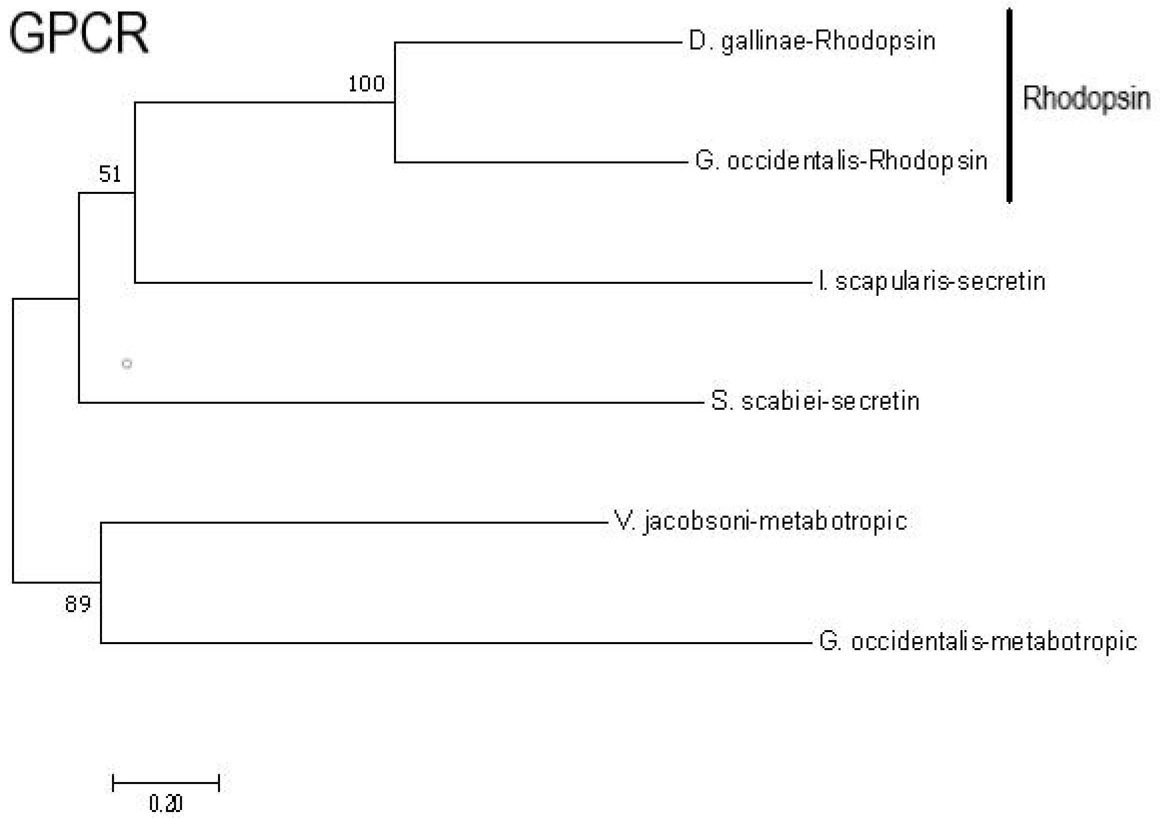


Figure S7. Phylogenetic tree of rhodopsin-like GPCR of *D. gallinae* and other mite species. The amino acid sequences are listed in Supplementary file 2, Table S8.

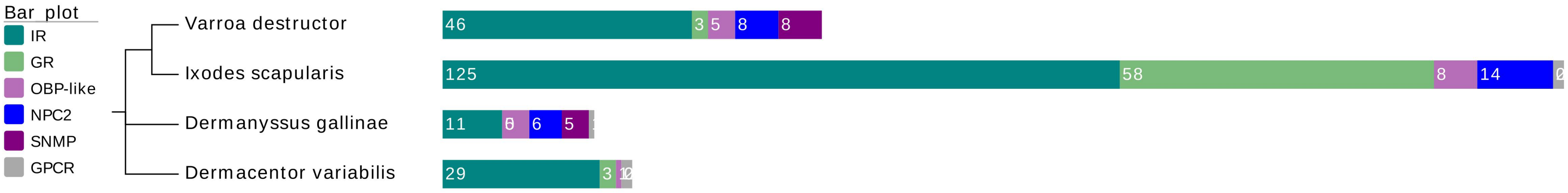


Figure S8. The number of chemosensory genes in different mite and tick species are presented in bar plot. A phylogenetic tree showing evolutionary relationships between various species are illustrated on the left. The number of chemosensory genes are obtained from *V. destructor, I. scapularis, D. variabilis.* The tree image was further viewed and graphically edited using the iTOL online tool with the number of identified chemosensory genes.