# Supplementary Figures

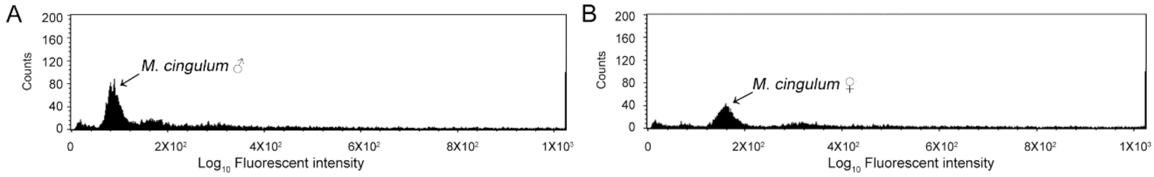

Figure S1. Flow cytometry estimation of the genome size for the *M. cingulum*. A) The Flow cytometry estimation of the male. B) Flow cytometry estimation of the female.

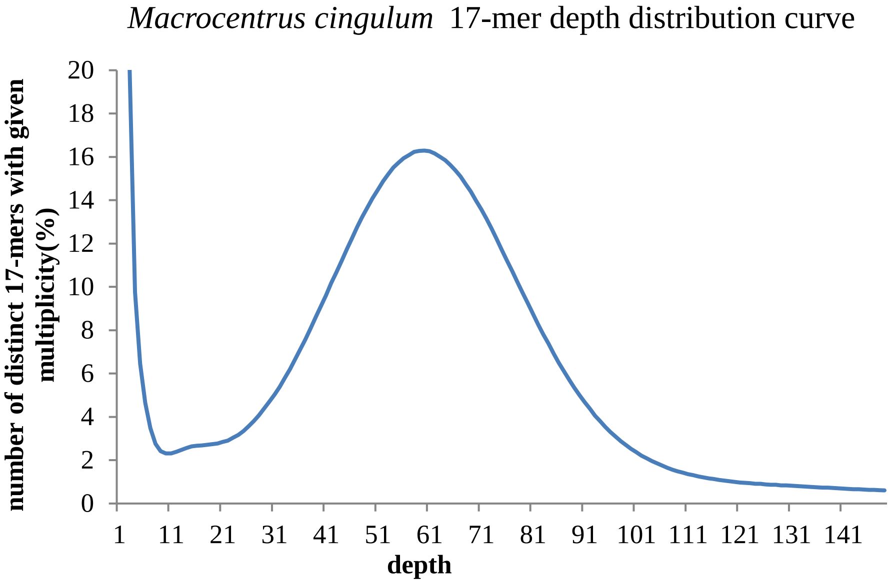

Figure S2. The distribution of 17-mer frequency in *M. cingulum* genome sequencing reads

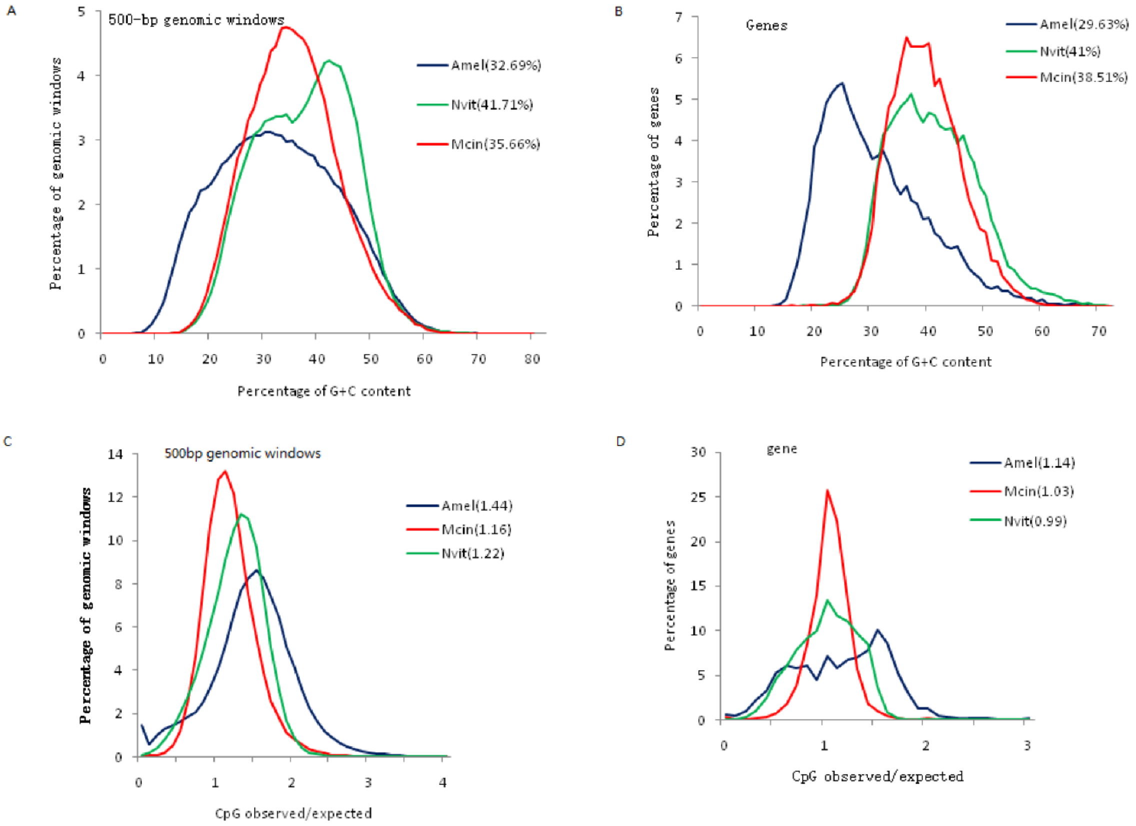

Figure S3. Distribution of GC content, CpG Obs/ExpRatios of *M. cingulum*(Mcin)*, N. vitripennis*(Nvit) and *A. mellifer*(Amel)

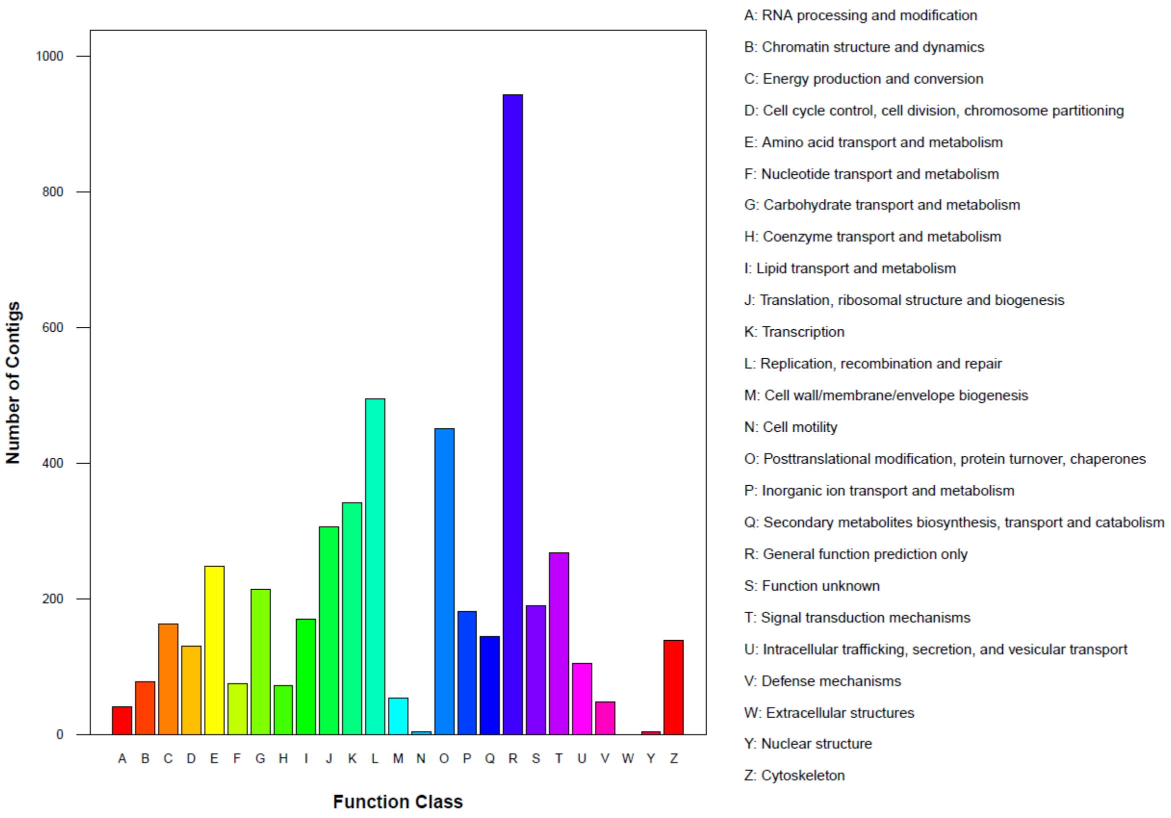

Figure S4. COG function classification of the OGS in *M. cingulum*

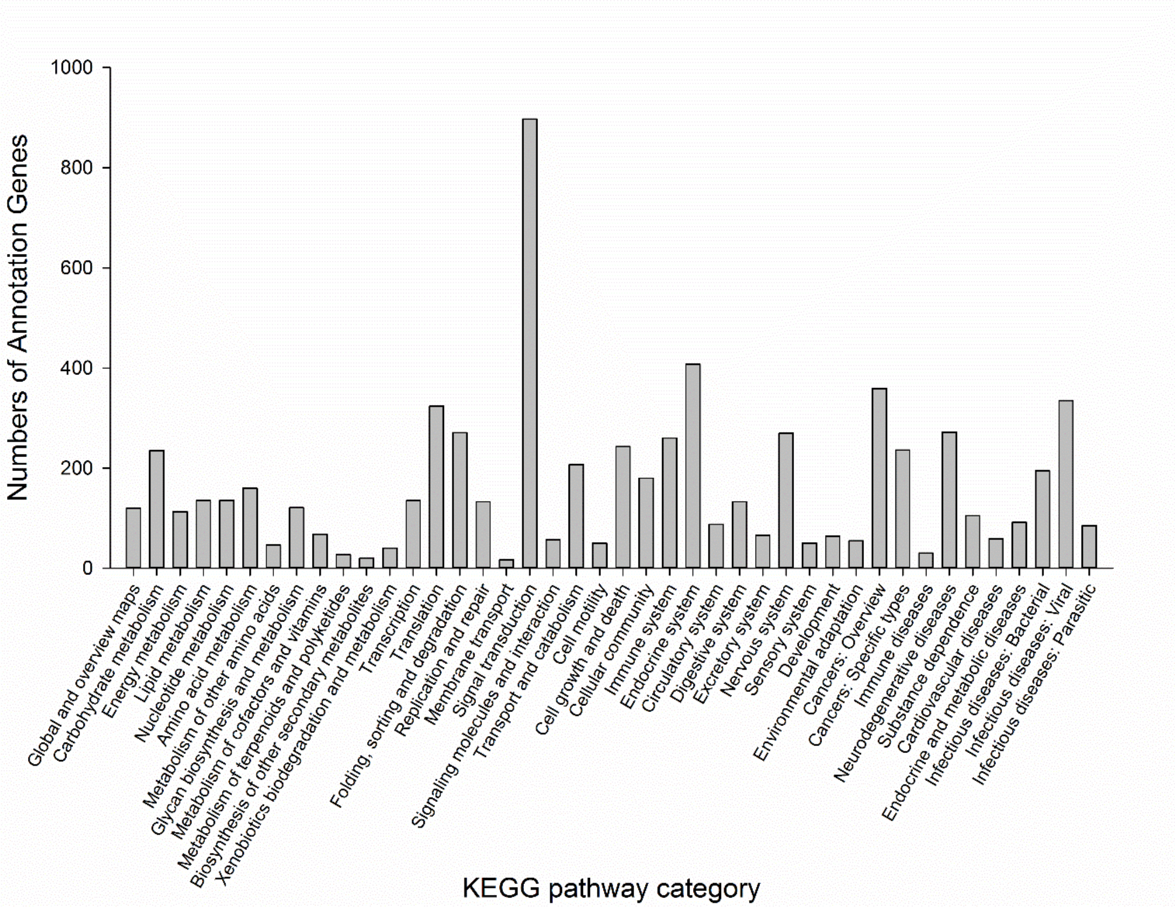

Figure S5. KEGG pathway analysis of the OGS in *M. cingulum*

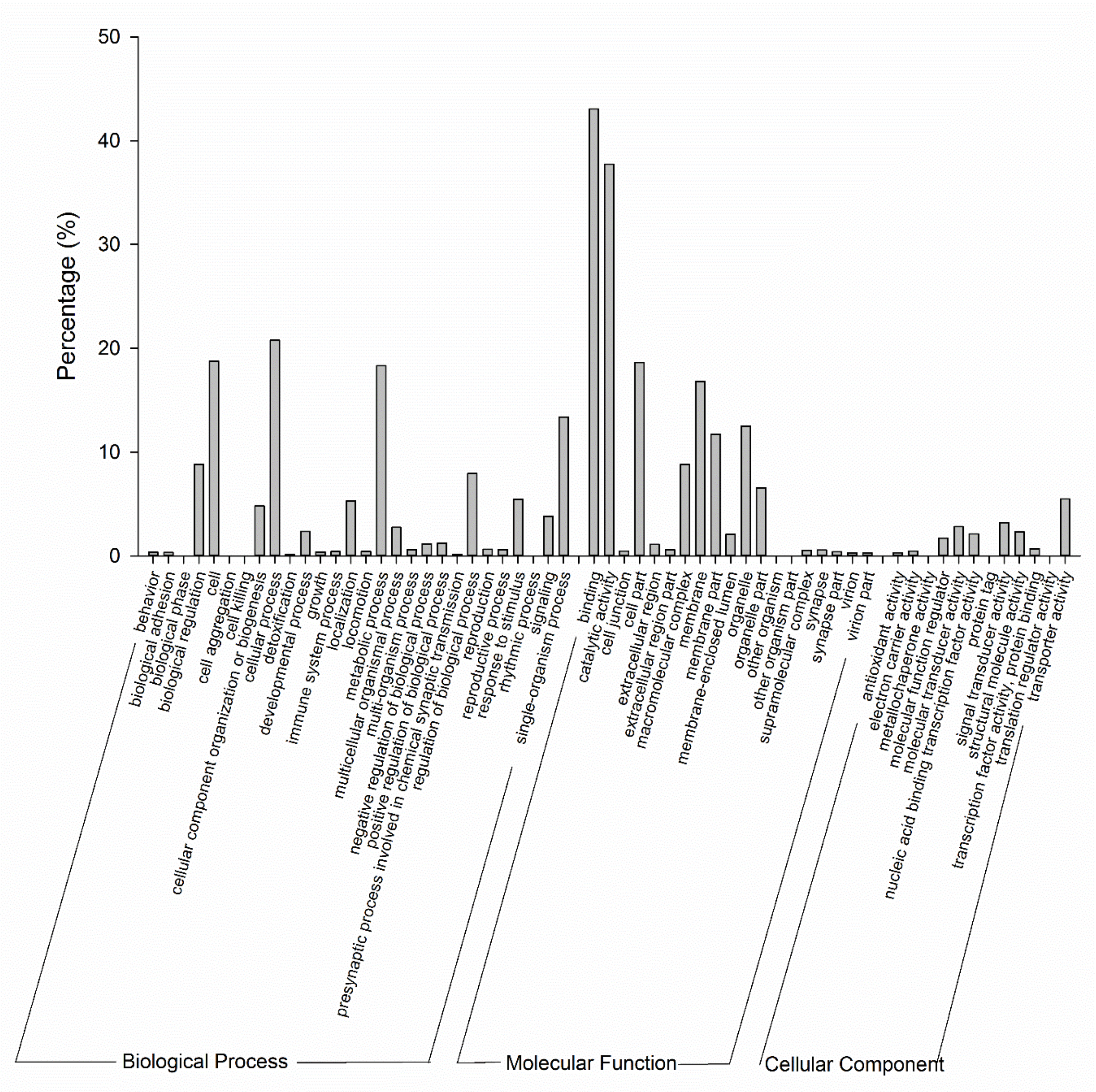

Figure S6. GO classification of the OGS in *M. cingulum*

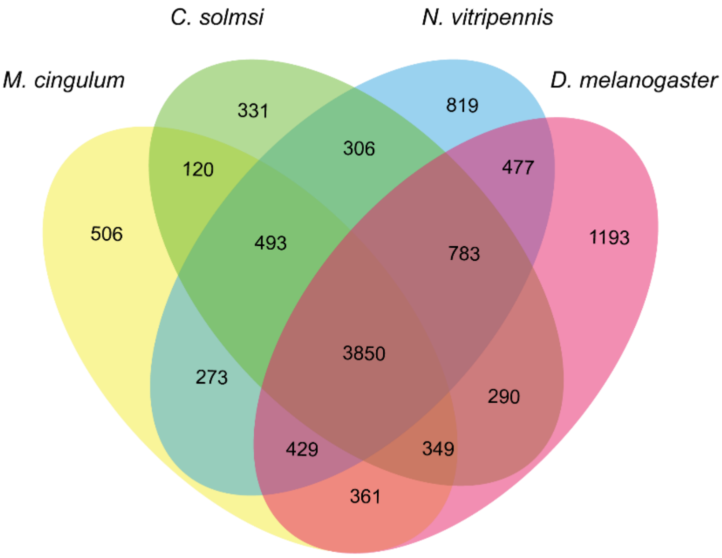

Figure S7. Venn diagram of the homologous protein-coding genes among three wasps (*M. cingulum, C. solmsi, N. vitripennis*) and fruit fly (*D. melanogaster*)

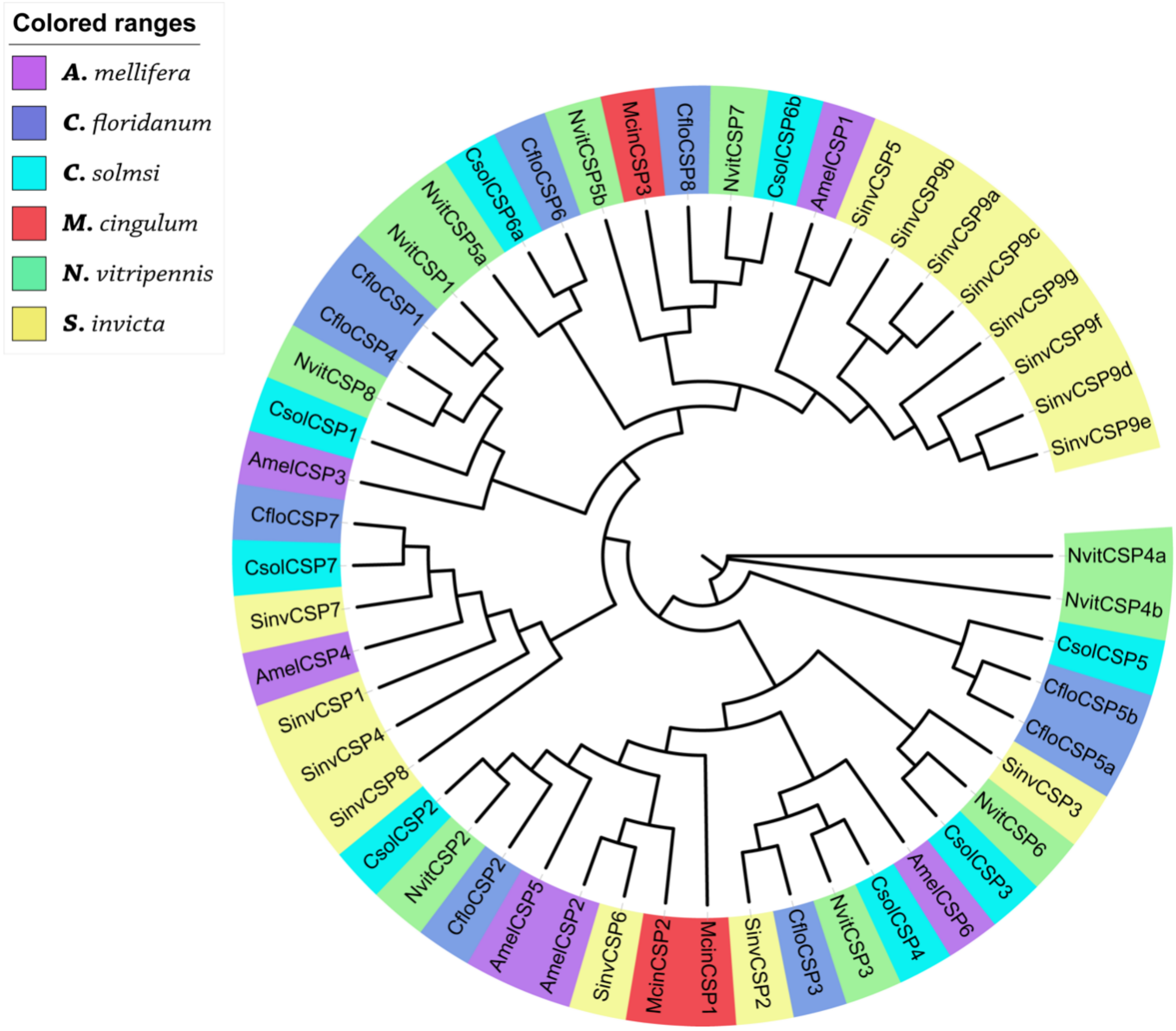

Figure S8. Phylogenetic relationship of CSP proteins from *A. mellifera*, *C. floridanum*, *C. solmsi*, *M. cingulum*, *N.vitripennis*, *S.invicta*

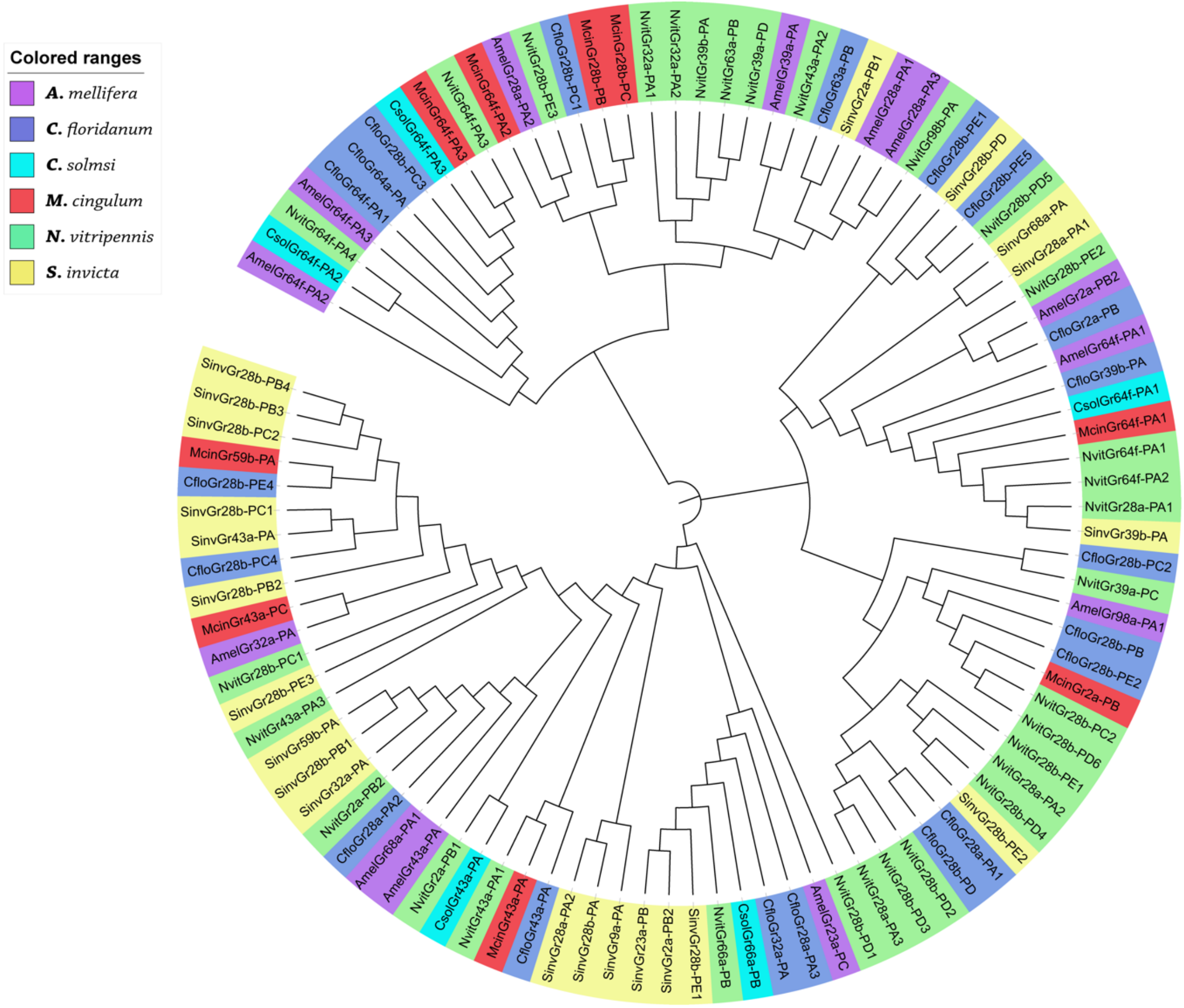

Figure S9. Phylogenetic relationship of GR proteins from *A. mellifera*, *C. floridanum*, *C. solmsi*, *M. cingulum*, *N.vitripennis*, *S.invicta*

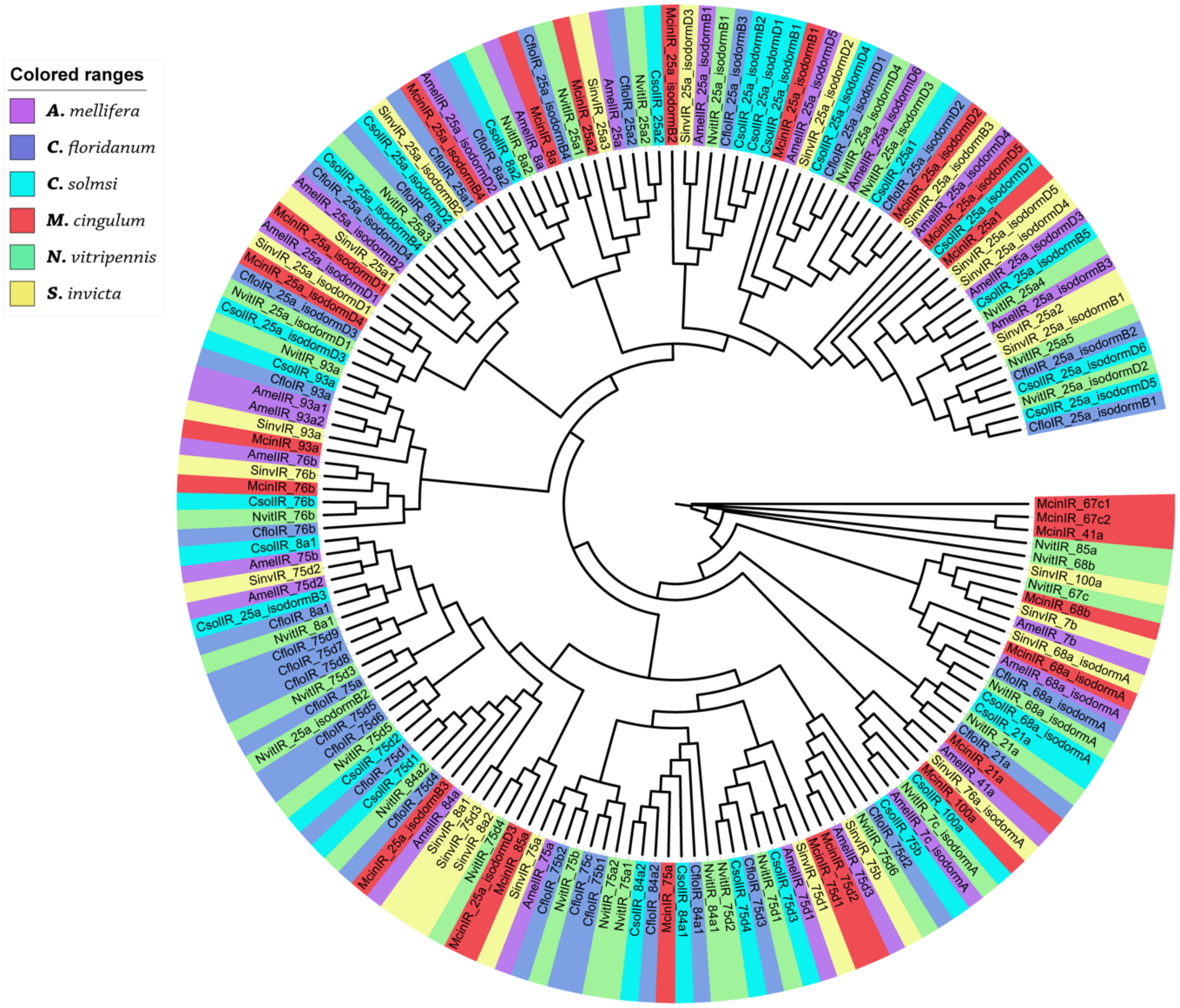

Figure S10. Phylogenetic relationship of IR proteins from *A. mellifera*, *C. floridanum*, *C. solmsi*, *M. cingulum*, *N.vitripennis*, *S.invicta*

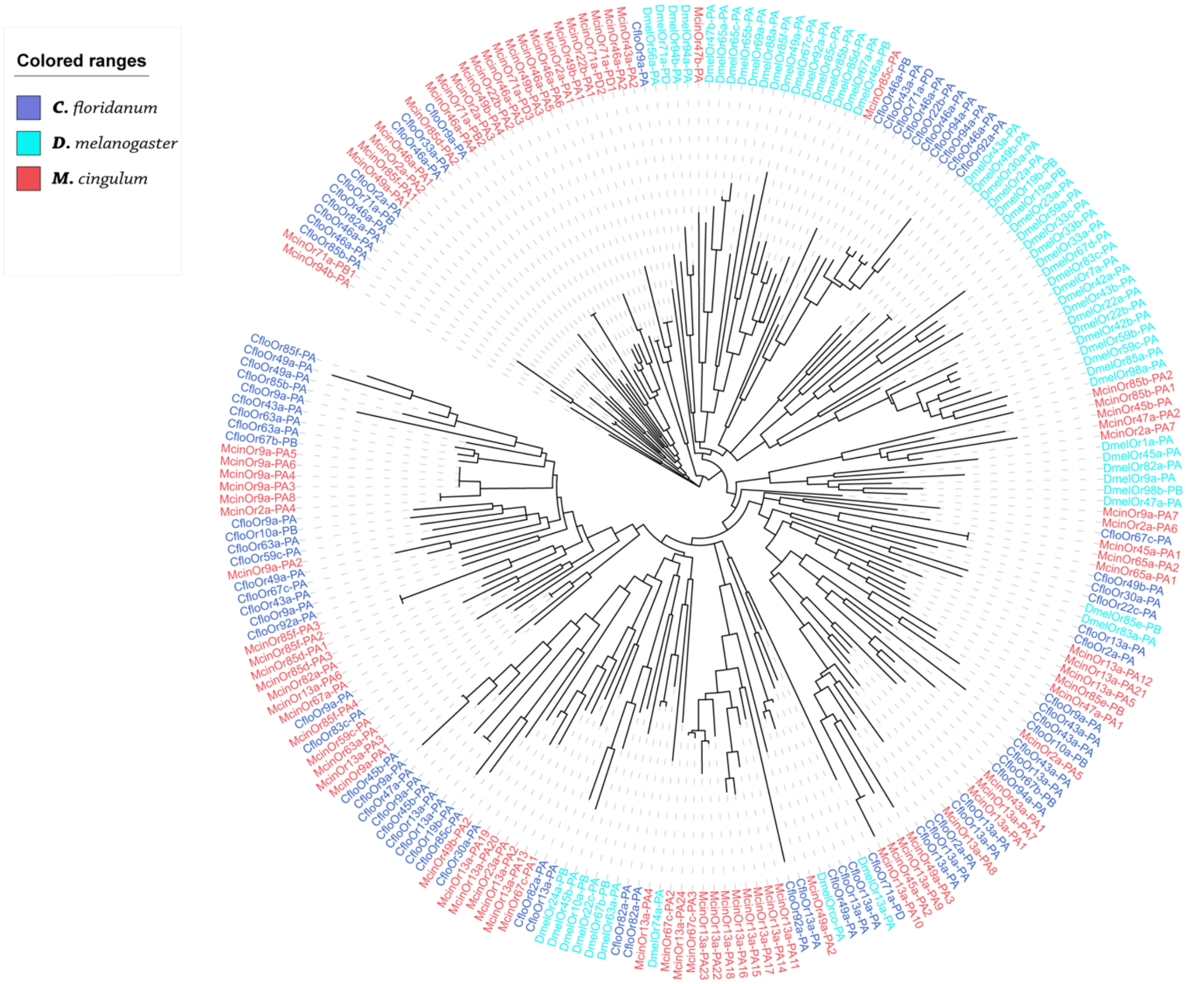

Figure S11. Phylogenetic relationship of OR proteins from *C. floridanum*, *D. melanogaster* and *M. cingulum*

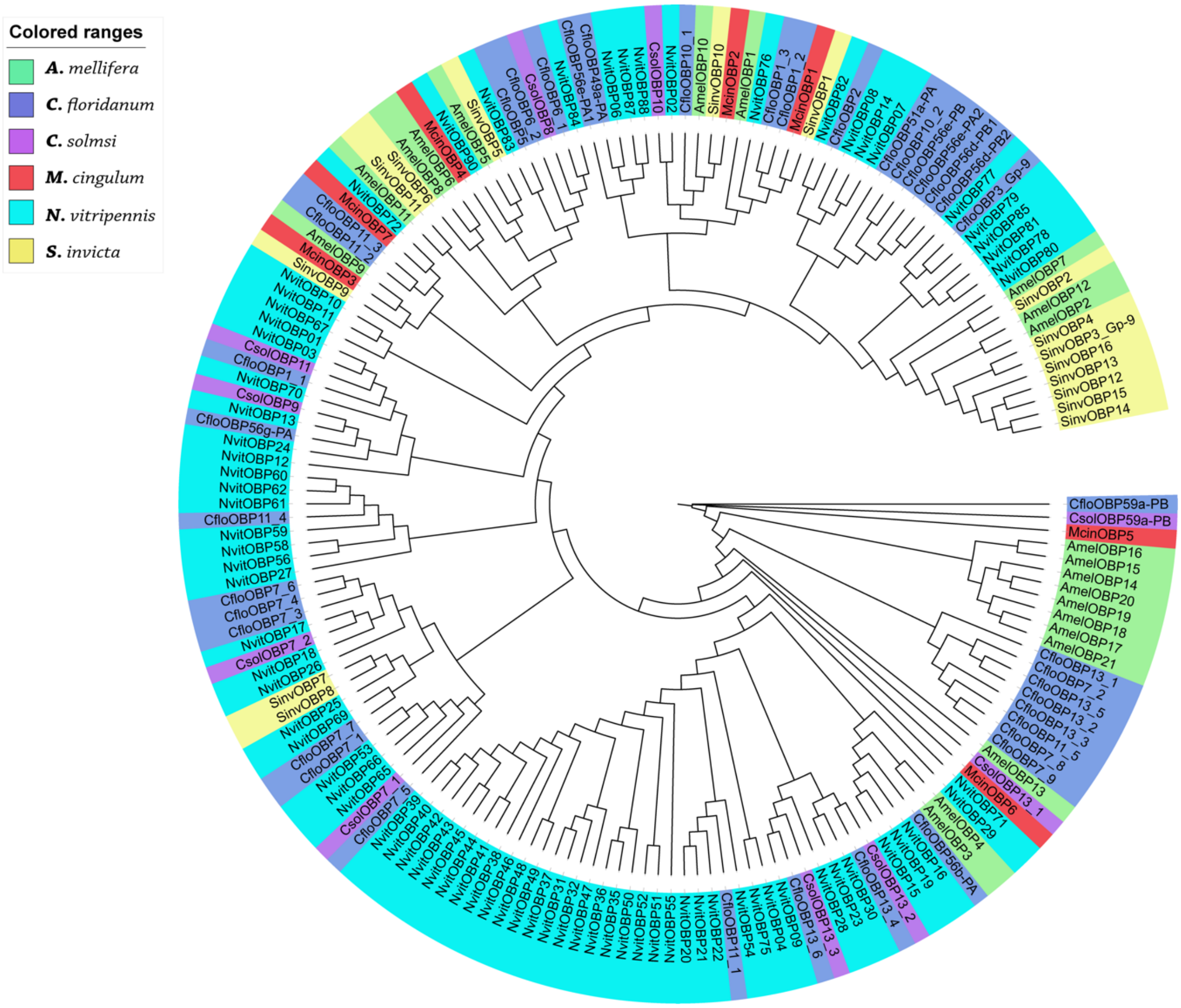

Figure S12. Phylogenetic relationship of OBP proteins from *A. mellifera*, *C. floridanum*, *C. solmsi*, *M. cingulum*, *N.vitripennis*, *S.invicta*

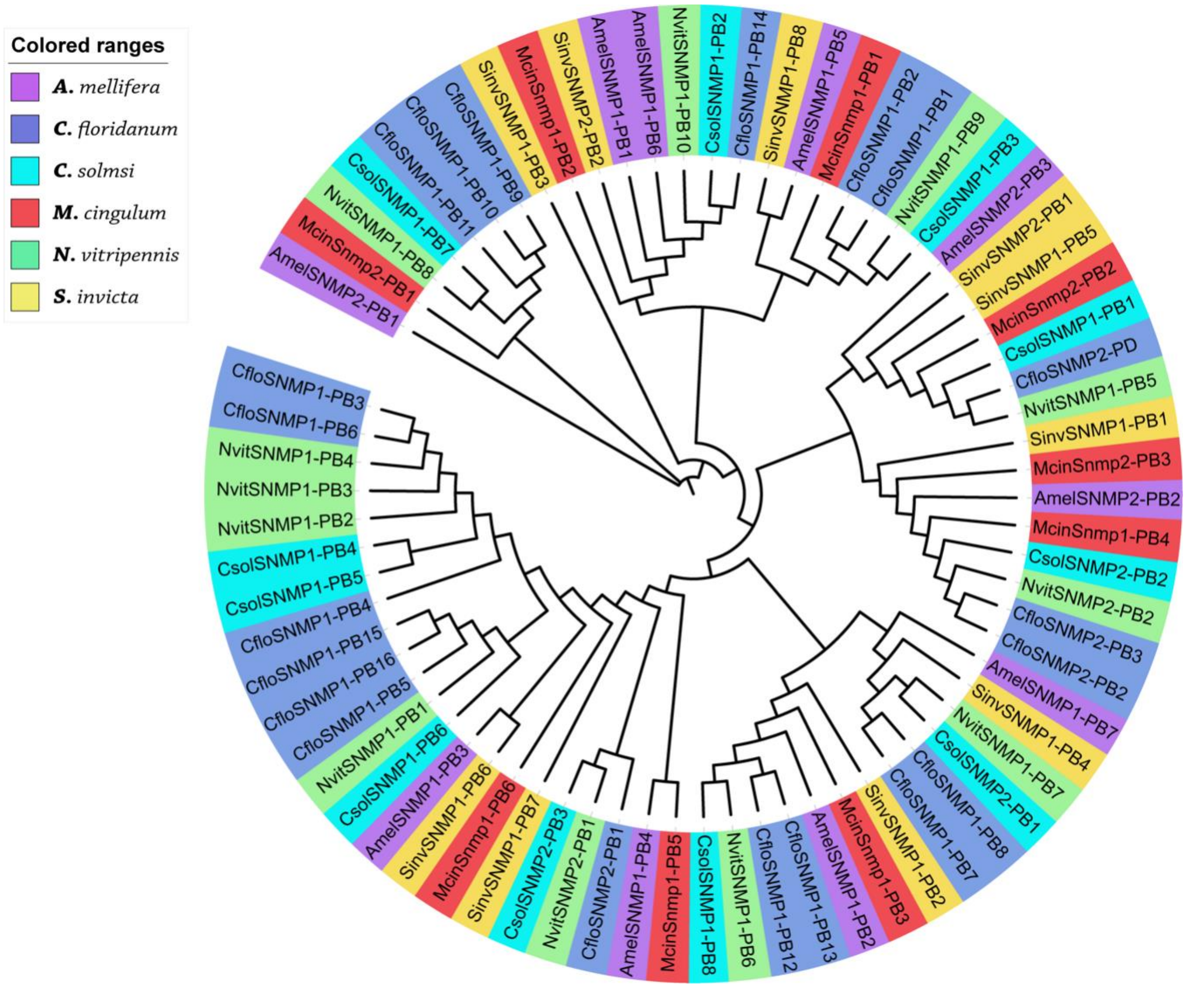

Figure S13. Phylogenetic relationship of SNMP proteins from *A. mellifera*, *C. floridanum*, *C. solmsi*, *M. cingulum*, *N.vitripennis*, *S.invicta*

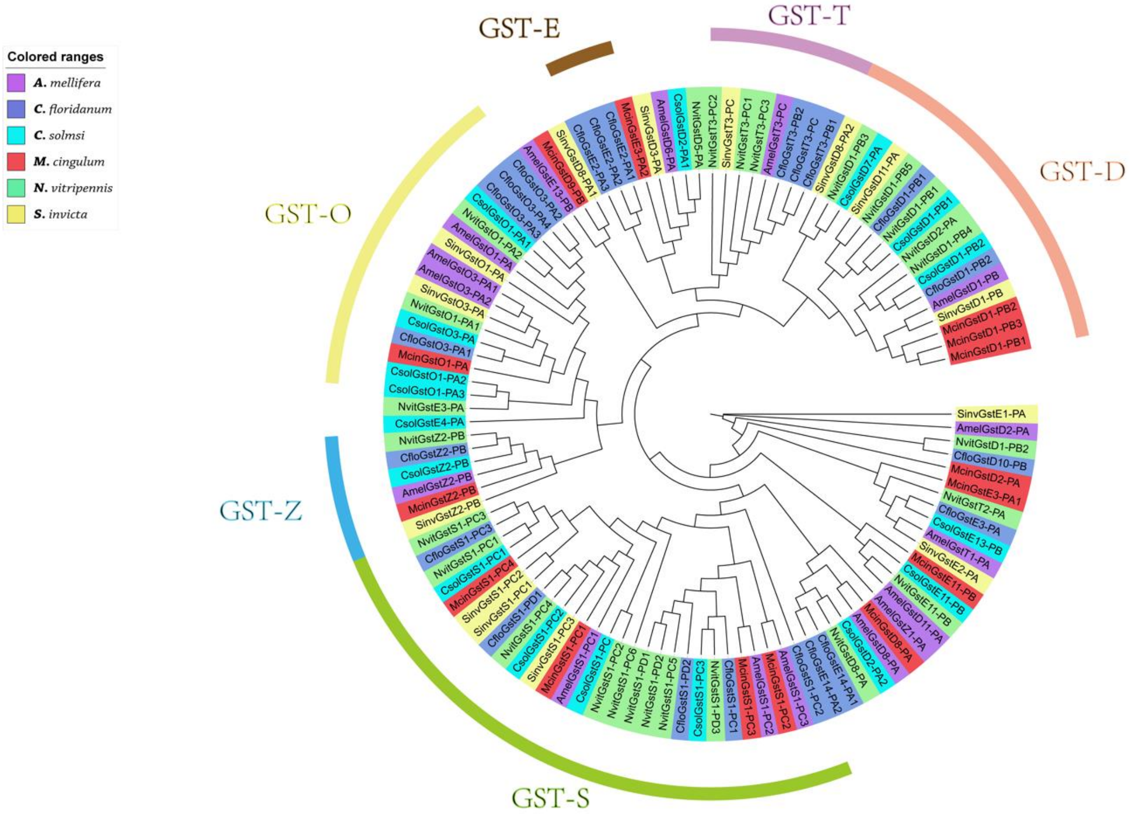

Figure S14. Phylogenetic relationship of GST proteins from *A. mellifera*, *C. floridanum*, *C. solmsi*, *M. cingulum*, *N.vitripennis*, *S.invicta*

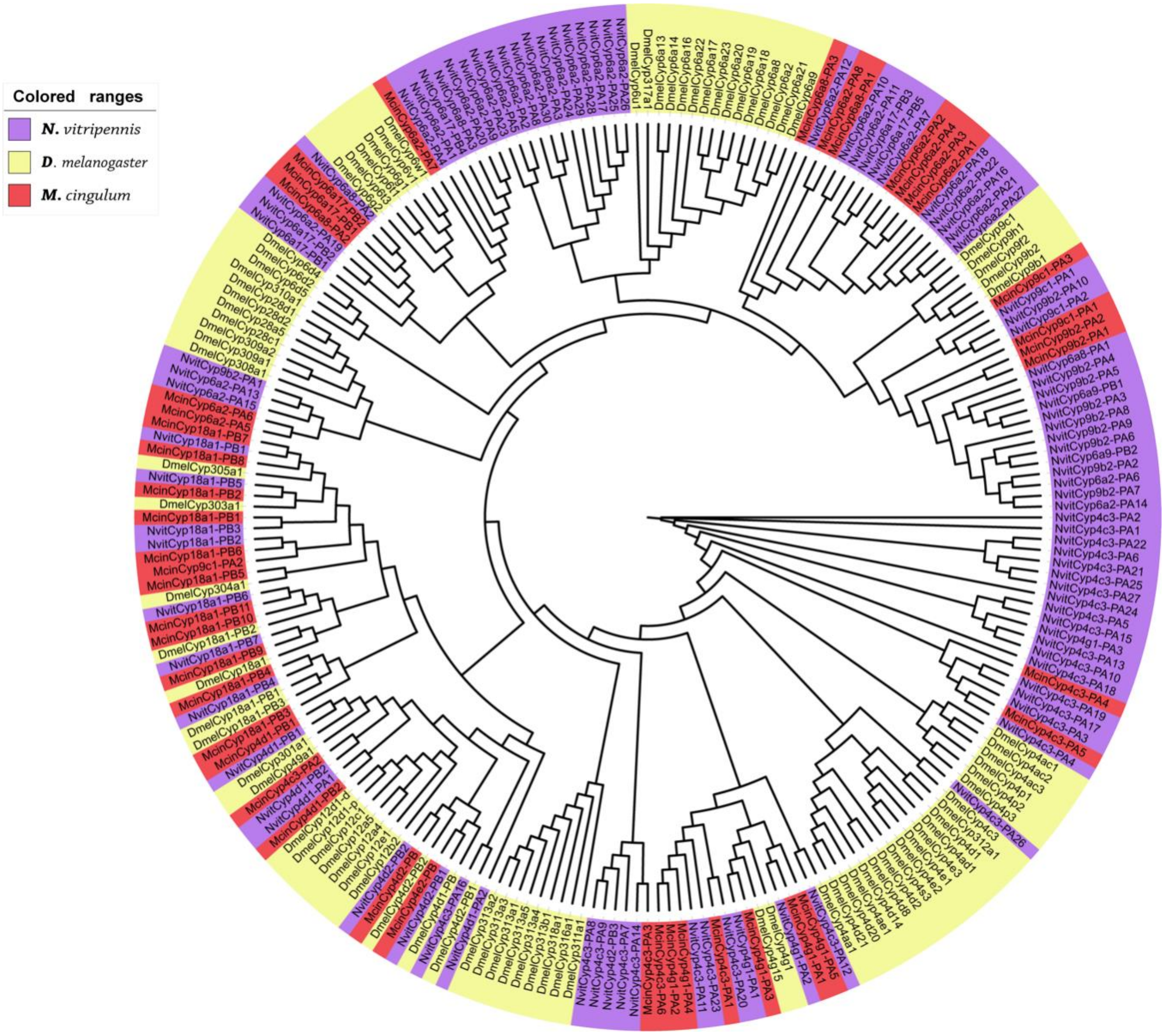

Figure S15. Phylogenetic relationship of P450 proteins from *N. vitripennis*, *D. melanogaster* and *M. cingulum*

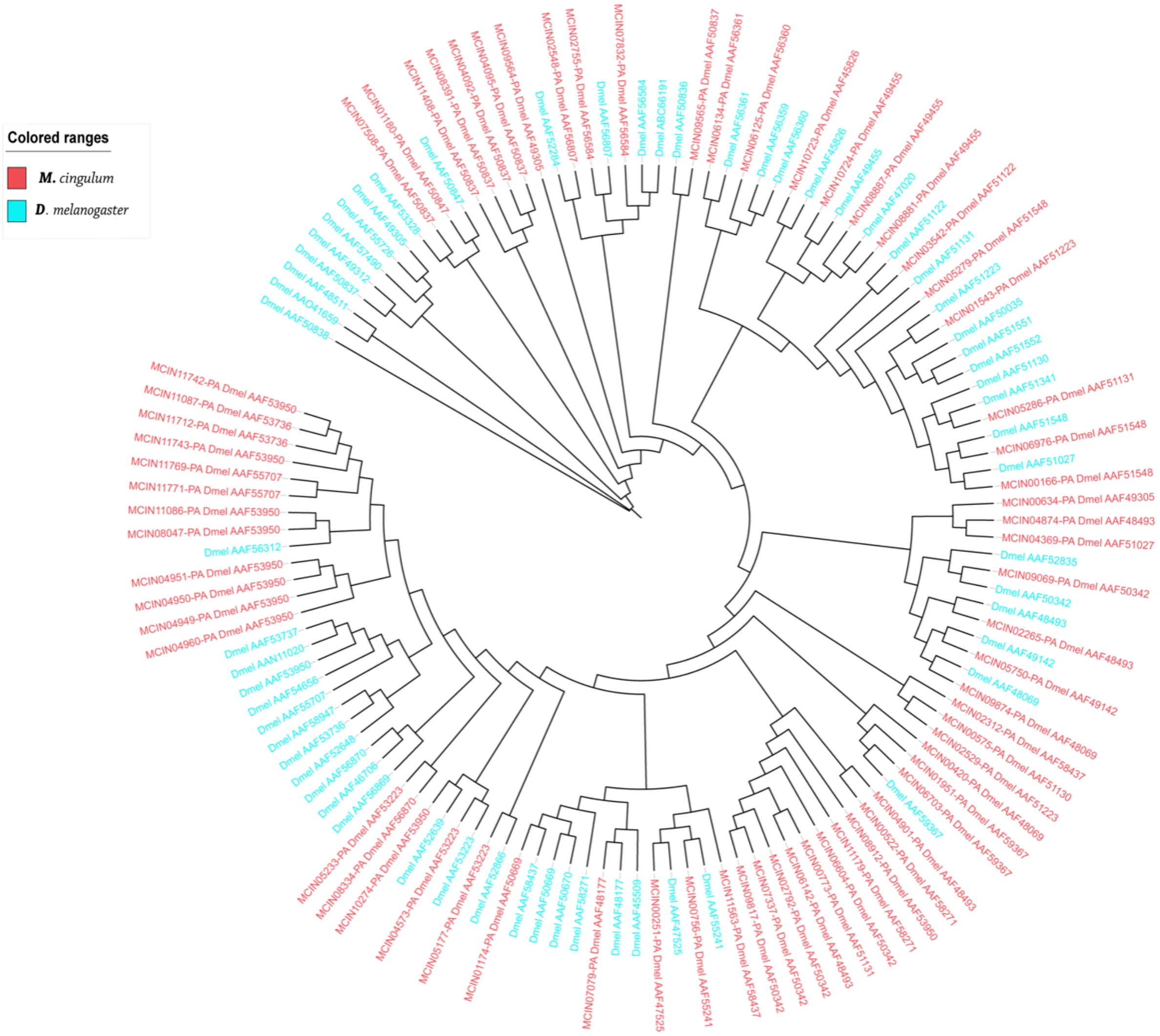

Figure S16. Phylogenetic relationship of ABC proteins from *M. cingulum* and *D. melanogaster*

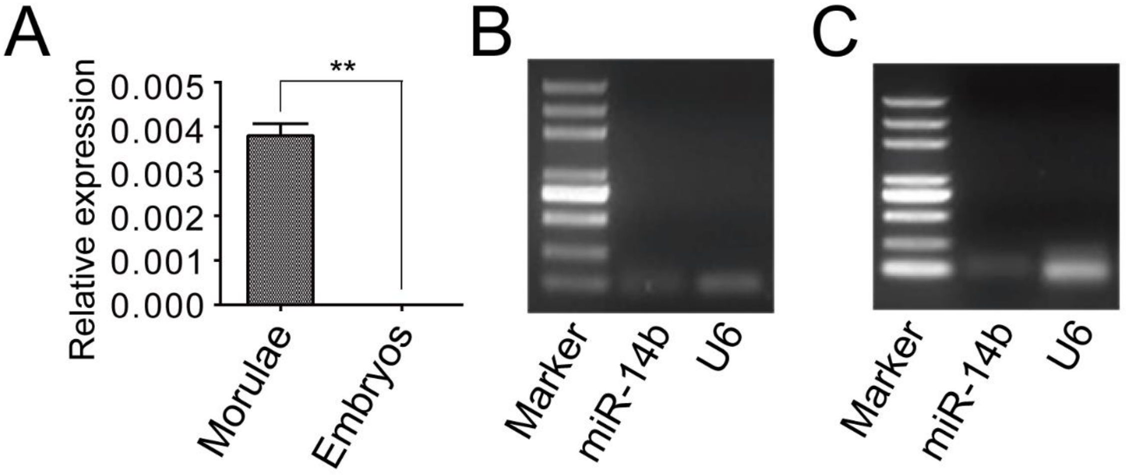

Figure S17. Different expression levels of miR-14b in different developmental stages of *M. cingulum*. A) The relative expression level of miR-14b in morulae and embryos. miR-14b is highly expressed in morulae. B) Results of quantitative real time PCR (qPCR) of miR-14b in morulae. C) Results of qPCR of miR-14b in embryos.

# Supplementary Tables

## Table S1. Genome sequencing data of *M. cingulum*

| Library | size | Raw-data(Coverage) | Clean-data (Gb) | Read length (bp) |
|---|---|---|---|---|
| Paired-end | 170bp | 22.60Gb(167X) | 20.78 | 101 |
| Paired-end | 500bp | 30.52Gb(226X) | 27.49 | 101 |
| Paired-end | 800bp | 26Gb(191X) | 23.61 | 101 |
| Mate-pair | 8Kb | 24.55Gb(167X) | 21.36 | 101 |

## Table S2. Estimation of *M. cingulum* genome size using K-mer analysis

| K-mer | K-mer number | K-mer depth | Genome size (bp) | Used bases (Gb) | Sequce depth (X) |
|---|---|---|---|---|---|
| 17 | 203,535 | 64.4926 | 135,068,848 | 10,332,487,725 | 76.4979 |

## Table S3. Summary of the *M. cingulum* genome assembly

| Category | Contigs | Scaffolds |
|---|---|---|
| Total length (bp) | 128,805,614 | 132,362,060 |
| Max length (bp) | 520,724 | 1,374,378 |
| Average length (bp) | 9,662 | 23,237 |
| N20 length | 146,572 | 458,231 |
| N50 length (bp) | 64,884 | 192,445 |
| N90 length (bp) | 6,290 | 21,264 |
| Total sequence numbers | 13,289 | 5,696 |

## Table S4. The published insect genomes

| Insects | GenBank | Source | References |
|---|---|---|---|
| *Drosophila melanogaster* | JSAE00000000 | flybase | Science, 2000, 287(5461): 2185-95 |
| *Anopheles gambiae* | ABKQ00000000 | vectorBase | Science, 2002, 298(5591): 129-49 |
| *Bombyx mori* | BABU00000000 | silkDB v2.0 | Science, 2004, 306(5703): 1937-40 |
| *Apis mellifera* | JSUV00000000 | beebase V4.5 | Nature, 2006, 443(7114): 931 |
| *Aedes aegypti* | AAGE00000000 | vectorBase | Science, 2007, 316(5832): 1718-23 |
| *Tribolium castaneum* | AAJJ00000000 | Ensembl release22 | Nature, 2008, 452(7190): 949-55 |
| *Culex quinquefasciatus* | AAWU00000000 | vectorBase | Science, 2010, 330(6000): 86-8 |
| *Camponotus floridanus* | AEAB00000000 | Ant Genomes Portal | Science, 2010, 329(5995): 1068-71 |
| *Harpegnathos saltator* | AEAC00000000 | Ant Genomes Portal | |
| *Acyrthosiphon pisum* | ABLF00000000 | aphidbase V2.1b | PLoS Biol, 2010, 8(2): e1000313 |
| *Pediculus humanus* | AAZO00000000 | vectorBase | PNAS, 2010, 107(27): 12168-12173 |
| *Nasonia vitripennis* | AAZX00000000 | NasoniaBase v1.2 | Science, 2010, 327(5963): 343-8 |
| *Acromyrmex echinatior* | AEVX00000000 | Ant Genomes Portal | Genome Res, 2011, 21(8): 1339-48 |
| *Linepithema humile* | ADOQ00000000 | Ant Genomes Portal | PNAS, 2011, 108(14): 5673-8 |
| *Pogonomyrmex barbatus* | ADIH00000000 | Ant Genomes Portal | PNAS, 2011, 108(14): 5667-72 |
| *Atta cephalotes* | ADTU00000000 | Ant Genomes Portal | PLoS genetics, 2011, 7(2): e1002007 |

| | | | |
|---|---|---|---|
| *Solenopsis invicta* | AEAQ00000000 | Ant Genomes Portal | PNAS, 2011, 108(14): 5679-84 |
| *Danaus plexippus* | AGBW00000000 | MonachBase v2.0 | Cell, 2011, 147(5): 1171-1185 |
| *Heliconius melpomene* | CAFA00000000 | Heliconius Genome project | Nature, 2012 |
| *Dendroctonus ponderosae* | APGL00000000 | Ensembl release22 | Genome Biol, 2013, 14(3): R27 |
| *Lasioglossum albipes* | ANOB01000000 | GCA_000346575.1 | Genome Biol, 2013, 14(12): R142 |
| *Plutella xylostella* | AHIO00000000 | DBM-DB v1.0 | Nature genetics, 2013, 45(2): 220-225 |
| *Ceratosolen solmsi* | ATAC00000000 | GCF_000503995.1 | Genome Biol, 2013, 14(12): R141 |
| *Antarctic midge (Belgica antarctica)* | JPYR00000000 | GCA_000775305.1 | Nat Commun, 2014, 54611 |
| *Cerapachys biroi* | JASI00000000 | GCF_000611835.1 | Curr Biol, 2014, 24(4): 451-8 |
| *Musca domestica* | AQPM00000000 | GCF_000371365.1 | Genome Biol, 2014, 15(10): 466 |
| *Stick insect (Timema cristinae)* | MSSY02000000 | Nosil Lab | Science, 2014, 344(6185): 738-42 |
| *Zootermopsis nevadensis* | AUST00000000 | GCA_000696155.1 | Nat Commun, 2014, 53636 |
| *Locusta migratoria* | AVCP00000000 | GCA_000516895.1 | Nat Commun, 2014, 52957 |
| *Nilaparvata lugens* | AOSB00000000 | OMIGA annotation project | Genome Biol, 2014, 15(12): 521 |
| *Spodoptera frugiperda* | JQCY00000000 | GCA_000753635.2 | Genomics, 2014, 104(2): 134-43 |
| *Diuraphis noxia* | MPKX01000000 | GCF_001186385.1 | BMC Genomics, 2015, 16(1): 429 |
| *Hypothenemus hampei* | LBGY00000000 | GCA_001012855.1 | Rep, 2015, 512525 |
| *Lucilia cuprina* | JHUJ00000000 | GCA_000699065.1 | Nat Commun, 2015, 67344 |

| | | | |
|---|---|---|---|
| *Operophtera brumata* | JTDY01000000 | GCA_001266575.1 | Genome Biol Evol, 2015, 7(8): 2321-32 |
| *Cimex lectularius* | JHUN00000000 | GCA_000648675.1 | Nat Commun, 2016, 710165 |
| *Ceratitis capitata* | AOHK00000000 | GCF_000347755.1 | Genome Biol, 2016, 17(1): 192 |

## Table S5. The genome assembly assessment on different insects

| **Insects** | **BUSCO** | | | | |
|---|---|---|---|---|---|
| | **Complete** | **Single** | **Duplication** | **Fragment** | **Missing** |
| *Drosophila melanogaster* | 0.996984 | 0.989747 | 0.00723764 | 0.001809 | 0.001206 |
| *Anopheles gambiae* | 0.995175 | 0.977081 | 0.01809409 | 0.001206 | 0.003619 |
| *Bombyx mori* | 0.95778 | 0.953559 | 0.00422195 | 0.018697 | 0.023522 |
| *Apis mellifera* | 0.971049 | 0.968034 | 0.00301568 | 0.014475 | 0.014475 |
| *Aedes aegypti* | 0.972256 | 0.897467 | 0.0747889 | 0.010253 | 0.017491 |
| *Tribolium castaneum* | 0.993366 | 0.990953 | 0.00241255 | 0.004222 | 0.002413 |
| *Culex quinquefasciatus* | 0.967431 | 0.917973 | 0.04945718 | 0.007841 | 0.024729 |
| *Camponotus floridanus* | 0.923402 | 0.916164 | 0.00723764 | 0.016285 | 0.060314 |
| *Harpegnathos saltator* | 0.586852 | 0.582027 | 0.00482509 | 0.001809 | 0.411339 |
| *Acyrthosiphon pisum* | 0.768396 | 0.731604 | 0.03679131 | 0.04041 | 0.191194 |
| *Pediculus humanus* | 0.872135 | 0.870326 | 0.00180941 | 0.009047 | 0.118818 |
| *Nasonia vitripennis* | 0.95959 | 0.950543 | 0.00904704 | 0.020507 | 0.019903 |
| *Acromyrmex echinatior* | 0.472256 | 0.471653 | 0.00060314 | 0.002413 | 0.525332 |
| *Linepithema humile* | 0.962002 | 0.957177 | 0.00482509 | 0.007841 | 0.030157 |
| *Pogonomyrmex barbatus* | 0.972859 | 0.970446 | 0.00241255 | 0.0193 | 0.007841 |

| | | | | | |
|---|---|---|---|---|---|
| *Atta cephalotes* | 0.973462 | 0.965621 | 0.00784077 | 0.013872 | 0.012666 |
| *Solenopsis invicta* | 0.962002 | 0.958987 | 0.00301568 | 0.021713 | 0.016285 |
| *Danaus plexippus* | 0.924005 | 0.905911 | 0.01809409 | 0.017491 | 0.058504 |
| *Heliconius melpomene* | 0.956574 | 0.951146 | 0.00542823 | 0.018094 | 0.025332 |
| *Dendroctonus ponderosae* | 0.930639 | 0.875151 | 0.05548854 | 0.028347 | 0.041013 |
| *Lasioglossum albipes* | 0.989144 | 0.969843 | 0.01930036 | 0.006634 | 0.004222 |
| *Plutella xylostella* | 0.878166 | 0.727986 | 0.15018094 | 0.026538 | 0.095296 |
| *Ceratosolen solmsi* | 0.375754 | 0.375151 | 0.00060314 | 0 | 0.624246 |
| *Antarctic midge (Belgica antarctica)* | 0.979493 | 0.974065 | 0.00542823 | 0.013269 | 0.007238 |
| *Cerapachys biroi* | 0.983112 | 0.970446 | 0.01266586 | 0.01146 | 0.005428 |
| *Musca domestica* | 0.987334 | 0.969843 | 0.01749095 | 0.004222 | 0.008444 |
| *Stick insect (Timema cristinae)* | 0.929433 | 0.924005 | 0.00542823 | 0.036791 | 0.033776 |
| *Zootermopsis nevadensis* | 0.886007 | 0.875754 | 0.01025332 | 0.001809 | 0.112183 |
| *Locusta migratoria* | 0.404704 | 0.387214 | 0.01749095 | 0.283474 | 0.311821 |
| *Nilaparvata lugens* | 0.854041 | 0.770808 | 0.08323281 | 0.033776 | 0.112183 |
| *Spodoptera frugiperda* | 0.781665 | 0.772618 | 0.00904704 | 0.062726 | 0.155609 |
| *Diuraphis noxia* | 0.642943 | 0.627865 | 0.01507841 | 0.042823 | 0.314234 |
| *Hypothenemus hampei* | 0.966224 | 0.963812 | 0.00241255 | 0.021713 | 0.012063 |

| | | | | | |
|---|---|---|---|---|---|
| *Lucilia cuprina* | 0.961399 | 0.945115 | 0.01628468 | 0.00965 | 0.028951 |
| *Operophtera brumata* | 0.946321 | 0.913148 | 0.0331725 | 0.03076 | 0.022919 |
| *Cimex lectularius* | 0.932449 | 0.908926 | 0.02352232 | 0.016888 | 0.050663 |
| *Ceratitis capitata* | 0.991556 | 0.987334 | 0.00422195 | 0.003619 | 0.004825 |
| *Macrocentrus cingulum* | 0.98854 | 0.941496 | 0.04704463 | 0.006031 | 0.005428 |

### Table S6. Classification of repeat sequences identified in the *M. cingulum* genome

| Repeat types | | Number of elements | Length occupied (bp) | Percentages of sequence (%) |
|---|---|---|---|---|
| Interspersed repeats | SINE | 236 | 67,958 | 0.1 |
| | LINE | 1,101 | 423,211 | 0.32 |
| | LTR | 5,229 | 1,465,193 | 1.11 |
| | DNA elements | 9,034 | 3,180,053 | 2.41 |
| | Unclassified | 59,557 | 15,510,372 | 11.71 |
| Satellites | | 2 | 1,283 | 0 |
| Simple repeats | | 47,377 | 1,843,813 | 1.39 |
| Low complexity | | 9,452 | 1,843,813 | 0.33 |
| Total base masked | | 131,988 | 24,335,696 | 17.37 |

**Table S7. Genome features of the *M. cingulum, N. vitripennis and A. mellifera***

| Genomic features | M. cingulum (version 1.0) | N. vitripennis (version 1.0) | A. mellifera（version 3.2） |
|---|---|---|---|
| genome size (Mb) | 132 | 295 | 227 |
| CEGMA genes (%) | 99.2 | 97.6 | 98.8 |
| Contig N50 (bp) | 64,884 | 18,500 | 42,850 |
| Scaffold N50(bp) | 192,445 | 709,000 | 986,335 |
| GC content (%) | 35.7 | 40.6 | 32.8 |
| Repeat rate (%) | 24.9 | 42.1 | 13.6 |
| Coding gene number | 11,993 | 18,822 | 15,314 |
| Number of genes with Go terms | 7,254 | 8,778 | 8,162 |
| Number of genes with InterPro domains | 9,094 | 17,079 | 9,249 |
| Number of miRNA | 111 | 953 | 314 |
| Number of tRNA | 144 | 756 | 271 |
| Number of snoRNA | 16 | 71 | 53 |
| Number of rRNA | 148 | 47 | 38 |

## Table S8. Gene features of *M. cingulum*, *N. vitripennis* and *A. mellifera*

| **Species** | **Exon Number** | **Exon Length** | **Gene Length** | **Intron Length** | **CDS Length** |
|---|---|---|---|---|---|
| *M. cingulum* | 5.13 | 299.61 | 3127.88 | 385.13 | 1536.40 |
| *N. vitripennis* | 5.46 | 284.13 | 7281.90 | 1285.14 | 1289.52 |
| *A. mellifera* | 5.53 | 320.29 | 7615.47 | 1291.25 | 1260.73 |

## Table S9. The insects with OGSs in InsectBase

| Species name | Genome size (Mb) | Gene number | Protein number |
|---|---|---|---|
| *Acromyrmex echinatior* | 289 | 17,277 | 17,277 |
| *Acyrthosiphon pisum* | 525 | 36,194 | 36,938 |
| *Aedes aegypti* | 1,434 | 18,800 | 17,142 |
| *Anopheles coluzzii* | 219 | 14,702 | 14,702 |
| *Anopheles darlingi* | 133 | 10,947 | 10,456 |
| *Anopheles gambiae* | 265 | 15,423 | 14,666 |
| *Anopheles sinensis* | 215 | 19,352 | 19,351 |
| *Anopheles stephensi* | 219 | 13,787 | 13,250 |
| *Apis dorsata* | 223 | 20,601 | 18,144 |
| *Apis florea* | 224 | 19,333 | 17,663 |
| *Apis mellifera* | 227 | 15,313 | 15,313 |
| *Athalia rosae* | 159 | 20,151 | 19,484 |
| *Atta cephalotes* | 309 | 18,092 | 18,092 |
| *Bactrocera cucurbitae* | 363 | 21,640 | 20,753 |
| *Bactrocera dorsalis* | 402 | 18,231 | 17,744 |
| *Bombus impatiens* | 242 | 21,775 | 20,895 |
| *Bombus terrestris* | 241 | 21,558 | 20,320 |
| *Bombyx mori* | 467 | 14,622 | 14,622 |
| *Camponotus floridanus* | 228 | 17,063 | 17,063 |
| *Cerapachys biroi* | 206 | 26,315 | 23,802 |
| *Ceratitis capitata* | 469 | 23,710 | 23,087 |
| *Ceratosolen solmsi* | 268 | 13,200 | 12,833 |
| *Chilo suppressalis* | 362 | 10,221 | 10,221 |
| *Culex quinquefasciatus* | 562 | 19,389 | 19,018 |
| *Danaus plexippus* | 238 | 15,129 | 15,129 |
| *Dendroctonus ponderosae* | 246 | 13,456 | 13,459 |
| *Diaphorina citri* | 492 | 21,985 | 20,995 |
| *Drosophila ananassae* | 225 | 15,588 | 15,070 |
| *Drosophila erecta* | 148 | 15,615 | 15,048 |
| *Drosophila grimshawi* | 196 | 15,415 | 14,986 |
| *Drosophila melanogaster* | 139 | 17,717 | 30,439 |
| *Drosophila mojavensis* | 188 | 14,990 | 14,595 |
| *Drosophila persimilis* | 184 | 17,351 | 16,878 |
| *Drosophila pseudoobscura* | 149 | 17,382 | 16,857 |
| *Drosophila sechellia* | 163 | 17,049 | 16,483 |
| *Drosophila simulans* | 135 | 15,912 | 15,427 |
| *Drosophila virilis* | 201 | 15,343 | 14,491 |
| *Drosophila willistoni* | 230 | 16,008 | 15,513 |
| *Drosophila yakuba* | 161 | 16,515 | 16,094 |

| | | | |
|---|---|---|---|
| *Fopius arisanus* | 149 | 20,056 | 18,906 |
| *Harpegnathos saltator* | 288 | 18,563 | 18,563 |
| *Heliconius melpomene* | 266 | 15,201 | 12,828 |
| *Linepithema humile* | 213 | 16,115 | 16,115 |
| *Manduca sexta* | 367 | 27,529 | 27,402 |
| *Mayetiola destructor* | 180 | 22,630 | 22,024 |
| *Megachile rotundata* | 264 | 28,682 | 26,024 |
| *Megaselia scalaris* | 489 | 11,638 | 11,462 |
| *Microplitis demolitor* | 243 | 18,954 | 18,169 |
| *Musca domestica* | 728 | 20,165 | 19,549 |
| *Nasonia vitripennis* | 287 | 18,940 | 18,821 |
| *Nilaparvata lugens* | 1,324 | 36,723 | 36,723 |
| *Pediculus humanus* | 108 | 11,664 | 10,775 |
| *Plutella xylostella* | 383 | 18,072 | 18,072 |
| *Pogonomyrmex barbatus* | 228 | 17,737 | 17,188 |
| *Rhodnius prolixus* | 683 | 15,642 | 15,440 |
| *Solenopsis invicta* | 343 | 16,521 | 16,521 |
| *Tribolium castaneum* | 222 | 16,542 | 16,525 |
| *Vollenhovia emeryi* | 280 | 27,195 | 26,071 |
| *Wasmannia auropunctata* | 323 | 26,070 | 24,085 |
| *Zootermopsis nevadensis* | 472 | 14,610 | 14,610 |

## Table S10. Hemomucin genes in eight wasps

| Order | Species | SRA | Length | O-glycosylation positions |
|---|---|---|---|---|
| Braconidae | Microplitis bicoloratus | 9 | 443 | 7 |
| Braconidae | Microplitis demolitor | 4 | 444 | 9 |
| Braconidae | Macrocentrus cingulum |  | 579 | 56 |
| Braconidae | Cotesia vestalis | 12 | 439 | 3 |
| Braconidae | Cotesia rubecula | 12 | 439 | 4 |
| Braconidae | Cotesia glomerata | 30 | 439 | 5 |
| Ichneumonidae | Venturia canescens | 1 | 616 | 65 |
| Ichneumonidae | Diadromus collaris | 2 | 436 | 5 |

Table S11. The different gene expression of Embryo and Pseudogerm transcriptomes in KEGG pathway

| Pathway map 1-categories | Pathway map 2-categories | Embryo upregulate | Pseudogerm upregulate |
|---|---|---|---|
| Metabolism | Global and overview maps | 2 | 1 |
| | Carbohydrate metabolism | 6 | 12 |
| | Energy metabolism | 1 | 2 |
| | Lipid metabolism | 3 | 4 |
| | Nucleotide metabolism | 5 | 1 |
| | Amino acid metabolism | 9 | 4 |
| | Metabolism of other amino acids | 4 | 2 |
| | Glycan biosynthesis and metabolism | 5 | 4 |
| | Metabolism of cofactors and vitamins | 0 | 1 |
| | Metabolism of terpenoids and polyketides | 0 | 3 |
| | Biosynthesis of other secondary metabolites | 0 | 0 |
| | Xenobiotics biodegradation and metabolism | 0 | 1 |
| Genetic Information Processing | Transcription | 0 | 0 |
| | Translation | 2 | 1 |
| | Folding, sorting and degradation | 2 | 3 |
| | Replication and repair | 3 | 0 |

|  |  |  |  |
|---|---|---|---|
| Environmental Information Processing | Membrane transport | 2 | 1 |
|  | Signal transduction | 39 | 28 |
|  | Signaling molecules and interaction | 4 | 1 |
| Cellular Processes | Transport and catabolism | 7 | 7 |
|  | Cell motility | 1 | 0 |
|  | Cell growth and death | 8 | 1 |
|  | Cellular community | 15 | 5 |
| Organismal Systems | Immune system | 9 | 5 |
|  | Endocrine system | 14 | 14 |
|  | Circulatory system | 4 | 1 |
|  | Digestive system | 5 | 6 |
|  | Excretory system | 1 | 2 |
|  | Nervous system | 3 | 7 |
|  | Sensory system | 0 | 4 |
|  | Development | 3 | 1 |
|  | Environmental adaptation | 0 | 3 |
| Human Diseases | Cancers: Overview | 14 | 9 |
|  | Cancers: Specific types | 15 | 6 |

| | | |
|---|---|---|
| Immune diseases | 1 | 2 |
| Neurodegenerative diseases | 1 | 4 |
| Substance dependence | 0 | 1 |
| Cardiovascular diseases | 3 | 0 |
| Endocrine and metabolic diseases | 2 | 3 |
| Infectious diseases: Bacterial | 9 | 5 |
| Infectious diseases: Viral | 8 | 2 |
| Infectious diseases: Parasitic | 6 | 0 |

# Table S12. The differently expressed miRNAs in embryo and mixed embryo transcriptomes

| miRNA | Normal embryo | Mixed embryo | log2FoldChange | pvalue |
|---|---|---|---|---|
| mci-miR-14b | 20.21998745 | 1991.692631 | 4.924602884 | 0.015168939 |
| mci-miR-9a-5p | 1.263749216 | 42.72999685 | 3.446504164 | 0.111932706 |
| mci-miR-9a-3p | 1.263749216 | 34.02573823 | 3.189647009 | 0.144792447 |
| mci-miR-1a | 0 | 14.24333228 | 3.218293882 | 0.174454125 |
| mci-miR-6038 | 10.10999373 | 0 | -2.944791637 | 0.217081046 |
| mci-bantam-5p | 7.582495294 | 0 | -2.553140638 | 0.285047192 |
| mci-miR-210a-3p | 7.582495294 | 0 | -2.553140638 | 0.285047192 |
| mci-miR-iab-4-3p | 0 | 7.912962379 | 2.472083197 | 0.300695842 |
| mci-miR-1175 | 1.263749216 | 15.03462852 | 2.23409632 | 0.327254046 |
| mci-miR-932 | 6.318746078 | 0 | -2.308083435 | 0.332374298 |
| mci-miR-2491c | 41.70372412 | 7.912962379 | -1.828253143 | 0.366669062 |
| mci-miR-3770 | 17.69248902 | 69.63406894 | 1.563998291 | 0.419362271 |
| mci-miR-6065 | 115.0011786 | 317.3097914 | 1.207760725 | 0.505654641 |
| mci-miR-2779a | 0 | 3.165184952 | 1.398284856 | 0.537206421 |
| mci-miR-993-3p | 0 | 3.165184952 | 1.398284856 | 0.537206421 |
| mci-miR-iab-8-3p | 0 | 3.165184952 | 1.398284856 | 0.537206421 |
| mci-bantam-3p | 2.527498431 | 0 | -1.36733031 | 0.541944226 |
| mci-miR-210b | 2.527498431 | 0 | -1.36733031 | 0.541944226 |
| mci-miR-281-5p | 5.054996863 | 0.791296238 | -1.312692382 | 0.582609892 |
| mci-miR-4945 | 11.37374294 | 3.165184952 | -1.219072159 | 0.588920427 |
| mci-miR-996 | 0 | 2.373888714 | 1.195588272 | 0.593695913 |
| mci-miR-2779f | 1.263749216 | 0 | -1.081479192 | 0.62619967 |
| mci-miR-3477-3p | 1.263749216 | 0 | -1.081479192 | 0.62619967 |

| | | | | |
|---|---|---|---|---|
| mci-miR-971 | 1.263749216 | 0 | -1.081479192 | 0.62619967 |
| mci-miR-2491a | 34.12122882 | 15.03462852 | -0.918543525 | 0.644005878 |
| mci-miR-252-3p | 0 | 1.582592476 | 0.970549781 | 0.664401652 |
| mci-miR-263a | 0 | 1.582592476 | 0.970549781 | 0.664401652 |
| mci-miR-71a | 2.527498431 | 7.912962379 | 1.005734728 | 0.665065447 |
| mci-miR-2491b | 69.50620686 | 34.81703447 | -0.805577886 | 0.668644215 |
| mci-miR-6067 | 61.92371157 | 30.86055328 | -0.808089568 | 0.669759722 |
| mci-miR-33 | 5.054996863 | 1.582592476 | -0.899790707 | 0.705393978 |
| mci-miR-276 | 0 | 0.791296238 | 0.596960254 | 0.788636567 |
| mci-miR-277-5p | 0 | 0.791296238 | 0.596960254 | 0.788636567 |
| mci-miR-305 | 0 | 0.791296238 | 0.596960254 | 0.788636567 |
| mci-miR-307-5p | 0 | 0.791296238 | 0.596960254 | 0.788636567 |
| mci-miR-87 | 0 | 0.791296238 | 0.596960254 | 0.788636567 |
| mci-miR-iab-4-5p | 0 | 0.791296238 | 0.596960254 | 0.788636567 |
| mci-miR-6001 | 8.846244509 | 4.747777427 | -0.594455326 | 0.792409835 |
| mci-miR-6006 | 5.054996863 | 8.704258617 | 0.519521645 | 0.817976599 |
| mci-miR-2491d | 5.054996863 | 7.912962379 | 0.423834485 | 0.851835053 |
| mci-miR-308 | 18.95623823 | 15.03462852 | -0.253592447 | 0.901323242 |
| mci-miR-278 | 1.263749216 | 0.791296238 | -0.22379999 | 0.92081334 |
| mci-miR-277-3p | 2.527498431 | 3.165184952 | 0.16854129 | 0.943714403 |
| mci-miR-34 | 1.263749216 | 1.582592476 | 0.123433982 | 0.957577503 |
| mci-miR-281-3p | 5.054996863 | 5.539073665 | 0.082910259 | 0.971353955 |
| mci-miR-184 | 5.054996863 | 4.747777427 | -0.0557747 | 0.980846862 |
| mci-miR-12 | 2.527498431 | 2.373888714 | -0.044437896 | 0.985157543 |
| mci-miR-282 | 32.85747961 | 33.23444199 | 0.01309561 | 0.994576002 |
| mci-miR-375 | 24.0112351 | 23.73888714 | -0.012839458 | 0.994822876 |

## Table S13. Comparison of gene numbers for Chemoreception in *A.mellifera, C. floridanum, C. solmsi, M. cingulum, N. vitripennis* and *S. invicta*

| Organisms | IR | SNMP | CSP | OR | OBP | GR |
|---|---|---|---|---|---|---|
| *A. mellifera* | 24 | 10 | 9 | 142 | 21 | 18 |
| *C. floridanum* | 33 | 20 | 9 | 80 | 40 | 24 |
| *C. solmsi* | 29 | 11 | 8 | 49 | 10 | 6 |
| *M. cingulum* | 25 | 9 | 3 | 89 | 7 | 14 |
| *N. vitripennis* | 32 | 12 | 10 | 131 | 90 | 31 |
| *S. invicta* | 24 | 10 | 15 | 125 | 16 | 28 |

**Table S14. Comparison of gene numbers for Gene families associated with insecticide resistance and detoxification in *D. melanogaster*, *A. mellifera*, *C. floridanum*, *C. solmsi*, *M. cingulum*, *N. vitripennis* and *S. invicta***

| Organisms | P450 | GST | ABC |
|---|---|---|---|
| *D. melanogaster* | 85 | 35 | 59 |
| *A. mellifera* | 56 | 14 | 64 |
| *C. floridanum* | 108 | 22 | 99 |
| *C. solmsi* | 46 | 17 | 78 |
| *M. cingulum* | 43 | 15 | 68 |
| *N. vitripennis* | 96 | 26 | 62 |
| *S. invicta* | 133 | 12 | 69 |

**Table S15. Comparison of gene numbers of insect immune in *A. mellifera*, *C. floridanum*, *C. solmsi*, *M. cingulum*, *N. vitripennis* and *S. invicta***

| Genes | *A. mellifera* | *C. floridanum* | *C. solmsi* | *D. melanogaster* | *M. cingulum* | *N. vitripennis* | *S. invicta* |
|---|---|---|---|---|---|---|---|
| **CASPs** | **5** | **6** | **9** | **7** | **2** | **8** | **7** |
| **CASPAs** | **1** | **1** | **1** | **1** | **2** | **1** | **1** |
| **CATs** | **3** | **1** | **1** | **2** | **0** | **1** | **1** |
| **CTLs** | **13** | **22** | **25** | **41** | **19** | **32** | **11** |
| **FREPs** | **2** | **1** | **1** | **14** | **2** | **1** | **2** |
| **GALEs** | **3** | **5** | **3** | **6** | **3** | **3** | **2** |
| **IAPs** | **5** | **12** | **5** | **4** | **4** | **17** | **6** |
| **LYSs** | **2** | **3** | **1** | **12** | **1** | **1** | **1** |
| **MLs** | **4** | **2** | **3** | **8** | **4** | **7** | **5** |
| **PRRs** | **6** | **11** | **6** | **17** | **6** | **12** | **5** |
| **GNBPs** | **2** | **1** | **0** | **6** | **2** | **2** | **2** |
| **PGRPs** | **4** | **10** | **6** | **11** | **4** | **10** | **3** |
| **PPOs** | **5** | **5** | **8** | **10** | **3** | **10** | **4** |
| **RELs** | **4** | **7** | **7** | **4** | **3** | **6** | **2** |
| **SPZs** | **7** | **13** | **9** | **6** | **5** | **9** | **4** |
| **TEPs** | **4** | **5** | **3** | **6** | **3** | **3** | **3** |
| **TOLLs** | **8** | **9** | **6** | **9** | **13** | **10** | **4** |

CASPs: caspases, CASPAs: caspase Activators, CATs:Catalases, CTLs: C-type lectins, FREPs: Fibrinogen-Related Proteins, GALEs: Galactoside-Binding Lectins, IAPs: Inhibitors of Apoptosis, LYSs: Lysozymes, MLs: MD2-Like Receptors, PPOs: prophenoloxidases, PRRs: Pattern Recognition Receptors, GNBPs: Gram-negative binding proteins, PGRPs: peptidoglycan recognition proteins, RELs: Relish-like Proteins, SODs: Superoxide Dismutatses, Spzs: Spaetzle-like Proteins, TEPs: thioester-containing protein, TOLLs: Toll-Receptors

## Table S16. The PCR primer for target genes of mci-miR-14b

| Genes | Primer |
|---|---|
| *MBP-1* | TTTACGGTTCGGCTATCACTTT |
| | CGGCATCGCTAGATCCTCA |
| *KMT2E* | TTGAAATCGTGCGTGGTCTT |
| | GAGGGCTCATCGTAGGTGGTA |
| *RUNT* | CCAGTCCAACACCCACGAA |
| | TGGCTTACACTTATGCCTCCTAT |