# Supplementary Table

**Supplementary Table 1.** Gene losses in Aculeate (63 losses) and Chalcidoid/Ichneumonoid (77 losses) clades.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Amino acid synthetic pathway | Observed gene loss in Aculeata clade | Observed gene loss in Parasitoida clade | Expected gene loss in Aculeata clade | Expected gene loss in Parasitoida clade | P value (Chi-square test) | Significant |
| All pathways | 63 | 77 | 63 | 77 | 1 | ns |

Note: Gene loss in each amino acid synthetic pathway was identified using a KEGG online tool, KEGG Mapper (https://www.kegg.jp/kegg/mapper.html) with all predicted pathway genes. Observed gene loss events were documented based on the genes involved and phylogenetic position. We regraded the percent of branches relative to total as the random expected proportion of gene loss events. To compare the gene loss frequency between Aculeates (Aculeata) and Parasitoida (Chalcidoidea/Ichneumonoidea), we used Chi-square test to perform the statistical test, and ns indicates no significant difference.

**Supplementary Table 2.** Gene losses in pathways that have been disrupted in the most recent common ancestor (MRCA) of Holomentabola (HOL), MRCA of Hymenoptera (HYM), and the pathways are complete in the common ancestor of Hymenoptera.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Amino acid synthetic pathway | Observed gene loss in MRCA-HOL | Observed gene loss in MRCA-HYM | Observed gene loss in HYM | Expected gene loss in MRCA-HOL | Expected gene loss in MRCA-HYM | Expected gene loss in HYM | P value (Chi-square test) | Significant |
| All pathways | 125 | 15 | 24 | 85 | 49 | 30 | <0.01 | yes |

**Supplementary Table 3.** Gene losses in Aculeate (6 losses) and Chalcidoid/Ichneumonoid (17 losses) clades in the pathways that are complete in the common ancestor of Hymenoptera.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Observed gene loss in Aculeata clade | Observed gene loss in Parasitoida clade | Expected gene loss in Aculeata clade | Expected gene loss in Parasitoida clade | P value (Chi-square test) | Significant |
| 6 | 17 | 10 | 13 | 0.207 | ns |

**Supplementary Table 4.** Lost genes in the KEGG amino acid biosynthesis pathways in the *C. chilonis* genome (100 ko ids).

|  |  |
| --- | --- |
| **Ko\_id** | **Annotation** |
| K09758 | asdA; aspartate 4-decarboxylase [EC:4.1.1.12] |
| K11358 | yhdR; aspartate aminotransferase [EC:2.6.1.1] |
| K01914 | asnA; aspartate--ammonia ligase [EC:6.3.1.1] |
| K02203 | thrH; phosphoserine / homoserine phosphotransferase [EC:3.1.3.3 2.7.1.39] |
| K00928 | lysC; aspartate kinase [EC:2.7.2.4] |
| K12524 | thrA; bifunctional aspartokinase / homoserine dehydrogenase 1 [EC:2.7.2.4 1.1.1.3] |
| K12525 | metL; bifunctional aspartokinase / homoserine dehydrogenase 2 [EC:2.7.2.4 1.1.1.3] |
| K12526 | lysAC; bifunctional diaminopimelate decarboxylase / aspartate kinase [EC:4.1.1.20 2.7.2.4] |
| K00003 | E1.1.1.3; homoserine dehydrogenase [EC:1.1.1.3] |
| K00872 | thrB1; homoserine kinase [EC:2.7.1.39] |
| K00640 | cysE; serine O-acetyltransferase [EC:2.3.1.30] |
| K17069 | MET17; O-acetylhomoserine/O-acetylserine sulfhydrylase [EC:2.5.1.49 2.5.1.47] |
| K07173 | luxS; S-ribosylhomocysteine lyase [EC:4.4.1.21] |
| K00651 | metA; homoserine O-succinyltransferase [EC:2.3.1.46] |
| K14155 | patB, malY; cystathione beta-lyase [EC:4.4.1.8] |
| K00549 | metE; 5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase [EC:2.1.1.14] |
| K01653 | E2.2.1.6S, ilvH, ilvN; acetolactate synthase I/III small subunit [EC:2.2.1.6] |
| K11258 | ilvM; acetolactate synthase II small subunit [EC:2.2.1.6] |
| K00053 | ilvC; ketol-acid reductoisomerase [EC:1.1.1.86] |
| K09011 | cimA; D-citramalate synthase [EC:2.3.1.182] |
| K17989 | SDS, SDH, CHA1; L-serine/L-threonine ammonia-lyase [EC:4.3.1.17 4.3.1.19] |
| K01754 | E4.3.1.19, ilvA, tdcB; threonine dehydratase [EC:4.3.1.19] |
| K01702 | LEU1; 3-isopropylmalate dehydratase [EC:4.2.1.33] |
| K00215 | dapB; 4-hydroxy-tetrahydrodipicolinate reductase [EC:1.17.1.8] |
| K01778 | dapF; diaminopimelate epimerase [EC:5.1.1.7] |
| K05822 | dapH, dapD; tetrahydrodipicolinate N-acetyltransferase [EC:2.3.1.89] |
| K05823 | dapL; N-acetyldiaminopimelate deacetylase [EC:3.5.1.47] |
| K03340 | dapdh; diaminopimelate dehydrogenase [EC:1.4.1.16] |
| K00838 | ARO8; aromatic amino acid aminotransferase I [EC:2.6.1.57 2.6.1.27 2.6.1.5] |
| K16792 | aksD; methanogen homoaconitase large subunit [EC:4.2.1.114] |
| K16793 | aksE; methanogen homoaconitase small subunit [EC:4.2.1.114] |
| K05826 | lysW; alpha-aminoadipate/glutamate carrier protein LysW |
| K05827 | lysX; [lysine-biosynthesis-protein LysW]---L-2-aminoadipate ligase [EC:6.3.2.43] |
| K05828 | lysZ, argB; LysW-gamma-L-alpha-aminoadipate/LysW-L-glutamate kinase [EC:2.7.2.-] |
| K05829 | lysY, argC; LysW-gamma-L-alpha-aminoadipyl-6-phosphate/LysW-L-glutamyl-5-phosphate reductase [EC:1.2.1.-] |
| K05831 | lysK, argE; LysW-gamma-L-lysine/LysW-L-ornithine carboxypeptidase |
| K00618 | E2.3.1.1; amino-acid N-acetyltransferase [EC:2.3.1.1] |
| K14681 | argHA; argininosuccinate lyase / amino-acid N-acetyltransferase [EC:4.3.2.1 2.3.1.1] |
| K14682 | argAB; amino-acid N-acetyltransferase [EC:2.3.1.1] |
| K00930 | argB; acetylglutamate kinase [EC:2.7.2.8] |
| K00145 | argC; N-acetyl-gamma-glutamyl-phosphate reductase [EC:1.2.1.38] |
| K12659 | ARG56; N-acetyl-gamma-glutamyl-phosphate reductase / acetylglutamate kinase [EC:1.2.1.38 2.7.2.8] |
| K19412 | argX; glutamate--LysW ligase ArgX [EC:6.3.2.-] |
| K00611 | OTC, argF, argI; ornithine carbamoyltransferase [EC:2.1.3.3] |
| K01750 | E4.3.1.12, ocd; ornithine cyclodeaminase [EC:4.3.1.12] |
| K00765 | hisG; ATP phosphoribosyltransferase [EC:2.4.2.17] |
| K01523 | hisE; phosphoribosyl-ATP pyrophosphohydrolase [EC:3.6.1.31] |
| K01496 | hisI; phosphoribosyl-AMP cyclohydrolase [EC:3.5.4.19] |
| K11755 | hisIE; phosphoribosyl-ATP pyrophosphohydrolase / phosphoribosyl-AMP cyclohydrolase [EC:3.6.1.31 3.5.4.19] |
| K14152 | HIS4; phosphoribosyl-ATP pyrophosphohydrolase / phosphoribosyl-AMP cyclohydrolase / histidinol dehydrogenase [EC:3.6.1.31 3.5.4.19 1.1.1.2 |
| K02501 | hisH; glutamine amidotransferase [EC:2.4.2.-] |
| K02500 | hisF; cyclase [EC:4.1.3.-] |
| K01663 | HIS7; glutamine amidotransferase / cyclase [EC:2.4.2.- 4.1.3.-] |
| K01693 | hisB; imidazoleglycerol-phosphate dehydratase [EC:4.2.1.19] |
| K00817 | hisC; histidinol-phosphate aminotransferase [EC:2.6.1.9] |
| K04486 | E3.1.3.15B; histidinol-phosphatase (PHP family) [EC:3.1.3.15] |
| K18649 | IMPL2; inositol-phosphate phosphatase / L-galactose 1-phosphate phosphatase / histidinol-phosphatase [EC:3.1.3.25 3.1.3.93 3.1.3.15] |
| K01089 | hisB; imidazoleglycerol-phosphate dehydratase / histidinol-phosphatase [EC:4.2.1.19 3.1.3.15] |
| K00013 | hisD; histidinol dehydrogenase [EC:1.1.1.23] |
| K01626 | E2.5.1.54, aroF, aroG, aroH; 3-deoxy-7-phosphoheptulonate synthase [EC:2.5.1.54] |
| K03856 | AROA2, aroA; 3-deoxy-7-phosphoheptulonate synthase [EC:2.5.1.54] |
| K13853 | aroG, aroA; 3-deoxy-7-phosphoheptulonate synthase / chorismate mutase [EC:2.5.1.54 5.4.99.5] |
| K01735 | aroB; 3-dehydroquinate synthase [EC:4.2.3.4] |
| K13829 | aroKB; shikimate kinase / 3-dehydroquinate synthase [EC:2.7.1.71 4.2.3.4] |
| K03785 | aroD; 3-dehydroquinate dehydratase I [EC:4.2.1.10] |
| K03786 | aroQ, qutE; 3-dehydroquinate dehydratase II [EC:4.2.1.10] |
| K00014 | aroE; shikimate dehydrogenase [EC:1.1.1.25] |
| K13832 | aroDE, DHQ-SDH; 3-dehydroquinate dehydratase / shikimate dehydrogenase [EC:4.2.1.10 1.1.1.25] |
| K13830 | ARO1; pentafunctional AROM polypeptide [EC:4.2.3.4 4.2.1.10 1.1.1.25 2.7.1.71 2.5.1.19] |
| K00800 | aroA; 3-phosphoshikimate 1-carboxyvinyltransferase [EC:2.5.1.19] |
| K01736 | aroC; chorismate synthase [EC:4.2.3.5] |
| K01657 | trpE; anthranilate synthase component I [EC:4.1.3.27] |
| K13503 | trpEG; anthranilate synthase [EC:4.1.3.27] |
| K13501 | TRP1; anthranilate synthase / indole-3-glycerol phosphate synthase / phosphoribosylanthranilate isomerase [EC:4.1.3.27 4.1.1.48 5.3.1.24] |
| K01656 | TRP3; anthranilate synthase / indole-3-glycerol phosphate synthase [EC:4.1.3.27 4.1.1.48] |
| K00766 | trpD; anthranilate phosphoribosyltransferase [EC:2.4.2.18] |
| K13497 | trpGD; anthranilate synthase/phosphoribosyltransferase [EC:4.1.3.27 2.4.2.18] |
| K01817 | trpF; phosphoribosylanthranilate isomerase [EC:5.3.1.24] |
| K01609 | trpC; indole-3-glycerol phosphate synthase [EC:4.1.1.48] |
| K13498 | trpCF; indole-3-glycerol phosphate synthase / phosphoribosylanthranilate isomerase [EC:4.1.1.48 5.3.1.24] |
| K01695 | trpA; tryptophan synthase alpha chain [EC:4.2.1.20] |
| K01696 | trpB; tryptophan synthase beta chain [EC:4.2.1.20] |
| K06001 | trpB; tryptophan synthase beta chain [EC:4.2.1.20] |
| K01694 | TRP; tryptophan synthase [EC:4.2.1.20] |
| K01850 | E5.4.99.5; chorismate mutase [EC:5.4.99.5] |
| K04092 | tyrA1; chorismate mutase [EC:5.4.99.5] |
| K14187 | tyrA; chorismate mutase / prephenate dehydrogenase [EC:5.4.99.5 1.3.1.12] |
| K04093 | pheA1; chorismate mutase [EC:5.4.99.5] |
| K04516 | AROA1, aroA; chorismate mutase [EC:5.4.99.5] |
| K06208 | aroH; chorismate mutase [EC:5.4.99.5] |
| K06209 | pheB; chorismate mutase [EC:5.4.99.5] |
| K01713 | pheC; cyclohexadienyl dehydratase [EC:4.2.1.51 4.2.1.91] |
| K04518 | pheA2; prephenate dehydratase [EC:4.2.1.51] |
| K05359 | ADT, PDT; arogenate/prephenate dehydratase [EC:4.2.1.91 4.2.1.51] |
| K14170 | pheA; chorismate mutase / prephenate dehydratase [EC:5.4.99.5 4.2.1.51] |
| K00832 | tyrB; aromatic-amino-acid transaminase [EC:2.6.1.57] |
| K15849 | PAT, AAT; bifunctional aspartate aminotransferase and glutamate/aspartate-prephenate aminotransferase [EC:2.6.1.1 2.6.1.78 2.6.1.79] |
| K00220 | tyrC; cyclohexadieny/prephenate dehydrogenase [EC:1.3.1.43 1.3.1.12] |
| K15226 | tyrAa; arogenate dehydrogenase (NADP+) [EC:1.3.1.78] |
| K15227 | TYRAAT; arogenate dehydrogenase (NADP+), plant [EC:1.3.1.78] |

**Supplementary Table 5.** Ability of amino acid biosynthesis in *C. chilonis* based on the pathway completeness analysis.

|  |  |
| --- | --- |
| **Amino acids** | ***C. chilonis*** |
| Alanine | YES |
| Aspartate | YES |
| Glutamate | YES |
| Glutamine | YES |
| Glycine | YES |
| Serine | YES |
| Threonine | YES |
| Cysteine | YES |
| Methionine | YES |
| Valine | NO |
| Leucine | NO |
| Isoleucine | NO |
| Lysine | NO |
| Arginine | YES |
| Proline | YES |
| Histidine | NO |
| Tyrosine | NO |
| Phenylalanine | NO |
| Tryptophan | NO |

**Supplementary Table 6. Up-regulated protease genes in the host *C. suppressalis* after parasitism (log2Foldchange > 2).**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Gene ID** | **FPKM** | **log2Foldchange** | **P value** | **FDR** | **Annotation** |
| **Control** | **Parasitized** |  |  |
| **Hemocytes** |  |  |  |  |  |  |
| evm.model.scaffold28.len4000090.46 | 2.2 | 128.9 | 5.9 | 6.00E-11 | 1.23E-09 | Putative serine protease F56F10.1 |
| evm.model.scaffold517.len147688.1 | 27.1 | 1215.0 | 5.5 | 1.35E-88 | 1.10E-86 | Carboxypeptidase B |
| evm.model.scaffold66.len2634640.29 | 178.0 | 7693.2 | 5.4 | 0 | 0 | Trypsin, alkaline C |
| evm.model.scaffold2.len10331172.65 | 3.6 | 101.5 | 4.8 | 4.82E-07 | 7.11E-06 | Aminoacylase-1 |
| evm.model.scaffold228.len915547.11 | 7.2 | 169.3 | 4.6 | 1.51E-20 | 4.50E-19 | Aminopeptidase N |
| evm.model.scaffold38.len3557774.35 | 151.0 | 3039.1 | 4.3 | 0 | 0 | Trypsin, alkaline C |
| evm.model.scaffold962.len52592.2 | 17.8 | 223.4 | 3.7 | 3.01E-06 | 4.13E-05 | Seminal metalloprotease 1 |
| evm.model.scaffold35.len3613038.30 | 36.6 | 312.0 | 3.1 | 6.08E-23 | 1.93E-21 | Chymotrypsin BI |
| evm.model.scaffold71.len2573062.19 | 27.5 | 167.2 | 2.6 | 1.85E-10 | 3.67E-09 | Serine protease 3 |
| evm.model.scaffold83.len2443179.25 | 14.2 | 68.1 | 2.3 | 0.00027932 | 0.0028881 | Trypsin-like protease |
| **Fat body** |  |  |  |  |  |  |
| evm.model.scaffold517.len147688.1 | 91.5 | 1947.8 | 4.4 | 5.34E-159 | 4.35E-157 | Carboxypeptidase B |
| evm.model.scaffold486.len177729.1 | 5310.2 | 77454.9 | 3.9 | 0 | 0 | Venom serine protease 34 |
| evm.model.scaffold2310.len18893.1 | 1514.9 | 16465.9 | 3.4 | 0 | 0 | Serine protease snake |
| evm.model.scaffold167.len1327927.16 | 8900.9 | 76627.2 | 3.1 | 0 | 0 | Serine protease easter |
| evm.model.scaffold2895.len14054.1 | 4.3 | 36.6 | 3.1 | 0.00027611 | 0.00168133 | Calpain-B |
| evm.model.scaffold38.len3557774.35 | 56.1 | 356.6 | 2.7 | 2.07E-173 | 1.84E-171 | Trypsin, alkaline C |
| evm.model.scaffold525.len142691.1 | 1877.6 | 9029.6 | 2.3 | 1.12E-120 | 7.26E-119 | Transmembrane protease serine 9 |
| evm.model.scaffold962.len52592.2 | 29.4 | 118.0 | 2.0 | 0.0023592 | 0.01210314 | Seminal metalloprotease 1 |
| evm.model.scaffold281.len686280.8 | 6221.4 | 24655.9 | 2.0 | 0 | 0 | Serine protease 33 |

**Supplementary Table 7.** Comparison of ABC transporter gene numbers among 37 species.

|  |  |
| --- | --- |
| Organisms | ABC |
| *Bombus terrestris* | 97 |
| *Bombus impatiens* | 120 |
| *Melipona quadrifasciata* | 41 |
| *Apis mellifera* | 49 |
| *Eufriesea mexicana* | 84 |
| *Habropoda laboriosa* | 57 |
| *Megachile rotundata* | 140 |
| *Dufourea novaeangliae* | 46 |
| *Atta cephalotes* | 58 |
| *Acromyrmex echinatior* | 101 |
| *Solenopsis invicta* | 54 |
| *Pogonomyrmex barbatus* | 83 |
| *Camponotus floridanus* | 100 |
| *Linepithema humile* | 105 |
| *Ooceraea biroi* | 82 |
| *Harpegnathos saltator* | 119 |
| *Polistes dominula* | 40 |
| *Pteromalus puparum* | 57 |
| *Nasonia vitripennis* | 58 |
| *Anisopteromalus calandrae* | 55 |
| *Pachycrepoideus vindemmiae* | 43 |
| *Ceratosolen solmsi* | 47 |
| *Copidosoma floridanum* | 81 |
| *Trichogramma pretiosum* | 85 |
| *Cotesia chilonis* | 103 |
| *Microplitis demolitor* | 51 |
| *Macrocentrus cingulum* | 52 |
| *Lysiphlebus fabarum* | 57 |
| *Aphidius ervi* | 53 |
| *Diachasma alloeum* | 82 |
| *Fopius arisanus* | 47 |
| *Venturia canescens* | 59 |
| *Diadromus collaris* | 53 |
| *Orussus abietinus* | 94 |
| *Cephus cinctus* | 182 |
| *Neodiprion lecontei* | 85 |
| *Athalia rosae* | 51 |

**Supplementary Table 8.** Amino acid transporter (AAT) gene (including the amino acid/polyamine/organocation (APC) family and the amino acid/auxin permease (AAAP) gene family) numbers among 37 species.

|  |  |  |
| --- | --- | --- |
| Organisms | APC | AAAP |
| *Bombus terrestris* | 51 | 31 |
| *Bombus impatiens* | 46 | 26 |
| *Melipona quadrifasciata* | 13 | 14 |
| *Apis mellifera* | 17 | 13 |
| *Eufriesea mexicana* | 25 | 24 |
| *Habropoda laboriosa* | 21 | 22 |
| *Megachile rotundata* | 54 | 33 |
| *Dufourea novaeangliae* | 18 | 18 |
| *Atta cephalotes* | 18 | 18 |
| *Acromyrmex echinatior* | 37 | 24 |
| *Solenopsis invicta* | 17 | 12 |
| *Pogonomyrmex barbatus* | 26 | 25 |
| *Camponotus floridanus* | 30 | 26 |
| *Linepithema humile* | 32 | 26 |
| *Ooceraea biroi* | 37 | 33 |
| *Harpegnathos saltator* | 42 | 37 |
| *Polistes dominula* | 14 | 12 |
| *Pteromalus puparum* | 14 | 14 |
| *Nasonia vitripennis* | 14 | 14 |
| *Anisopteromalus calandrae* | 15 | 13 |
| *Pachycrepoideus vindemmiae* | 15 | 14 |
| *Ceratosolen solmsi* | 14 | 12 |
| *Copidosoma floridanum* | 26 | 28 |
| *Trichogramma pretiosum* | 24 | 22 |
| *Cotesia chilonis* | 10 | 8 |
| *Microplitis demolitor* | 16 | 12 |
| *Macrocentrus cingulum* | 16 | 12 |
| *Lysiphlebus fabarum* | 17 | 12 |
| *Aphidius ervi* | 15 | 14 |
| *Diachasma alloeum* | 29 | 21 |
| *Fopius arisanus* | 14 | 10 |
| *Venturia canescens* | 16 | 17 |
| *Diadromus collaris* | 15 | 19 |
| *Orussus abietinus* | 34 | 34 |
| *Cephus cinctus* | 66 | 35 |
| *Neodiprion lecontei* | 21 | 20 |
| *Athalia rosae* | 13 | 14 |

**Supplementary Table 9. Up-regulated amino acid transporters in the host *C. suppressalis* after parasitism (log2Foldchange > 2).**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Gene ID** | **FPKM** | **log2Foldchange** | **P value** | **FDR** | **Annotation** |
|  | **Control** | **Parasitized** |  |  |  |  |
| **Hemocytes** |  |  |  |  |  |  |
| evm.model.scaffold4018.len9498.1 | 0.0 | 1033.0 | 15.7 | 0.0004 | 0.0025 | Vacuolar amino acid transporter 5 |
| evm.model.scaffold1.len11383612.58 | 111.2 | 6832.6 | 5.9 | 5.25E-34 | 2.13E-32 | Vesicular glutamate transporter 2 |
| evm.model.scaffold84.len2437568.19 | 327.0 | 3984.5 | 3.6 | 0.0005 | 0.0032 | Proton-coupled amino acid transporter-like protein CG1139 |
| **Fat body** |  |  |  |  |  |  |
| evm.model.scaffold1243.len41907.1 | 395.4 | 4039.1 | 3.4 | 0.0034 | 0.0171 | Vesicular glutamate transporter 2 |
| evm.model.scaffold33.len3817196.43 | 209.4 | 972.7 | 2.2 | 0.01296 | 0.0463 | Proton-coupled amino acid transporter-like protein CG1139 |

**Supplementary Table 10.** Genome sequencing data of *C. chilonis*.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Pair-end libraries** | **Insert size** | **Total data (G)** | **Sequence coverage (X)** | **Read length(bp)** |
| Illumina reads | 250bp | 33 | 174.6 | 150 |
| 2K | 9.62 | 50.9 |
| 5K | 5.92 | 31.32 |
| 10K | 15.9 | 84.13 |
| Pacbio reads | - | 7.63 | 40.37 | - |

**Supplementary Table 11.** Overview of the assembly genome and gene set of *C. chilonis*.

|  |  |
| --- | --- |
| **Assembled genome** |  |
| Total length (Contigs) | 189,230,571 bp |
| N50 length (Contigs) | 1,215,404 bp |
| Total length (Scaffolds) | 189,527,638 bp |
| N50 length (Scaffolds) | 2,208,775 bp |
| Quantity of scaffolds | 365 |
| Largest scaffold | 10,923,184 bp |
| **Quality control** |  |
| CEGMA genes (%) | 95.56 |
| BUSCO v3 genes (%)a | C:96.6[D:1.2], F:1.2, M:2.2, n=1658 |
| **Genome features** |  |
| Repeat (%) | 36.18 |
| GC content (%) | 30.36 |
| Protein coding genesb | 14,142 |

aBUSCO score in BUSCO annotation (C: complete [D: duplicated], F: fragmented, M: missing, n: number of genes used).

bOGS predicted based on the Optimized Maker-based Insect Genome Annotation (OMIGA) pipeline.

**Supplementary Table 12.** Classification of repeat sequences identified in the *C. chilonis* genome.

|  |  |  |  |
| --- | --- | --- | --- |
| **Repeat types** | **Number of elements** | **Length occupied (bp)** | **Percentages of sequence (%)** |
| Interspersed repeats | SINE | 1,819 | 373,936 | 0.2 |
| LINE | 6,090 | 3,594,665 | 1.9 |
| LTR | 7,446 | 7,303,168 | 3.85 |
| DNA elements | 30,274 | 12,839,055 | 6.77 |
| Unclassified | 129,866 | 36,233,310 | 19.12 |
| Satellites |   | 126 | 42,912 | 0.02 |
| Simple repeats |   | 153,716 | 6,957,160 | 3.67 |
| Low complexity |   | 24,312 | 1,229,228 | 0.65 |
| Total base masked |   | 353,649 | 68,573,434 | 36.18 |

**Supplementary Table 13.** Species used for phylogenetic and comparative analyses.

|  |  |  |  |
| --- | --- | --- | --- |
| **Species**  | **Family** | **Superfamily**  | **Data sources** |
| *Cotesia chilonis* | Braconidae  | Ichneumonoidea  | This study |
| *Microplitis demolitor* | Braconidae  | Ichneumonoidea  | https://www.ncbi.nlm.nih.gov/assembly/GCF\_000572035.2 |
| *Macrocentrus cingulum* | Braconidae  | Ichneumonoidea  | https://www.ncbi.nlm.nih.gov/assembly/GCA\_002156465.1 |
| *Diachasma alloeum* | Braconidae  | Ichneumonoidea  | https://www.ncbi.nlm.nih.gov/assembly/GCF\_001412515.1 |
| *Fopius arisanus* | Braconidae  | Ichneumonoidea  | https://www.ncbi.nlm.nih.gov/assembly/GCF\_000806365.1 |
| *Nasonia vitripennis* | Pteromalidae  | Chalcidoidea  | http://hymenopteragenome.org/nasonia/ |
| *Ceratosolen solmsi* | Agaonidae  | Chalcidoidea  | https://www.ncbi.nlm.nih.gov/assembly/GCF\_000503995.1 |
| *Copidosoma floridanum* | Encyrtidae  | Chalcidoidea  | https://www.ncbi.nlm.nih.gov/assembly/GCF\_000648655.2 |
| *Trichogramma pretiosum* | Trichogrammatidae  | Chalcidoidea  | https://www.ncbi.nlm.nih.gov/assembly/GCF\_000599845.2 |
| *Polistes dominula* | Vespidae | Vespoidea | https://www.ncbi.nlm.nih.gov/assembly/GCF\_001465965.1 |
| *Atta cephalotes* | Formicidae | Formicoidea | http://hymenopteragenome.org/ant\_genomes/ |
| *Solenopsis invicta*  | Formicidae | Formicoidea | http://hymenopteragenome.org/ant\_genomes/ |
| *Apis mellifera* | Apidae  | Apoidea | http://hymenopteragenome.org/beebase |
| *Orussus abietinus* | Orussidae  | Orussoidea  | https://www.ncbi.nlm.nih.gov/assembly/GCF\_000612105.2 |
| *Cephus cinctus* | Cephidae | Cephoidea | https://www.ncbi.nlm.nih.gov/assembly/GCF\_000341935.1 |
| *Athalia rosae* | Tenthredinidae  | Tenthredinoidea  | https://www.ncbi.nlm.nih.gov/assembly/GCF\_000344095.2 |
| *Tribolium castaneum*  | Tenebrionidae | Tenebrionoidea | https://www.ncbi.nlm.nih.gov/assembly/GCF\_000002335.3 |

**Supplementary Table 14.** Information on fossils used to calibrate the inferred phylogenetic tree and to estimate divergence times between major lineages.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Calibration** | **Calibration node** | **Fossil** | **Country or state** | **Time interval** | **Formation age range (Ma)** | **Reference** |
| 1 | stem Chalcidoidea | *Khutelchalcis gobiensis* | Mongolia | Barremian | 129.4-125.0 | https://www.paleobiodb.org/ |
| 2 | stem Braconidae  | *Cretobraconus maculatus* | Mongolia | Tithonian | 152.1-145.0 | https://www.paleobiodb.org/ |
| 3 | stem Hymenoptera | *Triassoxyela foveolata* | Kyrgyzstan (Osh) | Carnian | 237.0-228.0 | https://www.paleobiodb.org/ |

**Supplementary Table 15.** Composition of Grace's Insect Medium, according to Thermo Fisher, catalog number: 11605.

|  |  |  |  |
| --- | --- | --- | --- |
| Components | Molecular Weight | Concentration (mg/L) | mM |
| Amino Acids |  |  |  |
| Glycine | 75 | 650 | 8.666667 |
| L-Alanine | 89 | 225 | 2.52809 |
| L-Arginine hydrochloride | 211 | 700 | 3.3175356 |
| L-Asparagine | 132 | 350 | 2.6515152 |
| L-Aspartic acid | 133 | 350 | 2.631579 |
| L-Cystine 2HCl | 313 | 28.68 | 0.09162939 |
| L-Glutamic Acid | 147 | 600 | 4.0816326 |
| L-Glutamine | 146 | 600 | 4.109589 |
| L-Histidine | 155 | 2500 | 16.129032 |
| L-Isoleucine | 131 | 50 | 0.3816794 |
| L-Leucine | 131 | 75 | 0.57251906 |
| L-Lysine hydrochloride | 183 | 625 | 3.4153006 |
| L-Methionine | 149 | 50 | 0.33557048 |
| L-Phenylalanine | 165 | 150 | 0.90909094 |
| L-Proline | 115 | 350 | 3.0434783 |
| L-Serine | 105 | 550 | 5.2380953 |
| L-Threonine | 119 | 175 | 1.4705882 |
| L-Tryptophan | 204 | 100 | 0.49019608 |
| L-Tyrosine disodium salt | 225 | 62.14 | 0.27617776 |
| L-Valine | 117 | 100 | 0.85470086 |
| beta-Alanine | 89 | 200 | 2.247191 |
| Vitamins |  |  |  |
| Biotin | 244 | 0.01 | 4.10E-05 |
| Choline chloride | 140 | 0.2 | 0.00142857 |
| D-Calcium pantothenate | 477 | 0.02 | 4.19E-05 |
| Folic Acid | 441 | 0.02 | 4.54E-05 |
| Nicotinic acid (Niacin) | 123 | 0.02 | 1.63E-04 |
| Para-Aminobenzoic Acid | 137 | 0.02 | 1.46E-04 |
| Pyridoxine hydrochloride | 206 | 0.02 | 9.71E-05 |
| Riboflavin | 376 | 0.02 | 5.32E-05 |
| Thiamine hydrochloride | 337 | 0.02 | 5.93E-05 |
| i-Inositol | 180 | 0.02 | 1.11E-04 |
| Inorganic Salts |  |  |  |
| Calcium Chloride (CaCl2) (anhyd.) | 111 | 500 | 4.5045047 |
| Magnesium Chloride (anhydrous) | 95 | 1070 | 11.263158 |
| Magnesium Sulfate (MgSO4) (anhyd.) | 120 | 1358 | 11.316667 |
| Potassium Chloride (KCl) | 75 | 2800 | 37.333332 |
| Sodium Bicarbonate (NaHCO3) | 84 | 350 | 4.1666665 |
| Sodium Phosphate monobasic (NaH2PO4-H2O) | 138 | 1013 | 7.3405795 |
| Other Components |  |  |  |
| Alpha-Ketoglutaric acid | 146 | 370 | 2.5342467 |
| D-Fructose | 180 | 400 | 2.2222223 |
| D-Glucose (Dextrose) | 180 | 700 | 3.8888888 |
| Fumaric acid | 116 | 55 | 0.47413793 |
| Lactalbumin hydrolysate |  | 3303 | Infinity |
| Malic acid | 134 | 670 | 5 |
| Succinic acid | 118 | 60 | 0.5084746 |
| Sucrose | 342 | 26680 | 78.011696 |
| Yeastolate |  | 3330 | Infinity |

**Supplementary Table 16.** Parameters for quantiﬁcation of 28 amino acids by UPLC-MS/MS.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Abbreviation** | **Full name** | **Retention Time** | **Transition** | **DP** | **CE** | **liner range** | **Calibration Curves \*** | ***R*2** |
|  |  | **(min)** | **(m/z)** | **(V)** | **(eV)** | **(ng/mL)** |  |  |
| 4A3HBA | 4-Amino-3-hydrocybutyricacid | 5.57 | 120>84 | 30 | 16 | 2.5-500 | y = 0.0225x - 0.0562 | 0.9989 |
| 5HLys | 5-Hydroxylysine | 9.61 | 163>85 | 110 | 19 | 2.5-500 | y = 0.0067x - 0.0051 | 0.9998 |
| 5HydroxyTry | 5-HydroxyTryptophan | 4.49 | 221>204 | 60 | 14 | 2.5-500 | y = 0.0006x - 0.0022 | 0.9991 |
| ADMA | Asymmetric dimethylarginine | 7.38 | 203>70 | 80 | 30 | 1-500 | y = 0.0196x - 0.0482 | 0.9982 |
| Arg | Arginine | 8.33 | 175>70 | 50 | 27 | 10-500 | y = 0.0059x + 0.1849 | 0.9985 |
| Asn | Asparagine | 6.36 | 133>74 | 40 | 22 | 5-500 | y = 0.0267x + 0.238 | 0.9983 |
| Asp | Aspartate | 2.88 | 134>74 | 40 | 18 | 1-500 | y = 0.0106x + 0.0128 | 0.9993 |
| bAla | β-Alanine | 4.76 | 90>30.1 | 40 | 21 | 1-500 | y = 0.0037x - 0.0046 | 0.9996 |
| Carn | Carnosine | 8.73 | 227>110 | 50 | 30 | 1-500 | y = 0.0124x - 0.0287 | 0.999 |
| Cys | Cystine | 9.01 | 241>74 | 40 | 38 | 5-500 | y = 0.0022x - 0.0094 | 0.9986 |
| DMGly | N,N-dimethylglycine | 4.69 | 104.1>58 | 40 | 19 | 1-500 | y = 0.0732x - 0.1486 | 0.9992 |
| Gln | Glutamine | 6.15 | 147>84 | 40 | 40 | 5-500 | y = 0.014x + 0.1012 | 0.9991 |
| Glu | Glutamate | 5.81 | 148>84 | 40 | 40 | 5-500 | y = 0.0116x + 0.172 | 0.9987 |
| Gly | Glycine  | 5.71 | 76>30 | 30 | 19 | 5-500 | y = 0.0037x + 0.0522 | 0.9986 |
| His | Histidine | 8.49 | 156>110 | 40 | 17 | 10-500 | y = 0.0049x - 0.023 | 0.998 |
| Ile | Isoleucine | 3.58 | 132>86 | 40 | 13 | 5-500 | y = 0.0069x + 0.1028 | 0.9986 |
| Leu | Leucine | 3.38 | 132>86 | 40 | 13 | 10-500 | y = 0.0094x + 0.184 | 0.9995 |
| Lys | Lysine | 8.55 | 147.1>84 | 40 | 22 | 5-500 | y = 0.0073x - 0.0227 | 0.9989 |
| Met | Methionine | 3.92 | 150>104 | 40 | 25 | 10-500 | y = 0.0036x + 0.0183 | 0.9984 |
| N6-Acetyllysine | N6-Acetyllysine | 5.46 | 189>84 | 40 | 30 | 5-500 | y = 0.0074x + 0.0076 | 0.9992 |
| Orn | Ornithine | 8.68 | 133>70 | 40 | 21 | 5-500 | y = 0.007x + 0.1495 | 0.999 |
| Phe | Phenylalanine | 3.41 | 166>120 | 40 | 19 | 2.5-500 | y = 0.0151x - 0.0537 | 0.9982 |
| Pro | Proline | 4.26 | 116>70 | 50 | 20 | 1-500 | y = 0.0189x + 0.064 | 0.9985 |
| Ser | Serine | 6.2 | 106>60 | 40 | 15 | 5-500 | y = 0.0712x - 0.2962 | 0.9992 |
| Thr | Threonine | 5.56 | 120>74 | 30 | 15 | 5-500 | y = 0.005x + 0.0955 | 0.9987 |
| Try | Tryptophan | 3.53 | 205>188 | 50 | 13 | 5-500 | y = 0.0106x + 0.0388 | 0.9996 |
| Tyr | Tyrosine | 4.45 | 182>136 | 40 | 19 | 5-500 | y = 0.0021x + 0.024 | 0.9996 |
| Val | Valine | 4.15 | 118>72 | 30 | 20 | 10-500 | y = 0.0084x + 0.1015 | 0.9998 |
| Val13C5-15N |  | 4.15 |  | 30 | 15 |  |  |  |
| Ala13C3-15N |  | 5.22 |  | 40 | 15 |  |  |  |
| Arg13C6-15N4 |  | 8.35 |  | 50 | 27 |  |  |  |
| Gln13C5 |  | 6.15 |  | 40 | 40 |  |  |  |
| Glu13C5 |  | 5.82 |  | 30 | 21 |  |  |  |
| Ile13C6-15N |  | 3.58 |  | 30 | 15 |  |  |  |
| Leu13C6 |  | 3.36 |  | 40 | 13 |  |  |  |
| Lys13C6-15N2 |  | 8.57 |  | 40 | 22 |  |  |  |
| Phe13C9-15N |  | 3.41 |  | 40 | 19 |  |  |  |
| Tryptophan-13C11 | 　 | 3.53 | 　 | 50 | 13 | 　 | 　 | 　 |