Phylogenomics and the evolution of hemipteroid insects

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Abstract

Hemipteroid insects (Paraneoptera), with over 10% of all known insect diversity, are a major component of terrestrial and aquatic ecosystems. Previous phylogenetic analyses have not consistently resolved the relationships among major hemipteroid lineages. We provide maximum likelihood-based phylogenomic analyses of a taxonomically comprehensive dataset comprising sequences of 2,395 single-copy, protein-coding genes for 193 samples of hemipteroid insects and outgroups. These analyses yield a well-supported phylogeny for hemipteroid insects. Monophyly of each of the three hemipteroid orders (Psocodea, Thysanoptera, and Hemiptera) is strongly supported, as are most relationships among suborders and families. Thysanoptera (thrips) is strongly supported as sister to Hemiptera. However, as in a recent large-scale analysis sampling all insect orders, trees from our data matrices support Psocodea (bark lice and parasitic lice) as the sister group to the holometabolous insects (those with complete metamorphosis). In contrast, four-cluster likelihood mapping of these data does not support this result. A molecular dating analysis using 23 fossil calibration points suggests hemipteroid insects began diversifying before the Carboniferous, over 365 million years ago. We also explore implications for understanding the timing of diversification, the evolution of morphological traits, and the evolution of mitochondrial genome organization. These results provide a phylogenetic framework for future studies of the group.

Significance Statement

Hemipteroid insects constitute a major fraction of insect diversity, comprising three orders and over 120,000 described species. We used a comprehensive sample of the diversity of this group involving 193 genome-scale datasets and sequences from 2,395 genes to uncover the evolutionary tree for these insects and provide a timescale for their diversification. Our results indicated that thrips (Thysanoptera) are the closest living relatives of true bugs and allies (Hemiptera) and that these insects started diversifying before the Carboniferous period, over 365 million years ago. The evolutionary tree from this research provides a backbone framework for future studies of this important group of insects.

Introduction

The hemipteroid insect orders, Psocodea (bark lice and parasitic lice), Thysanoptera (thrips), and Hemiptera (true bugs and allies; i.e. hemipterans), with over 120,000 described species, comprise well over 10% of known insect diversity. However, the evolutionary relationships among the major lineages of these insects are not yet resolved. Recent phylogenomic analyses questioned the monophyly of this group (1) demanding a reconsideration of the evolution of hemipteroid and holometabolous insects. We assess these prior results, which placed Psocodea as the sister taxon to Holometabola (insects with complete metamorphosis; e.g. wasps, flies, beetles, butterflies), and uncover relationships within and among hemipteroid insect orders by analyzing a large phylogenomic data set covering all major lineages of hemipteroid insects.

Knowledge of the phylogeny of these insects is important for several reasons. First, major transitions between the mandibulate (chewing) mouthpart insect groundplan and piercing-sucking mouthparts occurred in this group. In particular, thrips and hemipterans, and some ectoparasite lice in Psocodea, have highly modified mouthparts adapted for feeding on fluids and, hence, differ markedly from their mandibulate ancestors. Through a series of remarkable modifications, hemipteroids acquired a "piercing-sucking" mode of feeding in both immature and adult stages that enabled them to feed not only on plant vascular fluids, but also on blood and other liquid diets. Resolution of the evolutionary tree of hemipteroid insects is needed to provide a framework for understanding morphological transitions that occurred in this group, as well as to provide a timeframe over which these changes occurred.

In addition, several lineages of hemipteroid insects (particularly thrips and Psocodea) underwent major reorganizations of their mitochondrial genomes, including the emergence of

minicircles (2). Understanding how these changes in mitochondrial genome organization occurred requires knowledge of evolutionary relationships to document in which lineages these changes first arose. Finally, hemipteroids are among the most abundant insects (3) and are therefore key components of terrestrial and aquatic food webs (4). Thus, a robust backbone phylogenetic framework is needed to place ecological studies in their evolutionary context and for use in comparative genomic and macroevolutionary analyses.

Despite their importance, relatively few studies have addressed the relationships among the major groups of hemipteroid insects (Paraneoptera, *sensu stricto* [excluding Zoraptera], also termed Acercaria). While a recent large transcriptome-based phylogenomic analysis of insects (1) provided a well resolved and strongly supported phylogenetic framework for the insect orders in general, it did not sample intensively within individual orders and recovered some unexpected relationships. Among the most puzzling was the non-monophyly of the hemipteroid insects, with Psocodea as the sister taxon of holometabolous insects rather than as sister to thrips plus hemipterans (Condylognatha). Although this result was congruent with one earlier analysis based on three nuclear protein-coding genes (5), it had not been proposed in other molecular phylogenetic or morphological studies. Previous morphological studies indicated monophyly of hemipteroid insects with Psocodea sister to thrips plus hemipterans (6–9); or sometimes a group comprising thrips plus Psocodea (10, 11).

Another unexpected relationship recovered by Misof et al. (1) was the placement of moss bugs (Coleorrhyncha) as sister to a group comprising leafhoppers, cicadas, and relatives (Auchenorrhyncha) instead of sister to true bugs (Heteroptera). A recent morphological study also found some support for moss bugs sister to Auchenorrhyncha (12). In contrast, prior analyses based on morphology (e.g. 9) and DNA sequence data (e.g. 13) consistently placed

moss bugs as sister to true bugs. An analysis of a reduced gene set from transcriptome data (14) also recovered moss bugs as sister to true bugs, while the full gene set placed moss bugs as sister to Auchenorrhyncha. Analysis of mitochondrial genomes (15) produced an even more unconventional result, with moss bugs placed as the sister taxon of planthoppers (Fulgoroidea), making Auchenorrhyncha paraphyletic. Thus, it is important to investigate the placement of moss bugs in more detail with both expanded taxon and gene sampling.

We evaluated these possible conflicts among analyses by analyzing a more comprehensive dataset comprising an increased number of clusters of orthologous sequence groups (2,395 protein-coding, single-copy genes) as well as an increased taxon sample within hemipteroid insects: 160 samples vs. 22 sampled by Misof et al. (1). We included representatives of all major hemipteroid lineages (sub- and infraorders). Outgroups comprised 33 species of holometabolous and non-holometabolous insect orders. This data set enabled us to test the hypothesis of non-monophyly of hemipteroid insects and also provides a more detailed backbone framework for the hemipteroid phylogeny. We evaluate the implications of this phylogeny for understanding the evolution of feeding strategy, morphology, and mitochondrial genome organization of this major group of insects.

Results

Phylogeny of Hemipteroid Insect Orders

Separate amino-acid sequence alignments of the 2,395 single-copy genes across 193 terminal taxa (Supplemental Materials and Methods; Tables S1-S4) yielded a concatenated supermatrix of 859,518 aligned amino-acid positions, which was used in subsequent phylogenetic analyses. A concatenated nucleotide sequence supermatrix of only first and second codon positions resulted in ~1.72 million aligned nucleotide sequence sites. Tree reconstructions based on the nucleotide sequence data supported a phylogenetic tree (Fig. 1, S1, S2) with 172/190 (~90%) of all nodes supported in 100% of bootstrap replicates. The tree based on amino-acid sequence data (Fig. S3) was highly concordant with that based on nucleotide data. Analysis of an optimized amino-acid dataset (see Materials and Methods) produced a tree (Fig. S4) that was identical to that based on all amino-acids with respect to relationships among orders, suborders, infraorders, and superfamilies, but had some minor rearrangements within these groups.

Considering relationships within and among orders in more detail, the thrips (Thysanoptera) were recovered with 100% bootstrap support as the sister taxon of Hemiptera (i.e., monophyletic Condylognatha), although only 68% of quartets supported this result in Fourcluster Likelihood Mapping (FcLM; Tables S5, S6). As in the study of Misof et al. (1), Psocodea were placed as the sister taxon of Holometabola in 100% of bootstrap replicates, rendering hemipteroid insects paraphyletic. However, only 25% of quartets supported Psocodea as sister to Holometabola, compared to 67% of the quartets supporting hemipteroid insect monophyly. Results from the FcLM imply that the placement of Psocodea as sister to Holometabola is unstable and may be due to confounding phylogenetic signal (e.g., from heterogeneous composition of amino-acid sequences, non-stationarity of substitution processes, or non-random distribution of missing data) and is also dependent on the taxon sample. However, permutation tests of these results suggested the impact of these potential confounding signals on the topology was minor (Table S6). To evaluate whether the parasitic lice in particular (Phthiraptera), which have elevated substitution rates compared to other hemipteroids (16), were a possible source of conflicting signal, we compared quartets with and without these ectoparasitic insects as the

representative of Psocodea. However, the support from FcLM for monophyly of hemipteroid insects was highly similar whether parasitic lice were included (66%) or not (67%).

Morphological character mapping over three possible alternative topologies (Fig. S5) revealed no apomorphies supporting Psocodea + Holometabola. In contrast, there are 14 potential apomorphies for the monophyly of Paraneoptera. These results indicate that there is more agreement between morphology and the FcLM results, as compared to the supermatrix analyses with all taxa. For Coleorrhyncha (moss bugs), three characters are apomorphies for a sister relationship to Auchenorrhyncha (leafhoppers and relatives) but two other characters appear to support a sister relationship to Heteroptera (true bugs).

In general, the phylogenetic results from transcriptomes are congruent with the generally accepted classification schemes within these insect orders. Bark lice and parasitic lice (Psocodea) together are monophyletic. As has been suggested based on both morphological (17) and molecular (16, 18) analyses, the parasitic lice are embedded within free-living bark lice, being the sister taxon of book lice (Liposcelididae), which makes the bark lice ("Psocoptera") paraphyletic. In contrast to results based on 18S rDNA sequences (18), parasitic lice (Phthiraptera) were supported as a monophyletic group in our analyses, which included representatives of all four suborders of parasitic lice.

The thrips (Thysanoptera) were found to be monophyletic. The thrips family Phlaeothripidae was recovered as the sister taxon to the remaining thrips (Aeolothripidae + Thripidae), congruent with previous molecular analyses and the current classification of Thysanoptera into the suborders Tubulifera (i.e. Phlaeothripidae) and Terebrantia (all other thrips) (19).

The order Hemiptera was also monophyletic. Within Hemiptera, Sternorrhyncha (whiteflies, psyllids, scales, and aphids) was recovered as the sister taxon of the remaining hemipterans. Recent classification schemes (20) and prior molecular studies (13, 21) have placed the enigmatic moss bugs as the sister taxon of true bugs. However, our results recover moss bugs as the sister taxon of Auchenorrhyncha (leafhoppers, planthoppers, and relatives), which was also found by Misof et al. (1). In FcLM analyses, 96% of quartets placed moss bugs with Auchenorrhyncha, suggesting little underlying conflict in the data for this result (Table S6).

Within Sternorrhyncha, whiteflies (Aleyrodoidea) were sister to the remainder of the suborder, and psyllids (Psylloidea) were sister to a clade composed of aphids (Aphidoidea) + scale insects (Coccoidea), also supported by 91% of quartets in FcLM analyses. Previous phylogenetic analyses of Sternorrhyncha have tended to focus within particular superfamilies or families (e.g. 22–24) rather than addressing relationships among major lineages (superfamilies).

The earliest molecular phylogenetic analyses of Hemiptera (e.g. 25, 26) failed to recover Auchenorrhyncha as a monophyletic group, as has a more recent analysis of mitochondrial genomes (15). However, our analyses provided strong support for monophyly of this group, corroborating results of other studies based on multiple loci (13, 14). Within Auchenorrhyncha, our results strongly support the taxonomic status of the two recognized infraorders Fulgoromorpha (i.e. Fulgoroidea, planthoppers) and Cicadomorpha (leafhoppers/treehoppers, spittlebugs, and cicadas) as monophyletic, as found previously (13). However, relationships among the three superfamilies of Cicadomorpha were inconsistently resolved. Cicadas (Cicadoidea) plus spittlebugs (Cercopoidea) were sister to leafhoppers/treehoppers (Membracoidea) in the analysis of nucleotide sequences (Fig. 1, FcLM 52% of quartets), but cicadas were sister to spittlebugs plus leafhoppers/treehoppers in the analysis of amino-acid

sequence data (Supplemental Figure S1), which was also found in 48% of quartets of nucleotide data in FcLM analyses.

Relationships among the earlier diverging lineages of true bugs (Heteroptera) have not been resolved consistently across previous analyses (14, 27–29), in which the deepest divergences received low statistical branch support and recovered different relationships among infraorders. In our analysis, which included representatives of all seven currently recognized infraorders, the four infraorders for which more than one species was included were found to be monophyletic. Like two recent studies based on combined molecular and morphological data (29) and transcriptome data (14), we found 100% bootstrap support for 1) a clade comprising litter bugs (Dipsocoromorpha), unique-headed bugs (Enicocephalomorpha), and water striders (Gerromorpha) (also found in 100% of quartets in FcLM analyses) and 2) shore bugs (Leptopodomorpha) as the sister to Cimicomorpha + Pentatomomorpha (also found in 100% of quartets in FcLM analyses).

Divergence Time Analysis

The estimate of the root age for our tree, the split between Palaeoptera (dragonflies, damselflies, and mayflies) and Neoptera (all other insects) at 437 million years ago (mya) (95% CI 401-486) was only slightly older than that estimated for this node by Misof et al. (1), at 406 mya. Divergence dates for more interior nodes tended to be older than those estimated by Misof et al. (1) and more similar to those of Tong et al. (30), possibly due either to much denser sampling of minimum age fossil calibration points throughout this part of the insect tree or to different methodology (e.g., MCMCtree versus BEAST; or different prior distributions of expected ages for Bayesian analyses). Analyses of divergence times postulated a common

ancestor of thrips and hemipterans as early as the Devonian (~407 mya, 95% CI 373-451). Radiation within Hemiptera is also inferred to have begun in this period (~386 mya, 95% CI 354-427), with radiations within Sternorrhyncha, Auchenorrhyncha, and Heteroptera having commenced by the late Carboniferous (all before 300 mya). Radiation within modern Psocodea dates to the Carboniferous (328 mya, 95% CI 292-376), with divergence of this lineage from other insects as early as 404 mya (95% CI 367-451).

Discussion

Analysis of 2,395 protein-coding, single-copy genes derived from transcriptomes of hemipteroid insects and outgroups provided strong support for a backbone tree of hemipteroid insects largely congruent with previous analyses and classification schemes. In particular, we recovered with strong support monophyly of the three orders of hemipteroid insects: Psocodea, Thysanoptera, and Hemiptera. We also recovered monophyly of most currently recognized suborders, infraorders, and superfamilies within these groups as well as resolving relationships among these major groups. Although the unconventional result of a sister relationship between Psocodea and Holometabola of Misof et al. (1) appeared to be robust to our substantially increased taxon sampling based on maximum likelihood bootstrapping, it was not supported by Four-cluster Likelihood Mapping analyses. FcLM, which can detect potentially confounding signal, suggests extensive underlying conflict for this result, with the majority of quartets placing Psocodea with thrips and hemipterans, which would imply monophyly of Paraneoptera in rooted trees. However, permutations appear to rule out several possible types of confounding signal (e.g. among-lineage heterogeneity or non-random distribution of missing data) in our dataset. Recent work has suggested that bootstrap support from very large data sets may provide an

overestimate of confidence for phylogenetic results (31–33). Thus, the position of Psocodea in the insect tree is still an open question. Monophyly of hemipteroid insects is supported by several morphological autapomorphies (34); therefore, non-monophyly of the group would imply homoplasy in these traits. In addition, there is no known morphological apomorphy supporting Psocodea + Holometabola (Fig. S5). In contrast, the other less conventional relationship, a clade comprising Coleorrhyncha and Auchenorrhyncha uncovered by Misof et al. (1), was recovered by our trees with increased taxon sampling and is supported by 96% of quartets in the FcLM analyses and three morphological apomorphies, suggesting that this result is robust.

Divergence time estimates using a dense sampling of 23 fossil calibration points suggest that the radiation of the hemipteroid insect orders is relatively ancient, beginning before the early Carboniferous, considerably older than initial expectations based on available fossils. However, the insect fossil record of this period is extremely fragmentary, and relatively old fossils of modern lineages that are used as calibration points imply that branches uniting these lineages must be older still, given that fossil ages represent minimum ages.

Implications for Evolution of Feeding Strategy

Although inferences regarding the evolution of feeding strategies based solely on data from extant species must be interpreted with caution given the high diversity of extinct hemipteroid faunas, our phylogenetic results agree with evidence from the fossil record that the earliest hemipteroids fed on detritus, pollen, fungi, or spores (as in most modern barklice and some modern thrips). Plant-fluid feeding probably coincided with the origin of Hemiptera and was independently derived in thrips. Today, Hemiptera is the fifth largest insect order, surpassed only by the four major holometabolous orders (Hymenoptera, Coleoptera, Lepidoptera, and Diptera). It remains one of the most abundant and diverse groups of plant-feeding insects. Within Hemiptera, the origin of true bugs apparently coincided with a shift from herbivory to predation, with subsequent shifts back to herbivory (29, 35) in the more derived lineages (Pentatomomorpha and Cimicomorpha). The two other large suborders of Hemiptera (Auchenorrhyncha and Sternorrhyncha) feed almost exclusively on vascular plant fluids.

Our results also suggest that the earliest hemipterans fed preferentially on phloem. Phloem feeding remains predominant in extant plant-feeding hemipterans, including nearly all Sternorrhyncha and most Auchenorrhyncha (36), while modern moss bugs feed on phloem-like tissues in mosses (37). A shift to xylem feeding appears to have coincided with the origin of Cicadomorpha (at least the crown group of this lineage), in which all cicadas and spittlebugs retain this preference. This is also supported by the fossil record in which the earliest leafhoppers had inflated faces (38), indicating a preference for xylem feeding, despite the predominance of phloem feeding among modern leafhoppers and trechoppers (Membracoidea). A shift to phloem feeding apparently occurred early in the evolution of Membracoidea but at least one reversal to xylem feeding (in Cicadellinae–sharpshooters) has been inferred previously (39), consistent with our results.

Implications for Morphological Evolution

Based on the conflicting statistical support between the supermatrix analysis and Fourcluster Likelihood Mapping, the position of lice (Psocodea) appears to be unstable. Morphological evidence, in contrast, supports the monophyly of hemipteroid insects (Paraneoptera). Our parsimony mapping of 142 morphological characters (Fig. S5) found no apomorphies supporting Psocodea + Holometabola but 14 apomorphies supporting hemipteroid insect monophyly. Some of these are reductions or losses, including the reduced number of tarsomeres (three in modern hemipteroids), reduced number of Malpighian tubules (four), and presence of only one abdominal ganglionic complex. Nevertheless, these characters, together with characters of the forewing base, still appear to support the sister group relationship between Psocodea and thrips plus hemipterans (11, 34, 40). Thus, the phylogenetic position of Psocodea requires further study of morphological and molecular data.

In contrast to the equivocal support for Paraneoptera, Condylognatha is strongly supported not only in the phylogenomic analyses, but also with six morphological apomorphies. The origin of this group apparently coincided with a distinct shift in mouthpart morphology and feeding habits toward piercing and sucking. These changes include anterior shifting of tentorial pits, elongated and slender mandibles, stylet-like laciniae, and a narrowed labium (Fig. S5). Subsequent evolutionary transformations led to the very distinct and unique piercing-sucking mouthparts of hemipterans that facilitate ingestion of liquid from plant or animal tissues.

The sister-group relationship that we found between moss bugs (Coleorrhyncha) and Auchenorrhyncha has not, to our knowledge, been proposed previously in any explicit phylogenetic analysis other than in recent phylogenomic analyses of transcriptomes (1, 14). Traditionally, moss bugs were treated as one of three suborders of "Homoptera" (along with Sternorrhyncha and Auchenorrhyncha), largely based on the structure of the head. The mouthparts of moss bugs arise posteroventrally (41), as in leafhoppers and relatives, rather than anteriorly as in true bugs (42). Nevertheless, morphological evidence from fossil and living moss bugs, primarily from wing structure and musculature, suggested a closer relationship to true bugs (9, 41, 43). However, a recent comparative morphological study (12) revealed that moss bugs share a unique derived feature of the wing base with Auchenorrhyncha; a membranous proximal

median plate. The same study also showed that some previously suggested morphological synapomorphies of moss bugs and true bugs (Fig. S5C) are either ambiguous or have been misinterpreted (12). Prior molecular evidence supporting moss bugs plus true bugs was also somewhat equivocal (13: ML bootstrap 83% and MP bootstrap 63%). Our results support those of other transcriptome studies (1, 14) in placing Coleorrhyncha sister to Auchenorrhyncha.

Implications for Evolution of Mitochondrial Genome Organization

Several groups of hemipteroid insects have been shown to have highly rearranged mitochondrial genomes (2). The sister relationship between thrips and hemipterans indicates that the heightened rates of mitochondrial (mt) genome rearrangements observed in the lice (44) and thrips (45) are the result of convergence between these two clades. Even if Psocodea is sister to thrips plus hemipterans, and not to holometabolous insects, recent analyses indicating that the ancestor of all Psocodea had a generally standard insect mitochondrial gene order still result in an interpretation involving convergence (46). This phylogenetic evidence is also consistent with the absence of any shared, derived gene arrangements between Psocodea and thrips, as both have independently diverged from the inferred ancestral insect mt genome arrangement (2, 45).

An interpretation involving convergence is also consistent with the varying degrees of rearrangement observed within each order. Within Psocodea, mt genomes vary wildly across different taxonomic scales, from a single derived arrangement found in all Psocomorpha (46), to wide variation within a single genus (*Liposcelis*, 47), and between closely related species of parasitic lice. In contrast, for the thrips, mitochondrial genome arrangements are relatively consistent at the family level (with only tRNA rearrangements observed), albeit still highly rearranged relative to the ancestral insect mt genome (48). Very few rearrangements of any type

are observed in the Hemiptera, with the vast majority of families possessing the inferred ancestral arrangement (2).

In summary, although the exact phylogenetic position of Psocodea remains to be resolved convincingly, our results based on transcriptomes for hemipteroid insects provide a strong new phylogenetic framework for future studies of genomic, morphological, ecological, and behavioral characteristics of this important group of insects.

Materials and Methods

Our general approach closely followed methods described previously by Misof et al. (1) and Peters et al. (49) for phylogenomic analyses of insect transcriptomes (see also Supplemental Material and Methods). Transcriptomes of 140 samples of Paraneoptera were newly sequenced with 100 bp paired-end reads for this study using Illumina HiSeq2000 or HiSeq2500 machines to achieve at least 2.5 Gbp per taxon. These were combined with previously published transcriptomes of 48 species (18 additional Paraneoptera, 5 Palaeoptera, 16 Polyneoptera, and 9 Holometabola [from 1]). Gene sequences from annotated genomes of five species were also included (*Zootermopsis nevadensis, Pediculus humanus, Rhodnius prolixus, Acromyrmex echinatior*, and *Tribolium castaneum*) (Table S1). In total, our analyzed data included 193 samples. The taxon sample includes representatives of 97 hemipteroid families with several larger families represented by multiple subfamily representatives.

All paired-end reads were assembled with SOAPdenovo-Trans (version 1.01; 50) and the assembled transcripts were filtered for possible contaminants (Table S2) as described in Peters et al. (49). The raw reads and filtered assemblies were submitted to the NCBI SRA and TSA archives (Table S1). We searched the assemblies for transcripts of 2,395 protein-coding genes

that the OrthoDB v7 database (51) suggested to be single-copy across the species *Acromyrmex echinatior, Daphnia pulex, Pediculus humanus, Rhodnius prolixus, Tribolium castaneum,* and *Zootermopsis nevadenis* (Table S3) with official gene sets from these (draft) genomes using the software Orthograph (version beta4, 52; results of orthology search see Table S4). Orthologous transcripts were aligned with MAFFT (version 7.123; 53) at the translational (amino acid) level. Subsequently, all multiple sequence alignments (MSAs) were quality checked and, if necessary, improved using the procedure outlined by Misof et al. (1). Corresponding nucleotide MSAs were generated with a modified version of the software Pal2Nal (54) (version 14; see Misof et al. [1] for details on the software modification) using the amino-acid alignments as a blueprint.

Alignment sections that could not be discriminated from randomly aligned regions at the amino-acid level of each gene were identified with Aliscore version 1.2 (55, 56). To maximize the fit of our substitution models, we used a domain-based approach to partition the data (1). We did this by identifying for each gene the protein domains (clans, families) and unannotated regions (also called voids) using the Pfam database (57; Supplemental Materials and Methods). In a simultaneous step, information from Aliscore and from protein domain identification was processed to yield a concatenated partitioned supermatrix with ambiguous alignment regions removed. The phylogenetic information content of each data block was assessed with MARE (version 0.1.2-rc) (58), and all uninformative data blocks (IC=0) were removed. We subsequently used PartitionFinder (developer version 2.0.0-pre14, 59) to simultaneously infer the best partitioning scheme and amino acid substitution models, using the relusterf algorithm. We applied the same partitioning scheme when analyzing the corresponding supermatrix at the nucleotide level, except that we modeled the first and second codon positions of each partition

separately. Third codon positions were excluded from our analyses because of more pronounced compositional heterogeneity (Fig. S6).

Phylogenetic trees were inferred using a Maximum Likelihood approach with ExaML vers. 3.0.17 (60) for both the nucleotide and amino-acid data sets. We conducted 50 ML tree searches (25 with a completely random and 25 with a parsimony starting tree). We performed 50 non-parametric bootstrap replicates mapping the support on the best ML tree after checking for bootstrap convergence with the default bootstopping criteria (61). An optimized dataset, which requires the presence of at least one species from a given taxonomic group (Table S5) in each data block of the supermatrix (62), was used for testing the possible impact of missing data at the partition level.

Four-cluster likelihood mapping (63) was used for assessing the phylogenetic signal for alternative phylogenetic relationships. Permutation tests in these analyses assessed the impact of heterogeneous amino-acid sequence composition among lineages, non-stationarity of substitution processes, and non-random distribution of missing data on the inferred phylogenetic tree (1). Four-cluster likelihood mapping and permutation tests were used for a total of six phylogenetic hypotheses (Tables S5, S6).

To understand the morphological transformations underlying the evolution of the hemipteroid groups and to identify potential shared derived characters (synapomorphies), we used the morphological data matrix of Friedemann et al. (9) with 118 characters of the entire body (with modifications from 14) and additionally 25 characters associated with the wing base (8). Due to the different taxon sampling we analyzed each matrix separately. By tracing characters over the tree using maximum parsimony using Winclada (64), we evaluated three possible phylogenetic alternatives: 1) paraphyletic Paraneoptera and Coleorrhyncha sister to

Auchenorrhyncha (result from ML analysis of transcriptomes), 2) monophyletic Paraneoptera (as suggested by FcLM analyses), and 3) paraphyletic Paraneoptera, but with Coleorrhyncha sister to Heteroptera (as suggested in previous literature).

To estimate divergence dates, we used the topology resulting from ML analysis of first and second position nucleotides as the input tree and assigned 23 ingroup fossil calibration points (65) throughout the tree (Table S7). These calibrations were used as minimum ages in soft bound uniform priors with a root age of 406 mya (1) as a soft bound maximum. These priors were used in a Bayesian MCMCTree (66) molecular dating analysis of a first and second position nucleotide data set for which sites were present in at least 95% of taxa.

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Figure 1. Dated phylogeny of hemipteroid insects (Hemiptera, Thysanoptera, Psocodea) based on maximum likelihood analysis of a supermatrix of first and second codon position nucleotides corresponding to 859,518 aligned amino-acid positions from transcriptome or genome sequences of 193 samples. Colored circles indicate bootstrap support. Timescale in millions of years (bottom) estimated from MCMCTree Bayesian divergence time analyses using 23 fossil calibration points and a reduced dataset. Number of species sampled from each group indicated in parentheses. Higher taxa indicated as taxon labels and below branches; common names above branches. Images represent five major groups: Heteroptera, Auchenorrhyncha, Sternorrhyncha, Thysanoptera, and Psocodea.



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Supplementary Information for

Phylogenomics and the evolution of hemipteroid insects

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Supplemental text Figs. S1 to S6 Tables S1 to S7 References for SI reference citations

Supplemental Materials and Methods

Sampling of transcriptomes. We sampled RNA of 139 species of Paraneoptera for transcriptome sequencing. Collected samples were ground and preserved in RNAlater (Qiagen, Hilden, Germany) and stored at +4 °C or -80 °C or dry at -80 °C until further processing. For all species, we sampled the RNA from the entire body of adult specimens (in a few cases nymphs were also sampled or information on life-stage is unknown). Detailed information on the samples (e.g., collecting information and date) are provided in NCBI along with deposited data (Table S1). For one species (*Largus californicus*), we sequenced two samples because they had not been identified before sequencing. We also included in our study new assemblies of raw data from 48 whole body transcriptomes (18 additional Paraneoptera, 5 Palaeoptera, 16 Polyneoptera, and 9 Holometabola; marked with asterisks in Table S1) published in a preceding investigation (1). Our sampling of transcriptomes thus comprised 158 samples of Paraneoptera, comprising 157 different species, and 30 outgroup species.

Transcriptome sequencing. RNA extraction, next generation sequencing (NGS) library preparation, and sequencing of the prepared libraries on Illumina HiSeq 2000 or 2500 sequencers (Illumina, San Diego, CA, USA) followed the protocol given by Misof et al. (1). In one species (i.e., *Diaphorina citri*), from which a total RNA yield < 3 μ g was obtained, we constructed the NGS library using the TruSeq mRNA Library Prep Kit (Illumina). We sheared the purified mRNA into fragments of 160–170 bp in length using divalent cations at 98 °C. Fragment sizes and concentrations were determined with the aid of an Agilent Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA) and a StepOnePlus Real-Time PCR thermocycler (Applied Biosystems, Waltham, MA, USA). All NGS libraries were paired-end sequenced on a HiSeq 2000 or 2500 (Illumina) with 150 bp (all libraries except those prepared using the TruSeq kit) and 90 bp (libraries prepared with the TruSeq kit) read length. Per library, we collected at least 2.5 Gbp of raw data.

De novo assembly of transcriptomes. Transcript raw reads were assembled using the assembler SOAPdenovo-Trans 31 or 49 kmer (version 1.01) (2). All raw reads were quality checked and trimmed, and all remaining reads were used for *de novo* assembly. Quality check/trimming and *de novo* assembly were done as described by Peters et al. (3).

Identification and removal of contaminating sequences. Local installations of VecScreen (http://www.ncbi.nlm.nih.gov/tools/vecscreen/) and the UniVec database build 7.1 (or 9.0; Table S2) (http://www.ncbi.nlm.nih.gov/tools/vecscreen/univec/) were used to remove terminal and internal contamination with vector or linker/adapter sequences from transcriptome assemblies. The removal of internal contamination resulted in a split of contigs/scaffolds. We next searched the assembled transcriptomes for cross-library contamination, as it often occurs when index-tagged NGS libraries are pooled on the same Illumina NGS sequencer lane. The search strategy is outlined in Mayer et al. (4) and Peters et al. (3). Specifically, we compared each transcriptome assembly with all other assemblies sequenced in context of the 1KITE project using BLASTN of the BLAST+ (version 2.2.29) program suite (5). We explicitly refrained from restricting the contamination search to only those transcriptomes sequenced on the same lane, in order to be able to detect also contamination that may have occurred in pre-sequencing steps (e.g. library construction). In cases where BLASTN identified transcripts that shared a sequence similarity of at least 98% over a length of at least 180 bp, we used the coverage depth to decide which transcript is likely the original sequence (by being more than twice as abundant) and which one likely represents the contamination (by being less abundant). We used as coverage depth of a given transcript the average k-mer coverage statistic provided by the assembly software SOAPdenovo-Trans-31 or 49 kmer (2). Having identified transcripts sharing a high sequence identity, we applied the following procedure: (i) If two transcripts differed more than 2-fold in their relative coverage, we removed the transcript with the lower relative coverage from the corresponding assembly. (ii) If the coverage of the two transcripts in question differed 2fold or less, we conservatively removed both of them from the two corresponding assemblies. This procedure allowed us to remove putative foreign contamination (e.g., from third party libraries sequenced on the same lane, but not present in our analyses). In case of multiple highly similar sequences, we retained only the single transcript with the highest relative coverage, given that its coverage was more than 2-fold higher than the coverage of the second-best matching transcript. We additionally removed transcripts from the assemblies that NCBI identified as possibly foreign contamination when submitting the assemblies to the NCBI Transcriptome Shotgun Assembly (TSA) database (see below). Information on how many transcripts were removed from each transcript library is summarized in Table S2. Due to the different assembly strategies some of the assemblies had significantly shorter and less abundant contigs filtered out due to contamination issues. All assemblies and raw data (reads) are available at NCBI via the Umbrella BioProjects accession number NCBI: PRJNA183205 ("The 1KITE project: evolution of insects") or NCBI: PRJNA296358 ("Hemipteroid Insect Assembling the Tree of Life Project").

Identification of orthologous transcripts of single-copy protein-coding genes. We used the program Orthograph (version beta4) (6) to map transcripts to a set of target genes. For this search, Orthograph requires a set of reference gene groups in which all sequences within a group of genes are suggested to be orthologous among the reference taxa ("orthologous groups"). This set is also referred to as the "ortholog reference set". A customized profile query in OrthoDB7 (7) identified clusters of orthologous sequence groups (COGs) of singlecopy orthologs within Pancrustacea. Specifically, we selected the Pancrustacea node and required the presence of exactly one gene copy (=1) in the genomes of six reference species: Daphnia pulex (8), Zootermopsis nevadensis (9), Pediculus humanus (10), Rhodnius prolixus (11), Tribolium castaneum (12), and Acromyrmex echinatior (13). We also required the presence of zero or a single gene copy (<=1) in the genome of *Acyrthosiphon pisum* (14). We did not restrict the number of gene copies in the remaining of the Pancrustacean genomes. Our specific profile query returned 2,453 clusters of COGs. We did not include the sequences of Acyrthosiphon pisum in our ortholog clusters, so every cluster contained exactly six sequences, one from each of the six reference species. We next downloaded the official gene sets (OGSs) for each reference species, from the respective genome databases (see Table S3). OGSs were downloaded both on the amino acid and the corresponding nucleotide level (transcript sequences and cds). The OGS of Zootermopsis nevadensis was kindly provided by J. Liebig and R. Waterhouse prior to their official release. We modified the OGSs by the use of custom Perl scripts as following: 1) sequence headers were shortened so that they correspond with the public gene identifiers in the OrthoDB-tab-delimited and OrthoDB-fasta files, 2) sequences with ambiguous/duplicate headers were removed in the OGSs files, 3) amino acid sequences with no corresponding nucleotide sequence were removed and vice versa, 4) sequence headers were made corresponding on the amino acid and the nucleotide level, 5) alternative isoforms of the same gene were removed from the OGSs only if the gene belonged to our reference set of COGs, 6) terminal stop codons were removed from the protein OGSs, 7) If the protein sequence used by OrthoDB for inferring *de novo* orthology (fetched from the OrthoDB-fasta file) did not exactly match the sequence with the same identifier in the protein OGS, we replaced the sequence in the OGS with the corresponding OrthoDB sequence. The screening for alternative isoforms of the same gene was only possible in those cases where such information was available from the headers of the sequences. Next, we checked whether all gene identifiers in the OrthoDB-tab-delimited file were present in the respective OGS files. We found 58 COGs for which Daphnia pulex identifiers had no equivalent in the respective protein and nucleotide OGS files. We deleted those COGs from the ortholog reference set, which reduced the total number of orthologous groups to 2,395. The OrthoDB7 IDs of COGs which were deleted from the initial set are the following: EOG7JTJQ7, EOG7HFCC3, EOG7FNQ2F, EOG73RP58, EOG7HXQJZ, EOG79H519, EOG75XTFF, EOG7D8HQK, EOG7H7MX4, EOG7813F7, EOG7VXMQN, EOG7RZHHV, EOG7CKF38, EOG76B9C8, EOG73NSX7, EOG7WDZWG, EOG728MKZ, EOG7WXB40, EOG7M9M92, EOG7DZMFK. EOG7RVNBJ, EOG7RZHKM, EOG7WB3Q1, EOG7F873J, EOG7QZT5N, EOG7WDZWD, EOG74V7SB, EOG74NH9C, EOG75N6RK, EOG7GJJ72, EOG75BM0H, EOG7KDS50, EOG7RRS2S, EOG7JMT9D, EOG7XMDS3, EOG7HXQJW, EOG7B3CFV, EOG75R2XS, EOG72K79M, EOG7XT583, EOG7M9M97, EOG7XHJJH, EOG7H4RP4, EOG7D5NGJ, EOG7BGVGZ, EOG70GZ94, EOG7161Z2, EOG77DW2F, EOG7NH272, EOG7CGJST, EOG72VV23, EOG7QGFXZ, EOG7KMHN3, EOG74Z422, EOG7DZMDN, EOG7V7J1V, EOG7SFVQF, EOG73JXPV. The transcript libraries were searched for these 2,395 single-copy protein-coding target genes with Orthograph (6). Orthograph uses a graph-based approach with the best-reciprocal hit (BRH) criterion to extend clusters of known orthologous sequence groups in transcript sequences. The algorithm maps sequences from the transcript library to the globally best matching COG.

Orthograph depends on the following bioinformatics software packages, of which we used the hereafter specified versions: HMMER (version 3.1b2) (15), BLAST+ (version 2.2.26) (5), Exonerate (version 2.2) (16), and MAFFT (version 7.123) (17). We applied a non-strict reciprocal search. Thus, the BRH criterion was fulfilled if the first reciprocal hit was a protein sequence that was also part of the pHMM, irrespective of the species. We allowed frame shift-corrected transcriptional extension of each transcript beyond the region of the transcript for which the BRH criterion was established (option: extend-orf = 1), as long as this region was not longer than the region for which the BRH was established (see above). We used the accompanying helper script to summarize the Orthograph results from all taxa. When summarizing the results, we removed terminal and masked internal stop codons with X and NNN, respectively, in all amino acid and corresponding nucleotide sequences (option: -s). All other Orthograph parameters were left at the default values.

Searching for transcripts being orthologous to one of the 2,395 target genes in all 188 analyzed transcript libraries returned transcripts of, on average, 2,068 target genes (median: 2,113.5; minimum: 1,451; maximum 2,339; Table S4). All 2,395 target genes were present in at least one of the 188 transcriptomes.

Inference of multiple sequence alignments. We constructed multiple sequence alignments (MSA) using the amino acid sequences with MAFFT (version 7.123we) (17), using the L-INS-i algorithm. We subsequently checked the resulting MSAs and, if necessary, refined the MSAs as outlined by Misof et al. (1). Assessing the quality of the inferred Multiple Sequence Alignments (MSAs) revealed putatively misaligned amino acid transcripts (hereafter referred to as outliers) in 651 of the 2,395 investigated single copy genes. Alignment refinement of the 1,642 outlier sequences succeeded for 449 sequences. The remaining 1,193 sequences, referring to a total of 532 single copy genes, remained outliers and were consequently removed from the MSAs. We also removed the corresponding nucleotide sequences from the respective nucleotide file. In addition, we removed all sequences of *Daphnia pulex* from both the amino acid and nucleotide data as well as sites with gaps only.

We inferred the MSA of each OG also on the transcriptional (nucleotide) level using a modified version of the software Pal2Nal (18) (version 14; see Misof et al. [1] for details on the software modification). All MSAs have been deposited in and are available from Dryad Data Repository (Accession pending).

Protein domain identification. To infer a protein domain-based sequence data partitioning scheme, we applied the procedure described by Misof et al. (1). Protein domains and protein domain clans (i. e., evolutionary related protein domains) (19) were identified on the amino acid level in each MSA by using pHMMs from the Pfam database (release 29) and the software pfam_scan.pl (version 1.5, release date: May 24, 2016) (20) in conjunction with HMMER (version 3.1b2) (15). For additional details, see Misof et al. (1) and Peters et al. (3).

Search for protein domains in the refined amino acid sequence alignments of the 2,395 single-copy genes assigned 33.7% of the alignment sites to domains of Pfam-A. A total of 66.3% of the alignment sites remained unannotated. Based on the domain identification results, we split the 2,395 MSAs and rearranged their sites into 3,717 data blocks. More specifically, by pooling Pfam A domain regions according to their clan annotation (19), we obtained 295 clan-based data blocks. Pooling Pfam-A domains without a clan annotation according to the domain name, we obtained 1,027 data blocks. Finally, pooling unannotated regions (voids) according to their gene origin we obtained 2,395 data blocks corresponding to the 2,395 analyzed genes.

Multiple sequence alignment masking. All amino acid alignments were analyzed with Aliscore version 1.2 (21, 22) to identify blocks of putative ambiguously aligned sequence regions or regions indistinguishable from randomized data. We used the '-e' option, which is able to cope with transcript sequence alignments containing many gaps (23). Aliscore was run with the default sliding window size and the option –r in order to compare all sequence pairs in each sliding window. Alignment segments identified by Aliscore as being ambiguously aligned or randomized in the MSAs were subsequently removed from domain and void data blocks at the amino acid and corresponding nucleotide level. Gap symbols (-) at the beginning and at the end of the resulting MSAs were replaced with 'X' (translational level) and 'N' (transcriptional level), respectively. Finally, we concatenated protein domain-and gene-based data blocks (the latter comprising void regions) to supermatrices using custom Perl scripts.

Removal of phylogenetically non-informative data blocks. We assessed the potential phylogenetic signal of each data block with the software MARE (version 0.1.2-rc) (24). Data blocks whose information content (IC) was zero at the translational level were removed from the supermatrix on the translational and on the transcriptional level using custom Perl scripts.

After (*i*) removing ambiguous alignment sites in each data block resulting from steps outlined above, (*ii*) deleting data blocks that contained an IC = 0 (792 in total), and (*iii*) concatenating all remaining data blocks, the resulting supermatrices consisted of 859,518 amino acid and 1,719,036 nucleotide sites (1st and 2nd codon position only), respectively. The amino acid supermatrix comprised 2,902 data blocks (279 clan data blocks from pooled Pfam-A protein domains with clan association, 855 data blocks from pooled Pfam-A protein domains without clan association, 1,768 data blocks from void regions). Both supermatrices are deposited at Dryad Data Repository (Accession pending).

Partitioning and substitution model selection. We used PartitionFinder (developer version 2.0.0-pre14) (25, 26) to identify combinations of data blocks that can be modeled with the same substitution model and parameters and which therefore should be combined into

partitions. The corrected Akaike information criterion (AICc) (27) was used to assess whether or not to combine data blocks to partitions. PartitionFinder was run with the following settings: branchlengths [linked], models [LG+G, LG+G+F, WAG+G, WAG+G+F, BLOSUM62+G, BLOSUM62+G+F, DCMUT+G, DCMUT+G+F, JTT+G, JTT+G+F, LG4X], model_selection [AICc], search [rclusterf] (Lanfear et al. 2014), weights [rate = 1.0, base = 1.0, model = 0.0, alpha = 1.0], rcluster-percent [100.0], rcluster-max [10,000]. PartitionFinder was started with the following additional command line arguments: '--raxml --ml-tree --all-states --min-subset-size 50'. The restriction to the selected substitution matrices was done because of computational limitations.

PartitionFinder suggested merging the 2,902 data blocks of the amino acid supermatrix into 1,857 partitions. Furthermore, PartitionFinder determined the best amino acid substitution model for each partition. This partitioning scheme and the determined models were used for subsequent analysis.

We obtained a partitioning scheme on the nucleotide level by transferring the partitioning scheme that we used in the amino acid supermatix to the nucleotide supermatrix. An important extension of the partitioning scheme on the nucleotide level is that we treated all 1st and 2nd codon positions of each partition as separate partitions. Third codon positions were discarded and not considered in our analyses, since the nucleotide data set with third codon positions exhibits a much higher level of among lineage compositional heterogeneity. As a result, the nucleotide partitioning scheme had twice as many partitions (3,714) as the amino acid partitioning scheme. In the nucleotide phylogenetic analysis, we used the GTR+G substitution model for all partitions, which is the only model that can be analyzed with ExaML. The final data sets and files containing the inferred partition schemes and model selection results are deposited at Dryad Data Repository (Accession pending). To distinguish the supermatrices mentioned here from the optimized dataset (see below), we refer to the here-mentioned matrices as the "primary matrices".

Testing for compositional heterogeneity. We evaluated whether or not our datasets have evolved under globally stationary, reversible, and homogeneous (SRH) conditions (28). To test for possible compositional heterogeneity of amino acid and nucleotide datasets, we used SymTest version 2.0.47 (https://github.com/ottmi/symtest) (29), which uses matched-pairs tests of homogeneity (for details see Misof et al. [1]). Tests were applied on i) the amino acid supermatrix, ii) the nucleotide supermatrix with all codon positions and iii) the nt supermatrix including 1st and 2nd codon positions only. We generated heatmaps based on the p-values obtained from Bowker's matched-pairs test of symmetry in order to determine which sequence pairs matched SRH conditions. All tested datasets exhibit among-lineage compositions included and the 3rd codon positions excluded because 1st and 2nd codon positions showed less among-lineage heterogeneity than a dataset including all codon positions. We assessed the possible impact of compositional heterogeneity among lineages on the inferred relationships using quartet permutation tests (see below).

We additionally evaluated the coverage of the (amino acid) dataset with respect to pairwise sequence coverage of unambiguous data using AliStat v.1.6 (<u>https://github.com/thomaskf/AliStat</u>) (30, see also 1). The overall completeness score was 60%.

Phylogenetic tree inference and bootstrap analysis. We applied the maximum likelihood optimality criterion (ML) as implemented in ExaML (version 3.0.17) (31) for the phylogenetic inferences. We selected the tree with the best log-likelihood score found in 50
independent tree searches (25 randomized stepwise addition parsimony starting trees, 25 completely random starting trees) per dataset. All starting trees were inferred with RAxML (version 8.0.26) (32). We applied the GTR substitution model when analyzing the nucleotide sequence data and applied the partition-specific substitution models suggested by PartitionFinder when analyzing the amino acid sequences in ExaML. Rate heterogeneity was approximated with gamma distributed site rates, using four discrete rate categories and the median for each category. Node support was estimated with non-parametric bootstrap analyses (33). Bootstrap replicate alignments and random starting trees were generated with RAxML and a custom shell script. Subsequently, ExaML was used to infer one ML tree per bootstrap replicate, applying the original partitioning scheme suggested by PartitionFinder for the respective supermatrix. In order to determine the minimum number of replicates needed for a reliable estimation of bootstrap support values, we used the "autoMRE" bootstrap convergence criterion (34), as implemented in RAxML, with the default threshold setting of 0.03. Convergence was evaluated after analyzing each batch of 50 bootstrap replicates. All inferred trees were rooted with the palaeopteran outgroups.

The majority rule extended (MRE) bootstrap convergence criterion (34) indicated that 50 bootstrap replicates are sufficient to accurately assess node support irrespective of whether we analyzed the primary supermatrix containing amino acid sequences or the primary supermatrix containing the corresponding nucleotide sequences. The node support values in Fig. 1 and Figs. S2 and S3 are consequently based on 50 non-parametric bootstrap replicates each. The results from the phylogenetic tree inference and bootstrap analysis have been deposited at Dryad Data Repository (Accession pending).

Testing for impact of missing data via analysis of a decisive dataset. Following Dell'Ampio et al. (35) and Misof et al. (1), we generated an optimized dataset, which contained at least one species from a given taxonomic group in each partition of the analyzed supermatrix (also known as a "decisive dataset" [35]). We defined 23 taxonomic groups (see Table S5 for which species are part of a given group). Given that the generation of an optimized dataset resulted in some data blocks being excluded from subsequent analyses, we inferred new partitioning schemes and corresponding partition-specific substitution models with PartitionFinder and RAxML seperately for the decisive dataset (with the same versions and settings as outlined above for the primary supermatrix). Note that we considered only amino acid sequences for the phylogenetic analyses of the optimized dataset. The decisive dataset comprises 550,395 amino acid positions, analyzed in 852 partitions. We conducted 50 tree searches with ExaML (version 3.0.17) (25 with randomized stepwise addition parsimony starting trees, 25 with completely random starting trees), identical software and settings to what was used for analysis of the primary supermatrix. The results from analyzing the decisive dataset have been deposited at Dryad Data Repository (Accession pending).

Four-cluster Likelihood Quartet Mapping (FcLM). We applied FcLM to assess the phylogenetic support for conflicting hypotheses (1, 3, 36). We focused on six phylogenetic hypotheses: (*i*) Psocodea sister to Holometabola. This hypothesis was tested with three different analyses, including or excluding subgroups of Condylognatha to assess the impact of these subgroups. First, it was tested with all Condylognatha included (*ia*), second with Thysanoptera excluded and Hemiptera included (*ib*), and third with Hemiptera excluded and Thysanoptera included (*ic*) (*ii*) Thysanoptera sister to Hemiptera; (*iii*) Coleorrhyncha sister to Auchenorrhyncha; (*iv*) Myerslopiidae sister to remainder of Membracoidea; (*v*) Aphidoidea sister to Coccoidea; (*vi*) Nepomorpha sister to the clade including Leptopodomorpha, Cimicomorpha and Pentatomomorpha; (*viii*) Gerromorpha sister to a clade including

Dipsocoromorpha and Enicocephalomorpha; (ix) Cercopoidea sister to Cicadoidea.

For each hypothesis tested, we defined four taxonomic groups. Specific information about which species were part of which group is given in Table S5. For testing each hypothesis, we generated optimized datasets, including only those partitions of the primary supermatrix which contained sequences of at least one species from the four taxa specified for testing the respective hypothesis (see Table S5).

We used the PTHREADS implementation of RAxML version 8.2.8 (32) to generate parsimony start trees and used RAxML version 8.2.8 PTHREADS and Examl version 3.0.17 (31) to infer the support for each quartet using the partition scheme and substitution models inferred for the complete supermatrix at the translational (amino acid) level. Results were finally mapped into 2D simplex graphs with a custom-made Perl script. Results are provided in Table S6. The optimized analyzed amino acid data sets used for FcLM have been deposited along with partition information files at Dryad Data Repository (Accession pending).

Results from FcLM testing the putative sister group relationship of Psocodea and Holometabola did not support the ML tree topology, i.e., Psocodea and Holometabola as sister groups, irrespective of whether all condylognathan taxa were included or certain taxa (Thysanoptera, Hemiptera) were excluded. The majority of quartets in all analyses support monophyletic Acercaria, with about half to two thirds of the quartets. We examined these results in further detail: based on the results including all Condylognathan taxa we only mapped selected quartets excluding certain psocodean or condylognathan taxa *a posteriori* (see Tables S5; S6) to see if the support for monophyletic Acercaria could be associated with the inclusion of certain taxonomic groups. Specifically, we excluded quartets including representatives of the following taxa from the results of (*ia*): (*iaIV*) all Phthiraptera, (*iaII*) all Phthiraptera plus Liposcelididae, (*iaIII*) all "Psocoptera", (*iaIV*) all Phthiraptera plus all Thysanoptera (see Tables S5; S6). In all tests, the results only marginally differed from the analysis including all taxa, the percentage of quartets supporting monophyletic Acercaria varied between 66% and 68%. This implies that the incongruence between the ML tree and the FcLM results is not caused by signal of certain taxonomic groups.

FcLM results of testing whether Nepomorpha are sister group of the clade including Leptopodomorpha, Cimicomorpha, and Pentatomomorpha (hypothesis (*vi*)) are not in line with the results of the ML tree reconstructions. All three possible unambiguous placements of Nepomorpha are supported by roughly one third of the quartets. However, the placement of Nepomorpha as sister group of the clade including Leptopodomorpha, Cimicomorpha, and Pentatomomorpha is also not fully supported in the ML tree. The placement of Nepomorpha should be addressed again in future studies, using a more specifically designed dataset with a broader taxon sampling in the respective groups.

Testing for non-phylogenetic signal biases via permutation tests. To assess the possible impact of confounding signal, we adopted the permutation approach suggested by Misof et al. (1): the permutation tests were performed using FcLM (see above). Confounding signal could arise from a variety of sources: 1) heterogeneous composition of amino acid sequences (i.e., among-lineage heterogeneity), 2) non-stationary substitution processes, and 3) non-random distribution of missing data on our phylogenetic inferences. All three phenomena are present in our datasets. For more information on the applied permutation schemes and their explanatory power, see Misof et al. (1). FcLM was conducted on the translational level and using the same partition scheme as before, but using the LG substitution matrix across all partitions for permutation I, II and III. Permutation tests were done for the hypotheses and with the identical taxonomic groups as described above (section 14), using RAxML (versions 8.2.6 and 8.2.8) (32) and ExaML (version 3.0.17) (31). Results of the permutation tests are

provided in Table S6.

In none of the permutation tests did any of the three possible unambiguous topologies receive more than 20% support except when testing the sister group relationship of Aphidoidea and Coccoidea (hypothesis v). Here, support for the three unambiguous topologies was rather uniformly distributed in the permutation tests, with peaks of 33% (permutation II) and 37% (permutation III). However, this artificial signal supported only topologies incompatible with a sister group relationship of Aphidioidea and Coccoidea (which is supported by both the ML tree and the FcLM analysis on the original data). In summary, we interpret the impact of confounding signal on the inferred phylogeny shown in Figure 1 as minor.

Therefore, as also discussed in the main text, reasons for the observed incongruence between ML tree and FcLM results concerning the placement of Psocodea (hypothesis i) are unclear and the underlying phylogenetic question still remains unanswered.

Divergence time estimation. We assigned 23 calibration points throughout the tree (Fig. S1, Table S7). These included four calibration points used by Misof et al. (1) as well as 19 additional fossil calibration points based on best-practice recommendations (37) using only fossils that had an apomorphy for the taxon they represented. Minimum age soft bound (0.025 probability density of age below minimum) uniform priors were applied to these fossil calibration points. For computational efficiency, using the dataset comprising first and second codon positions, we discarded all sites that were not present in at least 95% of the taxa using trimA1 (38). The maximum root age (split between Palaeoptera and remaining pterygote insects) was set to be 406 mya (1) for baseml analysis of substitution rates as well as root calibration. Because this maximum age was a point estimate from a prior study, it is not appropriate to use it has a hard bound maximum, so we used a soft bound (0.025 probability density of maximum age exceeding this value) uniform maximum prior.

We used an approximate likelihood calibration by first estimating the branch lengths with maximum likelihood calculation in *baseml* and then estimating divergence times with a GTR + gamma model with Markov Chain Monte Carlo (MCMC). Priors for the lineage specific rate variation *rgene gamma* and *sigma2 gamma* were set to (1, 2.708) and (1, 4.5), respectively, under an independent rates model (39). MCMC approximations of branching times were derived from 10,000 samples taken every 50 cycles after burnin of 50,000 cycles. The parameters were checked for stationarity in Tracer (40). Two independent runs were performed which produced nearly identical estimates (not shown).



Fig. S1

Dated tree from MCMCTree Bayesian search of first and second position nucleotides filtered to include sites for which 95% of taxa are present (input topology as Fig. 1). Numbers to right of nodes indicate estimate of node age, with 95% confidence intervals noted by light blue bar. Green circles indicate nodes used as calibration points in the analysis, with

age of calibration point given to left of node (< is soft maximum, and > is soft minimum). *Indicate calibrations from Misof et al. (1), all other calibration points detailed in Table S7. Scale is in units of 100 million years.



Fig. S2: Tree from ExaML analysis of first and second position nucleotides (see Materials and Methods for details). Numbers indicate support from bootstrap replicates.



Fig. S3: Tree from ExaML analysis of amino acid sequences (see Materials and Methods for details). Numbers indicate support from bootstrap replicates.



Fig. S4: Phylogenetic tree from ExaML analysis of a "decisive dataset" (see Materials and Methods) for amino acid sequences.



B monophyletic Paraneoptera



C Coleorrhyncha as sistergroup to Heteroptera



Characters:

- 1. jugal bar present
- 2. ocelli of immature stages absent
- 3. cerci of immature stages absent
- 4. paired ocelli in nymphs or larvae absent
- 5. polytrophic ovarioles
- 6. insertion of lacinia detached from stipes
- 7. lacinia elongate and stylet-like
- 8. abdominal ganglia one single mass
- 9. anterior region of 2. axillary sclerite inflated
- 10. abdominal sternite 1 absent 11. four or less Malpighian tubules
- 12. two tarsal segments
- 13. humeral plate and basisubcostale united with each other
- 14. anterior region of 2. axillary sclerite swollen
- 15. posterior median plate located posterodistally to 2.
- axillary sclerite
- 16. posterior median plate deeply concave 17. distal margin of posterior median plate sclerotized
- more strongly than its other regions
- 18. dorsal median plate divided into two sclerites
- 19. distal arm of 3. axillary sclerite and dorsal median plate do not articulate with each other
- 20 basalare and posterior median plate fused with each other
- 21. mandibles elongated
- 22. dorsal median plate placed next to 2. axillary sclerite, articulating along a convex hinge
- 23. proboscis not shifted posteriorly between bases of procoxae
- 24. anterior tentorial pits shifted dorsally
- 25. labrum narrowed
- 26. basisubcostale fused with anteroproximal part of 2. axillary sclerite
- 27. maxillary palps absent or reduced number of seaments
- 28. cardo strongly reduced or absent
- 29. labial rostrum present
- 30. Jabial palps absent or strongly reduced
- 31. second axillary sclerite divided into two sclerites
- 32. labial palp absent
- 33. tubular labium with three segments
- 34. posterior median plate reduced, often completely membranous
- 36. arolium present
- 37. complex tymbal acoustic system present
- 38. aristate antennal flagellum present 39. three tarsal segments
- 40. tubular labium with four segments
- 41. four or less antennal flagellomeres

Fig. S5: Maximum parsimony character mapping of 41 key morphological characters across three phylogenetic scenarios for major lineages of Paraneoptera (A-C). Only characters that are synapomorphic for non-terminal clades are shown. Some identical clades have different synapomorphies shown on different trees because some of the character changes were ambiguous (i.e., may have occurred earlier or later) under that particular phylogenetic scenario under parsimony optimization. Note that, in contrast to previous studies, there are three apomorphies supporting Coleorrhyncha + Auchenorrhyncha but only two supporting Coleorrhyncha + Heteroptera. The full morphological data matrix and character list are available in Dryad.

35. Evan's organ present





Fig. S6A





Fig. S6B



Fig. S6C

Fig. S6 Test for among lineage compositional heterogeneity. Heat map showing pairwise Bowker's tests of the full supermatrix of aligned amino acid sequences (A) and of nucleotide sequences of 1.+2. codon position (B) and of 1.,2.+3. codon position (C). P-values > 0.05 are colored in white and indicate sequence pairs that fully match SRH conditions.

Table S1. NCBI accession numbers of the sequenced and assembled transcriptomes. (*raw data from Misof et al. [1])

Library ID	Species	NCBI Tax ID	BioProject No.	BioSample No.	Experiment No.	Run No.	TSA Project No.	TSA Version
INSobdTACRAAPEI-89	Aaroniella sp.	1585309	PRJNA272159	SAMN03331946	SRX1048894	SRR2051465	GDEY00000000	GDEY01000000
INSeqtTCCRAAPEI-13	Amphipsocus japonicus	297952	PRJNA272160	SAMN03331947	SRX1048895	SRR2051466	GDDX0000000	GDDX01000000
INSIUDTBMRAAPEI-34	Aphelocheirus aestivalis	280209	PRJNA272161	SAMN03331948	SRX1048896	SRR2051467	GDEQ0000000	GDEQ01000000
INSUPTAFRAAFEI-44	Aphrophora ann Aquarius paludum	95691	PR/INA272162	SAMN03331950	SRX1048898	SRR2051460	GDEN00000000	GDEN01000000
INSlupTALRAAPEI-62	Aradus betulae	1452765	PRJNA272164	SAMN03331951	SRX1048899	SRR2051470	GDFD00000000	GDFD01000000
INSgigTBURAAPEI-171	Arocatus melanocephalus	1561047	PRJNA272165	SAMN03331952	SRX1048900	SRR2051471	GDEZ00000000	GDEZ01000000
INSofmTBCRAAPEI-37	Badonnelia titei	297922	PRJNA272166	SAMN03331953	SRX1048901	SRR2051472	GDFG00000000	GDFG01000000
INSobdTABRAAPEI-88	Bertkauia sp.	1585311	PRJNA272167	SAMN03331954	SRX1048902	SRR2051473	GDET00000000	GDET01000000
INSCIPTION AND INSCIPTION AND INSCIPTION AND INSCIPTION AND INC.	Caliscelis bonellii	1585281	PRJNA2/2168	SAMN03331955	SRX1048903	SRR2051474	GDFE00000000	GDFE01000000
INSylvTAQRAAPEI-43	Cerobasis questfalica	209976	PRJNA272109	SAMN03331950	SRX1048904	SRR2051475	GDFA0000000	GDFF01000000
INSidsTAFRAAPEI-31	Cimex lectularius	79782	PRJNA272171	SAMN03331958	SRX1048906	SRR2051477	GDEK00000000	GDEK01000000
INSlupTAORAAPEI-79	Corixa punctata	1545103	PRJNA272172	SAMN03331959	SRX1048907	SRR2051478	GDDR0000000	GDDR01000000
INSbusTBLRAAPEI-77	Corythucha ciliata	369451	PRJNA272173	SAMN03331960	SRX1048908	SRR2051479	GDDW00000000	GDDW01000000
INSeqtTDYRAAPEI-20	Daktulosphaira vitifoliae	58002	PRJNA272174	SAMN03331961	SRX1048909	SRR2051480	GDEB00000000	GDEB01000000
INSpmbTABRAAPEI-227	Diaphorina citri	121845	PRJNA272175	SAMN03331962	SRX1048910	SRR2051481	GDFN00000000	GDFN01000000
INSIUP I ASKAAPEI-89	Dictyophara europaea	1240369	PRJNA272176	SAMN03331963	SRX1048911 SRX1048912	SRR2051482	GDEP00000000	GDEP01000000
INSinITASRAAPEI-36	Echinophthirius horridus	138816	PRJNA272178	SAMN03331965	SRX1048913	SRR2051484	GDEJ00000000	GDEJ01000000
RINSinITAERACPEI-57	Elipsocus kuriliensis	1585294	PRJNA272179	SAMN03331966	SRX1048914	SRR2051485	GDFL00000000	GDFL0100000
INSeqtTCBRAAPEI-12	Embidopsocus sp.	1585315	PRJNA272180	SAMN03331967	SRX1048915	SRR2051486	GDFK00000000	GDFK01000000
INSqiqTBZRAAPEI-21	Franklinothrips vespiformis	297892	PRJNA272181	SAMN03331968	SRX1048916	SRR2051487	GDFM0000000	GDFM01000000
INSytvTAERAAPEI-14	Glycaspis brimblecombei	121847	PRJNA272182	SAMN03331969	SRX1048917	SRR2051488	GDFP00000000	GDFP01000000
INShauTAORAAPEI-8	Graphocephala fennahi	1464911	PRJNA272183	SAMN03331970	SRX1048918	SRR2051489	GDEF00000000	GDEF01000000
INSeqUCERAAPEI-15	Haematomyzus elephantis	239255	PRJNA272185	SAMN03331971	SRX1048919	SRR2051490	GDE100000000	GDE101000000
INSegtTCFRAAPEI-16	Hemipsocus chroloticus	209955	PRJNA272186	SAMN03331973	SRX1048921	SRR2051492	GDDZ00000000	GDDZ01000000
INSobdTADRAAPEI-90	Heterocaecilius solocipennis	297992	PRJNA272187	SAMN03331974	SRX1048922	SRR2051493	GDEU00000000	GDEU01000000
INShkeTCCRAAPEI-37	Hydrometra stagnorum	446445	PRJNA272188	SAMN03331975	SRX1048923	SRR2051494	GDEG0000000	GDEG01000000
INSlupTBERAAPEI-18	llyocoris cimicoides	280203	PRJNA272189	SAMN03331976	SRX1048924	SRR2051495	GDEW0000000	GDEW01000000
INSkzdTABRAAPEI-136	Lachesilla abiesicola	1585296	PRJNA272191	SAMN03331978	SRX1048926	SRR2051497	GDEL0000000	GDEL01000000
INSobdTAGRAAPEI-109	Longivalvus nubilus	469694	PRJNA272192	SAMN03331979	SRX1048927	SRR2051498	GDEV00000000	GDEV01000000
INSGIGTBYRAAPEI-19	Lygaeus equestris Mateumuraialla radiopieta	696229	PRJNA2/2193	SAMN03331980	SRX1048928	SRR2051499	GDFJ00000000	GDFJ0100000
INSeqUCDRAAPEI-14	Malsumuralella radiopicia Mesonsocus uninunctatus	297957	PRJNA272194	SAMN03331981	SRX1040929	SRR2051500	GDDQ0000000	GDDQ01000000
INSvtvTAVRAAPEI-87	Metatronis rufescens	1545162	PR.INA272190	SAMN03331984	SRX1048932	SRR2051502	GDEA00000000	GDEA01000000
INSgigTBERAAPEI-57	Metcalfa pruinosa	1185500	PRJNA272198	SAMN03331985	SRX1048933	SRR2051504	GDFH00000000	GDFH01000000
INSobdTAHRAAPEI-118	Neoblaste papillosa	1585345	PRJNA272199	SAMN03331986	SRX1048934	SRR2051505	GDFC00000000	GDFC01000000
INShkeTCJRAAPEI-41	Notonecta glauca	260537	PRJNA272200	SAMN03331987	SRX1048935	SRR2051506	GDEH00000000	GDEH01000000
INSobdTAFRAAPEI-95	Peripsocus phaeopterus	1585301	PRJNA272201	SAMN03331988	SRX1048936	SRR2051507	GDDU0000000	GDDU01000000
INShtrTABRAAPEI-14	Plea minutissima	280219	PRJNA272202	SAMN03331989	SRX1048937	SRR2051508	GDES00000000	GDES01000000
INSDUSTBURAAPEI-85	Pyrrnocoris apterus Saldula saltatoria	37000	PRJNA272203	SAMN03331990	SRX1048938	SRR2051509	GDF100000000	GDFI01000000
INSobdTAARAAPEI-87	Stimulopalpus japonicus	209965	PRJNA272205	SAMN03331992	SRX1048940	SRR2051511	GDDS00000000	GDDS01000000
INSlupTBKRAAPEI-31	Tachycixius pilosus	166225	PRJNA272206	SAMN03331993	SRX1048941	SRR2051512	GDEX00000000	GDEX01000000
INSlupTAKRAAPEI-57	Ulopa reticulata	139497	PRJNA272207	SAMN03331994	SRX1048942	SRR2051513	GDEO0000000	GDEO01000000
INSytvTCGRAAPEI-39	Valenzuela badiostigma	1407767	PRJNA272208	SAMN03331995	SRX1048943	SRR2051514	GDFB00000000	GDFB01000000
INSjdsTBFRAAPEI-57	Xenophyes metoponcus	1585308	PRJNA272209	SAMN03331996	SRX1048944	SRR2051515	GDEM0000000	GDEM01000000
Accon	Acanalonia conica	1585284	PRJNA272210	SAMN03341923	SRX893485	SRR1821891	GCXC00000000	GCXC01000000
AcspC	Aclerda sp.	1585310	PRJNA2/2211	SAMN03341924	SRX893486	SRR1821892	GCWU00000000	GCWU01000000
Acumb	Acutaspis unibonnera Agallia constricta	1585285	PR INA272212	SAMN03341925	SRX893488	SRR1821893	GCWT0000000	GCWT0100000
Alpil	Alvdus pilosus	1585286	PR.INA272213	SAMN03341927	SRX893489	SRR1821895	GCVZ00000000	GCVZ01000000
AntriUL	Anasa tristis	236421	PRJNA272215	SAMN03341928	SRX893490	SRR1821896	GCWG0000000	GCWG01000000
ScspS2	Anoplogonius nigricollis	1585353	PRJNA272216	SAMN03341929	SRX893562	SRR1821968	GCYN00000000	GCYN01000000
Appol	Aphalara polygoni	1585287	PRJNA272217	SAMN03341930	SRX893491	SRR1821897	GCWO0000000	GCWO01000000
ApspP	Aphelonotus fraterculus	1585355	PRJNA272218	SAMN03341931	SRX893492	SRR1821898	GCWA0000000	GCWA01000000
Arcri	Arilus cristatus	72517	PRJNA272219	SAMN03341932	SRX893493	SRR1821899	GCWE0000000	GCWE01000000
Bettu	Belostoma flumineum	236416	PRJNA272220	SAMN03341933	SRX893494	SRR1821900	GCWK0000000	GCWK01000000
Brocu	Bruchomorpha oculata	130607	PR.INA272221	SAMN03341935	SRX893496	SRR1821902	GCWE0000000	GCWE01000000
BuspN	Buenoa margaritacea	1585289	PRJNA272223	SAMN03341936	SRX893497	SRR1821903	GCXE00000000	GCXE01000000
Cacom	Campanulotes compar	135595	PRJNA272225	SAMN03341937	SRX893577	SRR1821983	GCWD0000000	GCWD01000000
Calat	Campylenchia latipes	54654	PRJNA272226	SAMN03341938	SRX893498	SRR1821904	GCWI0000000	GCWI01000000
CespC	Ceratocombus sp.	1585312	PRJNA272227	SAMN03341939	SRX893500	SRR1821906	GCWS0000000	GCWS01000000
Cecir	Ceroplastes cirripediformis	1585290	PRJNA272228	SAMN03341940	SRX893499	SRR1821905	GCWZ0000000	GCWZ01000000
TespT	Chalcocoris rutilans	1592895	PRJNA272224	SAMN03341941	SRX893565	SRR1821971	GCYS0000000	GCYS01000000
Chhil	Chinavia bilaric	244200	PR INA272229	SAMN03341942	SDX803502	SPR1821938	GC/WR0000000	GC/WR0100000
DispD1	Chondrodire chilensis	1585349	PR.INA272230	SAMN03341943	SRX893509	SRR1821909	GCYG0000000	GCYG0100000
Chaon	Chrysomphalus aonidum	340536	PRJNA272231	SAMN03341945	SRX893501	SRR1821907	GCVU00000000	GCVU01000000
CospC	Coccus sp.	1585313	PRJNA272232	SAMN03341946	SRX893505	SRR1821911	GCWW00000000	GCWW01000000
Cocol	Columbicola columbae	128991	PRJNA272233	SAMN03341947	SRX893578	SRR1821984	GCWB0000000	GCWB01000000
Colat	Corimelaena lateralis	1585293	PRJNA272234	SAMN03341948	SRX893504	SRR1821910	GCXA0000000	GCXA01000000
Crspl	Craspedorrhynchus sp.	1585369	PRJNA272235	SAMN03341949	SRX893506	SRR1821912	GCWN0000000	GCWN0100000
Chcha2	Cycadothrips chadwicki	1291257	PRJNA272236	SAMN03341950	SRX893502	SRR1821908	GCZM00000000	GCZM01000000

Cybel	Cyrpoptus belfragei	1464866	PRJNA272237	SAMN03341951	SRX893507	SRR1821913	GCWQ0000000	GCWQ01000000
Dacon	Dactylopius confusus	38117	PRJNA272238	SAMN03341952	SRX893508	SRR1821914	GCWV0000000	GCWV01000000
Damai	Dalbulus maidis	74065	PRJNA272239	SAMN03341953	SRX893575	SRR1821981	GCWP0000000	GCWP01000000
Echag	Echmepteryx hageni	209971	PRJNA272240	SAMN03341954	SRX893576	SRR1821982	GCWY00000000	GCWY01000000
Then	Empoasca labae	139595	PRJNA272241	SAMN03341955	SRX893511	SRR 1621917	GCVV00000000	GCVV01000000
Geocu	Gelastocoris oculatus	38104	PRJNA272243	SAMN03341957	SRX893514	SRR1821920	GCX100000000	GCXI01000000
GespG	Geocoris sp.	1585316	PRJNA272244	SAMN03341958	SRX893515	SRR1821921	GCWM00000000	GCWM01000000
Geewi	Geomydoecus ewingi	35641	PRJNA272245	SAMN03341959	SRX893513	SRR1821919	GCXD00000000	GCXD01000000
Hechi	Hespenedra chilensis	1585359	PRJNA272247	SAMN03341961	SRX893516	SRR1821922	GCXG00000000	GCXG01000000
Hecub	Heteropsylla cubana	121849	PRJNA272248	SAMN03341962	SRX893517	SRR1821923	GCXB0000000	GCXB01000000
Hoche	Holdgatiella chepuensis	1585361	PRJNA272249	SAMN03341963	SRX893518	SRR1821924	GCXW00000000	GCXW01000000
EnspE	Hopiitocoris sp.	1585371	PRJNA272250	SAMN03341964	SRX893512	SRR1821918	GCWH0000000	GCWH0100000
PespD	Idiosystatus acutiusculus Ischnodemus falicus	1080303	PRJNA272251	SAMN03341965	SRX893044	SRR1821950	GCX20000000	GCX201000000
JasoB	Jalvsus sp.	1585318	PRJNA272253	SAMN03341967	SRX893520	SRR1821926	GCXL00000000	GCXL01000000
Lacon	Lachesilla contraforcepeta	1585297	PRJNA272254	SAMN03341968	SRX893521	SRR1821927	GCWJ00000000	GCWJ01000000
Lasp	Ladella sp.	1585373	PRJNA272255	SAMN03341969	SRX893522	SRR1821928	GCXO00000000	GCXO01000000
LrspL1	Largus californicus	1585298	PRJNA272256	SAMN03341970	SRX893526	SRR1821932	GCXX00000000	GCXX01000000
LrspL2	Largus californicus	1585298	PRJNA272803	SAMN03341971	SRX893527	SRR1821933	GCXR00000000	GCXR01000000
Lican	Limnoporus canaliculatus	56088	PRJNA272257	SAMN03341972	SRX893523	SRR1821929	GCYA00000000	GCYA01000000
Lipil	Llanquihuea pilosa	1585299	PRJNA272258	SAMN03341973	SRX893524	SRR1821930	GCWX00000000	GCWX01000000
Lospivi	Lopidea amorphae	1201711	PRJNA272259	SAMN03341974	SRX893525	SKK1821931	GCXF0000000	GCXF01000000
Lyton	Lygaeus luncicus	50650	PR INA272261	SAMN03341975	SRX803528	SRR1821933	GCYM0000000	GCXM01000000
Magla	Macrohomotoma gladiata	1585365	PRJNA272262	SAMN03341977	SRX893530	SRR1821936	GCXQ00000000	GCXQ01000000
MaspM8	Mapuchea sp.	1585375	PRJNA272263	SAMN03341978	SRX893531	SRR1821937	GCXN00000000	GCXN01000000
PlspP	Megacopta cribraria	299257	PRJNA272264	SAMN03341979	SRX893550	SRR1821956	GCXV00000000	GCXV01000000
Olpla	Melanoliarus placitus	491322	PRJNA272269	SAMN03341980	SRX893537	SRR1821943	GCZE00000000	GCZE01000000
Memul	Mesovelia mulsanti	236398	PRJNA272265	SAMN03341981	SRX893534	SRR1821940	GCXS00000000	GCXS01000000
Megra	Mezira granulata	236326	PRJNA272266	SAMN03341982	SRX893533	SRR1821939	GCYM0000000	GCYM0100000
Nasub	Nabis subcoleoptratus	1594575	PRJNA272267	SAMN03341983	SRX893535	SRR1821941	GCVW00000000	GCVW01000000
Nogih	Neotibicen dorsatus	1404820	PRJNA272295	SAMN03342009	SKX893507	SRR1821973	GCY V00000000	GCYV01000000
Onfas	Onconeltus fasciatus	7536	PR INA272200	SAMN03341985	SRX893538	SRR1821942	GCXX0000000	GCXY01000000
Orins	Orius insidiosus	83647	PRJNA272271	SAMN03341986	SRX893539	SRR1821945	GCYL00000000	GCYL01000000
Orkel	Orothrips kelloggi	1291281	PRJNA272273	SAMN03341988	SRX893540	SRR1821946	GCXT00000000	GCXT01000000
Pacel	Pachypsylla celtidismamma	121851	PRJNA272274	SAMN03341989	SRX893542	SRR1821948	GCYC00000000	GCYC01000000
PespN	Pagasa sp.	1592893	PRJNA272275	SAMN03341990	SRX893545	SRR1821951	GCXU00000000	GCXU01000000
Peham	Peloridium pomponorum	1172124	PRJNA272276	SAMN03341991	SRX893543	SRR1821949	GCZG00000000	GCZG01000000
Phspu	Philaenus spumarius	36667	PRJNA272277	SAMN03341992	SRX893549	SRR1821955	GCZA00000000	GCZA01000000
PhspP	Phlaeothripidae sp.	1585319	PRJNA272278	SAMN03341993	SRX893548	SRR1821954	GCYQ00000000	GCYQ01000000
Phatr	Phylioscells atra	1405150	PRJNAZ72279	SAMN03341994	SKX893540	SRR1821952	GCYHUUUUUUUU	GCYH0100000
DispD2	Piezosternum calidum	1585282	PRJNA272280	SAMN03341995	SRX893510	SRR1821916	GCWL0000000	GCWL01000000
Poqua	Ponana guadralaba	1465165	PRJNA272282	SAMN03341997	SRX893551	SRR1821957	GCZF00000000	GCZF01000000
Prtes	Prociphilus tesselatus	1585303	PRJNA272283	SAMN03341998	SRX893553	SRR1821959	GCZH00000000	GCZH01000000
Prbic	Prosapia bicincta	139605	PRJNA272284	SAMN03341999	SRX893552	SRR1821958	GCYJ00000000	GCYJ01000000
Psfax	Psyllopsis fraxinicola	1585347	PRJNA272285	SAMN03342000	SRX893554	SRR1821960	GCYW00000000	GCYW01000000
Ptjoh	Ptycta johnsoni	469707	PRJNA272286	SAMN03342001	SRX893556	SRR1821962	GCYD00000000	GCYD01000000
RespM	Reuteroscopus ornatus	1010780	PRJNA272288	SAMN03342003	SRX893558	SRR1821964	GCYK0000000	GCYK01000000
Rhobe	Rhagovella obesa Schizaphis graminum	1190598	PRJNA272289	SAMN03342004	SRX893559	SRR1821965	GCTP0000000	GCTP01000000
ScsnS1	Scutelleridae sp	1585322	PR.INA272290	SAMN03342006	SRX893561	SRR1821967	GCYE0000000	GCYE0100000
Secin	Sehirus cinctus	1585283	PRJNA272292	SAMN03342007	SRX893563	SRR1821969	GCZB00000000	GCZB01000000
Stbis	Stictocephala bisonia	1585304	PRJNA272293	SAMN03342008	SRX893564	SRR1821970	GCZK00000000	GCZK01000000
TrspC	Trichocorixa calva	1585305	PRJNA272296	SAMN03342010	SRX893569	SRR1821975	GCYZ00000000	GCYZ01000000
Trcar	Trionymus caricis	1585306	PRJNA272297	SAMN03342011	SRX893568	SRR1821974	GCYT00000000	GCYT01000000
Trurt	Trioza urticae	121826	PRJNA272298	SAMN03342012	SRX893570	SRR1821976	GCYX00000000	GCYX01000000
Tusal	Tuberolachnus salignus	96551	PRJNA272299	SAMN03342013	SRX893571	SRR1821977	GCYY00000000	GCYY01000000
Uneuo	Unaspis euonymi	340633	PRJNA272300	SAMN03342014	SRX893572	SRR1821978	GCZJ0000000	GCZJ01000000
Vifla	Videncene flevomeculete	1585307	PRJNA272301	SAMN03342015	SRA893373	SRR 1821979	GCZC00000000	GCZC01000000
INSvtvTALRAAPEL35*	Acanthocasuarina muelleriana	1323547	PR.INA219519	SAMN02047100	SRX314816	SRR921560	GAYY00000000	GAYY02000000
INSnfrTAPRAAPEI-36*	Acanthosoma haemorrhoidale	483950	PRJNA219520	SAMN02047154	SRX314817	SRR921561	GAUV00000000	GAUV02000000
INShauTBERAAPEI-33*	Aleochara curtula	135881	PRJNA219522	SAMN02047128	SRX314819	SRR921563	GATW00000000	GATW02000000
INSfrgTALRAAPEI-22*	Apachyus charteceus	1323532	PRJNA219524	SAMN02047175	SRX314821	SRR921565	GAUW00000000	GAUW02000000
INSfrgTAZRAAPEI-46*	Aposthonia japonica	911381	PRJNA219525	SAMN02047170	SRX314822	SRR921566	GAWU00000000	GAWU02000000
INShauTAKRAAPEI-90*	Baetis sp.	1476881	PRJNA219528	SAMN02047149	SRX314825	SRR921569	GATU00000000	GATU02000000
INStrgTAVRAAPEI-41*	Biaberus atropos	36942	PRJNA219531	SAMN02047121	SRX314828	SRR921572	GAYD0000000	GAYD02000000
INSTITUTI AARAAPEI-84*	Calopteryx spiendens	02012 070260	PRJNA219534	SAMN02047184	SKX314831	SKK921575	GAYMUUUUUUUUU	GAY M02000000
INSidsTRHRAAPEI-34	Cordulegaster holtoni	126173	PR.INA219537	SAMN02047159	SRX314830	SRR921582	GAYODODODOO	GAY002000000
INSbttTKRAAPEI-18*	Corydalus cornutus	559164	PRJNA219543	SAMN02047201	SRX314840	SRR921584	GATG00000000	GATG02000000
INSytvTCDRAAPEI-35*	Cryptocercus wrighti	89837	PRJNA219546	SAMN02047199	SRX314843	SRR921587	GAZN00000000	GAZN02000000
INSytvTCFRAAPEI-37*	Ectopsocus briggsi	322492	PRJNA219550	SAMN01801569	SRX215455	SRR645929	GAPT00000000	GAPT02000000
INSfrgTAHRAAPEI-18*	Epiophlebia superstes	126247	PRJNA219553	SAMN02047171	SRX314846	SRR921592	GAVW00000000	GAVW02000000
INSytvTBHRAAPEI-14*	Essigella californica	759921	PRJNA219554	SAMN02047099	SRX314848	SRR921594	GAZF00000000	GAZF02000000

INSjdsTATRAAPEI-57* INSytvTCERAAPEI-36* INSjdsTBNRAAPEI-89*	Euroleon nostras Eurylophella sp. Forficula auricularia	516507 1323562 13068	PRJNA219555 PRJNA219556 PRJNA219558	SAMN02047165 SAMN02047200 SAMN02047143	SRX314849 SRX314850 SRX314852	SRR921595 SRR921596 SRR921598	GAXW00000000 GAZG00000000 GAYQ00000000	GAXW02000000 GAZG02000000 GAYQ02000000
INSjdsTABRAAPEI-20* INSfrgTAKRAAPEI-21*	Frankliniella cephalica Galloisiana yuasai Gaylotelaa sa	407008 378494 1323563	PRJNA219559 PRJNA219560	SAMN02047110 SAMN02047172 SAMN02047167	SRX314853 SRX314854	SRR921599 SRR921600	GAYE00000000 GAWN00000000	GAYE02000000 GAWN02000000
INSjdsTADRAAPEI-22*	Gynaikothrips ficorum	59752	PRJNA219563	SAMN02047111	SRX314857	SRR921603	GAXG00000000	GAXG02000000
INSnfrTBERAAPEI-19*	Gyrinus marinus	1323538	PRJNA219564	SAMN02047132	SRX314858	SRR921604	GAUY0000000	GAUY02000000
INSytv1CFRAAPEI-43"	Inocellia crassicornis Leuctra sp	279416	PRJNA219567 PRJNA219571	SAMN02047103 SAMN02047153	SRX314861 SRX314865	SRR921607 SRR921611	GALIE00000000	GAUE02000000
INStmbTBGRAAPEI-33*	Liposcelis bostrychophila	185214	PRJNA219573	SAMN02047187	SRX314867	SRR921613	GAYV00000000	GAYV02000000
INShauTAARAAPEI-90*	Mantis religiosa	7507	PRJNA219575	SAMN02047157	SRX314869	SRR921615	GASW00000000	GASW02000000
INStrg I AFRAAPEI-31*	Menopon gallinae Metallyticus splendidus	328185 627751	PRJNA219579 PRJNA219580	SAMN02047141 SAMN02047174	SRX314873 SRX314874	SRR921619 SRR921620	GAWR0000000	GAWR02000000
INSfrgTBCRAAPEI-57*	Nilaparvata lugens	108931	PRJNA219582	SAMN02047185	SRX314876	SRR921622	GAYF00000000	GAYF02000000
INShauTAPRAAPEI-9*	Notostira elongata	537464	PRJNA219583	SAMN02047151	SRX314877	SRR921623	GASV00000000	GASV02000000
INSnfrTBLRAAPEI-11*	Okanagana villosa	1323542	PRJNA219585	SAMN02047193	SRX314879	SRR921625	GAWQ0000000	GAWQ02000000
INShiri AARAAPEI-13" INSidsTB.IRAAPEI-79*	Orussus abletirius Osmylus fulvicenhalus	446453	PRJNA219580 PRJNA219587	SAMN02047118 SAMN02047166	SRX314880 SRX314881	SRR921620	GAU300000000	GAUJ02000000
INShauTALRAAPEI-93*	Perla marginata	227363	PRJNA219591	SAMN02047115	SRX314885	SRR921631	GATV00000000	GATV02000000
INSfrgTAORAAPEI-33*	Peruphasma schultei	614134	PRJNA219592	SAMN02047114	SRX314886	SRR921632	GAWJ0000000	GAWJ02000000
INSjdsTAIRAAPEI-35*	Planococcus citri	170843	PRJNA219593	SAMN02047127	SRX314887	SRR921633	GAXF0000000	GAXF02000000
INSytVI ANRAAPEI-3/*	Ranatra linearis Subilla sp	280158	PRJNA219599 PRJNA219617	SAMN02047138 SAMN02047106	SRX314893 SRX314911	SRR921639 SRR921657	GAY200000000	GAY202000000
INSfrgTBBRAAPEI-56*	Tanzaniophasma sp.	1323568	PRJNA219604	SAMN02047176	SRX314898	SRR921644	GAXB00000000	GAXB02000000
INSfrgTATRAAPEI-37*	Tenthredo koehleri	1323543	PRJNA219605	SAMN02047159	SRX314899	SRR921645	GAWW00000000	GAWW02000000
INShauTANRAAPEI-95*	Tetrix subulata	288127	PRJNA219606	SAMN02047150	SRX314900	SRR921646	GASQ0000000	GASQ02000000
INSIGSTACRAAPEI-21*	Timema cristinae	101013	PRJNA219609	SAMN02047112 SAMN02047191	SRX314903 SRX314904	SRR921649	GAXC00000000	GAXC02000000
INSfrgTAPRAAPEI-33*	Trialeurodes vaporariorum	88556	PRJNA219611	SAMN02047126	SRX314905	SRR921651	GAWX00000000	GAWX02000000
INSnfrTAORAAPEI-35*	Velia caprai	301293	PRJNA219616	SAMN02047131	SRX314910	SRR921656	GAUO00000000	GAUO02000000
INSjdsTARRAAPEI-47* INSjdsTAQRAAPEI-46*	Xenophysella greensladeae Zorotypus caudelli	1254495 1323545	PRJNA219618 PRJNA219620	SAMN02047181 SAMN02047173	SRX314912 SRX314914	SRR921658 SRR921660	GAY100000000 GAYA00000000	GAYI02000000 GAYA02000000

Table S2. Sequences removed during various contamination filtering steps.

		No. seg. after	No. seg. omitted after	No. seg. filtered	No. sea.	Local UniVec
Library-ID	Species	assembly	cross-contam. screen	by NCBI	published	version
INSobdTACRAAPEI-89	Aaroniella sp.	27,402	239	16	27,147	7.1
INSeqtTCCRAAPEI-13	Amphipsocus japonicus	29,615	412	1	29,202	7.1
INSlupTBMRAAPEI-34	Aphelocheirus aestivalis	40,934	316	5	40.613	7.1
INSlupTAFRAAPEI-44	Aphrophora alni	40.522	544	16	39,962	7.1
INSvtvTBARAAPEI-94	Aquarius paludum	71.669	163	3	71,503	7.1
INSlupTALRAAPEI-62	Aradus betulae	55.623	62	2	55,559	7.1
INSgigTBURAAPEI-171	Arocatus melanocenhalus	45,590	387	47	45,156	7.1
INSofmTBCRAAPEI-37	Badonnelia titei	33 779	186	10	33 583	71
INSobdTABRAAPEI-88	Bertkauja sp	27 453	177	1	27 275	71
INSgigTBMRAAPEI-84	Caliscelis bonellii	32 529	135	7	32 387	71
INSvtvTAORAAPEI-43	Centrotus cornutus	40,730	201	20	40.509	7.1
INSectTCGRAAPEI-17	Cerobasis questfalica	51 021	167	4	50,850	71
INSIde TAERAAREL 31	Cimex lectularius	33 465	74	1	33 300	71
INSIgnTAORAAPEL-79	Corixa nunctata	22 917	77	0	22,840	7.1
INShusTBI RAAPEL77	Condhucha ciliata	19 467	1 477	5	17 985	7 1
INSectTDYRAAPEL20	Daktulosphaira vitifoliae	26 964	450	9	26 505	7.1
INSombTABRAAPEL227	Dianhorina citri	20,004	220	10	28,868	7.1
INSUDTASRAAPEL89	Dictyophara europaea	50 399	243	4	50 152	7.1
	Diplonychuo rusticuo	40.277	510	55	49 710	7.1
	Entransition Contractions	49,277	140	35	40,710	7.1
	Elippopula kurilionoio	47.052	142	20	22,107	7.1
RINSINITAERACPEI-57	Elipsocus kunilensis	47,053	120	43	40,890	7.1
INSEQUICERAAPEI-12	Embluopsocus sp.	39,243	190	1,041	36,007	7.1
INSQIQTBZRAAPEI-21	Pranklinotrinps vespirormis	32,090	110	3	32,571	7.1
INSytv I AERAAPEI-14	Giycaspis brimblecombei	81,857	80	5	81,772	7.1
INShauTAORAAPEI-8	Graphocephala tenhani	31,250	12	3	31,175	7.1
INSeqt I CERAAPEI-15	Graphopsocus cruciatus	22,995	163	<i>(</i>	22,825	7.1
INSIII I AJRAAPEI-75	Haematomyzus elephantis	22,032	120	114	21,798	7.1
INSeqtTCFRAAPEI-16	Hemipsocus chroloticus	28,341	185	6	28,150	7.1
INSobd TADRAAPEI-90	Heterocaecilius solocipennis	31,242	1,018	1	30,223	7.1
INShkeTCCRAAPEI-37	Hydrometra stagnorum	33,023	738	1,276	31,009	7.1
INSlupTBERAAPEI-18	llyocoris cimicoides	30,092	153	1	29,938	7.1
INSkzdTABRAAPEI-136	Lachesilla abiesicola	18,822	770	125	17,927	7.1
INSobdTAGRAAPEI-109	Longivalvus nubilus	25,942	402	1	25,539	7.1
INSqiqTBYRAAPEI-19	Lygaeus equestris	56,420	61	1	56,358	7.1
INSeqtTCDRAAPEI-14	Matsumuraiella radiopicta	25,357	136	0	25,221	7.1
INSobdTAERAAPEI-93	Mesopsocus unipunctatus	24,933	107	0	24,826	7.1
INSytvTAVRAAPEI-87	Metatropis rufescens	24,505	649	8	23,848	7.1
INSqiqTBERAAPEI-57	Metcalfa pruinosa	59,425	793	5	58,627	7.1
INSobdTAHRAAPEI-118	Neoblaste papillosa	30,050	254	12	29,784	7.1
INShkeTCJRAAPEI-41	Notonecta glauca	22,739	94	9	22,636	7.1
INSobdTAFRAAPEI-95	Peripsocus phaeopterus	25,252	1,578	0	23,674	7.1
INSnfrTABRAAPEI-14	Plea minutissima	42,153	954	23	41,176	7.1
INSbusTBJRAAPEI-85	Pyrrhocoris apterus	57,571	2,813	7	54,751	7.1
INSlupTBPRAAPEI-37	Saldula saltatoria	29,656	396	13	29,247	7.1
INSobdTAARAAPEI-87	Stimulopalpus japonicus	22,728	163	0	22,565	7.1
INSlupTBKRAAPEI-31	Tachycixius pilosus	33,958	289	1	33,668	7.1
INSlupTAKRAAPEI-57	Ulopa reticulata	47,519	207	5	47,307	7.1
INSytvTCGRAAPEI-39	Valenzuela badiostigma	19,269	69	12	19,188	7.1
INSjdsTBFRAAPEI-57	Xenophyes metoponcus	76,720	616	28	76,076	7.1
Accon	Acanalonia conica	186,341	710	74,923	110,708	9.0
AcspC	Aclerda sp.	82,501	42	34,766	47,693	9.0
Acumb	Acutaspis umbonifera	80,407	541	29,101	50,765	9.0
Agcon	Agallia constricta	133,937	141	75,416	58,380	9.0
Alpil	Alydus pilosus	77,309	169	31,305	45,835	9.0
AntriUL	Anasa tristis	96,206	76	37,903	58,227	9.0
ScspS2	Anoplogonius nigricollis	27,094	55	7,660	19,379	9.0
Appol	Aphalara polygoni	136,201	101	73,995	62,105	9.0
ApspP	Aphelonotus fraterculus	52,518	48	16,998	35,472	9.0
Arcri	Arilus cristatus	50,450	146	17,710	32,594	9.0
Beflu	Belostoma flumineum	54,409	109	19,878	34,422	9.0
Botri	Boisea trivittata	86,679	192	33,080	53,407	9.0
Brocu	Bruchomorpha oculata	137,320	172	65,978	71,170	9.0

BuspN	Buenoa margaritacea	99,056	91	50,075	48,890	9.0
Cacom	Campanulotes compar	86,458	496	25,271	60,691	9.0
Calat	Campylenchia latipes	140,739	547	66,396	73,796	9.0
CespC	Ceratocombus sp.	65,213	25	24,322	40,866	9.0
Cecir	Ceroplastes cirripediformis	54,822	141	17,407	37,274	9.0
TespT	Chalcocoris rutilans	52,061	61	14,027	37,973	9.0
McspM	Chauliops fallax	44,369	133	15,922	28,314	9.0
Chhil	Chinavia hilaris	83,065	693	31,674	50,698	9.0
DispD1	Chondrodire chilensis	73.874	95	22.229	51.550	9.0
Chaon	Chrvsomphalus aonidum	78.237	229	27.923	50.085	9.0
CospC	Coccus sp.	72,792	174	28,209	44,409	9.0
Cocol	Columbicola columbae	64,966	1.659	24,868	38,439	9.0
Colat	Corimelaena lateralis	82.865	1.020	38,196	43.649	9.0
Crspl	Craspedorrhynchus sp.	61,147	71	21,971	39,105	9.0
Chcha2	Cycadothrins chadwicki	27 167	29	8 942	18 196	9.0
Cybel	Cvrpoptus belfragei	67.604	45	22,692	44,867	9.0
Dacon	Dactylonius confusus	66 214	44	21 570	44 600	9.0
Damai	Dalbulus maidis	26 176	0	514	25,662	9.0
Echag	Echmenteryx hageni	22 137	0	499	21,638	9.0
Emfah	Empoasca fabae	112 926	60	59 323	53 543	9.0
Then	Frankliniella occidentalis	54 713	241	24 912	29 560	9.0
Geocu	Gelestocoris oculatus	73 286	261	29,504	43 521	9.0
GeenG	Geocoris en	59,200	37	20,004	36,872	0.0
Geopti	Geocons sp.	45 022	102	16 500	29,420	0.0
Hoobi	Hosponodra chilonsia	62 011	170	10,000	42 704	0.0
Heaub	Heteropsylla cubana	74 761	96	31 476	42,754	9.0
Hoche	Holdgatiella chenyensis	67 / 17	402	23 730	43,105	0.0
EnenE	Honditagoria en	49.200	492	19 177	43,195	9.0
BoopD	discustatus soutiussulus	40,230	49	56 029	50,004	9.0
respu	laobadomus folious	100,120	194	50,930	60,966 56 101	9.0
Islai	Ischnodernus falicus	109,409	110	04,412	67 492	9.0
Jaspo	Jalysus sp.	70.044	000	94,412	07,402	9.0
Lacon	Lacriesilla contraiorcepeta	72,041	230	43,105	29,498	9.0
Lasp	Ladella sp.	203,450	525	107,149	95,762	9.0
LrspL1	Largus californicus	120,350	95	52,101	08,104	9.0
LrspL2	Largus californicus	118,794	81	48,568	70,145	9.0
Lican	Limnoporus canaliculatus	209,846	287	111,792	97,767	9.0
Lipii	Lianquinuea pilosa	/1,/2/	325	24,842	46,560	9.0
LospM	Lopidea amorphae	60,119	160	24,819	35,140	9.0
Lytur	Lygaeus turcicus	117,789	358	44,815	72,616	9.0
Lylin	Lygus lineolaris	121,382	114	75,718	45,550	9.0
Magla	Macrohomotoma gladiata	83,386	92	37,223	46,071	9.0
MaspM8	Mapuchea sp.	123,033	181	52,373	70,479	9.0
PlspP	Megacopta cribraria	35,716	34	9,547	26,135	9.0
Olpla	Melanoliarus placitus	143,486	273	59,160	84,053	9.0
Memul	Mesovelia mulsanti	183,199	506	102,766	79,927	9.0
Megra	Mezira granulata	111,446	65	51,021	60,360	9.0
Nasub	Nabis subcoleoptratus	55,264	92	22,481	32,691	9.0
Tidor	Neotibicen dorsatus	113,041	159	49,640	63,242	9.0
Negib	Nessorhinus gibberulus	67,149	462	26,966	39,721	9.0
Onfas	Oncopeltus fasciatus	84,660	174	30,974	53,512	9.0
Orins	Orius insidiosus	60,138	160	34,065	25,913	9.0
Orkel	Orothrips kelloggi	54,987	168	22,111	32,708	9.0
Pacel	Pachypsylla celtidismamma	246,968	50	162,680	84,238	9.0
PespN	Pagasa sp.	51,885	62	20,506	31,317	9.0
Peham	Peloridium pomponorum	112,698	154	49,839	62,705	9.0
Phspu	Philaenus spumarius	204,741	313	115,426	89,002	9.0
PhspP	Phlaeothripidae sp.	88,571	1,653	28,867	58,051	9.0
Phatr	Phylloscelis atra	92,285	66	39,666	52,553	9.0
Phpen	Phymata pennsylvanica	117,819	106	62,328	55,385	9.0
DispD2	Piezosternum calidum	38,887	53	11,388	27,446	9.0
Poqua	Ponana quadralaba	150,261	403	69,097	80,761	9.0
Prtes	Prociphilus tesselatus	76,345	35	24,111	52,199	9.0
Prbic	Prosapia bicincta	75,730	66	27,430	48,234	9.0

Defex	Poullonaia fravinicala	140 101	114	79 504	62 542	0.0
Plat	Psyliopsis Iraxinicola	50.004	114	70,324	00,040	0.0
Ptjon	Ptycta jonnsoni	50,831	284	27,768	28,779	9.0
RespM	Reuteroscopus ornatus	50,102	45	16,610	33,447	9.0
Rhobe	Rhagovelia obesa	78,443	52	28,093	50,298	9.0
Scgra	Schizaphis graminum	48,786	103	17,148	31,535	9.0
ScspS1	Scutelleridae sp.	45,583	25	13,708	31,850	9.0
Secin	Sehirus cinctus	88,121	91	32,383	55,647	9.0
Stbis	Stictocephala bisonia	103,720	210	41,014	62,496	9.0
TrspC	Trichocorixa calva	104.655	31	57.173	47.451	9.0
Trear	Trionymus caricis	42 615	29	14 341	28 245	9.0
Truct	Trioza urticae	162 /0/	112	105.037	57 345	0.0
Tuest	Tubereleebaue eeligaue	F0 700	56	100,007	20,750	0.0
lusar	ruberolacrinus salignus	07,700	30	10,955	39,739	9.0
Uneuo	Unaspis euonymi	97,153	451	37,495	59,207	9.0
Uramb	Uroleucon ambrosiae	52,904	25	17,345	35,534	9.0
Vifla	Vidanoana flavomaculata	92,727	944	35,285	56,498	9.0
INSytvTALRAAPEI-35	Acanthocasuarina muellerianae	43,114	212	23	42,879	7.1
INSnfrTAPRAAPEI-36	Acanthosoma haemorrhoidale	28,356	138	0	28,218	7.1
INSnfrTANRAAPEI-34	Cercopis vulnerata	34,017	133	12	33,872	7.1
INSytvTCFRAAPEI-37	Ectopsocus briggsi	35,614	116	4	35,494	7.1
INSytvTBHRAAPEI-14	Essigella californica	58,003	1,347	0	56,656	7.1
INSidsTABRAAPEI-20	Frankliniella cephalica	28,220	2.201	3	26.016	7.1
INSidsTADRAAPEI-22	Gynaikothrips ficorum	111 931	543	138	111 250	7 1
INStmbTBCBAAPEL33	Liposcelis bostrychonhila	33 056	211	234	33 511	7.1
	Mananan gallinaa	05,000	106	204	05,011	7.1
	Nienopon gainnae	20,201	100	0	20,140	7.1
INSIIGTBCRAAPEI-57	Nilaparvata lugeris	40,064	198	0	39,800	7.1
INShauTAPRAAPEI-9	Notostira elongata	27,842	415	14	27,413	7.1
INSnfrTBLRAAPEI-11	Okanagana villosa	51,383	69	0	51,314	7.1
INSjdsTAIRAAPEI-35	Planococcus citri	37,278	120	9	37,149	7.1
INSytvTANRAAPEI-37	Ranatra linearis	32,052	84	11	31,957	7.1
INSjdsTACRAAPEI-21	Thrips palmi	24,671	305	0	24,366	7.1
INSfrgTAPRAAPEI-33	Trialeurodes vaporariorum	50,050	155	0	49,895	7.1
INSnfrTAORAAPEI-35	Velia caprai	25,734	70	0	25,664	7.1
INSjdsTARRAAPEI-47	Xenophysella greensladeae	75,081	460	37	74,584	7.1
INShauTBERAAPEI-33	Aleochara curtula	21,787	113	0	21,674	7.1
INSjdsTATRAAPEI-57	Euroleon nostras	40,636	352	63	40,221	7.1
INSytvTCFRAAPEI-43	Inocellia crassicornis	36,526	133	15	36,378	7.1
INSidsTBJRAAPEI-79	Osmvlus fulvicephalus	23,176	144	0	23.032	7.1
INShttTKRAAPEI-18	Corvdalus cornutus	18,806	142	0	18,664	7.1
INSnfrTBERAAPEI-19	Gvrinus marinus	23 637	127	19	23 491	7 1
	Orussus abietinus	26,005	253	3	26,649	71
	Subilla sp	50,000	1 513	155	57 801	7.1
	Subila Sp.	05,405	400	100	37,001	7.1
INSIIGTATRAAPEI-37	Destis as	20,400	499	0	24,957	7.1
INShauTAKRAAPEI-90	Baetis sp.	23,766	6/	2	23,697	7.1
INStmb I AARAAPEI-84	Calopteryx splendens	21,389	511	0	20,878	7.1
INSjdsTBHRAAPEI-74	Cordulegaster boltoni	30,660	132	1	30,527	7.1
INSfrgTAHRAAPEI-18	Epiophlebia superstes	30,449	61	0	30,388	7.1
INSytvTCERAAPEI-36	Eurylophella sp.	36,421	43	6	36,372	7.1
INSfrgTALRAAPEI-22	Apachyus charteceus	33,943	175	13	33,755	7.1
INSfrgTAZRAAPEI-46	Aposthonia japonica	54,664	204	2	54,458	7.1
INSfrgTAVRAAPEI-41	Blaberus atropos	63,927	258	17	63,652	7.1
INSytvTCDRAAPEI-35	Cryptocercus wrighti	56,337	63	0	56,274	7.1
INSjdsTBNRAAPEI-89	Forficula auricularia	48,947	1,106	5	47,836	7.1
INSfrgTAKRAAPEI-21	Galloisiana yuasai	49,615	93	0	49,522	7.1
INSfrgTAXRABPEI-44	Gryllotalpa sp.	45,804	73	6	45,725	7.1
INSnfrTALRAAPEI-31	Leuctra sp.	27,748	108	1	27,639	7.1
INShauTAARAAPEI-90	Mantis religiosa	45,453	59	0	45,394	7.1
INShauTAMRAAPEI-94	Metallyticus splendidus	62,765	341	0	62,424	7.1
INShauTALRAAPEI-93	Perla marginata	34,553	269	0	34,284	7.1
INSfrgTAORAAPEI-33	Peruphasma schultei	48,023	54	0	47,969	7.1
INSfrgTBBRAAPEI-56	Tanzaniophasma sp.	36.828	221	0	36.607	7.1
INShauTANRAAPEI-95	Tetrix subulata	30.056	248	0	29.808	7.1
INSnfrTBPRAAPEI-15	Timema cristinae	41.806	845	10	40.951	7.1
INSidsTAQRAAPEI-46	Zorotypus caudelli	54.883	420	129	54.334	7.1
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Table S3. Official gene sets used to search for single-copy genes in the assembled transcriptomes.

Species name	No. aa seq (adj.)	No. nt seq. (adj.)	Version	Source	URL	Date of download
Daphnia pulex	30940	30940	jgi060905	wFleabase	http://wfleabase.org/	2015-12-09
Zootermopsis nevadensis	17737	17737	2.1	J. Liebig and R. Waterhouse		2012-03-06
Rhodnius prolixus	15437	15437	1.1	Vectorbase	https://www.vectorbase.org/	2015-12-09
Pediculus humanus	10773	10773	1.2	Vectorbase	https://www.vectorbase.org/	2015-12-09
Acromyrmex echinatior	17277	17277	3.8	Hymenoptera genome database	http://hymenopteragenome.org/acromyrmex/	2015-12-09
Tribolium castaneum	16643	16643	3.0 (GLEAN set)	Beetlebase	http://beetlebase.org/	2012-09-24

Table S4. Assembly statistics of and number of identified target genes in the analyzed transcriptomes.

species name	library ID	orthologs	assembly size	no. of contigs	longest contig	GC content
Aaroniella_sp	INSobdTACRAAPEI-89	1927	23766864	27404	16212	38.0
Acanalonia_conica	Accon	2238	78405065	110708	26636	33.5
Acanthocasuarina_muellerianae	INSytvTALRAAPEI-35	1948	32423115	43114	26973	40.7
Acanthosoma_haemorrhoidale	INSnfrTAPRAAPEI-36	2038	21839689	28363	21664	37.2
Aclerda_sp	AcspC	2194	52568191	47693	28558	36.3
Acutapsis_umbonifera	Acumb	2204	56800197	50765	34303	37.9
Agallia_constricta	Agcon	2224	43799808	58380	16244	38.5
Aleochara_curtula	INShauTBERAAPEI-33	2205	26220864	21790	32053	42.7
Alydus_pilosus		2152	34803354	45835	15679	36.2
Ampnipsocus_japonicus	INSeqUICCRAAPEI-13	2205	29689878	29617	15982	41.3
Anasa_tristis	AntriUL	2232	41/3/148	58227	1/616	36.2
Anopiogonius_nigricollis		1001	13070490	19379	19262	30.1
Apachyus_chaneceus	Appel	2137	22030423	53934 62105	26200	34.4
Aphalara_polygoni Aphalochairus aastivalis		1740	24403060	40938	31110	37.1
Aphelonotus fraterculus		1876	28334676	35472	11008	35.8
Aphrophora alni		2029	24934951	40530	14315	34.6
Aposthonia japonica	INSfraTAZRAAPEI-46	1977	38245803	54667	28491	35.0
Aquarius paludum	INSvtvTBARAAPEI-94	1768	40444876	71674	19731	36.4
Aradus betulae	INSINDTALRAAPEI-62	2059	36374496	55635	20022	40.1
Arilus cristatus	Arcri	2099	26079491	32594	12288	33.0
Arocatus melanocephalus	INSgigTBURAAPEI-171	1624	23080808	45600	23878	36.4
Badonnelia titei	INSofmTBCRAAPEI-37	2213	31991002	33784	12278	40.4
Baetis sp	INShauTAKRAAPEI-90	2187	24843866	23767	15601	47.7
Belostoma_flumineum	Beflu	2043	31958003	34422	25271	38.7
Bertkauia_sp	INSobdTABRAAPEI-88	1967	24712390	27453	15801	39.9
Blaberus_atropos	INSfrgTAVRAAPEI-41	1630	42412602	63930	43709	35.5
Boisea_trivittata	Botri	2239	44811122	53407	40947	34.7
Bruchomorpha_oculata	Brocu	2245	44927908	71170	25096	34.3
Buenoa_margaritacea	BuspN	2209	42732228	48890	26142	39.7
Caliscelis_bonelli	INSqiqTBMRAAPEI-84	2098	24859751	32533	22272	34.7
Calopteryx_splendens	INStmbTAARAAPEI-84	1681	16132415	21395	12927	40.6
Campanulotes_compar	Cacom	2279	37003730	60691	10662	42.3
Campylenchia_latipes	Calat	2248	53986922	73796	26892	36.2
Centrotus_cornutus	INSytvTAQRAAPEI-43	2041	29417059	40732	10054	38.3
Ceratocombus_sp	CespC	2119	32858075	40866	26482	35.3
Cercopis_vulnerata	INSnfrTANRAAPEI-34	2144	29859012	34019	19358	36.5
Cerobasis_guestfalica	INSeqt1CGRAAPEI-17	2219	35966251	51022	8101	40.2
Ceroplastes_cirripediformis		2107	39278582	37274	16944	37.4
Chalcocoris_rutilans	lespl	21/6	32002254	37973	10167	34.6
Chauliops_fallax	MCSPM Chhil	1523	18071929	28314	10202	38.4
Chinavia_niians	Chaop	2200	40040690	50095	10490	34.1
Cirrysomphalus_aonidum		2210	32072011	33466	20073	30.0
		2202	54712771	44400	23700	37.8
Columbicola columbae	Cocol	2263	23469018	38439	12188	39.2
Cordulegaster boltoni	INSidsTBHRAAPEI-74	2123	29371935	30664	33264	40.9
Corimelaena lateralis	Colat	2193	34742355	43649	20654	35.3
Corixa punctata	INSINDTAORAAPEI-79	1812	14994749	22921	26887	40.9
Corvdalus cornutus	INSbttTKRAAPEI-18	2184	20260587	18806	35896	37.3
Corvthucha ciliata	INSbusTBLRAAPEI-77	2261	23062128	19469	13226	44.2
Craspedorrhynchus_sp	Crspl	2329	43044996	39105	47085	36.8
Cryptocercus_wrighti	INSytvTCDRAAPEI-35	2170	48853407	56338	54303	39.4
Cycadothrips_chadwicki	Chcha2	1451	13337924	18196	8752	40.2
Cyrpoptus_belfragei	Cybel	2121	33202685	44867	27489	32.6
Dactylopius_confuses	Dacon	2210	48584386	44600	31937	33.8
Daktulosphaira_vitifoliae	INSeqtTDYRAAPEI-20	2169	23156504	26967	11232	34.0
Dalbulus_maidis	Damai	1708	28305469	25662	9283	36.9
Diaphorina_citri	INSpmbTABRAAPEI-227	1853	25035188	29097	10258	38.7
Dictyophara_europaea	INSlupTASRAAPEI-89	1715	27538128	50408	45016	32.0
Diplonychus_rusticus	INSerITAHRAAPEI-72	1549	23091850	49286	14091	39.7
Echinophthirius_horridus	INSINITASRAAPEI-36	1657	11524277	22275	6618	38.0
Echmepteryx_hageni	Echag	1890	26911408	21638	11802	36.3
Ectopsocus_briggsi	INSytvTCFRAAPEI-37	2153	25227588	35615	9531	43.4

Elipsocus_kuriliensis	RINSinITAERACPEI-57	2191	40016327	47063	8877	44.1
Embidopsocus sp	INSeqtTCBRAAPEI-12	1758	21905982	59250	10702	40.4
Empoasca fabae	Emfab	2088	38163234	53543	15054	39.2
Enionhlehia superstes	INSfraTAHRAAPEI-18	2151	31652091	30452	23676	39.9
Enthrothring arizonao	Phon	2267	50086664	58051	23741	12.1
Engine colifornico		2207	42076090	50031	14679	92.1
		2170	42970009	36013	14070	30.4
Euroleon_nostras	INSIDSTATRAAPEI-57	2149	25501862	40647	12436	34.1
Eurylophella_sp	INSytvTCERAAPEI-36	2255	33582280	36423	24697	38.5
Forficula_auricularia	INSjdsTBNRAAPEI-89	2034	32695435	48954	13835	36.4
Frankliniella_cephalica	INSjdsTABRAAPEI-20	2175	27030171	28223	11155	45.2
Frankliniella_occidentalis	Thsp	2210	32367596	29560	15554	43.9
Franklinothrips_vespiformis	INSgigTBZRAAPEI-21	2084	31768835	32692	14897	40.6
Galloisiana vuasai	INSfrgTAKRAAPEI-21	2061	41946837	49619	40128	39.3
Gelastocoris oculatus	Geocu	2161	32973781	43521	36273	37.9
Geocoris sp	GespG	1850	28981020	36872	10545	37.3
Georgydoecus ewingi	Geewi	2330	30368452	28429	27623	35.1
Chapania brimblosomboi		1024	45276001	01074	2/020	22.7
Grycaspis_briniblecomber	INSYLVI AERAAPEI-14	1924	45576001	01074	24000	33.7
Grapnocepnala_tennani	INShauTAORAAPEI-8	1953	26595304	31251	14969	43.1
Graphopsocus_cruciatus	INSeqt1CERAAPEI-15	2127	24293525	22995	11545	44.3
Gryllotalpa_sp	INSfrgTAXRABPEI-44	2093	35496701	45808	12684	43.4
Gynaikothrips_ficorum	INSjdsTADRAAPEI-22	2064	66024375	111941	16079	40.9
Gyrinus_marinus	INSnfrTBERAAPEI-19	2039	20417344	23637	13197	41.7
Haematomyzus_elephantis	INSinITAJRAAPEI-75	1926	12833632	22048	10375	34.2
Hemipsocus chroloticus	INSeatTCFRAAPEI-16	2059	20416847	28340	10115	38.5
Hespenedra chilensis	Hechi	1973	34845676	42794	16141	37.3
Heterocaecilius solocipennis	INSobdTADRAAPEI-90	2156	28503963	31247	27624	41.0
Heteropsylla cubana	Hecub	2074	31314294	43189	27561	31.6
Heldrotielle, chequencie	Hecho	2074	27742750	43105	10196	20.2
Holugaliella_chepuerisis		2009	37743759	43195	12100	39.3
Hopinocoris_sp		1731	23360899	30064	8092	31.0
Hydrometra_stagnorum	INShkeTCCRAAPEI-37	1835	19418800	33031	7700	41.2
ldiosystatus_acutiusculus	PespD	2186	58492799	60988	27335	37.4
llyocoris_cimicoides	INSlupTBERAAPEI-18	2076	23705280	30096	23234	39.5
Inocellia_crassicornis	INSytvTCFRAAPEI-43	2045	29780369	36528	26505	37.2
lschnodemus_falicus	Isfal	2227	46332497	56131	19540	35.8
Jalysus_sp	JaspB	2145	48312152	67482	26786	37.5
Lachesilla abiesicola	INSkzdTABRAAPEI-136	2144	15723521	18834	75872	43.6
Lachesilla contraforcepeta	Lacon	2305	30598256	29498	23120	43.6
ladella sp	Lasp	2029	56403614	95782	22438	32.0
Largus californicus 1	I rspl 1	2223	52822694	68154	27408	34.3
Largue californicue ?	I repl 2	2284	57081533	70145	30587	34.4
		2204	34596469	27740	16609	44.4
Leucua_sp		2202	54500400	27749	10090	44.4
Limnoporus_canaliculatus		2116	5//21/25	97767	23521	34.8
Liposcelis_bostrycnophila	INStmb1BGRAAPEI-33	2234	30313310	33962	11842	37.0
Llanquihuea_pilosa	Llpil	1913	37598185	46560	14020	38.9
Longivalvus_nubilus	INSobdTAGRAAPEI-109	1748	20513523	25943	27936	40.8
Lopidea_amorphae	LospM	2236	42042747	35140	45764	44.1
Lygaeus_equestris	INSqiqTBYRAAPEI-19	2082	33073221	56428	23814	37.7
Lygaeus_turcicus	Lytur	2209	57465981	72616	20964	33.4
Lygus_lineolaris	Lylin	2197	41384616	45550	16081	41.4
Macrohomotoma gladiata	Magla	1663	28762605	46071	14458	36.8
Mantis religiosa	INShauTAARAAPEI-90	1941	32398303	45459	30306	38.3
Manuchea sn	MaspM8	2196	58929596	70479	23498	37.0
Mapucnou_sp Matsumuraiella, radionicta	INSectTCDRAAPEL14	2236	20010145	25357	12003	44.2
	Dise D	2230	23010143	20007	14075	22.0
wegacopta_cribaria	PISP	2107	23100014	20135	14075	33.9
Menopon_gallinae	INStrg AFRAAPEI-31	2289	30203641	25285	22431	44.1
Mesopsocus_unipunctatus	INSobdTAERAAPEI-93	2015	21829923	24932	20770	44.4
Mesovelia_mulsanti	Memul	2081	51536645	79927	26120	36.9
Metallyticus_splendidus	INShauTAMRAAPEI-94	1879	41919841	62765	28076	38.5
Metatropis_rufescens	INSytvTAVRAAPEI-87	1545	17936255	24507	12345	38.2
Metcalfa_pruinosa	INSqiqTBERAAPEI-57	2057	39141482	59431	46763	37.3
Mezira_granulata	Megra	2270	52025883	60360	23101	34.2
Nabis subcoleoptratus	Nasub	2231	31787638	32691	31678	35.9
Neoblaste papillosus	INSobdTAHRAAPEI-118	2080	26179780	30050	21400	39.8
Nessorhinus aibberulus	Negib	2051	26821667	39721	14307	38.8
Nilananyata Jugans	INSfraTBCDAADELE7	2177	34001040	40068	14006	41.0
i inapai vala_luyono	Intolig I DORMAF LI-37	2111	34301043	+0000	14000	41.0

Notonecta_glauca	INShkeTCJRAAPEI-41	1861	18412270	22742	17892	39.5
Notostira elongata	INShauTAPRAAPEI-9	2010	27035672	27842	20340	42.6
Okanagana_villosa	INSnfrTBLRAAPEI-11	2034	41652891	51387	27820	36.6
Oliarus placitus	Olpla	2079	65922569	84053	23378	37.2
Oncopeltus fasciatus	Onfas	2229	43620981	53512	26876	34.6
Orius insidiosus	Orins	2218	26926668	25913	25544	38.9
Orothrips kelloggi	Orkel	2145	35528455	32708	14480	42.0
Orussus abietinus	INSnfrTAARAAPEI-13	2250	41103769	26905	30157	45.0
Osmylus fulvicenhalus	INSidsTB IRAAPEI-79	2146	26355321	23179	19514	41.5
Pachynsylla, celtidismamma	Pacel	2102	61715315	84238	24914	34.6
Pagasa sp	PospN	2280	30300488	31317	23075	37.6
Payasa_sp Deleridium hommonierum	Pespin	2200	50390400	60705	23975	37.0
Pelondium_nammoniorum		2111	01/20140	02705	13029	37.1
Peripsocus_priaeopierus		2000	21477391	20202	9465	41.4
Peria_marginata	INSTAULALRAAPEI-93	1648	25914117	34552	49442	40.1
Peruphasma_schulter	INSTIGIAORAAPEI-33	1801	39144177	48026	29663	41.5
Philaenus_spumarius	Phspu	2248	65543966	89002	17088	34.4
Phylloscelis_atra	Phatr	2199	36849431	52553	27734	32.2
Phymata_pennsylvanica	Phpen	2176	38379194	55385	17434	35.0
Piezosternum_callidum	DispD2	1985	22251963	27446	13970	34.0
Planococcus_citri	INSjdsTAIRAAPEI-35	2073	33725955	37278	19452	37.4
Plea_minutissima	INSnfrTABRAAPEI-14	1985	29142902	42160	17548	39.6
Ponana_quadralaba	Poqua	2267	64045803	80761	37426	36.3
Prociphilus_tesselatus	Prtes	2192	47009400	52199	20954	32.8
Prosapia_bicincta	Prbic	2156	39526530	48234	26849	34.3
Psyllopsis_faxinicola	Psfax	2012	46898347	63543	26501	39.6
Ptycta_johnsoni	Ptjoh	2039	25929616	28779	8858	37.8
Pyrrhocoris_apterus	INSbusTBJRAAPEI-85	2211	50904712	57571	17493	45.8
Ranatra linearis	INSvtvTANRAAPEI-37	1879	25565273	32053	20494	39.2
Reuteroscopus ornatus	RespM	2229	38334317	33447	28771	45.4
Rhagovelia obesa	Rhobe	2053	37228276	50298	27798	34.3
Saldula saltatoria	INSlupTBPRAAPEI-37	1923	17750697	29659	8499	41.4
Schizaphis graminum	Scora	2200	36200869	31535	28132	34.3
Scutelleridae sp	ScsnS1	2189	29353446	31850	27028	34.6
Sebirus cincutus	Secin	2296	45570966	55647	26998	34.3
Stictocenhala hisonia	Sthis	2230	53718825	62496	27522	36.7
Stimulopalnus, japonicus		2097	22669149	22727	10168	30.7
		1648	2001/042	33060	20868	38.4
Tanzanionhasma sn		1040	34502030	36833	56132	42.1
Tanzaniophasma_sp	DispD1	1908	37591600	50655	19105	42.1
Taracterius_crimensis		1995	37301099	01000	00470	20.0
Tentriredo_koenien		2259	34190669	20400	20472	39.9
Tetrix_subulata	INSTAULANRAAPEI-95	2168	2/2/2/30	30059	26747	41.2
i nrips_paimi	INSIGSTACRAAPEI-21	2156	26952606	24676	9288	46.5
libicen_dorsata		2062	46190617	63242	50110	34.3
Timema_cristinae	INSnfrTBPRAAPEI-15	1667	28302605	41810	18112	37.4
Trialeurodes_vaporariorum	INSfrgTAPRAAPEI-33	2093	41324811	50053	26890	38.4
Trichocorixa_calva	TrspC	2202	37866798	47451	29164	38.5
Trionymus_caricis	Trcar	2169	34841673	28245	16367	37.2
Trioza_urticae	Trurt	2009	41034766	57345	14025	39.7
Tuberolachnus_salignus	Tusal	2177	42294348	39759	24256	34.9
Ulopa_reticulata	INSlupTAKRAAPEI-57	1730	27100790	47527	7949	39.3
Unaspis_euonymi	Uneuo	2241	54887968	59207	24867	40.4
Uroleucon_ambrosiae	Uramb	2197	42211539	35534	27583	34.4
Valenzuela_badiostigma	INSytvTCGRAAPEI-39	2204	21728266	19271	19268	46.4
Velia_caprai	INSnfrTAORAAPEI-35	1796	19253870	25735	16929	39.1
Vidanoana_flavomaculata	Vifla	2109	45681665	56498	26349	39.0
Subilla sp	INSnfrTAGRAAPEI-19	2162	42967462	59482	40221	36.0
Xenophyes metoponcus	INSidsTBFRAAPEI-57	2001	54285874	76720	12828	38.5
Xenophysella greensladeae	INSidsTARRAAPEI-47	1951	48507682	75086	9682	39.6
Zorotypus caudelli	INSidsTAQRAAPEI-46	2003	35582821	54890	15225	35.6

Table S5. Group composition for Four-Cluster Likelihood Mapping (FcLM).

Hypothesis (i): Psocodea are the sister group of Holometabola

(ia) All Condylognatha included

Group 1 (G1): Psocodea (31species)

Echmepteryx_hageni, Cerobasis_guestfalica, Bertkauia_sp, Amphipsocus_japonicus, Valenzuela_badiostigma, Matsumuraiella_radiopicta, Graphopsocus_cruciatus, Aaroniella_sp, Heterocaecilius_solocipennis, Lachesilla_abiesicola, Mesopsocus_unipunctatus, Peripsocus_phaeopterus, Hemipsocus_chroloticus, Longivalvus_nubilus, Neoblaste_papillosus, Elipsocus_kuriliensis, Lachesilla_contraforcepeta, Ectopsocus_briggsi, Ptycta_johnsoni, Stimulopalpus_japonicus, Embidopsocus_sp, Badonnelia_titei, Liposcelis_bostrychophila, Pediculus_humanus, Haematomyzus_elephantis, Echinophthirius_horridus, Columbicola_columbae, Campanulotes_compar, Craspedorrhynchus_sp, Geomydoecus_ewingi, Menopon_gallinae

Group 2 (G2): Condylognatha (129 species)

Franklinothrips vespiformis, Thrips palmi, Gynaikothrips ficorum, Frankliniella cephalica, Erythrothrips arizonae, Frankliniella occidentalis, Orothrips kelloggi, Cycadothrips chadwicki, Diaphorina citri, Trionymus caricis, Macrohomotoma gladiata, Psyllopsis_faxinicola, Heteropsylla_cubana, Pachypsylla_celtidismamma, Aphalara_polygoni, Trioza_urticae, Glycaspis_brimblecombei, Acanthocasuarina_muellerianae, Trialeurodes_vaporariorum, Daktulosphaira_vitifoliae, Planococcus_citri, Aclerda_sp, Ceroplastes cirripediformis, Coccus sp, Dactylopius confuses, Chrysomphalus aonidum, Acutapsis umbonifera, Unaspis euonymi, Essigella californica, Prociphilus tesselatus, Schizaphis graminum, Uroleucon ambrosiae, Tuberolachnus salignus, Cercopis vulnerata, Aphrophora_alni, Philaenus_spumarius, Prosapia_bicincta, Tibicen_dorsata, Okanagana_villosa, Dalbulus_maidis, Ponana_quadralaba, Agallia constricta, Hespenedra chilensis, Empoasca fabae, Vidanoana flavomaculata, Llanquihuea pilosa, Stictocephala bisonia, Campylenchia latipes, Nessorhinus gibberulus, Holdgatiella chepuensis, Centrotus cornutus, Graphocephala fennahi, Ulopa reticulata, Mapuchea sp, Dictyophara_europaea, Tachycixius_pilosus, Nilaparvata_lugens, Metcalfa_pruinosa, Caliscelis_bonelli, Acanalonia_conica, Bruchomorpha_oculata, Oliarus_placitus, Idiosystatus_acutiusculus, Phylloscelis_atra, Taractellus_chilensis, Cyrpoptus belfragei, Ladella sp, Xenophyes metoponcus, Xenophysella greensladeae, Peloridium hammoniorum, Hoplitocoris sp, Ceratocombus sp, Mesovelia mulsanti, Rhagovelia obesa, Limnoporus canaliculatus, Hydrometra stagnorum, Aquarius paludum, Velia caprai, Belostoma flumineum, Gelastocoris oculatus, Trichocorixa calva, Buenoa margaritacea, Ranatra linearis, Aphelocheirus aestivalis, Diplonychus rusticus, Corixa punctata, Ilyocoris cimicoides, Notonecta glauca, Plea minutissima, Saldula saltatoria, Cimex lectularius, Arilus cristatus, Phymata pennsylvanica, Rhodnius prolixus, Corythucha ciliata, Aphelonotus fraterculus, Lygus lineolaris, Reuteroscopus ornatus, Lopidea amorphae, Notostira elongata, Nabis subcoleoptratus, Pagasa sp, Orius_insidiosus, Arocatus_melanocephalus, Lygaeus_equestris, Metatropis_rufescens, Pyrrhocoris_apterus, Aradus_betulae, Mezira granulata, Sehirus cincutus, Corimelaena lateralis, Piezosternum callidum, Chinavia hilaris, Megacopta cribaria, Scutelleridae sp, Anoplogonius_nigricollis, Chalcocoris_rutilans, Jalysus_sp, Lygaeus_turcicus, Oncopeltus_fasciatus, Ischnodemus falicus, Geocoris sp, Chauliops fallax, Largus californicus 1, Largus californicus 2, Alydus pilosus, Anasa tristis, Boisea_trivittata, Acanthosoma_haemorrhoidale

Group 3 (G3): Holometabola (11 species)

Corydalus_cornutus, Osmylus_fulvicephalus, Euroleon_nostras, Inocellia_crassicornis, Subilla_sp, Aleochara_curtula, Tribolium_castaneum, Gyrinus_marinus, Orussus_abietinus, Tenthredo_koehleri, Acromyrmex_echinatior

Group 4 (G4): outgroups (17 species)

Blaberus_atropos, Cryptocercus_wrighti, Apachyus_charteceus, Forficula_auricularia, Aposthonia_japonica, Galloisiana_yuasai, Mantis_religiosa, Metallyticus_splendidus, Tanzaniophasma_sp, Tetrix_subulata, Gryllotalpa_sp, Peruphasma_schultei, Timema_cristinae, Leuctra_sp, Perla_marginata, Zorotypus_caudelli, Zootermopsis_nevadensis

(ib) With Thysanoptera excluded

Group 1 (G1): Psocodea (31 species)

Echmepteryx_hageni, Cerobasis_guestfalica, Bertkauia_sp, Amphipsocus_japonicus, Valenzuela_badiostigma, Matsumuraiella_radiopicta, Graphopsocus_cruciatus, Aaroniella_sp, Heterocaecilius_solocipennis, Lachesilla_abiesicola, Mesopsocus_unipunctatus, Peripsocus_phaeopterus, Hemipsocus_chroloticus, Longivalvus_nubilus, Neoblaste_papillosus, Elipsocus_kuriliensis, Lachesilla_contraforcepeta, Ectopsocus_briggsi, Ptycta_johnsoni, Stimulopalpus_japonicus, Embidopsocus_sp, Badonnelia_titei, Liposcelis_bostrychophila, Pediculus_humanus, Haematomyzus_elephantis, Echinophthirius_horridus, Columbicola_columbae, Campanulotes_compar, Craspedorrhynchus_sp, Geomydoecus_ewingi, Menopon_gallinae

Group 2 (G2): Hemiptera (121 species)

Diaphorina_citri, Trionymus_caricis, Macrohomotoma_gladiata, Psyllopsis_faxinicola, Heteropsylla_cubana, Pachypsylla_celtidismamma, Aphalara_polygoni, Trioza_urticae, Glycaspis_brimblecombei, Acanthocasuarina_muellerianae, Trialeurodes_vaporariorum, Daktulosphaira_vitifoliae, Planococcus_citri, Aclerda_sp, Ceroplastes_cirripediformis, Coccus_sp, Dactylopius_confuses, Chrysomphalus_aonidum, Acutapsis_umbonifera, Unaspis_euonymi, Essigella_californica, Prociphilus_tesselatus, Schizaphis_graminum, Uroleucon_ambrosiae, Tuberolachnus_salignus, Cercopis_vulnerata, Aphrophora_alni, Philaenus_spumarius, Prosapia_bicincta, Tibicen_dorsata, Okanagana_villosa, Dalbulus_maidis, Ponana_quadralaba, Agallia_constricta, Hespenedra_chilensis,Empoasca_fabae, Vidanoana_flavomaculata, Llanquihuea_pilosa, Stictocephala_bisonia, Campylenchia_latipes, Nessorhinus_gibberulus, Holdgatiella_chepuensis, Centrotus_cornutus, Graphocephala_fennahi, Ulopa_reticulata, Mapuchea_sp, Dictyophara_europaea, Tachycixius_pilosus, Nilaparvata_lugens, Metcalfa_pruinosa, Caliscelis_bonelli, Acanalonia_conica, Bruchomorpha_oculata, Oliarus_placitus, Idiosystatus_acutiusculus, Phylloscelis_atra, Taractellus_chilensis, Cyrpoptus_belfragei, Ladella_sp, Xenophyes_metoponcus, Xenophysella_greensladeae, Peloridium_hammoniorum, Hoplitocoris_sp, Ceratocombus_sp, Mesovelia_mulsanti, Rhagovelia_obesa, Limnoporus_canaliculatus, Hydrometra_stagnorum, Aquarius_paludum, Velia_caprai, Belostoma_flumineum, Gelastocoris_oculatus, Trichocorixa_calva, Buenoa_margaritacea, Ranatra_linearis, Aphelocheirus_aestivalis, Diplonychus_rusticus, Corixa_punctata, Ilyocoris_cimicoides, Notonecta_glauca, Plea_minutissima, Saldula_saltatoria, Cimex_lectularius, Arilus_cristatus, Phymata_pennsylvanica, Rhodnius_prolixus, Corythucha_ciliata, Aphelonotus_fraterculus, Lygus_lineolaris, Reuteroscopus_ornatus, Lopidea_amorphae, Notostira_elongata, Nabis_subcoleoptratus, Pagasa_sp, Orius_insidiosus, Arocatus_melanocephalus, Lygaeus_equestris, Metatropis_rufescens, Pyrrhocoris_apterus, Aradus_betulae, Mezira_granulata, Sehirus_cincutus, Corimelaena_lateralis, Piezosternum_callidum, Chinavia_hilaris, Megacopta_cribaria, Scutelleridae_sp, Anoplogonius_nigricollis, Chalcocoris_rutilans, Jalysus_sp, Lygaeus_turcicus, Oncopeltus_fasciatus, Ischnodemus_falicus, Geocoris_sp, Chauliops_fallax, Largus californicus 1, Largus californicus 2, Alydus pilosus, Anasa tristis, Boisea trivittata, Acanthosoma haemorrhoidale

Group 3 (G3): Holometabola (11 species)

Corydalus_cornutus, Osmylus_fulvicephalus, Euroleon_nostras, Inocellia_crassicornis, Subilla_sp, Aleochara_curtula, Tribolium_castaneum, Gyrinus_marinus, Orussus_abietinus, Tenthredo_koehleri, Acromyrmex_echinatior

Group 4 (G4): outgroups (17 species)

Blaberus_atropos, Cryptocercus_wrighti, Apachyus_charteceus, Forficula_auricularia, Aposthonia_japonica, Galloisiana_yuasai, Mantis_religiosa, Metallyticus_splendidus, Tanzaniophasma_sp, Tetrix_subulata, Gryllotalpa_sp, Peruphasma_schultei, Timema_cristinae, Leuctra_sp, Perla_marginata, Zorotypus_caudelli, Zootermopsis_nevadensis

(ic) With Hemiptera excluded

Group 1 (G1): Psocodea (31 species)

Echmepteryx_hageni, Cerobasis_guestfalica, Bertkauia_sp, Amphipsocus_japonicus, Valenzuela_badiostigma, Matsumuraiella_radiopicta, Graphopsocus_cruciatus, Aaroniella_sp, Heterocaecilius_solocipennis, Lachesilla_abiesicola, Mesopsocus_unipunctatus, Peripsocus_phaeopterus, Hemipsocus_chroloticus, Longivalvus_nubilus, Neoblaste_papillosus, Elipsocus_kuriliensis, Lachesilla_contraforcepeta, Ectopsocus_briggsi, Ptycta_johnsoni, Stimulopalpus_japonicus, Embidopsocus_sp, Badonnelia_titei, Liposcelis_bostrychophila, Pediculus_humanus, Haematomyzus_elephantis, Echinophthirius_horridus, Columbicola_columbae, Campanulotes_compar, Craspedorrhynchus_sp, Geomydoecus_ewingi, Menopon_gallinae

Group 2 (G2): Thysanoptera (8 species)

Franklinothrips_vespiformis, Thrips_palmi, Gynaikothrips_ficorum, Frankliniella_cephalica, Erythrothrips_arizonae, Frankliniella occidentalis, Orothrips kelloggi, Cycadothrips chadwicki

Group 3 (G3): Holometabola (11 species)

Corydalus cornutus, Osmylus fulvicephalus, Euroleon_nostras, Inocellia crassicornis, Subilla_sp, Aleochara_curtula, Tribolium castaneum, Gyrinus marinus, Orussus abietinus, Tenthredo koehleri, Acromyrmex echinatior

Group 4 (G4): outgroups (17 species)

Blaberus_atropos, Cryptocercus_wrighti, Apachyus_charteceus, Forficula_auricularia, Aposthonia_japonica, Galloisiana_yuasai, Mantis_religiosa, Metallyticus_splendidus, Tanzaniophasma_sp, Tetrix_subulata, Gryllotalpa_sp, Peruphasma_schultei, Timema_cristinae, Leuctra_sp, Perla_marginata, Zorotypus_caudelli, Zootermopsis_nevadensis

Hypothesis (ii): Thysanoptera are the sister group of Hemiptera

Group 1 (G1): Psocodea (31 species)

Echmepteryx_hageni, Cerobasis_guestfalica, Bertkauia_sp, Amphipsocus_japonicus, Valenzuela_badiostigma, Matsumuraiella_radiopicta, Graphopsocus_cruciatus, Aaroniella_sp, Heterocaecilius_solocipennis, Lachesilla_abiesicola, Mesopsocus_unipunctatus, Peripsocus_phaeopterus, Hemipsocus_chroloticus, Longivalvus_nubilus, Neoblaste_papillosus, Elipsocus_kuriliensis, Lachesilla_contraforcepeta, Ectopsocus_briggsi, Ptycta_johnsoni, Stimulopalpus_japonicus, Embidopsocus_sp, Badonnelia_titei, Liposcelis_bostrychophila, Pediculus_humanus, Haematomyzus_elephantis, Echinophthirius_horridus, Columbicola_columbae, Campanulotes_compar, Craspedorrhynchus_sp, Geomydoecus_ewingi, Menopon_gallinae

Group 2 (G2): Thysanoptera (8 species)

Franklinothrips_vespiformis, Thrips_palmi, Gynaikothrips_ficorum, Frankliniella_cephalica, Erythrothrips_arizonae, Frankliniella_occidentalis, Orothrips_kelloggi, Cycadothrips_chadwicki

Group 3 (G3): Hemiptera (121 species)

Diaphorina_citri, Trionymus_caricis, Macrohomotoma_gladiata, Psyllopsis_faxinicola, Heteropsylla_cubana, Pachypsylla_celtidismamma, Aphalara_polygoni, Trioza_urticae, Glycaspis_brimblecombei, Acanthocasuarina_muellerianae, Trialeurodes_vaporariorum, Daktulosphaira_vitifoliae, Planococcus_citri, Aclerda_sp, Ceroplastes_cirripediformis, Coccus_sp, Dactylopius_confuses, Chrysomphalus_aonidum, Acutapsis_umbonifera, Unaspis_euonymi, Essigella_californica, Prociphilus_tesselatus, Schizaphis_graminum, Uroleucon_ambrosiae, Tuberolachnus_salignus, Cercopis_vulnerata, Aphrophora_alni, Philaenus_spumarius, Prosapia_bicincta, Tibicen_dorsata, Okanagana_villosa, Dalbulus_maidis, Ponana_quadralaba, Agallia_constricta, Hespenedra_chilensis,Empoasca_fabae, Vidanoana_flavomaculata, Llanquihuea_pilosa, Stictocephala_bisonia, Campylenchia_latipes, Nessorhinus_gibberulus, Holdgatiella_chepuensis, Centrotus_cornutus, Graphocephala_fennahi, Ulopa_reticulata, Mapuchea_sp, Dictyophara_europaea, Tachycixius_pilosus, Nilaparvata_lugens, Metcalfa_pruinosa, Caliscelis_bonelli, Acanalonia_conica, Bruchomorpha_oculata, Oliarus_placitus, Idiosystatus_acutiusculus, Phylloscelis_atra, Taractellus_chilensis, Cyrpoptus_belfragei, Ladella_sp, Xenophyes_metoponcus, Xenophysella_greensladeae, Peloridium_hammoniorum, Hoplitocoris_sp, Ceratocombus_sp, Mesovelia_mulsanti, Rhagovelia_obesa, Limnoporus_canaliculatus, Hydrometra_stagnorum, Aquarius_paludum, Velia_caprai, Belostoma_flumineum, Gelastocoris_oculatus, Trichocorixa_calva, Buenoa_margaritacea, Ranatra_linearis, Aphelocheirus_aestivalis, Diplonychus_rusticus, Corixa_punctata, Ilyocoris_cimicoides, Notonecta_glauca, Plea_minutissima, Saldula_saltatoria, Cimex_lectularius, Arilus_cristatus, Phymata_pennsylvanica, Rhodnius_prolixus, Corythucha_ciliata, Aphelonotus_fraterculus, Lygus_lineolaris, Reuteroscopus_ornatus, Lopidea_amorphae, Notostira_elongata, Nabis_subcoleoptratus, Pagasa_sp, Orius_insidiosus, Arocatus_melanocephalus, Lygaeus_equestris, Metatropis_rufescens, Pyrrhocoris_apterus, Aradus_betulae, Mezira_granulata, Sehirus_cincutus, Corimelaena_lateralis, Piezosternum_callidum, Chinavia_hilaris, Megacopta_cribaria, Scutelleridae_sp, Anoplogonius_nigricollis, Chalcocoris_rutilans, Jalysus_sp, Lygaeus_turcicus, Oncopeltus fasciatus, Ischnodemus falicus, Geocoris_sp, Chauliops_fallax, Largus_californicus_1, Largus_californicus_2, Alydus_pilosus, Anasa_tristis, Boisea_trivittata, Acanthosoma_haemorrhoidale

Group 4 (G4): Holometabola (11 species)

Corydalus_cornutus, Osmylus_fulvicephalus, Euroleon_nostras, Inocellia_crassicornis, Subilla_sp, Aleochara_curtula, Tribolium_castaneum, Gyrinus_marinus, Orussus_abietinus, Tenthredo_koehleri, Acromyrmex_echinatior

Hypothesis (iii): Coleorrhyncha are the sister group of Auchenorrhyncha

Group 1 (G1): Coleorrhyncha (3 species)

Xenophyes_metoponcus, Xenophysella_greensladeae, Peloridium_hammoniorum

Group 2 (G2): Auchenorrhyncha (34 species)

Cercopis_vulnerata, Aphrophora_alni, Philaenus_spumarius, Prosapia_bicincta, Tibicen_dorsata, Okanagana_villosa, Dalbulus_maidis, Ponana_quadralaba, Agallia_constricta, Hespenedra_chilensis,Empoasca_fabae, Vidanoana_flavomaculata, Llanquihuea_pilosa, Stictocephala_bisonia, Campylenchia_latipes, Nessorhinus_gibberulus, Holdgatiella_chepuensis, Centrotus_cornutus, Graphocephala_fennahi, Ulopa_reticulata, Mapuchea_sp, Dictyophara_europaea, Tachycixius_pilosus, Nilaparvata_lugens, Metcalfa_pruinosa, Caliscelis_bonelli, Acanalonia_conica, Bruchomorpha_oculata, Oliarus_placitus, Idiosystatus_acutiusculus, Phylloscelis_atra, Taractellus_chilensis, Cyrpoptus_belfragei, Ladella_sp

Group 3 (G3): Heteroptera (59 species)

Hoplitocoris_sp, Ceratocombus_sp, Mesovelia_mulsanti, Rhagovelia_obesa, Limnoporus_canaliculatus, Hydrometra_stagnorum, Aquarius_paludum, Velia_caprai, Belostoma_flumineum, Gelastocoris_oculatus, Trichocorixa_calva, Buenoa_margaritacea, Ranatra_linearis, Aphelocheirus_aestivalis, Diplonychus_rusticus, Corixa_punctata, Ilyocoris_cimicoides, Notonecta_glauca, Plea_minutissima, Saldula_saltatoria, Cimex_lectularius, Arilus_cristatus, Phymata_pennsylvanica, Rhodnius_prolixus, Corythucha_ciliata, Aphelonotus_fraterculus, Lygus_lineolaris, Reuteroscopus_ornatus, Lopidea_amorphae, Notostira_elongata, Nabis_subcoleoptratus, Pagasa_sp, Orius_insidiosus, Arocatus_melanocephalus, Lygaeus_equestris, Metatropis_rufescens, Pyrrhocoris_apterus, Aradus_betulae, Mezira_granulata, Sehirus_cincutus, Corimelaena_lateralis, Piezosternum_callidum, Chinavia_hilaris, Megacopta_cribaria, Scutelleridae_sp, Anoplogonius_nigricollis, Chalcocoris_rutilans, Jalysus_sp, Lygaeus_turcicus, Oncopeltus_fasciatus, Ischnodemus_falicus, Geocoris_sp, Chauliops_fallax, Largus_californicus_1, Largus_californicus_2, Alydus pilosus, Anasa tristis, Boisea trivittata, Acanthosoma haemorrhoidale

Group 4 (G4): Sternorrhyncha (25 species)

Diaphorina_citri, Trionymus_caricis, Macrohomotoma_gladiata, Psyllopsis_faxinicola, Heteropsylla_cubana, Pachypsylla_celtidismamma, Aphalara_polygoni, Trioza_urticae, Glycaspis_brimblecombei, Acanthocasuarina_muellerianae, Trialeurodes_vaporariorum, Daktulosphaira_vitifoliae, Planococcus_citri, Aclerda_sp, Ceroplastes_cirripediformis, Coccus_sp, Dactylopius_confuses, Chrysomphalus_aonidum, Acutapsis_umbonifera, Unaspis_euonymi, Essigella_californica, Prociphilus_tesselatus, Schizaphis_graminum, Uroleucon_ambrosiae, Tuberolachnus_salignus

Hypothesis (iv): Myerslopiidae are the sister group of the remainder of Membracoidea

Group 1 (G1): Cercopoidea (4 species)

Cercopis_vulnerata, Aphrophora_alni, Philaenus_spumarius, Prosapia_bicincta

Group 2 (G2): Cicadoidea (2 species)

Tibicen dorsata, Okanagana villosa

Group 3 (G3): Membracoidea minus Myerslopiidae (14 species)

Dalbulus_maidis, Ponana_quadralaba, Agallia_constricta, Hespenedra_chilensis, Empoasca_fabae, Vidanoana_flavomaculata, Llanquihuea_pilosa, Stictocephala_bisonia, Campylenchia_latipes, Nessorhinus_gibberulus, Holdgatiella_chepuensis, Centrotus_cornutus, Graphocephala_fennahi, Ulopa_reticulata

Group 4 (G4): Myerslopiidae (1 species)

Mapuchea_sp

Hypothesis (v): Aphidoidea are the sister group of Coccoidea

Group 1 (G1): Psylloidea (10 species)

Diaphorina_citri, Trionymus_caricis, Macrohomotoma_gladiata, Psyllopsis_faxinicola, Heteropsylla_cubana, Pachypsylla_celtidismamma, Aphalara polygoni, Trioza urticae, Glycaspis brimblecombei, Acanthocasuarina muellerianae

Group 2 (G2): Aleyrodoidea (1 species)

Trialeurodes_vaporariorum

Group 3 (G3): Coccoidea (9 species)

Daktulosphaira_vitifoliae, Planococcus_citri, Aclerda_sp, Ceroplastes_cirripediformis, Coccus_sp, Dactylopius_confuses, Chrysomphalus aonidum, Acutapsis umbonifera, Unaspis euonymi

Group 4 (G4): Aphidoidea (5 species)

Essigella californica, Prociphilus tesselatus, Schizaphis graminum, Uroleucon ambrosiae, Tuberolachnus salignus

Hypothesis (vi): Nepomorpha are the sister group of a clade including Leptopodomorpha, Cimicomorpha, and Pentatomomorpha

Group 1 (G1): Auchenorrhyncha+Coleorrhyncha (37 species)

Cercopis_vulnerata, Aphrophora_alni, Philaenus_spumarius, Prosapia_bicincta, Tibicen_dorsata, Okanagana_villosa, Dalbulus_maidis, Ponana_quadralaba, Agallia_constricta, Hespenedra_chilensis, Empoasca_fabae, Vidanoana_flavomaculata, Llanquihuea_pilosa, Stictocephala_bisonia, Campylenchia_latipes, Nessorhinus_gibberulus, Holdgatiella_chepuensis, Centrotus_cornutus, Graphocephala_fennahi, Ulopa_reticulata, Mapuchea_sp, Dictyophara_europaea, Tachycixius_pilosus, Nilaparvata_lugens, Metcalfa_pruinosa, Caliscelis_bonelli, Acanalonia_conica, Bruchomorpha_oculata, Oliarus_placitus, Idiosystatus_acutiusculus, Phylloscelis_atra, Taractellus_chilensis, Cyrpoptus_belfragei, Ladella_sp, Xenophyes_metoponcus, Xenophysella_greensladeae, Peloridium_hammoniorum

Group 2 (G2): Enicocephalomorpha+Dipsocoromorpha+Gerromorpha (8 species)

Hoplitocoris_sp, Ceratocombus_sp, Mesovelia_mulsanti, Rhagovelia_obesa, Limnoporus_canaliculatus, Hydrometra_stagnorum, Aquarius_paludum, Velia_caprai

Group 3 (G3): Nepomorpha (11 species)

Belostoma flumineum, Gelastocoris_oculatus, Trichocorixa_calva, Buenoa_margaritacea, Ranatra_linearis, Aphelocheirus_aestivalis, Diplonychus_rusticus, Corixa_punctata, Ilyocoris_cimicoides, Notonecta_glauca, Plea_minutissima

Group 4 (G4): Leptopodomorpha+Cimicomorpha+Pentatomomorpha (40 species)

Saldula_saltatoria, Cimex_lectularius, Arilus_cristatus, Phymata_pennsylvanica, Rhodnius_prolixus, Corythucha_ciliata, Aphelonotus_fraterculus, Lygus_lineolaris, Reuteroscopus_ornatus, Lopidea_amorphae, Notostira_elongata, Nabis_subcoleoptratus, Pagasa_sp, Orius_insidiosus, Arocatus_melanocephalus, Lygaeus_equestris, Metatropis_rufescens, Pyrrhocoris_apterus, Aradus_betulae, Mezira_granulata, Sehirus_cincutus, Corimelaena_lateralis, Piezosternum_callidum, Chinavia_hilaris, Megacopta_cribaria, Scutelleridae_sp, Anoplogonius_nigricollis, Chalcocoris_rutilans, Jalysus_sp, Lygaeus_turcicus, Oncopeltus_fasciatus, Ischnodemus_falicus, Geocoris_sp, Chauliops_fallax, Largus_californicus_1, Largus_californicus_2, Alydus_pilosus, Anasa_tristis, Boisea_trivittata, Acanthosoma_haemorrhoidale

Hypothesis (vii): Leptopodomorpha sister to a clade comprising Cimicomorpha and Pentatomomorpha

Group 1 (G1): Cimicomorpha+Pentatomomorpha (39 species)

Arocatus _melanocephalus, Lygaeus _equestris, Metatropis _rufescens, Pyrrhocoris _apterus, Aradus _betulae, Mezira _granulata, Sehirus _cincutus, Corimelaena _lateralis, Piezosternum _callidum, Chinavia _hilaris, Megacopta _cribaria, Scutelleridae _sp, Anoplogonius _nigricollis, Chalcocoris _rutilans, Jalysus _sp, Lygaeus _turcicus, Oncopeltus _fasciatus, Ischnodemus _falicus, Geocoris _sp, Chauliops _fallax, Largus _californicus _1, Largus _californicus _2, Alydus _pilosus, Anasa _tristis, Boisea _trivittata, Acanthosoma _haemorrhoidale, Cimex _lectularius, Arilus _cristatus, Phymata _pennsylvanica, Rhodnius _prolixus, Corythucha _ciliata, Aphelonotus _fraterculus, Lygus _lineolaris, Reuteroscopus _ornatus, Lopidea _amorphae, Notostira _elongata, Nabis _subcoleoptratus, Pagasa _sp, Orius _insidiosus

Group 2 (G2): Leptopodomorpha (1 species)

Saldula_saltatoria

Group 3 (G3): Nepomorpha (11 species)

Belostoma_flumineum, Gelastocoris_oculatus, Trichocorixa_calva, Buenoa_margaritacea, Ranatra_linearis, Aphelocheirus_aestivalis, Diplonychus_rusticus, Corixa_punctata, Ilyocoris_cimicoides, Notonecta_glauca, Plea_minutissima

Group 4 (G4): Enicocephalomorpha+Dipsocoromorpha+Gerromorpha (8 species)

_Hoplitocoris_sp, Ceratocombus_sp, Mesovelia_mulsanti, Rhagovelia_obesa, Limnoporus_canaliculatus, Hydrometra_stagnorum, Aquarius_paludum, Velia_caprai

Hypothesis (viii): Gerromorpha sister to a clade including Dipsocoromorpha and Enicocephalomorpha

Group 1 (G1): Enicocephalomorpha+Dipsocoromorpha (2 species)

Hoplitocoris_sp, Ceratocombus_sp

Group 2 (G2): Gerromorpha (6 species)

Mesovelia_mulsanti, Rhagovelia_obesa, Limnoporus_canaliculatus, Hydrometra_stagnorum, Aquarius_paludum, Velia_caprai

Group 3 (G3): Nepomorpha+Letopodomorpha+Cimicomorpha+Pentatomomorpha (51 species)

Belostoma flumineum, Gelastocoris oculatus, Trichocorixa calva, Buenoa margaritacea, Ranatra linearis, Aphelocheirus aestivalis, Diplonychus rusticus, Corixa punctata, Ilyocoris cimicoides, Notonecta glauca, Plea minutissima, Saldula saltatoria, Arocatus melanocephalus, Lygaeus equestris, Metatropis rufescens, Pyrrhocoris apterus, Aradus betulae, Mezira granulata, Sehirus cincutus, Corimelaena lateralis, Piezosternum callidum, Chinavia hilaris, Megacopta cribaria, Scutelleridae sp, Anoplogonius nigricollis, Chalcocoris rutilans, Jalysus sp, Lygaeus turcicus, Oncopeltus fasciatus, Ischnodemus falicus, Geocoris sp, Chauliops fallax, Largus californicus 1, Largus californicus 2, Alydus pilosus, Anasa tristis, Boisea trivittata, Acanthosoma haemorrhoidale, Cimex lectularius, Arilus cristatus, Phymata pennsylvanica, Rhodnius prolixus, Corythucha ciliata, Aphelonotus fraterculus, Lygus lineolaris, Reuteroscopus ornatus, Lopidea amorphae, Notostira elongata, Nabis subcoleoptratus, Pagasa sp, Orius insidiosus

Group 4 (G4): Auchenorrhyncha+Coleorrhyncha (37 species)

Cercopis_vulnerata, Aphrophora_alni, Philaenus_spumarius, Prosapia_bicincta, Tibicen_dorsata, Okanagana_villosa, Dalbulus_maidis, Ponana_quadralaba, Agallia_constricta, Hespenedra_chilensis,Empoasca_fabae, Vidanoana_flavomaculata, Llanquihuea_pilosa, Stictocephala_bisonia, Campylenchia_latipes, Nessorhinus_gibberulus, Holdgatiella_chepuensis, Centrotus_cornutus, Graphocephala_fennahi, Ulopa_reticulata, Mapuchea_sp, Dictyophara_europaea, Tachycixius_pilosus, Nilaparvata_lugens, Metcalfa_pruinosa, Caliscelis_bonelli, Acanalonia_conica, Bruchomorpha_oculata, Oliarus_placitus, Idiosystatus_acutiusculus, Phylloscelis_atra, Taractellus_chilensis, Cyrpoptus_belfragei, Ladella_sp, Xenophyes_metoponcus, Xenophysella_greensladeae, Peloridium_hammoniorum

Hypothesis (ix): Cercopoidea sister to Cicadoidea

Group 1 (G1): Membracodea (15 species)

Dalbulus_maidis, Ponana_quadralaba, Agallia_constricta, Hespenedra_chilensis, Empoasca_fabae, Vidanoana_flavomaculata, Llanquihuea_pilosa, Stictocephala_bisonia, Campylenchia_latipes, Nessorhinus_gibberulus, Holdgatiella_chepuensis, Centrotus_cornutus, Graphocephala_fennahi, Ulopa_reticulata, Mapuchea_sp

Group 2 (G2): Cercopoidea (4 species)

Cercopis vulnerata, Aphrophora alni, Philaenus spumarius, Prosapia bicincta

Group 3 (G3): Cicadoidea (2 species)

Tibicen dorsata, Okanagana villosa

Group 4 (G4): Fulgoroidea (13 species)

Dictyophara_europaea, Tachycixius_pilosus, Nilaparvata_lugens, Metcalfa_pruinosa, Caliscelis_bonelli, Acanalonia_conica, Bruchomorpha_oculata, Oliarus_placitus, Idiosystatus_acutiusculus, Phylloscelis_atra, Taractellus_chilensis, Cyrpoptus_belfragei, Ladella_sp

Table S6. Results from Four-Cluster Likelihood Mapping (FcLM), including permutation tests.

Hypothesis (i): Psocodea are the sister group of Holometabola

(ia) All Condylognatha included

- # quartets: 747,813
- # aa sites: 859,005
- # partitions: 1,856

Groups: G1: Psocodea, G2: Condylognatha, G3: Holometabola, G4: outgroups

T1: Psocodea, Condylognatha | Holometabola, Outgroups

T2: Psocodea, Holometabola | Condylognatha, Outgroups

T3: Psocodea, Outgroups | Condylognatha, Holometabola

data	T1: G1,G2 G3,G4	T2: G1,G3 G2,G4	T3: G1,G4 G2,G3	T12	T13	T23	T*
original	67%	25%	8%	0	0	0	0
Perm I	15%	7%	5%	8%	2%	1%	61%
Perm II	6%	4%	4%	5%	6%	6%	68%
Perm III	0%	0%	0%	4%	4%	4%	86%

Results from modifications excluding quartets including representatives of the following taxa from the results of (*ia*) (original data only):

(ial) Excluding all Phthiraptera

quartets: 554,829

T1: 67%, T2: 26%, T3: 7%, T12, T13, T23, T*: 0%

(iall) Excluding all Phthiraptera plus Liposcelidae

quartets: 506,583

T1: 67%, T2: 27%, T3: 6%, T12, T13, T23, T*: 0%

(iaIII) Excluding all "Psocoptera"

quartets: 192,984

T1: 66%, T2: 22%, T3: 12%, T12, T13, T23, T*: 0%

(iaIV) Excluding all Phthiraptera from G1 and all Thysanoptera from G2

quartets: 520,421 T1: 68%, T2: 25%, T3: 7%, T12, T13, T23, T*: 0%

(ib) With Thysanoptera excluded

quartets: 701,437

aa sites: 858,762

partitions: 1,856

Groups: G1: Psocodea, G2: Hemiptera, G3: Holometabola, G4: outgroups

T1: Psocodea, Hemiptera | Holometabola, Outgroups

T2: Psocodea, Holometabola | Hemiptera, Outgroups

T3: Psocodea, Outgroups | Hemiptera, Holometabola

data	T1: G1,G2 G3,G4	T2: G1,G3 G2,G4	T3: G1,G4 G2,G3	T12	T13	T23	T*
original	68%	24%	8%	0%	0%	0%	0%
Perm I	14%	8%	6%	8%	2%	1%	60%
Perm II	5%	5%	5%	5%	6%	6%	67%
Perm III	0%	0%	0%	4%	4%	4%	86%

(ic) With Hemiptera excluded

quartets: 46,376

aa sites: 846,928

partitions: 1,831

Groups: G1: Psocodea, G2: Thysanoptera, G3: Holometabola, G4: outgroups

T1: Psocodea, Thysanoptera | Holometabola, Outgroups

T2: Psocodea, Holometabola | Thysanoptera, Outgroups

T3: Psocodea, Outgroups | Thysanoptera, Holometabola

data	T1: G1,G2 G3,G4	T2: G1,G3 G2,G4	T3: G1,G4 G2,G3	T12	T13	T23	T*
original	51%	46%	3%	0%	0%	0%	0%
Perm I	20%	5%	4%	5%	1%	1%	64%
Perm II	11%	17%	16%	11%	9%	12%	24%
Perm III	0%	0%	0%	6%	5%	5%	84%

Hypothesis (ii): Thysanoptera are the sister group of Hemiptera

quartets: 330,088

aa sites: 846,234

partitions: 1,806

Groups: G1: Psocodea, G2: Thysanoptera, G3: Hemiptera, G4: Holometabola

T1: Psocodea, Thysanoptera | Hemiptera, Holometabola

T2: Psocodea, Hemiptera | Thysanoptera, Holometabola

T3: Psocodea, Holometabola | Thysanoptera, Hemiptera

data	T1: G1,G2 G3,G4	T2: G1,G3 G2,G4	T3: G1,G4 G2,G3	T12	T13	T23	T*
original	7%	25%	68%	0%	0%	0%	0%
Perm I	19%	6%	10%	2%	3%	10%	51%
Perm II	5%	4%	4%	5%	5%	6%	70%
Perm III	0%	0%	0%	5%	5%	4%	85%

Hypothesis (iii): Coleorrhyncha are the sister group of Auchenorrhyncha

quartets: 150,450

aa sites: 833,738

partitions: 1,757

Groups: G1: Coleorrhyncha, G2: Auchenorrhyncha, G3: Heteroptera, G4: Sternorrhyncha

T1: Coleorrhyncha, Auchenorrhyncha | Heteroptera, Sternorrhyncha

T2: Coleorrhyncha, Heteroptera | Auchenorrhyncha, Sternorrhyncha

T3: Coleorrhyncha, Sternorrhyncha | Auchenorrhyncha, Heteroptera

data	T1: G1,G2 G3,G4	T2: G1,G3 G2,G4	T3: G1,G4 G2,G3	T12	T13	T23	T*
original	96%	4%	0%	0%	0%	0%	0%
Perm I	13%	8%	7%	5%	2%	10%	56%
Perm II	6%	7%	8%	8%	8%	8%	54%
Perm III	0%	0%	0%	4%	4%	4%	86%

Hypothesis (iv): Myerslopiidae are the sister group of the remainder of Membracoidea

quartets: 112

aa sites: 779,558

partitions: 1,659

Groups: G1: Cercopoidea, G2: Cicadoidea, G3: Membracoidea minus Myerslopiidae, G4: Myerslopiidae

T1: Cercopoidea, Cicadoidea | Membracoidea minus Myerslopiidae, Myerslopiidae

T2: Cercopoidea, Membracoidea minus Myerslopiidae | Cicadoidea, Myerslopiidae

T3: Cercopoidea, Myerslopiidae | Cicadoidea, Membracoidea minus Myerslopiidae

data	T1: G1,G2 G3,G4	T2: G1,G3 G2,G4	T3: G1,G4 G2,G3	T12	T13	T23	T*
original	100%	0%	0%	0%	0%	0%	0%
Perm I	7%	12%	2%	2%	0%	10%	68%
Perm II	21%	18%	46%	4%	6%	1%	5%
Perm III	31%	37%	11%	6%	3%	11%	2%

Hypothesis (v): Aphidoidea are the sister group of Coccoidea

quartets: 450

aa sites: 780,773

partitions: 1,610

Groups: G1: Psylloidea, G2: Aleyrodoidea, G3: Coccoidea, G4: Aphidoidea

T1: Psylloidea, Aleyrodoidea | Coccoidea, Aphidoidea

T2: Psylloidea, Coccoidea | Aleyrodoidea, Aphidoidea

T3: Psylloidea, Aphidoidea | Aleyrodoidea, Coccoidea

data	T1: G1,G2 G3,G4	T2: G1,G3 G2,G4	T3: G1,G4 G2,G3	T12	T13	T23	T*
original	91%	9%	0%	0%	0%	0%	0%
Perm I	5%	5%	5%	1%	0%	1%	82%
PermII	23%	19%	37%	5%	5%	8%	4%
PermIII	23%	33%	21%	8%	7%	8%	2%

Hypothesis (vi): Nepomorpha are the sister group of a clade including Leptopodomorpha, Cimicomorpha, and Pentatomomorpha

quartets: 130,240

aa sites: 841,766

partitions: 1,817

Groups: G1: Auchenorrhyncha+Coleorrhyncha,

G2: Enicocephalomorpha+Dipsocoromorpha+Gerromorpha, G3: Nepomorpha,

G4: Leptopodomorpha+Cimicomorpha+Pentatomomorpha

 $T1: Nepomorpha, Enicocephalomorpha+Dipsocoromorpha+Gerromorpha \mid Auchenorrhyncha+Coleorrhyncha, Leptopodomorpha+Cimicomorpha+Pentatomomorpha$

T2: Nepomorpha, Auchenorrhyncha+Coleorrhyncha | Enicocephalomorpha+Dipsocoromorpha+Gerromorpha, Leptopodomorpha+Cimicomorpha+Pentatomomorpha

 $T3: Nepomorpha, Leptopodomorpha+Cimicomorpha+Pentatomomorpha \mid Enicocephalomorpha+Dipsocoromorpha+Gerromorpha, Auchenorrhyncha+Coleorrhyncha$

data	T1: G1,G2 G3,G4	T2: G1,G3 G2,G4	T3: G1,G4 G2,G3	T12	T13	T23	Τ*
original	31%	37%	32%	0%	0%	0%	0%
Perm I	13%	6%	20%	3%	6%	15%	38%
Perm II	7%	7%	10%	8%	9%	10%	49%
Perm III	0%	0%	0%	4%	4%	4%	87%

Hypothesis (vii): Leptopodomorpha sister to a clade comprising Cimicomorpha and Pentatomomorpha

quartets: 3432

aa sites: 770,296

partitions: 1,502

Groups: G1: Cimicomorpha+Pentatomomorpha, G2: Leptopodomorpha, G3: Nepomorpha, G4: Gerromorpha+Dipsocoromorpha+Enicocephalomorpha

T1: Cimicomorpha+Pentatomomorpha, Leptopodomorpha | Nepomorpha, Gerromorpha+Dipsocoromorpha+Enicocephalomorpha

T2: Cimicomorpha+Pentatomomorpha, Nepomorpha | Leptopodomorpha, Gerromorpha+Dipsocoromorpha+Enicocephalomorpha

 $T3: Cimicomorpha + Pentatomomorpha, Gerromorpha + Dipsocoromorpha + Enicocephalomorpha \mid Leptopodomorpha, Nepomorpha + Dipsocoromorpha +$

data	T1: G1,G2 G3,G4	T2: G1,G3 G2,G4	T3: G1,G4 G2,G3	T12	T13	T23	T*
original	100%	0%	0%	0%	0%	0%	0%
Perm I	6%	5%	12%	20%	3%	3%	61%
Perm II	21%	8%	15%	10%	15%	11%	21%
Perm III	9%	15%	24%	9%	9%	10%	23%

Hypothesis (viii): Gerromorpha sister to a clade including Dipsocoromorpha and Enicocephalomorpha

quartets: 22,032

aa sites: 828,041

partitions: 1,725

Groups: G1: Enicocephalomorpha+Dipsocoromorpha, G2: Gerromorpha, G3: Nepomorpha+Leptopodomorpha+Cimicomorpha+Pentatomomorpha G4: Auchenorrhyncha+Coleorrhyncha

 $T1: Enicocephalomorpha+Dipsocoromorpha, Gerromorpha \mid Nepomorpha+Leptopodomorpha+Cimicomorpha+Pentatomomorpha, Auchenorrhyncha+Coleorrhyncha$

 $T2: Enicocephalomorpha+Dipsocoromorpha, Nepomorpha+Leptopodomorpha+Cimicomorpha+Pentatomomorpha \mid Gerromorpha, Auchenorrhyncha+Coleorrhyncha$

T3: Enicocephalomorpha+Dipsocoromorpha, Auchenorrhyncha+Coleorrhyncha | Gerromorpha, Nepomorpha+Leptopodomorpha+Cimicomorpha+Pentatomomorpha

data	T1: G1,G2 G3,G4	T2: G1,G3 G2,G4	T3: G1,G4 G2,G3	T12	T13	T23	Τ*
original	100%	0%	0%	0%	0%	0%	0%
Perm I	11%	5%	10%	12%	5%	7%	51%
Perm II	8%	10%	11%	9%	10%	14%	39%
Perm III	0%	0%	0%	4%	4%	4%	87%

Hypothesis (ix): Cercopoidea sister to Cicadoidea

quartets: 1,456

aa sites: 829,781

partitions: 1,718

Groups: G1: Membracoidea, G2: Cercopoidea, G3: Cicadoidea G4: Fulgoroidea

T1: Membracoidea, Cercopoidea | Cicadoidea, Fulgoroidea

T2: Membracoidea, Cicadoidea | Cercopoidea, Fulgoroidea

T3: Membracoidea, Fulgoroidea | Cercopoidea, Cicadoidea

data	T1: G1,G2 G3,G4	T2: G1,G3 G2,G4	T3: G1,G4 G2,G3	T12	T13	T23	T*
original	48%	0%	52%	0%	0%	0%	0%
Perm I	16%	6%	6%	2%	3%	0%	68%
Perm II	14%	18%	36%	6%	12%	8%	6%
Perm III	14%	18%	29%	7%	9%	10%	13%

Table S7. Information on fossils used to calibrate the inferred phylogenetic tree and to estimate divergence times between major lineages.

		Age	
Fossil Taxon	Current Placement	Estimate	Citation
Liposcelis sp.	Troctomorpha: Liposcelididae	53 MYA	41
Psocidus multiplex	Psocomorpha: Psocidae	33.9 MYA	42
Liposcelis sp.	Troctomorpha: Liposcelididae	53 MYA	41
Psocidus multiplex	Psocomorpha: Psocidae	33.9 MYA	42
Leaphis prima	Hemiptera: Sternorrhyncha: Aphidomorpha	245 MYA	43
Surijokocixius tomiensis	Hemiptera: Fulgoromorpha: Surijokocixiidae	251 MYA	44
Paraprosbole rotruda	Cicadoidea: Tettigarctidae	200 MYA	45
Undescribed	Hemiptera: Fulgoromorpha: Cixiidae	150 MYA	46
Priscoflata subvexa	Hemiptera: Fulgoromorpha: Flatidae	60 MYA	47
Wedelphus dichopteroides	Hemiptera: Fulgoromorpha: Fulgoridae	44 MYA	48
Triassojassus proavitus	Archijassidae: Archijassinae	220 MYA	49
Proerrhomus rugosus	Cicadellidae: Proerrhominae	118 MYA	50
Enicocephalinus acragrimaldii	Hemiptera: Enicocephalomorpha: Enicocephalidae	120 MYA	51
Gallomesovelia grioti	Hemiptera: Gerromorpha: Mesoveliidae	150MYA	52
Cretogerris albianus	Hemiptera: Gerromorpha: Gerridae	100 MYA	53
Shurabella lepyroniopsis	Hemiptera: Nepmorpha: Corixoidea: Shurabellidae	180 MYA	54
Mesolygaeus laiyangensis	Hemiptera: Leptopodomorpha: Saldoidea: Archegocimicidae	200 MYA	55
Ceresopsis costalis	Hemiptera: Cimicomorpha: Reduvioidea: Ceresopsidae	180 MYA	45
Vetanthocoris decorus	Hemiptera: Cimicomorpha: Cimoicoidea: Vetanthocoridae	150 MYA	56
Miridoides mesozoicus	Hemiptera: Cimicomorpha: Miridae	150 MYA	57
Cretopiesma suukyae	Hemiptera: Pentatomomorpha: Aradidae	99 MYA	58

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