



Mantophasmatodea and phylogeny of the lower neopterous insects

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Abstract

Polyneoptera is a name sometimes applied to an assemblage of 11 insect orders comprising the lower neopterous or “orthopteroid” insects. These orders include familiar insects such as Orthoptera (grasshoppers), Blattodea (roaches), Isoptera (termites) (Mantodea) praying mantises, Dermaptera (earwigs), Phasmatodea (stick insects), Plecoptera (stoneflies), as well as the more obscure, Embiidina (web-spinners), Zoraptera (angel insects) and Grylloblattodea (ice-crawlers). Many of these insect orders exhibit a high degree of morphological specialization, a condition that has led to multiple phylogenetic hypotheses and little consensus among investigators. We present a phylogenetic analysis of the polyneopteran orders representing a broad range of their phylogenetic diversity and including the recently described Mantophasmatodea. These analyses are based on complete 18S rDNA, 28S rDNA, Histone 3 DNA sequences, and a previously published morphology matrix coded at the ordinal level. Extensive analyses utilizing different alignment methodologies and parameter values across a majority of possible ranges were employed to test for sensitivity of the results to ribosomal alignment and to explore patterns across the theoretical alignment landscape. Multiple methodologies support the paraphyly of Polyneoptera, the monophyly of Dictyoptera, Orthopteroidea (*sensu* Kukalova-Peck; i.e. Orthoptera + Phasmatodea + Embiidina), and a group composed of Plecoptera + Dermaptera + Zoraptera. Sister taxon relationships between Embiidina + Phasmatodea in a group called “Eukinolabia”, and Dermaptera + Zoraptera (“Haplocercata”) are also supported by multiple analyses. This analysis also supports a sister taxon relationship between the newly described Mantophasmatodea, which are endemic to arid portions of southern Africa, and Grylloblattodea, a small order of cryophilic insects confined to the north-western Americas and north-eastern Asia, in a group termed “Xenonomia”. This placement, coupled with the morphological disparity of the two groups, validates the ordinal status of Mantophasmatodea.

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All neopterous insects, those that can fold their wings, can be placed into one of three groups: Holometabola, insects that have complete metamorphosis; Paraneoptera, true bugs and their allies; or a third group sometimes called “Polyneoptera” or the “orthopteroid” insects (for the sake of clarity the term Polyneoptera will be used hereafter when referring to these orders collectively). There has been extensive discussion among entomologists regarding the monophyly of the polyneopteran lineages, a group that includes Blattodea (roaches), Isoptera (termites), Mantodea (praying

mantises), Dermaptera (earwigs), Embiidina (web-spinners), Grylloblattodea (ice crawlers), Orthoptera (crickets and grasshoppers), Phasmatodea (stick insects), Plecoptera (stoneflies), Zoraptera (angel insects), and the recently described Mantophasmatodea (gladiators). These orders represent one of the largest and earliest insect radiations, yet there has been no clear answer regarding the monophyly of Polyneoptera and the phylogenetic relationships of the individual orders (for a summary see: Kristensen, 1995). Hennig (1981) placed all of the polyneopteran orders except Plecoptera in a monophyletic group (Paurometabola), and considered Plecoptera as sister-taxon to the remainder of Neoptera. Boudreaux (1979) depicted a monophyletic Polyneoptera with Embiidina + Plecoptera as the sister taxon to the remaining orders; a group he called “Orthopterodida.” Kukalova-Peck (1991) presented a paraphyletic

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Polyneoptera divided into three major groups: the “Plecopteroid” orders, which consist of extant Plecoptera and extinct relatives; the “Orthopteroid” orders, which are comprised of Orthoptera, Phasmatodea, Embiidina, and extinct relatives; and the “Blattoid” orders, which include the remaining orders and appear as sister-taxon to the higher insects (Paraneoptera + Endopterygota). Kristensen (1991) underscored the lack of consensus regarding the relationships among these orders in his summary of insect phylogeny, by representing Polyneoptera as a nearly unresolved “comb”, with Dictyoptera (Blattodea + Mantodea + Isoptera) as the only supported monophyletic group. An analysis of insect phylogeny including 24 polyneopteran taxa and incorporating both morphological characters and multiple molecular markers (Wheeler et al., 2001b) supported Polyneoptera as a monophyletic group; however, the relationships within Polyneoptera differ significantly from previously cited studies.

Polyneopterous insects inhabit a wide range of ecological niches, exhibit incredible morphological diversity, and include many species that have a significant impact on human activities (i.e. grasshoppers, roaches, and termites). Entire orders within Polyneoptera are represented by a handful of species (Zoraptera, Grylloblattodea, and Mantophasmatodea), although amber and compression fossils demonstrate a much wider prehistoric distribution and hint at past diversity (Rasnitsyn, 1976; Engel and Grimaldi, 2000; Zompro et al., 2002). Polyneoptera represents nearly a third of extant ordinal diversity among insects, and an insight into their evolutionary relationships would shed considerable light on the early radiation and diversification of insects, yet this has been hampered by the lack of consensus regarding relationships among these groups. The major reason for this disagreement is the lack of morphological synapomorphies between orders (Kristensen, 1991); a problem further exacerbated by the extinction of at least five major lineages (Grimaldi, 2001).

The recent discovery and description of Mantophasmatodea (gladiator insects), the first new insect order to be described in nearly a century, has elicited considerable excitement and controversy. Klass et al. (2002) stated “mantophasmatodeans are phenetically ‘orthopteroid’ insects”, and listed several morphological similarities that they share with other polyneopteran orders, including Phasmatodea (stick insects), Orthoptera (grasshoppers), and Grylloblattodea (ice-crawlers). However, they did not propose any specific sister group for Mantophasmatodea and did not perform a phylogenetic analysis; and although the name of the new order suggests general similarity with both praying mantids (“Manto-”) and stick insects (“-phasmatodea”), it is unclear whether mantophasmatodeans are closely related to either of these distinct orders.

Mantophasmatodeans exhibit several characters which are similar to members of other polyneopteran insect orders; for instance, they have an enlarged arolium (a cushion-like pad between the tarsal claws) which is superficially similar to *Timema*, a basal genus of stick insects (Tilgner, 2002; Whiting et al., 2003). They also possess a “configuration of sclerites and lobes” in the proventriculus (a small region of the digestive tract) that is similar to Grylloblattodea, but also paralleled in other insects (Klass et al., 2002). A recent investigation detailing the sperm structure of *Mantophasmatodea zephyra* (Dallai et al. 2003) suggested similarities with Mantodea. Tilgner (2002) suggested that mantophasmatodeans may simply be “aberrant members of the order Orthoptera” and argued that the characters used to exclude them from this group were misinterpreted, however, he also failed to provide any formal phylogenetic analysis supporting this conclusion. The difficulty in placing the Mantophasmatodea among insect orders highlights the confusion regarding the phylogenetic relationships among polyneopteran insects in general.

Klass et al. (2002) argued that mantophasmatodeans are deserving of ordinal status because they do not exhibit any morphological features that can unambiguously place them within a described order. However, rather than performing a standard phylogenetic analysis to place Mantophasmatodea among extant insect orders, Klass et al. argued that “the only feasible way to discuss the phylogenetic position of Mantophasmatodea was by evaluating pertinent characters ‘mentally’”. This approach ignores recent strides in insect ordinal phylogeny which utilize formal analyses of morphological and molecular data (Beutel and Gorb, 2001; Wheeler et al., 2001b), and we question the utility of mental analyses in modern phylogenetics. Thus we consider the new ordinal designation a hypothesis that has yet to be rigorously tested, and this work represents the first formal test of the validity of Mantophasmatodea.

Sensitivity analysis, in the context of phylogenetic studies, is the exploration of how variation in underlying assumptions can affect the outcome of analyses (Giribet, 2003), given the same initial data. Sensitivity analyses examining the effect of alignment parameters have become more and more common, particularly when dealing with ribosomal data (Shull et al., 2001; Giribet et al., 2002; Ogden and Whiting, 2003; Whiting et al., 2003). Results presented here represent a portion of a large sensitivity analysis focusing on alignment parameters and utilizing two programs commonly used for the alignment of molecular data: ClustalX (Thompson et al., 1997) and POY (Wheeler et al., 1996–2003). Results relevant to the phylogeny of the polyneopterous orders are presented here, while specific details and patterns of the overall analysis can

be found in Terry and Whiting (Terry and Whiting, 2005).

Materials and methods

Taxon sampling

To increase the accuracy of phylogenetic estimation, we extensively sampled taxa (Zwickl and Hillis, 2002) by including exemplars of most major polyneopteran lineages representing the range of extant biological diversity. This includes 15 of 30 extant families for Orthoptera, six of nine for Blattodea, seven of eight for Isoptera, five of 10 for Mantodea, eight of 10 for Dermaptera, 10 of 10 for Phasmatodea, 16 of 16 for Plecoptera, 10 of 11 for Embiidina, and 1 of 1 for both Grylloblattodea and Zoraptera, for a total of 79 of 116 described families, multiple exemplars from major subordinal groups (i.e. Arctoperlaria, Antarctoperlaria, Ensifera, Caelifera), and two of three extant, described genera of Mantophasmatodea (*Sclerophasma* and *Tyrannophasma*). The excluded families are generally minor lineages, and this data set represents by far the most comprehensive treatment of polyneopteran relationships to date. Outgroup taxa include Archaeognatha (bristletails; one sp.), Zygentoma (silverfish; three spp.), Ephemeroptera (mayflies; three spp.), Odonata (dragonflies; four spp.), Hemiptera (true bugs; five spp.) and exemplars of most holometabolon orders (16 taxa). See Appendix Table A1 for a complete list of all taxa included in this analysis. DNA sequences are deposited in GenBank under accession numbers as listed in Appendix Table A1.

DNA extraction, amplification and sequencing

For larger specimens, a small portion of wing or leg muscle was dissected from the mesothorax. For smaller specimens, the thorax was cut length-wise, any gut contamination was removed and the entire thorax was used. Individual vouchers as well as conspecific specimens for specimens collected in series are deposited in the Brigham Young University Insect Genomics Collection (IGC). Tissue dissected from specimens was subjected to either a phenol/chloroform extraction (Whiting et al., 1997) or extraction via Qiagen's DNeasy tissue kit. Purified DNA was amplified for 18S, 28S and H3 via polymerase chain reaction using previously published primers (see Appendix Table A2 for a complete list of primers) and amplification profiles (Whiting et al., 1997; Colgan et al., 1998; Whiting, 2001). Due to their large size, each of the ribosomal genes was amplified using three separate regions with sufficient overlap to ensure continuity. These regions were approximately 1000, 800 and 600 nucleotides long

for 18S and 1200, 600 and 1000 nucleotides long for 28S. Yield and potential contamination were monitored by agarose gel electrophoresis. Target products were purified and cycle-sequenced using the ABI dRhodamine cycle sequencing kit via flanking and, for long PCR products, internal primers. These sequencing reactions were then column purified and subjected to automated sequencing on ABI's 377, 3100 or 3730xl automated sequencers. Complementary strands were independently sequenced and chromatographs were checked visually using Sequencher 4.1 (Sequencher, 2002).

Phylogenetic analyses

Direct optimization (DO). For the ribosomal sequences, we performed initial alignments using Sequencher 4.1, by manually aligning the conserved domains across all taxa. Sequences were then subdivided to facilitate finding an optimal solution during DO (Giribet, 2001). This yielded 26 and 28 separate sections for the 18S and 28S rDNA, respectively. Two small sections of 18S—corresponding to helices E21-3 and E21-4 of region V4 and a portion of helix 47 of region V9 (De Rijk et al., 1992)—and five small sections of 28S—corresponding to portions of the D2 expansion region and the D5, D6 and D7b expansion regions—were judged non-homologous across taxa and excluded from the analysis. To better estimate the polyneopteran relationships, portions of sequences for outgroup taxa that were judged to be unalignable relative to the polyneopteran taxa were excluded and treated as missing data. This was done for two regions of 18S—corresponding to portions of helix E10-1 of region V2 and helix 41 of region V7 (De Rijk et al., 1992)—and five regions of 28S—corresponding to portions of the D2 expansion region and the D3, D4 and D7a expansion regions.

The morphology matrix from Wheeler et al. (2001), a matrix coded for all extant insect orders, was stripped of characters uninformative below the ordinal level for the taxa included in this analysis. These characters were also coded for Mantophasmatodea. This resulted in a reduction from 275 to 125 characters: 10 ordered and 115 unordered. These characters were coded to reflect the “groundplan” morphology of the individual orders and all methods of analyses were performed using total evidence and without morphology, with significant differences discussed below. Three unordered tarsal characters from Beutel and Gorb (2001) were also included. The matrix can be downloaded from <http://www.inbio.byu.edu/faculty/mfw2/whitinglab>.

Analyses were performed for each individual data set (morphology, 18S, 28S, H3) and a combined total-evidence data set using the program POY, version 3.0, on an IBM SP2 supercomputer. The ribosomal data sets were analyzed for 90 parameter combinations (see ILD analysis below). The protein reading frame was

conserved across the H3 data set and it was designated as “pre-aligned” and analyzed for the ten transversion to transition parameter sets. For each analysis, POY was run in parallel across four nodes on the supercomputer. Each analysis produced a topology and implied alignments (for the ribosomal data) using the commands:

```
-fitchtrees -maxprocessors 8 -onan -onannum 1
-parallel -noleading -norandomizeoutgroup -sprmax-
trees 1 -tbrmaxtrees 1 -maxtrees 5 -holdmaxtrees 50
-slop 5 -buildspr -buildmaxtrees 2 -random 10 -multi-
random -treefuse -fuselimit 10 -fusemingroup 5 -fuse-
maxtrees 100 -numdriftchanges 30 -driftspr
-numdriftspr 10 -drifttbr -numdrifttbr 10 -slop 10
-checkslop 10 -impliedalignment -molecularmatrix *.txt
-seed -1
```

(*asterisk denotes filenames that varied between individual analyses).

Topologies generated from the most optimal parameter values, as judged by the ILD metric (Wheeler and Hayashi, 1998), were further searched by increasing the number of random additions to 200. The implied alignment for the parameter set with maximum congruence among data sets (1 : 1 : 1) can be downloaded at <http://www.inbio.byu.edu/faculty/mfw2/whitinglab/>.

Multiple sequence alignment via ClustalX. To compare equivalent data sets, the unalignable regions as described above were also excluded from the ClustalX alignment. Taxa missing data for specific regions were assigned a number of unknown character states (“N”) equivalent to the number of character states of the taxon with the longest known sequence for that region and the ribosomal data sets were then concatenated. We examined the behaviour of Clustal on 198 parameter combinations spanning the range of parameter values in ClustalX using the command line file:

```
clustalx *.txt -batch -gapopen= -gapext=* -trans-
weight=* -outfile=* -outorder= input -output=nexus
-type=dna.
```

(*asterisks denote filenames or parameters that varied between individual alignments)

Combined total-evidence data sets were then assembled for each parameter combination using the pre-aligned morphology and H3 data sets and the ClustalX aligned ribosomal data sets. Data sets constructed from the ribosomal alignments and combined total-evidence datasets were each analyzed in PAUP* with 50 random additions and tbr swapping.

All bootstrap support measures reported were performed with PAUP* using 1000 bootstrap replicates with 20 random additions per replicate. Bootstrap values for the DO analysis were done using the implied alignment generated by POY. Partitioned Bremer support values were calculated using a modified PAUP block generated by TreeRot (Sorenson, 1999) with 50 random additions for the parsimony topology. Bremer

support values for the DO analysis were performed via POY using the command “-bremer” and a constraint topology. Partitioned Bremer values for the DO analysis were calculated in the same manner as the parsimony analysis using the implied alignment. As this generates somewhat inflated support, partition values were given a proportional value so that the total of all support would equal the overall value obtained via POY.

Sensitivity analysis

For the DO analysis we sampled nine gap-transversion ratios ranging from 0.5 to 100, and 10 transversion-transition ratios ranging from 0.5 to infinity. Past studies have demonstrated that the lower ratio values tend to produce more congruent results (Wheeler, 1995; Giribet et al., 2001; Whiting et al., 2003) so we sampled this region of the search space more densely. Parameters for the ClustalX analysis are somewhat limited by how they are implemented within the program. Portions of the theoretical search space as defined above are unavailable and specific parameter combinations are not directly comparable with DO (Ogden and Whiting, 2003). However, parameter combinations unavailable in ClustalX are, for the most part, extreme values unlikely to yield reasonable alignments. ClustalX allows the user to choose a “gap opening penalty” ranging from 0 to 100, and 19 values spanning this range were used in this analysis, with values between 0 and 20 sampled more densely. “Transition weight” can be set between 0 and 1, with 0 signifying a mismatch between transitions (high cost) and 1 signifying a match (no cost). For the Clustal alignments we selected 11 values beginning at 0 and incrementally increasing by 0.1. We computed the incongruence length metric (Mickeyvich and Farris, 1981) for each parameter combination for both DO and parsimony analysis after the ClustalX alignment, by taking the difference between the length of the total tree, minus the sum of the lengths of the individual partitions (morphology, 18S, 28S, H3) and dividing by the length of the total tree. In the DO analysis we also calculated ILD metric values for the ribosomal data set and molecular data set using a subset of the parameter values described above to determine if the “pre-aligned” condition of the morphology and H3 data sets were skewing the ILD results. For a more complete description of the results from these analyses, and a discussion comparing the performance of DO versus alignment via ClustalX, see Terry and Whiting (2005).

Additional alignments and analyses

Parsimony analysis of ClustalX alignment. Alignments for each data partition were generated using ClustalX (Thompson et al., 1997) under the program’s default parameters. These individual alignments were

then assembled into a combined matrix and used for parsimony (PAUP*) and Bayesian (MrBayes) analyses. The parsimony analysis was performed using 200 random addition sequences incorporating TBR swapping with gaps treated as a 5th state. The resulting topology was subjected to 1000 bootstrap replicates with 25 random additions per replicate.

Bayesian analysis of ClustalX alignment. The ClustalX alignment described above was tested via ModelTest, and a Bayesian analysis was performed using the selected parameters. The matrix was analyzed over 500 000 generations using four chains and a sampling frequency of 100. The first 50 000 generations were discarded as the “burn-in” period and the remaining generations were assembled into a majority rule topology.

Results

Sequencing

All amplified H3 sequences had a conserved reading frame, with the exception of one dipteran (*Dolichozepea subalbipes*) that had a 67 base pair insert that was excised prior to analysis. The longest complete polyneopteran 18S sequence (*Grylloblatta* sp.) was 2125 base pairs in length with an average length of approximately 1900 base pairs. The longest complete polyneopteran 28S sequence was 2440 base pairs (also *Grylloblatta* sp.) with an average length of approximately 2300 base pairs.

DO sensitivity analysis

Of the 90 parameter sets investigated the set treating transitions, transversions and gaps equally (1 : 1 : 1) yields the most congruent result, with an ILD value of 0.03503. The single optimal topology (Fig. 1) has a length of 17054. The investigated parameter set with the least congruence between data partitions (ILD value of 0.12216) gives transversions a weight fourfold that of transitions, and gaps a weight 100-fold that of transversions. A complete listing of all ILD scores for the DO analysis can be found in Table 1. Three clades of ordinal status (Mantophasmatodea, Grylloblattodea, and Mantodea) are monophyletic across all combined analyses, and Blattodea is either paraphyletic or polyphyletic in all analyses. Other orders and supraordinal groupings show varying degrees of stability across the parameter space, however, the majority of clades are more robust to changes in the transition/transversion ratio than the gap/change ratio.

ClustalX sensitivity analysis

Of the 198 parameter sets investigated for the Clustal analysis, congruence between data sets was maximized

with a gap opening cost of 50 and a transition weight of 0.9 (ILD value of 0.03059); the greatest incongruence was obtained when the parameter set has gap opening set to one and a transition weight of zero (ILD value of 0.10242; see Table 2 for a complete list). The topology generated from the combined data sets using the default parameters for ClustalX appears in Fig. 2. A total of 19 most parsimonious trees were generated with a length of 20 478, a CI of 0.302, and an RI of 0.652. The orders Mantophasmatodea, Grylloblattodea, and Mantodea are monophyletic across all alignment parameters, as is Dictyoptera. Blattodea is either paraphyletic or polyphyletic in all analyses. The orders Plecoptera, Embiidina, Phasmatodea, Dermaptera and Zoraptera exhibit high levels of monophyly across analyses. The supraordinal groupings Mantophasmatodea + Grylloblattodea, Embiidina + Phasmatodea and Zoraptera + Dermaptera also display high levels of monophyly, despite varying parameters for the Clustal alignment.

Additional analyses

ModelTest selected GTR + I + Γ as the best justified model for the ClustalX alignment, and MrModeltest (Nylander, 2004) selected the same model when each gene partition was tested. Data sets, including morphology, were partitioned individually using a command block generated with MrModeltest, and each molecular partition had its own unique GTR + gamma + propinv model. The majority rule topology recovered from Bayesian analyses with posterior probabilities as estimated by majority rule percentages above nodes appears in Fig. 3. This analysis supports a monophyletic Polyneoptera, with Holometabola and Hemiptera as sister taxa.

Discussion

This analysis represents the most comprehensive analysis to date for the relationships among major lineages of polyneopteran insects. Results from the three separate methodologies (Figs 1–3) support a monophyletic Neoptera (folding-winged insects) and support Odonata (dragonflies) as its closest, extant relative. All analyses also support a monophyletic Holometabola and Hemiptera. Polyneoptera is a monophyletic clade in the Bayesian analysis, but is paraphyletic in the DO and parsimony analyses (Figs 1 and 2) with Plecoptera + Dermaptera + Zoraptera as sister to Holometabola, and the remainder of the polyneopterous orders as sister to this group in the DO analysis. Plecoptera + Dermaptera + Zoraptera also appear as sister to Holometabola in the parsimony analysis and Mantophasmatodea + Grylloblattodea are sister to this entire clade.

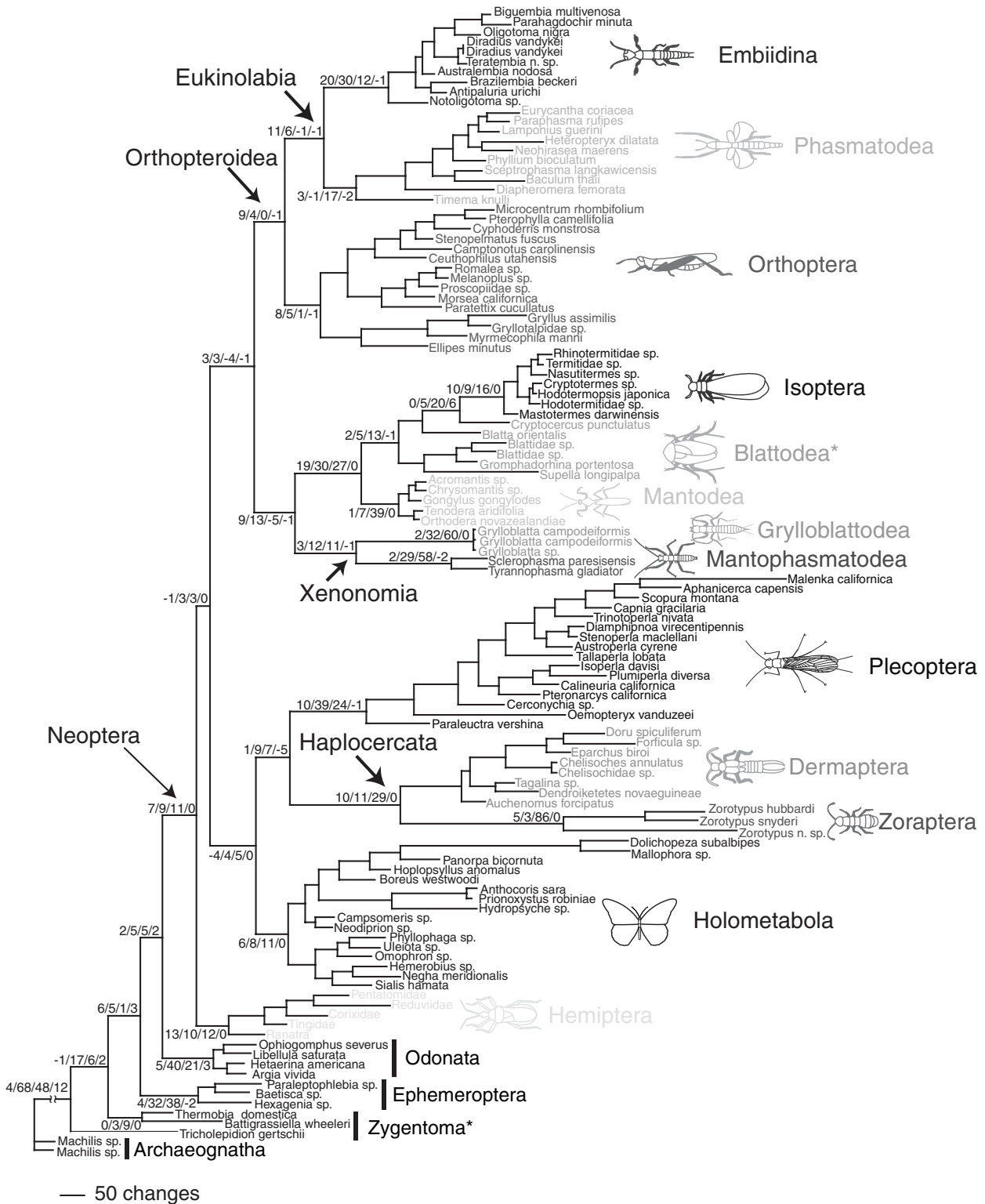


Fig. 1. Phylogeny of Polyneoptera and placement of Mantophasmatodea as sister group to Grylloblattodea (= Xenonomia). Direct Optimization topology from a combined analysis of 18S rDNA, 28S rDNA, Histone 3, and morphology, and all transformations weighted to unity. Branch lengths were calculated in PAUP* under ACCTRAN optimization criteria using the implied alignment from POY. Numbers above ordinal and interordinal nodes represent partitioned Bremer support values for Morphology/18S/28/H3, respectively. Tree length = 17 054, CI = 0.417, RI = 0.702.

Table 1
 Topology lengths and subsequent ILD values from DO analysis. Bold values represent global minimum ILD values

Gap:Tv	Tv:Ts	Data set									
		vvmorph.	28S	18S	rib.	rib. ILD	H3	mol.	mol. ILD	Total	Total ILD
0.5	0.5	238	18454	9651			5067			34816	0.04038
1	0.5	238	14505	7181			3468			26454	0.04015
2	0.5	238	18448	8416			3468			31907	0.04190
3	0.5	238	20938	9083			3468			35311	0.04486
4	0.5	238	23244	9671			3468			38455	0.04769
10	0.5	238	35384	12813			3468			55352	0.06231
20	0.5	238	54256	17526			3468			81888	0.07816
50	0.5	238	108609	31285			3468			158492	0.09396
100	0.5	238	197728	53577			3468			283770	0.10135
0.5	1	238	17043	8502			4596			31601	0.03867
1	1	238	11714	5298	17426	0.02376	2444	20163	0.03506	20409	0.03503
2	1	238	14644	5982	21198	0.02698	2444	23995	0.03855	24261	0.03928
3	1	238	16964	6571	24303	0.03160	2444	27130	0.04243	27410	0.04352
4	1	238	19430	7132	27285	0.02650	2444	30141	0.03766	30453	0.03970
10	1	238	31101	10100	43792	0.05917	2444	46499	0.06138	46958	0.06548
20	1	238	49741	14796			2444			72478	0.07256
50	1	238	104260	28822			2444			149534	0.09209
100	1	238	193383	49751			2444			277580	0.11443
0.5	2	238	13893	6479			3614			25111	0.03532
1	2	238	18466	7817	26999	0.02652	3614	31129	0.03958	31384	0.03980
2	2	238	23823	9166	33997	0.02965	3614	38195	0.04168	38486	0.04274
3	2	238	28389	10350	40116	0.03433	3614	44351	0.04505	44687	0.04690
4	2	238	32738	11341	45975	0.04124	3614	50170	0.04937	50488	0.05065
10	2	238	55443	17363	78650	0.07430	3614	83280	0.08237	83163	0.07822
20	2	238	92717	26705			3614			135590	0.09083
50	2	238	201120	53915			3614			291666	0.11239
100	2	238	381830	99067			3614			542762	0.10688
0.5	3	238	36885	16798			9481			66114	0.04102
1	3	238	24982	10257	36184	0.02612	4751	41597	0.03863	41873	0.03929
2	3	238	32785	12261	46577	0.03287	4751	52004	0.04244	52358	0.04437
3	3	238	39420	14074	55688	0.03940	4751	61111	0.04690	61473	0.04864
4	3	238	45936	15618	64375	0.04382	4751	69961	0.05226	70178	0.05180
10	3	238	80419	24804	113466	0.07265	4751	118677	0.07333	119829	0.08026
20	3	238	136299	38710			4751			198285	0.09223
50	3	238	298668	80489			4751			433227	0.11329
100	3	238	568333	147318			4751			811159	0.11159
0.5	4	238	22892	10266			5847			40970	0.04215
1	4	238	31389	12713	45259	0.02556	5847	52070	0.04073	52382	0.04190
2	4	238	41740	15321	59088	0.03430	5847	65818	0.04421	66150	0.04541
3	4	238	50768	17677	71073	0.03698	5847	77887	0.04616	78132	0.04610
4	4	238	59164	19764	82920	0.04814	5847	89576	0.05360	90218	0.05769
10	4	238	104864	31125	147986	0.08107	5847	154234	0.08038	155485	0.08625
20	4	238	179095	50496			5847			258662	0.08887
50	4	238	396597	105820			5847			567574	0.10408
100	4	238	753187	188234			5847			1079359	0.12216
0.5	10	238	48986	21309			12383			87052	0.04751
1	10	238	69772	27168	99834	0.02899	12383	114444	0.04475	114700	0.04480
2	10	238	95262	33794	133757	0.03515	12383	148159	0.04536	148561	0.04634
3	10	238	117160	39372	163685	0.04370	12383	178350	0.05290	178834	0.05413
4	10	238	138397	44708	192393	0.04828	12383	207547	0.05810	208066	0.05931
10	10	238	252027	73997	353512	0.07776	12383	368354	0.08130	367482	0.07847
20	10	238	435983	120499			12383			627388	0.09290
50	10	238	972439	253819			12383			1397900	0.11376
100	10	238	1879672	485520			12383			2628938	0.09552
0.5	20	238	92176	39675			23148			163306	0.04941
1	20	238	133157	51021			23148			218023	0.04797
2	20	238	183958	64378			23148			286421	0.05132
3	20	238	228360	75664			23148			345982	0.05368
4	20	238	268556	86543			23148			403947	0.06303
10	20	238	496955	145252			23148			722737	0.07907
20	20	238	858330	238855			23148			1235485	0.09301

Table 1
Continued

Gap:Tv	Tv:Ts	Data set									
		morph.	28S	18S	rib.	rib. ILD	H3	mol.	mol. ILD	Total	Total ILD
50	20	238	1937117	515641			23148			2752190	0.10030
100	20	238	3758134	964116			23148			5307895	0.10593
0.5	50	238	221441	94788			55642			391329	0.04911
1	50	238	325274	123546			55642			529389	0.04664
2	50	238	449337	156489			55642			698099	0.05213
3	50	238	558362	182598			55642			847803	0.06011
4	50	238	663053	210047			55642			991255	0.06282
10	50	238	1226657	357391			55642			1796706	0.08726
20	50	238	2143954	575659			55642			3084376	0.10014
50	50	238	4834661	1271191			55642			6922670	0.10992
100	50	238	9323658	2401340			55642			13259818	0.11154
0.5	100	238	437154	186092			109508			773250	0.05206
1	100	238	643142	244696			109508			1047862	0.04798
2	100	238	894977	308566			109508			1386168	0.05258
3	100	238	1112212	364968			109508			1684113	0.05771
4	100	238	1320594	416714			109508			1978663	0.06651
10	100	238	2455429	711105			109508			3568587	0.08191
20	100	238	4275103	1176009			109508			6116514	0.09085
50	100	238	9653891	2515246			109508			13881738	0.11547
100	100	238	18832288	4843214			109508			26669629	0.10815
0.5	8	238	8583	3615			2150			15408	0.05335
1	8	238	6297	2363			1078			10503	0.05018
2	8	238	8724	2921			1078			13777	0.05923
3	8	238	10731	3374			1078			16543	0.06782
4	8	238	12658	3839			1078			19143	0.06948
10	8	238	23345	6414			1078			34089	0.08842
20	8	238	40351	10334			1078			58624	0.11297
50	8	238	90633	22099			1078			128617	0.11327
100	8	238	178936	41704			1078			248784	0.10784

Abbreviations: Tv = transversion, Ts = transition, morph. = morphology dataset, rib. = combined ribosomal dataset, mol. = combined molecular data set.

For the DO analysis without the morphological data, all polyneopterous orders (with the exception of Blattodea) remain monophyletic; however, the clade Grylloblattodea + Mantophasmatodea is supported as sister taxon to Embiidina + Phasmatodea, Hemiptera appears as sister to Dictyoptera, and Plecoptera is placed as sister taxon to a large assemblage of orders. The parsimony analysis excluding morphology resulted in 20 equally parsimonious topologies, a consensus of which supports the paraphyly of Phasmatodea (Grylloblattodea + Mantophasmatodea nested within the order), the polyphyly of Orthoptera, and the placement of the two Diptera taxa included within the polyneopterous lineages rendering Dermaptera polyphyletic. Other relationships remain nearly identical to the combined analysis, but with a loss of resolution for many of the deeper nodes within Neoptera. When morphology is removed from the Bayesian analysis the monophyly of Polyneoptera is no longer supported, one Zoraptera taxon is placed within a clade composed of Hemiptera and Orthoptera, the remaining Zoraptera + Dermaptera are sister taxon to Holometabola, Plecoptera is sister taxon to Dictyoptera, and Grylloblattodea +

Mantophasmatodea are supported as sister to Embiidina + Phasmatodea.

Blattodea is rendered paraphyletic by Isoptera in all three of the methodological approaches presented here, and in each of these *Cryptocercus*, a wood feeding semi-social roach, is sister to Isoptera, with *Mastotermes darwinensis* supported as sister taxon to the remainder of Isoptera (Figs 1, 2 and 3). All other polyneopterous orders are supported as monophyletic in the combined analyses for each methodology.

Dictyoptera (Isoptera + Blattodea + Mantodea), the most universally accepted supra-ordinal grouping within Polyneoptera (Snodgrass, 1935; Boudreaux, 1979; Kristensen, 1991), is very strongly supported as monophyletic across all analyses, with Mantodea as sister taxon to termites and roaches. The paraphyly of Blattodea is supported by all three modes of analysis presented here, by previous molecular (Grandcolas and D'Haese, 2001; Lo et al., 2000) and behavioural data, and by paleontological evidence (Grimaldi, 2001). The preponderance of evidence confirms that termites are indeed "highly modified, social, myopic, wood-eating roaches" (Grimaldi, 2001), and molecular evidence

Table 2
 Topology lengths and subsequent ILD values from ClustalX alignments with PAUP* analyses

G.O.	T.S.W	Data set						G.O.	T.S.W	Data set					
		18S	28S	H3	morph	Total	ILD			18S	28S	H3	morph	Total	ILD
1	0	5589	11154	2400	234	21588	0.10242	20	0	5543	11671	2400	234	20855	0.04829
1	0.1	5513	10996	2400	234	21213	0.09758	20	0.1	5503	11765	2400	234	20932	0.04921
1	0.2	5435	10920	2400	234	21045	0.09770	20	0.2	5493	11725	2400	234	20853	0.04800
1	0.3	5427	10889	2400	234	21037	0.09921	20	0.3	5423	11716	2400	234	20758	0.04745
1	0.4	5423	10804	2400	234	19980	0.05601	20	0.4	5514	11686	2400	234	20796	0.04626
1	0.5	5365	10867	2400	234	19887	0.05134	20	0.5	5469	11801	2400	234	20831	0.04450
1	0.6	5352	10761	2400	234	19660	0.04644	20	0.6	5475	11876	2400	234	20968	0.04688
1	0.7	5358	10895	2400	234	19783	0.04529	20	0.7	5500	11698	2400	234	20809	0.04695
1	0.8	5387	11072	2400	234	20049	0.04768	20	0.8	5672	11913	2400	234	20967	0.03568
1	0.9	5498	11169	2400	234	20195	0.04427	20	0.9	5303	12059	2400	234	20904	0.04344
1	1.0	5783	12175	2400	234	21697	0.05093	20	1.0	5473	12010	2400	234	21199	0.05104
2	0	5488	10943	2400	234	20077	0.05041	30	0	5653	11946	2400	234	21295	0.04987
2	0.1	5397	10858	2400	234	19840	0.04793	30	0.1	5638	12096	2400	234	21504	0.05283
2	0.2	5348	10808	2400	234	19756	0.04890	30	0.2	5596	12065	2400	234	21311	0.04767
2	0.3	5292	10701	2400	234	19550	0.04721	30	0.3	5558	12257	2400	234	21556	0.05135
2	0.4	5289	10609	2400	234	19397	0.04459	30	0.4	5620	12231	2400	234	21535	0.04876
2	0.5	5226	10623	2400	234	19315	0.04308	30	0.5	5614	12216	2400	234	21485	0.04752
2	0.6	5249	10712	2400	234	19403	0.04164	30	0.6	5619	12280	2400	234	21532	0.04640
2	0.7	5253	10788	2400	234	19464	0.04054	30	0.7	5626	12286	2400	234	21566	0.04730
2	0.8	5293	11032	2400	234	19829	0.04388	30	0.8	5666	12330	2400	234	21503	0.04060
2	0.9	5382	11120	2400	234	19963	0.04143	30	0.9	5431	12542	2400	234	21568	0.04456
2	1.0	5625	11750	2400	234	20969	0.04578	30	1.0	5607	12598	2400	234	22061	0.05539
4	0	5259	10824	2400	234	19552	0.04271	40	0	5744	12349	2400	234	21766	0.04773
4	0.1	5239	10772	2400	234	19491	0.04340	40	0.1	5753	12506	2400	234	21946	0.04798
4	0.2	5203	10753	2400	234	19440	0.04372	40	0.2	5752	12517	2400	234	21990	0.04943
4	0.3	5146	10695	2400	234	19290	0.04225	40	0.3	5754	12548	2400	234	22059	0.05091
4	0.4	5140	10768	2400	234	19353	0.04191	40	0.4	5798	12662	2400	234	22188	0.04931
4	0.5	5192	10746	2400	234	19397	0.04253	40	0.5	5780	12473	2400	234	21894	0.04599
4	0.6	5176	10828	2400	234	19456	0.04204	40	0.6	5764	12558	2400	234	22001	0.04750
4	0.7	5157	10832	2400	234	19377	0.03891	40	0.7	5743	12563	2400	234	21923	0.04484
4	0.8	5167	10905	2400	234	19493	0.04037	40	0.8	5789	12573	2400	234	21957	0.04377
4	0.9	5119	11071	2400	234	19658	0.04243	40	0.9	5541	12641	2400	234	21779	0.04422
4	1.0	5348	11450	2400	234	20258	0.04077	40	1.0	5657	12802	2400	234	22101	0.04561
6	0	5239	10948	2400	234	19662	0.04277	50	0	5850	12899	2400	234	22408	0.04574
6	0.1	5238	10965	2400	234	19733	0.04541	50	0.1	5861	12833	2400	234	22347	0.04560
6	0.2	5227	10929	2400	234	19673	0.04488	50	0.2	5855	12825	2400	234	22356	0.04661
6	0.3	5135	10844	2400	234	19442	0.04264	50	0.3	5809	12897	2400	234	22359	0.04557
6	0.4	5236	10925	2400	234	19617	0.04190	50	0.4	5855	12857	2400	234	22382	0.04629
6	0.5	5168	10918	2400	234	19522	0.04108	50	0.5	5820	12893	2400	234	22400	0.04701
6	0.6	5218	10978	2400	234	19655	0.04197	50	0.6	5804	12902	2400	234	22379	0.04643
6	0.7	5222	11020	2400	234	19702	0.04192	50	0.7	5808	13012	2400	234	22541	0.04822
6	0.8	5259	11009	2400	234	19736	0.04226	50	0.8	5853	12872	2400	234	22370	0.04519
6	0.9	5202	11077	2400	234	19746	0.04219	50	0.9	5631	13348	2400	234	22295	0.03059
6	1.0	5413	11573	2400	234	20451	0.04063	50	1.0	5761	13322	2400	234	22771	0.04629
8	0	5288	11051	2400	234	19806	0.04206	60	0	5935	13206	2400	234	22867	0.04775
8	0.1	5251	11166	2400	234	19939	0.04454	60	0.1	5950	13141	2400	234	22865	0.04986
8	0.2	5251	11125	2400	234	19932	0.04626	60	0.2	5970	13145	2400	234	22893	0.04997
8	0.3	5288	11112	2400	234	19932	0.04505	60	0.3	5921	13255	2400	234	22957	0.04996
8	0.4	5283	11175	2400	234	19987	0.04478	60	0.4	5905	13259	2400	234	22942	0.04986
8	0.5	5252	11131	2400	234	19877	0.04327	60	0.5	5859	13194	2400	234	22809	0.04919
8	0.6	5235	11105	2400	234	19807	0.04206	60	0.6	5916	13125	2400	234	22772	0.04817
8	0.7	5301	11047	2400	234	19835	0.04300	60	0.7	5948	13170	2400	234	22779	0.04509
8	0.8	5261	11204	2400	234	19968	0.04352	60	0.8	5929	13274	2400	234	22898	0.04634
8	0.9	5173	11260	2400	234	19935	0.04354	60	0.9	5743	13491	2400	234	22893	0.04477
8	1.0	5319	11617	2400	234	20440	0.04256	60	1.0	5839	13778	2400	234	23461	0.05157
10	0	5416	11259	2400	234	20303	0.04896	70	0	6092	13440	2400	234	23310	0.04908
10	0.1	5398	11289	2400	234	20280	0.04729	70	0.1	6148	13445	2400	234	23397	0.05001
10	0.2	5373	11302	2400	234	20224	0.04524	70	0.2	6108	13449	2400	234	23389	0.05122
10	0.3	5376	11331	2400	234	20250	0.04489	70	0.3	5940	13549	2400	234	23256	0.04872
10	0.4	5280	11327	2400	234	20167	0.04592	70	0.4	6038	13588	2400	234	23474	0.05172
10	0.5	5400	11342	2400	234	20304	0.04571	70	0.5	6000	13779	2400	234	23604	0.05046

Table 2
Continued

		Data set						Data set							
G.O.	T.S.W	18S	28S	H3	morph	Total	ILD	G.O.	T.S.W	18S	28S	H3	morph	Total	ILD
10	0.6	5407	11354	2400	234	20318	0.04543	70	0.6	5982	13840	2400	234	23639	0.05004
10	0.7	5373	11358	2400	234	20257	0.04403	70	0.7	6057	13775	2400	234	23621	0.04890
10	0.8	5408	11391	2400	234	20343	0.04473	70	0.8	6054	13825	2400	234	23662	0.04856
10	0.9	5224	11404	2400	234	20146	0.04388	70	0.9	5859	13961	2400	234	23478	0.04362
10	1.0	5322	11722	2400	234	20579	0.04378	80	1.0	6032	14315	2400	234	24183	0.04970
12	0	5467	11308	2400	234	20343	0.04591	80	0	6220	13587	2400	234	23582	0.04838
12	0.1	5386	11362	2400	234	20300	0.04522	80	0.1	6170	13566	2400	234	23520	0.04889
12	0.2	5432	11401	2400	234	20410	0.04620	80	0.2	6210	13630	2400	234	23640	0.04932
12	0.3	5385	11376	2400	234	20354	0.04712	80	0.3	6086	13631	2400	234	23437	0.04634
12	0.4	5314	11409	2400	234	20333	0.04800	80	0.4	6111	13693	2400	234	23570	0.04803
12	0.5	5455	11432	2400	234	20415	0.04379	80	0.5	6097	13878	2400	234	23798	0.04996
12	0.6	5468	11426	2400	234	20457	0.04541	80	0.6	6194	13857	2400	234	23965	0.05341
12	0.7	5447	11619	2400	234	20678	0.04730	80	0.7	6180	13860	2400	234	23830	0.04851
12	0.8	5413	11507	2400	234	20415	0.04217	80	0.8	6104	14039	2400	234	23972	0.04985
12	0.9	5219	11606	2400	234	20314	0.04209	80	0.9	5898	14086	2400	234	23678	0.04477
12	1.0	5318	11799	2400	234	20649	0.04349	80	1.0	6233	14647	2400	234	24714	0.04856
15	0	5392	11577	2400	234	20543	0.04576	90	0	6331	13822	2400	234	23972	0.04943
15	0.1	5376	11580	2400	234	20506	0.04467	90	0.1	6234	13828	2400	234	23822	0.04727
15	0.2	5460	11464	2400	234	20571	0.04924	90	0.2	6216	13836	2400	234	23793	0.04653
15	0.3	5425	11477	2400	234	20526	0.04823	90	0.3	6088	13832	2400	234	23613	0.04485
15	0.4	5489	11529	2400	234	20623	0.04708	90	0.4	6200	14086	2400	234	24194	0.05266
15	0.5	5463	11564	2400	234	20621	0.04655	90	0.5	6217	14084	2400	234	24219	0.05302
15	0.6	5474	11514	2400	234	20610	0.04794	90	0.6	6215	14105	2400	234	24253	0.05356
15	0.7	5452	11535	2400	234	20611	0.04803	90	0.7	6337	14341	2400	234	24508	0.04880
15	0.8	5434	11702	2400	234	20682	0.04410	90	0.8	6313	14699	2400	234	24838	0.04799
15	0.9	5223	11739	2400	234	20482	0.04326	90	0.9	6141	14998	2400	234	24850	0.04334
15	1.0	5328	11870	2400	234	20692	0.04156	90	1.0	6234	15146	2400	234	25171	0.04597
18	0	5519	11745	2400	234	20926	0.04913	100	0	6329	13876	2400	234	23944	0.04615
18	0.1	5496	11684	2400	234	20873	0.05074	100	0.1	6319	14189	2400	234	24434	0.05288
18	0.2	5505	11674	2400	234	20846	0.04955	100	0.2	6307	14134	2400	234	24294	0.05018
18	0.3	5440	11634	2400	234	20746	0.05003	100	0.3	6223	14076	2400	234	24161	0.05083
18	0.4	5495	11721	2400	234	20856	0.04824	100	0.4	6354	14333	2400	234	24590	0.05161
18	0.5	5500	11679	2400	234	20792	0.04709	100	0.5	6383	14481	2400	234	24718	0.04936
18	0.6	5483	11707	2400	234	20808	0.04729	100	0.6	6386	14757	2400	234	24960	0.04740
18	0.7	5500	11634	2400	234	20803	0.04975	100	0.7	6689	14978	2400	234	25544	0.04866
18	0.8	5530	11803	2400	234	20908	0.04501	100	0.8	6691	15506	2400	234	26251	0.05409
18	0.9	5290	11830	2400	234	20675	0.04455	100	0.9	6293	16034	2400	234	26237	0.04863
18	1.0	5447	12016	2400	234	21038	0.04473	100	1.0	6477	15422	2400	234	25784	0.04852

supports this placement of termites relative to extant roaches. The term Blattaria, which is sometimes used interchangeably with Blattodea, is used more properly to refer to both extinct and extant roach lineages; a group which paleontological evidence supports as polyphyletic due to the inclusion of extant members of both Isoptera and Mantodea (Grimaldi, 2001). All extant exemplars of the blattarian lineage are more commonly referred to as Dictyoptera. While sensitivity analyses demonstrate a tendency for Mantodea to nest within Blattodea under suboptimal alignment parameters, the results from separate analyses on these data lend more support to a monophyletic Isoptera + Blattodea (Blattodea *sensu lato*).

The sister-relationship between Embiidina and Phasmatodea is well supported across multiple analyses and has been supported in other molecular analyses focusing

on different groups, but including exemplars of both these orders (Thomas et al., 2000; Whiting et al., 2003). Sensitivity analyses support this relationship under the entire range of Tv/Ts ratio values (although only at low gap/Tv ratios) for the DO analysis and across 96% of the parameter sets investigated for the Clustal/Parsimony analysis. In the morphological data set used here, Embiidina and Phasmatodea share several non-homoplasious characters including the presence of a secondary profurca-spinasternal muscle (Kristensen, 1975) and attachment of the first axillary sclerite close to the scutal margin (Kristensen, 1975). Both orders also have an aberrant primary flexor muscle and possess a secondary flexor muscle of the paraglossae (Rahle, 1970; Kristensen, 1975), a condition not seen in other orders. We have named the clade Embiidina + Phasmatodea as “Eukinolabia” from the Greek words

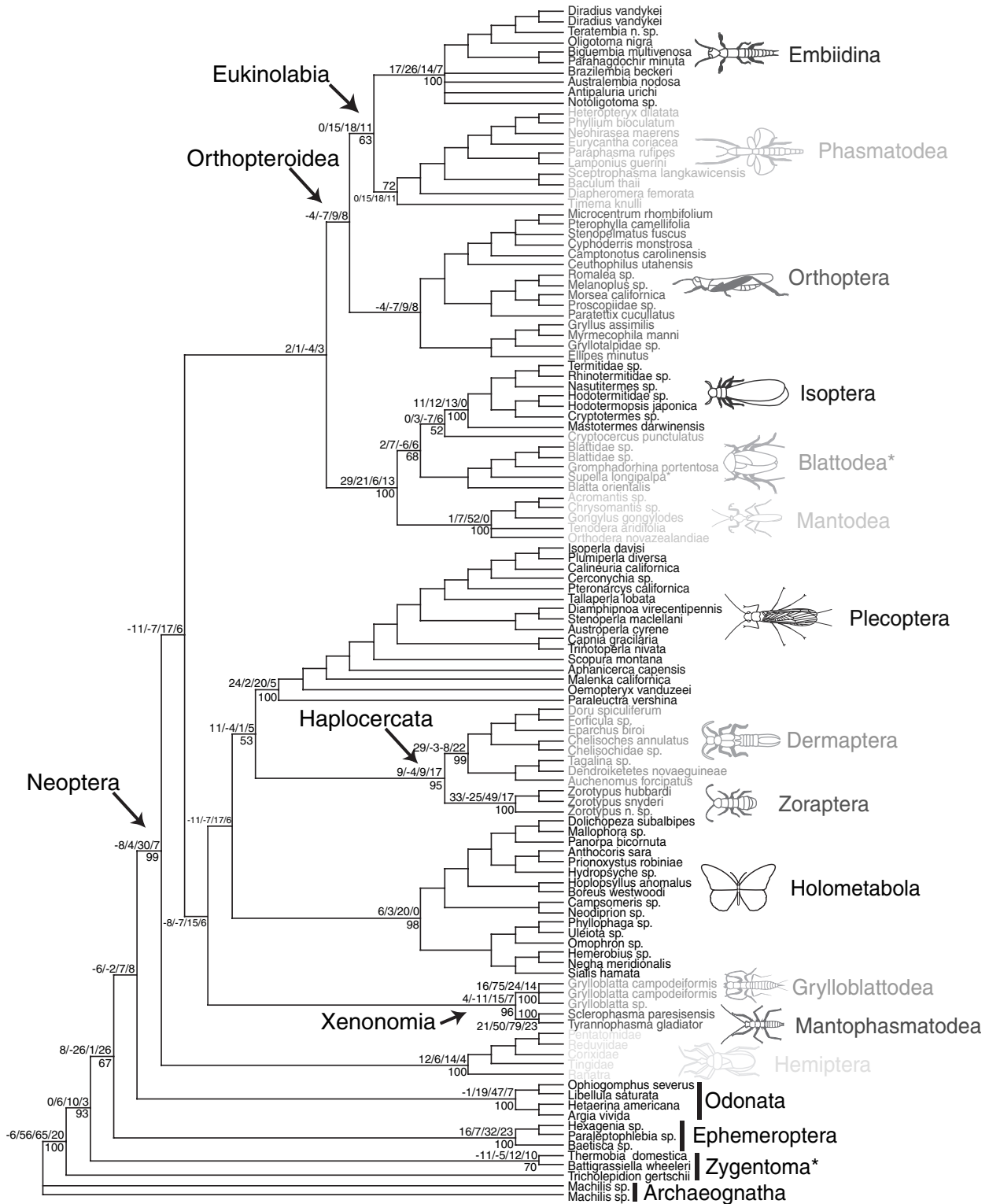


Fig. 2. Strict consensus of 19 optimal trees from parsimony analysis of ClustalX alignment. Generated via PAUP* with 200 random additions and TBR swapping from Clustal alignment under the default parameter set. Gaps were treated as a “5th position”. Single numbers on ordinal and interordinal nodes represent non-parametric bootstrap support percentages for 1000 replicates, while numbers separated by slashes represent partitioned bremer support values calculated via PAUP*, for Morphology/18S/28S/H3, respectively. Tree length = 20 478, CI = 0.331, RI = 0.661.

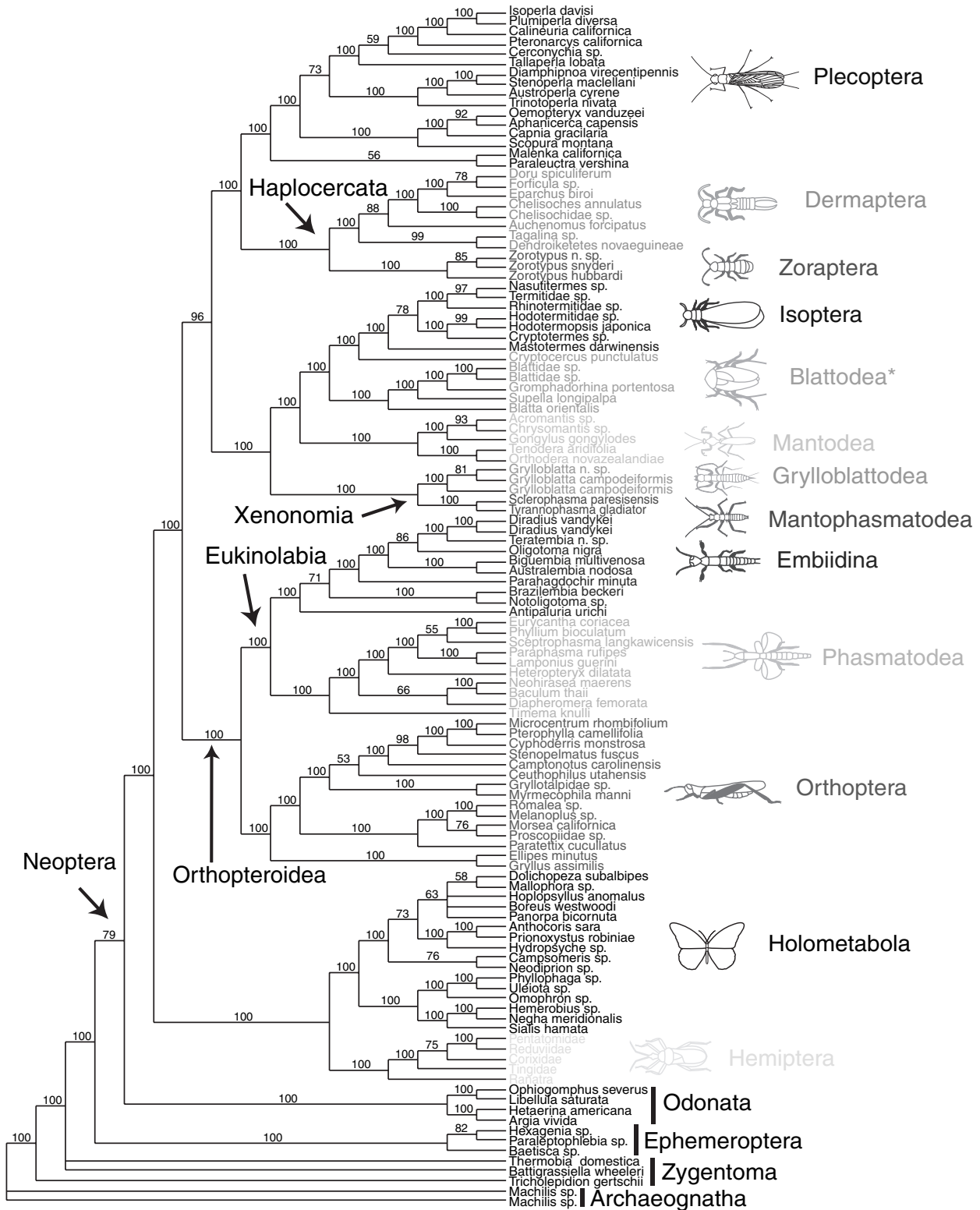


Fig. 3. Bayesian analysis of ClustalX alignment. Majority rule consensus of topologies generated via MrBayes with 500 000 generations (first 50 000 discarded as “burn-in”) using the model GTR + I + Γ . Individual data partitions were allowed their own unique GTR + gamma + propin model. Numbers above nodes represent percentage of group inclusion among all topologies generated after burn-in period.

“eukinetos”, meaning agile, and “labia”, meaning lips; in reference to the shared unique musculature of the paraglossae, a portion of the insect labium.

All combined analyses support the placement of Orthoptera as sister to Eukinolabia. The relationship between Orthoptera and Phasmatodea is a relatively catholic placement among past phylogenetic hypotheses (Boudreaux, 1979; Hennig, 1981; Kukulova-Peck, 1991; Wheeler et al., 2001a). Kukulova-Peck (1991) placed these three orders together (a group termed the “Orthopteroid orders”), but Hennig (1981) excluded Embiidna and nested Phasmatodea within Orthoptera as sister to Caelifera, a group he called Orthopteroidea. Based on this analysis and previous molecular analyses (Thomas et al., 2000; Whiting et al., 2003) we suggest a broadening of the definition Orthopteroidea to include Embiidina.

The clade Dermaptera + Zoraptera is conserved across the methodologies investigated here (Figs 1–3), is well supported across data set partitions (Fig. 1), and is supported in nearly all alignment parameter sets. All morphological characters included in this analysis and shared by Dermaptera and Zoraptera are homoplasious and sometimes very common across numerous polyneopteran orders (see morphological matrix); this is due to the highly derived status of Zoraptera, an order that has perhaps the most variation in placement across separate phylogenetic hypotheses (Kristensen, 1995; Rasnitsyn, 1998). Zoraptera and Dermaptera both possess single segmented cerci (terminal abdominal appendages present in many insects), although in appearance they are very different. These are small, oblong structures in nearly all Zoraptera species (although one extant and one extinct species have single-segmented elongate cerci), and are highly modified as forcep-like structures in the majority of Dermaptera species. For this reason we have chosen the term “Haplocercata”; derived from the Greek “haploos” meaning simple, and cercus, singular form of cerci, for this clade.

Plecoptera is supported as sister taxon to Haplocercata in all methodologies and has been linked to Dermaptera in the past (Kristensen, 1991). In sensitivity analyses under DO criteria, Plecoptera appears in multiple positions throughout the topology, a factor which is a major cause of the low levels of support for deeper nodes across the parameter landscape. The composition of relationships within Plecoptera also varies somewhat between analyses but is largely congruent within those recovered in a recent analysis including three additional molecular markers and a much better sampling of plecopteran taxa (Terry and Whiting, Manuscript in prep.). Taken together, all of these factors cast some doubt on its placement as sister to Haplocercata and suggest a large evolutionary distance between Plecoptera and its as-yet unidentified sister group.

Hennig (1981) hypothesized an Orthoptera rendered paraphyletic by Phasmatodea; however, these analyses support a monophyletic Orthoptera. The combined analyses render both suborders paraphyletic, with the bulk of Caelifera nesting within Ensifera and one caeliferan taxon (*Ellipes*, Tridactylidae) associated with various Ensifera.

These data demonstrate that Mantophasmatodea is neither the sister group of Mantodea nor Phasmatodea, and strongly support the sister taxon relationship of Grylloblattodea and Mantophasmatodea. This relationship is supported under multiple analytical methodologies (DO, standard parsimony and Bayesian analyses), across multiple parameter sets within Direct Optimization and Clustal/parsimony analysis, and across different alignment methodologies. Partitioned Bremer values (Baker and DeSalle, 1997) demonstrate support for this relationship among all data partitions of the combined analysis under DO criteria (18S, 28S, H3, and morphology).

Grylloblattodea is a small, cryophilic order of insect (25 extant species, five genera) confined to north-western North American and north-eastern Asia. Extant representatives are wingless scavengers and/or predators (Storozhenko, 1979) adapted to cool and cold environments. The most diverse genus (*Grylloblatta*) is distributed throughout the mountains of Western Canada and the North-western USA and is adapted to the extreme temperatures associated with glaciers and ice caves. On the other hand, extant Mantophasmatodea are confined to the Karoo-Namib region of southern Africa (Picker et al., 2002), and are adapted to the hot, arid conditions prevalent in this area. Although the paleontological evidence indicates a much wider historic distribution (Zompro et al., 2002), the fact that Mantophasmatodea remained undescribed for so long suggests that the limited records to date closely reflect the extent of their modern distribution. This disjunction of distributions and the small size of both groups suggest an ancient divergence with subsequent extinctions of intermediate lineages. This may be a common theme throughout Polyneoptera and could explain many of the difficulties regarding the phylogenetic reconstruction of relationships.

The goal of modern systematics is twofold: (1) to provide a biological “*lingua franca*” that facilitates an exchange of information among researchers, and (2) to provide a hierarchical system that is meaningful in the context of our understanding of evolutionary history. This makes the field of systematics both rigid in some respects—as it must operate within a historic system generally accepted by the scientific community—and plastic—as new data can alter specific designations within that system. In this context phylogeny is a critical factor in determining the validity of a new ordinal designation. For instance, phylogenetic placement of

Mantophasmatodea within a previously described order of insects, might indicate the secondary loss of morphological characters commonly used to unite that group, but would invalidate the erection of a new order merely to include a few newly discovered taxa. Placement as sister-taxon to an assemblage of two or more orders would require either the recognition of a new order or a drastic revision of the currently accepted groups. and finally, placement of Mantophasmatodea as sister-taxon to a single order would require a judgement as to whether mantophasmatodeans should be included within that order, or if they are somehow distinct enough to deserve ordinal recognition by themselves. This judgement would be affected by both the disparity (genetic, morphological, ecological, etc.) between the two groups in question and the historic inertia behind the previously recognized group.

The results of these phylogenetic analyses, coupled with the ecological disparity between these two groups and the formal recognition of Grylloblattodea for nearly a century, strongly supports the validity of the “gladiator” as a new insect order. We have named the clade comprising Grylloblattodea + Mantophasmatodea as “Xenonomia”, derived from the Greek “xenos” (stranger or outsider) and “onoma” (name). This is in reference to the fact that the ordinal names for both orders include portions of the names for orders to which they are not closely related.

Although this study makes great strides towards a full understanding of the phylogenetic relationships among the polyneopterous insect orders, there are still several outstanding issues. Among these is the exact nature of the paraphyly of Blattodea. Resolution of this issue will require analyses including taxon sampling focused on Dictyoptera lineages and will undoubtedly require revision of subordinal designations, with termites recognized at some level within the system. Moreover, the relatively low levels of support and vagility across sensitivity analyses for the deepest nodes within the polyneopteran orders is an issue that will need to be further examined as the number of molecular markers available increases and computational advances make large data sets more manageable.

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Appendix

Table A1
List of taxa included in this analysis. Higher level taxon names within quotation marks are of doubtful monophyly

"Polyneoptera"				GenBank accession no.		
Order	Family	Genus	Species	Histone 3	18S rDNA	28S rDNA
"Blattodea"	Blaberidae	<i>Gromphadorhina</i>	<i>portentosa</i>	AY125216	AY121129	AY125270
	Blattidae	undet.	sp.	AY521696	AY521827	AY521736
	Blattidae	undet.	sp.	AY521697	AY521828	AY521737
	Cryptocercidae	<i>Cryptocercus</i>	<i>punctulatus</i>	AY521698	AY521829	AY521738, AY521739
	Blattellidae	<i>Supella</i>	<i>longipalpa</i>	AY125217	AY121130	AY125271
Dermaptera	Blattidae	<i>Blatta</i>	<i>orientalis</i>	AY521699	AY521830	AY521740, AY521741
	Labiduridae	<i>Doru</i>	<i>spiculiferum</i>	AY125218	AY121131	AY125272, AY521751
	Forficulidae	<i>Forficula</i>	sp.	AY521703	AY521836	AY521752
	Forficulidae	<i>Eparchus</i>	<i>biroi</i>		AY521837	AY521753, AY521754, AY521755
	Chelisochidae	<i>Chelisoches</i>	<i>morio</i>	AY125220	AY121133	AY125273
	Pygidicranidae	<i>Tagalina</i>	sp.	AY521704	AY521838	AY521756, AY521757
	Apachyidae	<i>Denroiketetes</i>	<i>novaguineae</i>		AY521839	AY521758, AY521759, AY521760
	Spongiphoridae	<i>Auchenomus</i>	<i>forcipatus</i>		AY521840	AY521761, AY521762, AY521763
	Chelisochidae	undet.	sp.		AY521841	AY521764, AY521765
	Embiidina	Oligotomidae	<i>Oligotoma</i>	sp.	AY125221	AY121134
Teratembiiidae		<i>Diradius</i>	<i>vandykei</i>		AY521842	AY521766
Teratembiiidae		<i>Diradius</i>	<i>vandykei</i>		AY521843	AY521767
Embiidae		<i>Biguembia</i>	<i>multivenosa</i>	AY521705	AY521844	AY521768
Anisembiiidae		<i>Brazilembia</i>	<i>beckeri</i>		AY521845	AY521769
Embiidae		<i>Parahagdochir</i>	<i>minuta</i>		AY521846	AY521770
Teratembiiidae		<i>Teratembia</i>	n. sp.	AY125222	AY121135	AY125275
Australembiididae		<i>Australembia</i>	<i>nodosa</i>		AY521847	AY521771
Clothodidae		<i>Antipaluria</i>	<i>urichi</i>		AY521848	AY521772
Notoligotomidae		<i>Notoligotoma</i>	sp.	AY338617	AY338693	AY338650
Grylloblattodea	Gryllblattidae	<i>Grylloblatta</i>	<i>campodeiformis</i>	AY125225	AY121138	AY125278
	Gryllblattidae	<i>Grylloblatta</i>	<i>campodeiformis</i>		AY121139	AY125279
	Gryllblattidae	<i>Grylloblatta</i>	n. species		AY521850	AY521774
Isoptera	Rhinotermitidae	<i>Reticulitermes</i>	<i>tibialis</i>		AY521852	AY521777, AY521778
	Kalotermitidae	<i>Incisitermes</i>	<i>minor</i>		AY521853	AY521779, AY521780
	Kalotermitidae	<i>Cryptotermes</i>	sp.		AY521854	AY521781
	Termitidae	<i>Nasutitermes</i>	sp.	AY125226	AY121140	AY125280
	Termitidae	undet.	sp.		AY521855	AY521782
	Termopsidae	<i>Hodotermopsis</i>	<i>japonica</i>		AY521856	AY521783
	Mastotermitidae	<i>Mastotermes</i>	<i>darwinensis</i>		AY121141	AY125281
	Mantodea	Mantidae	<i>Tenodera</i>	<i>aridifolia</i>	AY125227	AY121142
Mantodea	Hymenopodidae	<i>Acromantis</i>	sp.	AY521709	AY521858	AY521787
	Hymenopodidae	<i>Chrysomantis</i>	sp.	AY521710	AY521859	AY521788
	Empusidae	<i>Gongylus</i>	<i>gongylodes</i>	AY521711	AY521860	AY521789
	Mantidae	<i>Orthodera</i>	<i>novazeylandi</i>		AY521861	AY521790
	Mantodea	Mantophasmatidae	<i>Sclerophasma</i>	<i>parensensis</i>	AY521712	AY521862
Mantophasmatodea	Mantophasmatidae	<i>Tyrannophasma</i>	<i>gladiator</i>	AY521713	AY521863	AY521792
	Mantophasmatidae	<i>Microcentrum</i>	<i>rhombifolium</i>	AY125229	AY521866	AY125284
	Tetrigidae	<i>Paratettix</i>	<i>cucullatus</i>	AY521717	AY521867	AY521796
	Tridactylidae	<i>Ellipes</i>	<i>minutus</i>	AY338640	AY338722	AY338678
	Stenopelmatidae	<i>Stenopelmatus</i>	<i>fuscus</i>	AY338641	AY338723	AY338679
	Romaleidae	<i>Romalea</i>	sp.	AY125230	AY121145	AY125285
	Gryllidae	<i>Gryllus</i>	<i>assimilis</i>	AY521718	AY521868	AY521797, AY521799
	Rhaphidiophoridae	<i>Ceuthophilus</i>	<i>utahensis</i>	AY521719	AY521869	AY521798
	Acrididae	<i>Melanoplus</i>	sp.	AY521720	AY521870	AY521800
	Tetrigoniidae	<i>Pterophylla</i>	<i>camellifolia</i>	AY125231	AY121146	AY125286
	Gryllotalpidae	undet.	sp.	AY125232	AY121147	AY125287
	Haglidae	<i>Cyphoderris</i>	<i>monstrosa</i>	AY521721	AY521871	AY521801, AY521802, AY521803
	Myrmecophilidae	<i>Myrmecophila</i>	<i>manni</i>	AY521722	AY521872	AY521804
	Eumastacidae	<i>Morsea</i>	<i>californica</i>		AY521873, AY521874	AY521805, AY521806
	Gryllacrididae	<i>Camptonotus</i>	<i>carolinensis</i>	AY521723	AY521875	AY521807
	Proscopiidae	<i>Anchotatus</i>	<i>janinae</i>	AY521724	AY521876	AY521808

Table A1
Continued

"Polyneoptera"				GenBank accession no.			
Order	Family	Genus	Species	Histone 3	18S rDNA	28S rDNA	
Plecoptera	Nemouridae	<i>Malenka</i>	<i>californica</i>	AY338642	AY338724	AY338680	
	Leuctridae	<i>Paraleuctra</i>	<i>vershina</i>		AY521878	AY521810	
	Taeniopterygidae	<i>Oemopteryx</i>	<i>vanduzeei</i>	AY521725	AY521879	AY521811	
	Pteronarcyidae	<i>Pteronarcys</i>	<i>californica</i>	AY521726	AY521880	AY521812	
	Perlidae	<i>Calineuria</i>	<i>californica</i>		AY521881	AY521813	
	Capniidae	<i>Capnia</i>	<i>gracilaria</i>		AY521882	AY521814	
	Gripopterygidae	<i>Trinotoperla</i>	<i>nivata</i>	AY521727	AY521883	AY521815	
	Notonemouridae	<i>Aphanicerca</i>	<i>capensis</i>	AY521728	AY521884	AY521816	
	Perlodidae	<i>Isoperla</i>	<i>davisi</i>	AY125233	AY121148	AY125288	
	Chloroperlidae	<i>Plumiperla</i>	<i>diversa</i>	AY125234	AY121149	AY125289	
	Peltoperlidae	<i>Tallaperla</i>	<i>lobata</i>	AY521729	AY521885	AY521817	
	Diamphipnoidae	<i>Diamphipnoa</i>	<i>virecentipennis</i>		AY521886	AY521818	
	Austroperlidae	<i>Austroperla</i>	<i>cyrene</i>		AY521887	AY521819	
	Eustheniidae	<i>Stenoperla</i>	<i>maclellani</i>		AY521882	AY521814	
	Scopuridae	<i>Scopura</i>	<i>montana</i>		AY521882	AY521814	
	Styloperlidae	<i>Cerconychia</i>	sp.	AY338643	AY338725	AY338681, AY338682	
	Phasmatodea	Heteronemiidae	<i>Diapheromera</i>	<i>femorata</i>	AY125235	AY121150	AY125290
		Phasmatidae	<i>Eurycantha</i>	<i>coriacea</i>	AY125238	AY121154	AY125294
		Bacillidae	<i>Heteropteryx</i>	<i>dilatata</i>	AY125241	AY121157	AY125297
		Pseudophasmatidae	<i>Paraphasma</i>	<i>rufipes</i>	AY125244	AY121160	AY125300
Phyllidae		<i>Phyllium</i>	<i>bioculatum</i>	AY125245	AY121161	AY125301	
Timematidae		<i>Timema</i>	<i>knully</i>	AY125246	AF423806, AY121162	AY125302	
Heteronemiidae		<i>Sceptrophasma</i>	<i>langkawicensis</i>	AY125249	AY121166	AY125306	
Heteronemiidae		<i>Neohirasea</i>	<i>maerens</i>	AY125251	AY121168	AY125308	
Phasmatidae		<i>Baculum</i>	<i>thaii</i>	AY125255	AY121172	AY125312	
Phasmatidae		<i>Lamponius</i>	<i>guerini</i>	AY125261	AY121178	AY125318	
Zoraptera	Zorotypidae	<i>Zorotypus</i>	<i>hubbardi</i>	AY521732	AY521890	AY521823	
	Zorotypidae	<i>Zorotypus</i>	<i>snyderi</i>	AY521734	AY521892	AY521825	
	Zorotypidae	<i>Zorotypus</i>	<i>n. species</i>	AY521733	AY521891	AY521824	
<hr/>							
"Apterygota"							
Archaegnatha	Machilidae	<i>Machilis</i>	sp.	AY338614	AY338689	AY338646	
	Machilidae	<i>Machilis</i>	sp.	AY521695	AY521826	AY521735	
Zygentoma	Lepismatidae	<i>Thermobia</i>	<i>domestica</i>	AY338644	AY338726	AY338683	
	Noticolliidae	<i>Battigrassiella</i>	<i>wheeleri</i>		AY338728	AY338685	
	Lepidotrichidae	<i>Tricholepidion</i>	<i>gertschii</i>	AY338645	AY338727	AY338684	
<hr/>							
"Paleoptera"							
Ephemeroptera	Ephemeridae	<i>Hexagenia</i>	sp.	AY125223	AY121136	AY125276	
	Leptophlebiidae	<i>Paraleptophlebia</i>	sp.	AY125224	AY121137	AY125277	
	Baetiscidae	<i>Baetisca</i>	sp.	AY338627	AY338707	AY338664	
Odonata	Gomphidae	<i>Ophiogomphus</i>	<i>severus</i>	AY125228	AY121143	AY125283	
	Libellulidae	<i>Libellula</i>	<i>saturata</i>	AY338637		AY338674	
	Coenagrionidae	<i>Hetaerina</i>	<i>americana</i>	AY338638	AY338718	AY338675	
	Ceonagrionidae	<i>Argia</i>	<i>vivida</i>	AY125229	AY521866	AY125284	
<hr/>							
Hemiptera	Pentatomidae	undet.			???	???	
	Phymatidae	<i>Phymata</i>	sp.		???	???	
	Nepidae	<i>Ranatra</i>	sp.		???	???	
	Corixidae	<i>Hesperocorixa</i>	sp.		???	???	
	Tingidae	<i>Corythucha</i>	sp.		???	???	
<hr/>							
Holometabola							
Coleoptera	Carabidae	<i>Omopron</i>	sp.		AY521831	AY521742, AY521743	
	Scarabaeidae	<i>Phyllophaga</i>	sp.	AY521700	AY521832	AY521744	
	Silvanidae	<i>Uleiota</i>	sp.	AY521701	AY521833	AY521745	
Diptera	Tipulidae	<i>Dolichocheza</i>	<i>subalbipes</i>	AY521702	AY521834	AY521746, AY521747	
	Asilidae	<i>Mallophora</i>	sp.		AY521835	AY521748, AY521749, AY521750	
Siphonaptera	Pulicidae	<i>Hoplopsyllus</i>	<i>anomalus</i>		AY521849	AY521773	
Hymenoptera	Scoliidae	<i>Capnsomeris</i>	sp.		AF423780	AY521775	
	Diprionidae	<i>Neodiprion</i>	sp.		AY521851	AY521776	
Lepidoptera	Pieridae	<i>Anthocharis</i>	<i>sara</i>		AF423785	AY521784	

Table A1
Continued

“Polyneoptera”				GenBank accession no.		
Order	Family	Genus	Species	Histone 3	18S rDNA	28S rDNA
Cossidae	<i>Prionoxystus</i>	<i>robiniae</i>	AY521706	AF423783	AY521785	
Mecoptera	Boreidae	<i>Boreus</i>	<i>westwoodi</i>	AY521707	AY521857	AY521786
	Panorpidae	<i>Panorpa</i>	<i>bicornuta</i>	AY521708	AF423902	AF423960
Neuroptera	Sialidae	<i>Sialis</i>	<i>hamata</i>	AY521714	AY521864	AY521793
	Hemerobiidae	<i>Hemerobius</i>	sp.	AY521715	AF423790	AY521794
	Inocellidae	<i>Negha</i>	<i>meridionalis</i>	AY521716	AY521865	AY521795
Trichoptera	Hydropsychidae	<i>Hydropsyche</i>	sp.	AY521731	AF286291	AF338267

Table A2
List of primers used to amplify molecular markers used in this analysis

Gene	Primer name	Sequence (5'–3')	Length	Direction	Relative position
18S	18S 1F	TACCTGGTTGATCCTGCCAGTAG	23	Forward	1
	18S ai	CCTGAGAAACGGCTACCACATC	22	Forward	2
	18S a0.7	ATTAAAGTTGTTGCGGTT	18	Forward	3
	18S a0.79	TTAGAGTGCYAAAGC	16	Forward	4
	18S a1.0	GGTAAAATTCTTGGAYCGTC	20	Forward	5
	18S a2.0	ATGGTTGCAAAGCTGAAAC	19	Forward	6
	18S a3.5	TGGTGCATGGCCGYTCTTAGT	21	Forward	7
	18S 7F	GCAATAACAGGTCTGTGATGCC	23	Forward	8
	18S 9R	GATCCTTCCGCAGGTTACCTAC	23	Reverse	1
	18S 7R	GCATCACAGACCTGTTATTGC	21	Reverse	2
	18S bi	GAGTCTCGTTCGTTATCGGA	20	Reverse	3
	18S b0.5	GTTTCAGCTTTCGAACCAT	19	Reverse	4
	18S b2.5	TCTTTGGCAAATGCTTTCGC	20	Reverse	5
	18S b2.9	TATCTGATCGCCTTCGAACCTCT	23	Reverse	6
	18S b3.9	TGCTTTRAGCACTCTAA	17	Reverse	7
	18S b5.0	TAACCGCAACAACCTTAAAT	19	Reverse	8
	18S b7.0	ATTTTRCGYGCCTGCTGCCTTCTCT	23	Reverse	9
28S	28 s Road 1a	CCCSCGTAAAYTTAGGCATAT	20	Forward	1
	28 s Road 3a	AGTACGTGAAACCGTTCAGG	20	Forward	2
	28S A	GACCCGTCTTGAAGCAGC	18	Forward	3
	28S Road 4a	GGAGTCTAGCATGTGYGCAAGTC	23	Forward	4
	28S Road 4.5a	AAGTTCCCTCAGGATAGCTG	21	Forward	5
	28S Road 4.8a	ACCTATTCTCAAACCTTAAATGG	23	Forward	6
	28S Road 5a	GGYGTGGTTGCTTAAGACAG	21	Forward	7
	28S Road 6a	GGCGAAAGGGAATCYGGTTC	20	Forward	8
	28S Road 7b1	GACTTCCCTTACCTACAT	18	Reverse	1
	28S Road 6b	AACCRGATFCCCTTTCGCC	19	Reverse	2
	28S Road 5b	CCACAGCGCCAGTTCTGCTTAC	22	Reverse	3
	28S B	TCGGAAGGAACCAGCTAC	18	Reverse	4
	28S C	ATAGTTCACCATCTYTCGGG	20	Reverse	5
	28S Road 4b	CCTTGGTCCGTGTTTCAAGAC	21	Reverse	6
	28S Road 3b	CCYTGAACGGTTTCACGTA	21	Reverse	7
H3	H3 AF	ATGGCTCGTACCAAGCAGACVGC	23	Forward	
	Hex AF	ATGGCTCGTACCAAGCAGACGGC	23	Forward	
	H3 AR	ATATCCTTRGGCATRATRGTGAC	23	Reverse	
	Hex AR	ATATCCTTGGGCATGATGGTGAC	23	Reverse	