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Higher-level phylogeny of the insect order Hemiptera: is Auchenorrhyncha really paraphyletic?

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Abstract. The higher-level phylogeny of the order Hemiptera remains a contentious topic in insect systematics. The controversy is chiefly centred on the unresolved question of whether or not the hemipteran suborder Auchenorrhyncha (including the extant superfamilies Fulgoroidea, Membracoidea, Cicadoidea and Cercopoidea) is a monophyletic lineage. Presented here are the results of a multilocus molecular phylogenetic investigation of relationships among the major hemipteran lineages, designed specifically to address the question of Auchenorrhyncha monophyly in the context of broad taxonomic sampling across Hemiptera. Phylogenetic analyses (maximum parsimony, maximum likelihood and Bayesian inference) were based on DNA nucleotide sequence data from seven gene regions (18*S* rDNA, 28*S* rDNA, histone *H3*, histone 2*A, wingless*, cytochrome *c* oxidase I and *NADH dehydrogenase subunit 4*) generated from 86 in-group exemplars representing all major lineages of Hemiptera (plus seven out-group taxa). All combined analyses of these data recover the monophyly of Auchenorrhyncha, and also support the monophyly of each of the following lineages: Hemiptera, Sternorrhyncha, Heteropterodea, Heteroptera, Fulgoroidea, Cicadomorpha, Membracoidea, Cercopoidea and Cicadoidea. Also presented is a review of the major lines of morphological and molecular evidence for and against the monophyly of Auchenorrhyncha.

Introduction

The insect orders Hemiptera Linnaeus (as described in Linné, 1758), Heteroptera Latreille, 1810 and Homoptera Latreille, 1810 have experienced a contentious history with regard to their higher-level classification (reviewed in detail by Bourgoin & Campbell, 2002 and Forero, 2008). The monophyly of Homoptera (that traditionally included the suborders Sternorrhyncha, Auchenorrhyncha and Coleorrhyncha) has been challenged based on investigations incorporating morphological (Goodchild, 1966; Schlee, 1969; Bourgoin, 1986a, b, 1993; Sweet, 1996) and molecular (Wheeler *et al*., 1993; Campbell *et al*., 1995; von Dohlen & Moran, 1995; Sorensen *et al*., 1995) evidence. Consensus (Bourgoin & Campbell, 2002) now suggests that the monophyletic order Hemiptera sensu lato (the largest nonholometablolous insect order, with approximately 82 000 described species) includes the following major monophyletic clades: Sternorrhyncha (scale insects, aphids, whiteflies, etc.; ~21 extant families); Heteroptera (true bugs sensu stricto; ~54 extant families); Coleorrhyncha (sometimes called 'moss bugs'; one extant family); Fulgoromorpha (planthoppers; ~20 extant families); and Cicadomorpha (leafhoppers, treehoppers, spittlebugs and cicadas; ~12 extant families). However, relationships among these higher-level hemipteran lineages have not yet been definitively resolved, and specifically, the phylogenetic positions of Fulgoromorpha and Cicadomorpha (traditionally classified together as the monophyletic group Auchenorrhyncha) remain controversial.

Fulgoromorpha (including the single extant planthopper superfamily Fulgoroidea) and Cicadomorpha (comprising Membracoidea, the leafhoppers and treehoppers; Cicadoidea, the cicadas; and Cercopoidea, the spittlebugs and froghoppers) constitute the suborder Auchenorrhyncha, although the monophyly and phylogenetic placement of Auchenorrhyncha have been debated for decades (see Bourgoin & Campbell, 2002; Forero, 2008). Several alternative phylogenetic hypotheses

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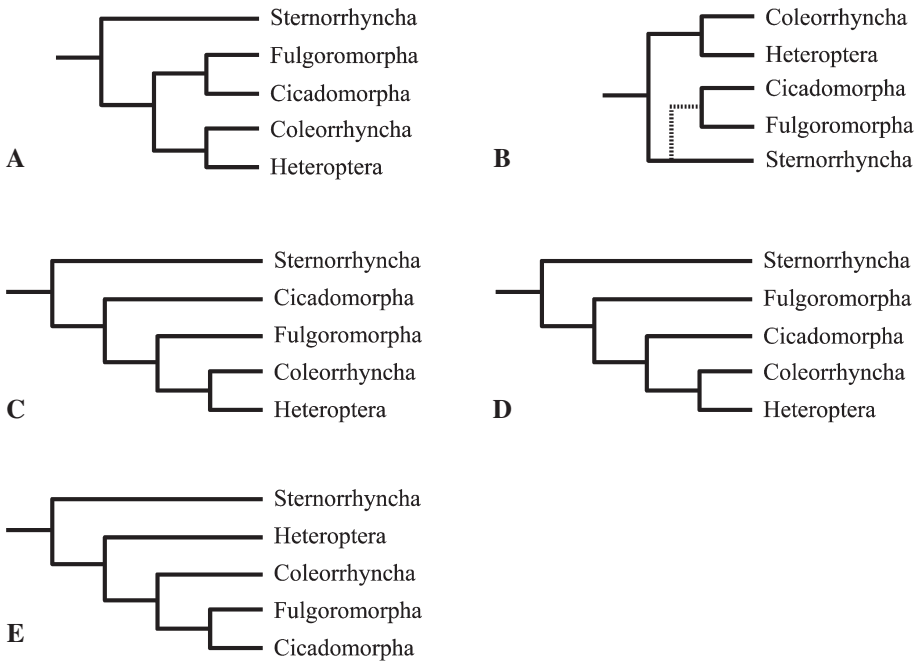

Fig. 1. Alternative hypotheses of higher-level relationships within Hemiptera: (A) monophyletic Auchenorrhyncha as sister group to Heteropterodea (Zrzavý, 1992); (B) monophyletic Auchenorrhyncha as putative sister group to Sternorrhyncha (consistent with Hennig, 1981); (C) nonmonophyletic Auchenorrhyncha, with Fulgoromorpha as sister group to Heteropterodea (consistent with Campbell *et al*., 1995; von Dohlen & Moran, 1995; Sorensen *et al*., 1995); (D) nonmonophyletic Auchenorrhyncha, with Cicadomorpha as sister group to Heteropterodea (Bourgoin & Campbell, 2002); and (E) monophyletic Auchenorrhyncha as sister group to Coleorrhyncha (consistent with Müller, 1962; Buchner, 1965).

have been advanced in which Auchenorrhyncha was: monophyletic, placed as the sister group to Heteropterodea (Fig. 1A; i.e. Heteroptera + Coleorrhyncha; Zrzavý, 1992); monophyletic, placed as the putative sister group to Sternorrhyncha (Fig. 1B; i.e. consistent with 'Homoptera', but included within Hemiptera; Hennig, 1981); or nonmonophyletic, with Fulgoromorpha and Cicadomorpha placed in separate phylogenetic positions within Hemiptera (Fig. 1C, D; Goodchild, 1966; Hamilton, 1981; Bourgoin & Campbell, 2002).

The 'Auchenorrhyncha question' gained widespread attention in 1995 with the publication of three phylogenetic studies (Campbell *et al*., 1995; von Dohlen & Moran, 1995; Sorensen *et al*., 1995) based on partial *18S* rDNA sequence data. Although not definitively refuting auchenorrhynchan monophyly, the analyses (reviewed in the Discussion, below), concluded that Fulgoromorpha and Cicadomorpha were likely to be separate lineages occupying independent (i.e. nonmonophyletic) positions within the phylogeny of Hemiptera.

The monophyly of Auchenorrhyncha has not been tested specifically or rigorously since those publications in 1995, and there remains no consensus – even among hemipterists – on the status of Auchenorrhyncha. Three subsequent re-analyses of Campbell *et al*.'s (1995) *18S* rDNA dataset resulted in consistent hypotheses of Auchenorrhyncha nonmonophyly (Bourgoin *et al*., 1997; Ouvrard *et al*., 2000; Xie *et al*., 2008). Conversely, Urban & Cryan (2007) found some support for the monophyly of Auchenorrhyncha in a phylogenetic analysis of the planthopper superfamily Fulgoroidea, thereby highlighting the potential power of multilocus molecular phylogenetic analyses to resolve this long-standing debate. Therefore, the present investigation is the first study designed specifically to evaluate the question of Auchenorrhyncha monophyly in the context of a higher-level phylogenetic reconstruction of Hemiptera sensu lato, using evidence from multiple genetic loci generated from a taxonomic sample representing all major extant hemipteran lineages.

Material and methods

Taxon sampling

Insect specimens were collected into 95–100% ethanol and stored at −80°C in the New York State Museum's Genome Bank (Albany, NY, U.S.A.). The 86 in-group specimens (Table S1) represent each of the major hemipteran suborders as follows: Sternorrhyncha (nine exemplars); Coleorrhyncha (two exemplars); Heteroptera (10 exemplars); Fulgoroidea (24 exemplars); Membracoidea (14 exemplars); Cicadoidea (9 exemplars); and Cercopoidea (18 exemplars). Nucleotide sequence data were obtained from GenBank for five of the exemplars of Heteroptera and two of Sternorrhyncha. All data from the remaining 79 in-group taxa were generated wholly or in part by the authors of the present study, with some sequences newly generated and others published in previous

studies (Cryan *et al*., 2000; Cryan, 2005; Urban & Cryan, 2007, 2009; Cryan & Svenson, 2010; Urban *et al*., 2010). Seven out-group specimens were included to represent other lineages within Paraneoptera, as well as the putatively more ancient lineages Blattaria, Isoptera and Mantodea (Table S1). Nucleotide sequence data for two of the out-group representatives were newly generated for the present study; data for the remaining out-group taxa were obtained from GenBank.

Molecular data

Nucleotide sequence data were generated from two nuclear ribosomal genes (*18S* and *28S* rDNA), three nuclear protein coding genes [histone *H3* (*H3*), histone *2A* (*H2A*) and *wingless* (*Wg*)] and two mitochondrial protein coding genes [cytochrome *c* oxidase I (*COI*) and *NADH dehydrogenase subunit 4* (*ND4*)]. Five of these genes [*18S*, *28S*, *H3*, *Wg* and *COI*] were chosen because they have been useful in reconstructing higher-level phylogenetic relationships within Auchenorrhyncha (Cryan *et al.*, 2000, 2004; Dietrich *et al.*, 2001; Cryan, 2005; Urban & Cryan, 2007, 2009; Cryan & Svenson, 2010; Urban *et al*., 2010). *ND4* has been used in reconstructing higher-level relationships within Mantodea (Svenson & Whiting, 2009); *H2A* is a novel locus for hemipteran phylogenetics.

DNA extraction and polymerase chain reaction amplification

DNA was extracted typically from either thoracic or leg muscle tissue (for some small specimens, like the exemplars of Delphacidae, DNA was extracted from the whole body) using FastDNA Extraction Kits (Qbiogene, Inc., Carlsbad, CA, U.S.A.) or Qiagen DNEasy Kits (Qiagen, Inc., Valencia, CA, U.S.A.). Polymerase chain reactions (PCRs) to amplify *18S*, *28S*, *H3*, *Wg* and *COI* were conducted following protocols used by Cryan (2005), Cryan *et al*. (2000), Urban & Cryan (2007, 2009) and Urban *et al.* (2010); *ND4* was amplified following protocols used by Svenson & Whiting (2009). *H2A* was amplified in 25-μL reactions using Qiagen DNA polymerase (Qiagen, Inc.) under the following cycling protocol: 3 min 'hot start' at 94°C, 30–35 cycles of 1 min at 46–54°C and 1 min at 72°C, with final extension at 72°C for 10 min. Oligonucleotide primers used in PCR reactions (Table S2) were synthesized by Wadsworth Laboratories (NY Department of Health, Albany, NY, U.S.A.) or by Integrated DNA Technologies, Inc. (Coralville, IA, U.S.A.). Amplified DNA was visualized using 1–2% agarose gel electrophoresis with ethidium-bromide staining. DNA products were purified using GeneClean (BIO 101, Vista, CA, U.S.A.) or ExoSAPIT (GE Healthcare, Piscataway, NJ, U.S.A.). Sequences were obtained from complementary strands using D-Rhodamine terminator cycle sequencing on ABI Prism 3 3100/3700 or ABI 3730XL DNA sequencers at Wadsworth Laboratories (Albany, NY, U.S.A.), the High Throughput Genomics Unit at the University of Washington (Seattle, WA, U.S.A.) and the Center for Functional Genomics Laboratory at the University at Albany (Albany, NY, U.S.A.).

Nucleotide sequence alignment

All chromatography data were inspected visually, assembled into contiguous sequences and edited using SEQUENCHER 4.10.1 for WINDOWS (GeneCodes, 2010). Initially, multiple sequence alignments for the *18S* and *28S* gene partitions were performed manually, and were then improved upon with the sequence alignment program MAFFT 6 (Katoh *et al.*, 2005), using the Q-INS-i iterative refinement algorithm. Highly variable regions of *18S* (14 regions with a combined length of 709 bp) and *28S* (10 regions with a combined length of 638 bp) that differed in base composition and sequence length among taxa were excluded from phylogenetic analysis because of extreme ambiguity in alignment. Multiple sequence alignments for *H3*, *H2A* and *ND4* were unambiguous and contained no gaps. The multiple sequence alignments for Wg and *COI* contained gaps, and therefore were aligned using the online version of MAFFT 6. For these gene partitions, sequences were exported in FASTA format and uploaded to the MAFFT server; the FFT-NS-i iterative refinement algorithm was used, with scoring matrix settings set to 200 point accepted mutations (PAM) (assigning scores to sequences based on amino acid alignment), and $k = 2$ (Kimura's two-parameter model setting the ratio of transitions to transversions at 2). The resulting multiple sequence alignments contained gaps, but none that interrupted or shifted the reading frame. Codon position for the three protein coding genes was determined by SEQUENCHER, and by comparison with translated sequences available on GenBank.

Phylogenetic reconstruction

Phylogenetic analyses were conducted under three optimality criteria: maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference. Under all reconstruction methodologies, gaps were treated as missing data.

MP analyses. MP analyses of the total data matrix (i.e. data from all seven genes, combined) were conducted using PAUP* 4.0b10 (Swofford, 2001). Heuristic tree searches were performed using 1000 random-addition replicates with the tree bisection and reconnection (TBR) option. Bootstrap support values for nodes on the MP tree were computed with 1000 standard replicates in PAUP*.

ML analysis. MODELTEST 3.7 (Posada & Crandall, 1998) was used to determine the best-fitting model for each of the seven gene partitions. Results of the Akaike information criterion (AIC, i.e. lowest criterion value; Akaike, 1974) indicated that the GTR + I + G model was the best-fitting model for *18S*, *28S*, *H3*, *Wg*, *COI* and *ND4*; the TvM + I + G model was indicated as the best fitting for *H2A*. Partitioned

ML analyses were conducted on the total data matrix using GARLI 2.0 for WINDOWS (Zwickl, 2006), with each partition set to its optimal model (as described above), with these models unlinked and employing their own rates (i.e. using the settings linkmodels = 0 and subsetspecificrates = 1). Twenty independent search replicates were run, with each replicate run for 1 000 000 generations. Bootstrap support values for nodes on the ML topology were computed with GARLI by running 100 bootstrap replicates. Reverse constraint searches were conducted, 'breaking' each of the 90 nodes in the ML tree using partitioned ML searches in GARLI. Four replicates were run for each reverse constraint search, with a termination of each replicate enforced under the default conditions of the program (terminate when no significant topology improvement is found in 20 000 generations, with score threshold set to 0.05, and significant topology change criterion set to 0.01). Likelihood support values were computed as the difference between the optimal ML tree score and the score of the best reverse-constrained tree (Lee & Hugall, 2003).

Bayesian analysis. A mixed-model Bayesian analysis of the total data matrix was conducted using MRBAYES 3.1.2 (Ronquist & Huelsenbeck, 2003) on the CIPRES Science Gateway (Miller *et al.*, 2010). As described previously, the GTR + I + G model was the best fitting model for each of six of the gene partitions. For the *H2A* data matrix, the best-fitting model was TvM + I + G, which differs only slightly from the GTR + I + G model in that there is a single transition rate (i.e. $b = e$). Because in MrBayes a five-rate model cannot be employed, the GTR + I + G was used for *H2A* as well.

The mixed-model Bayesian analysis was run for 25 million generations, with model parameters unlinked and estimated independently across partitions. Two independent runs were performed, each with four chains (three heated and one cold), uninformative priors and trees sampled at intervals of 1000 generations. To determine stationarity, log-likelihood scores were plotted across generations, and standard deviation of split frequencies between the two independent runs was examined for convergence. Of the 25 000 trees sampled in each run, the first 25% of sampled trees (i.e. 6250) were discarded as burn-in and the remaining 18 750 trees were used to construct a 50% majority rule consensus tree. The harmonic mean of likelihoods was estimated for post burn-in trees using the *sump* command in MRBAYES.

Examination of data conflict among genes

To examine potential conflict among genes, each of the seven individual gene datasets was analysed separately under the ML criterion using the best-fitting model for each gene, as determined via MODELTEST. Four replicates were run for each search, with the termination of each replicate enforced under the default conditions as described above. Because individual genes may not recover the same nodes as the combined data matrix (e.g. because of fewer numbers of informative sites and/or missing data for individual genes), the potential conflict among genes at various nodes in the ML topology (based on the combined data matrix) was examined by computing partitioned likelihood scores (PLSs; Lee & Hugall, 2003) for some deeper level nodes of interest (because of missing data, PLS scores could not be computed for all nodes in the topology). Log-likelihood values for the seven different genes were calculated in PAUP* using the site log-likelihood function for the optimal ML tree and the reverse-constrained trees for the nodes of interest. In the partitioned likelihood searches (of the optimal and reverse constrained trees), the model parameters estimated by GARLI resulted in an overestimate of total likelihood score (i.e. the sum of all site likelihoods exceeded the total tree score). Therefore, for computation of PLS for the selected nodes, unpartitioned ML searches were conducted under the GTR + I + G model for the total data matrix, with each search employing four independent replicates, and each replicate running for 1 000 000 generations.

Significance tests of alternative hypotheses of Hemipteran phylogeny

The alternative hypotheses of hemipteran phylogeny proposed in previous studies (Fig. 1B–E; Müller, 1962; Buchner, 1965; Hennig, 1981; Campbell *et al.*, 1995; Sorensen *et al.*, 1995; von Dohlen & Moran, 1995; Bourgoin & Campbell, 2002) were tested by comparing topologies artificially constrained to each hypothesis with the optimal ML topology. Constrained searches were conducted using unpartitioned ML searches in GARLI, with the hypothesized groups constrained to monophyly. Four independent search replicates were run for each constrained search, with each replicate run for 1 000 000 generations. Site log likelihoods for the best-constrained trees and the optimal unpartitioned ML tree were computed in PAUP*: these values were used to compute the approximately unbiased (AU) statistical test of topologies (Shimodaira, 2002) with the program CONSEL (Shimodaira & Hasegawa, 2001).

Results

The *18S* and *28S* ribosomal genes were amplified in three contiguous, overlapping fragments of approximately 600–700 bp each. The protein coding genes (*H3*, *H2A*, *Wg*, *COI* and *ND4*) were each amplified as a single fragment, with approximate lengths of *H3* ≈ 360 bp, *H2A* ≈ 300 bp, *Wg* ≈ 350 bp, *COI* ≈ 900 bp and *ND4* ≈ 440 bp. After ambiguously aligned regions of *18S* and *28S* were excluded (as described above), a combined dataset of approximately 7.0 kb for each taxon was retained for analyses. Descriptive information for each gene, including the number of variable/parsimony informative sites, is provided in Table S3.

Phylogenetic reconstruction

MP analysis. Parsimony analysis of the total data matrix yielded 1080 equally parsimonious trees (length = 21 186

Table 1. Nodal support for Fig. 2.

Node	ML boot (%)	LS	PP (%)	MP boot (%)	Node	ML boot (%)	LS	PP	MP boot (%)
1	98	16.9	100	74	46	100	10.6	100	88
2	100	29.8	100	100	47	<50	0.9	74	63
3	<50	2.0	89	<50	48	100	101.9	100	100
4	100	54.2	100	100	49	78	7.8	100	60
5	100	69.0	100	100	50	77	3.6	98	50
6	<50	2.4	80	<50	51	91	16.1	100	76
7	90	16.0	100	59	52	97	19.7	100	86
8	100	69.1	100	100	53	99	27.9	100	81
9	65	3.9	97	80	54	<50	1.0	<50	60
10	87	6.7	100	96	55	<50	1.2	<50	<50
11	91	5.5	100	62	56	97	1.2	56	92
12	100	40.8	100	<50	57	81	2.5	75	66
13	100	20.2	100	75	58	99	21.8	100	98
14	100	18.0	100	86	59	100	21.3	100	100
15	86	16.2	100	74	60	74	13.1	100	59
16	83	9.6	100	63	61	<50	2.7	94	<50
17	100	143.6	100	100	62	99	13.7	100	93
18	100	52.0	100	99	63	100	19.5	100	82
19	92	5.1	96	71	64	100	100.1	100	100
20	51	2.8	77	<50	65	74	9.3	100	69
21	52	3.9	82	<50	66	100	29.2	100	86
22	64	4.0	87	92	67	99	10.3	100	82
23	<50	4.0	87	<50	68	61	0.2	59	<50
24	66	6.9	100	<50	69	<50	0.8	<50	<50
25	73	13.2	100	<50	70	<50	0.8	61	<50
26	100	68.3	100	100	71	57	1.1	<50	<50
27	73	9.5	100	66	72	91	7.2	100	90
28	100	40.8	100	100	73	97	10.2	100	97
29	100	32.7	100	98	74	100	20.9	100	100
30	100	129.4	100	100	75	100	24.0	100	100
31	99	39.4	100	100	76	<50	0.7	<50	<50
32	99	1.6	96	<50	77	<50	0.8	58	<50
33	82	6.6	100	93	78	91	14.7	100	77
34	93	4.6	99	66	79	84	22.2	100	83
35	99	26.1	100	90	80	62	3.7	96	51
36	100	113.9	100	100	81	100	82.0	100	100
37	100	24.5	100	94	82	74	10.5	100	76
38	97	18.7	100	89	83	<50	0.4	<50	<50
39	100	47.8	100	100	84	100	22.5	100	97
40	100	53.2	100	100	85	59	3.8	98	77
41	100	48.9	100	100	86	89	14.6	100	86
42	97	13.2	100	90	87	95	14.0	100	69
43	99	21.9	100	87	88	100	115.1	100	100
44	54	7.4	100	<50	89	100	16.1	100	100
45	96	41.2	100	78	90	58	3.7	97	<50

LS, likelihood support (difference between optimal maximum likelihood, ML, tree score and score of each reverse constrained tree); ML Boot, bootstrap support for maximum likelihood tree (Fig. 2); MP Boot, bootstrap support for maximum parsimony tree; PP, Bayesian posterior probabilities.

steps). The strict consensus of these trees yielded a monophyletic Auchenorrhyncha, albeit with low bootstrap support (66%). Hemiptera was not recovered as monophyletic because of the placement of Sternorrhyncha in a clade with some non-hemipteran out-group taxa. Coleorrhyncha was placed as the sister group of Heteroptera; Heteropterodea was placed as the sister group of Auchenorrhyncha. Within Auchenorrhyncha, Fulgoroidea was placed as the sister group of Cicadomorpha; within the latter group, Membracoidea was the sister group to (Cicadoidea + Cercopoidea). Because the MP topology was highly similar to the ML topology, all support values (including bootstrap support values for the MP tree) are summarized on the ML topology, and provided in Table 1.

ML analysis. The likelihood scores of the 20 partitioned ML search replicates yielded trees with − ln scores ranging from

96512.17 to 96555.54, and with topologies nearly identical to the tree with the best likelihood score (Fig. 2). The ML analysis recovered a monophyletic Hemiptera, within which Sternorrhyncha was placed as the sister group to the remaining hemipterans. As under MP, Auchenorrhyncha was recovered as monophyletic (bootstrap support 73%, LS = 9.5). Fulgoroidea was placed sister to Cicadomorpha. Within Cicadomorpha, Membracoidea was sister to (Cicadoidea + Cercopoidea). Heteropterodea was recovered as monophyletic, with Coleorrhyncha placed as the sister group of Heteroptera; Heteropterodea was placed as the sister group of Auchenorrhyncha.

Bayesian analysis. The two independent runs yielded identical 50% consensus topologies, with harmonic means $-\ln =$ 96722.01 and 96722.96, respectively, and standard deviation of split frequencies (computed across two of the runs) <0.01. This Bayesian topology was, in turn, nearly identical to that recovered in the ML analysis (Fig. 2). The only exceptions were that relationships were not fully resolved among some exemplars of Cicadellidae, as well of some exemplars of Machaerotidae and of Aphrophoridae. The posterior probability value supporting the monophyly of Auchenorrhyncha was 100%.

Examination of data conflict among genes

Separate ML analysis of the *28S* dataset recovered the major relationships found in the analysis of the combined dataset. That is, separate analysis of *28S* recovered Hemiptera as monophyletic, with Sternorrhyncha placed as sister to the remaining hemipterans; Heteropterodea was monophyletic and recovered as sister to a monophyletic Auchenorrhyncha. Within Auchenorrhyncha, relationships among superfamilies were concordant with the topology reconstructed by analysis of the combined dataset: (Fulgoroidea + (Membracoidea + (Cicadoidea + Cercopoidea))).

The monophyly of Hemiptera was not supported in any of the other single-gene analyses. Indeed, separate analysis of each of the other loci yielded results that were topologically inconsistent with analysis of the combined molecular dataset. For example, separate analysis of the *18S* dataset placed the exemplars of out-groups Psocoptera and Phthiraptera within Hemiptera; topologies based on separate analyses of *H3*, *H2A*, *Wg*, *COI* and *ND4* did not support the monophyly of several families within Fulgoroidea, Membracoidea, Cicadoidea and Cercopoidea.

The PLS scores (Table S4) computed for nodes of particular interest (corresponding to higher-level taxonomic groups within Hemiptera) in the ML topologies resulting from these single-locus analyses indicated some degree of conflict among genes across most nodes examined. The only gene that did not show conflict (i.e. a negative support value) for any of these nodes was *28S*. Among the remaining genes, appreciable conflict did not appear to arise from any one particular gene. *18S* showed conflict with two of the 12 examined nodes, one of which corresponds to Auchenorrhyncha. *COI* showed conflict with three nodes, and the remaining genes each showed conflict with between five and eight nodes.

Significance tests of alternative hypotheses of Hemipteran phylogeny

Alternative hypotheses of hemipteran phylogeny based on morphological evidence (Fig. 1B, consistent with Hennig, 1981), *18S* rDNA evidence (Fig. 1C, consistent with Campbell *et al.*, 1995; von Dohlen & Moran, 1995; Sorensen *et al.*, 1995), *18S* rDNA and fossil evidence (Fig. 1D, consistent with Bourgoin & Campbell, 2002) and endosymbiont evidence (Fig. 1E, consistent with Müller, 1962; Buchner, 1965) were each evaluated for statistical concordance; results are summarized in Table S5. Results of AU tests significantly rejected the alternative hypotheses based on morphology (Fig. 1B) and on *18S* rDNA (Fig. 1C); AU tests failed to reject the remaining two alternative hypotheses of Hemipteran phylogeny.

Discussion

The hemipteran suborder Auchenorrhyncha, including such morphological extremes as the treehoppers (Membracidae) and the lanternflies (Fulgoridae), is arguably one of the most charismatic and diverse of insect groups. Many included species are considered major economic pests of worldwide agriculture (in terms of both feeding damage and plant disease transmission), making Auchenorrhyncha a group of significance in relation to human activity. Despite this importance, basic phylogenetic questions persist regarding Auchenorrhyncha: specifically, the monophyly of Auchenorrhyncha remains one of the most contentious questions in the higher-level systematics of Paraneoptera. As reviewed below, numerous studies have offered evidence (morphological, molecular and from the presence of bacterial endosymbionts) bearing on the question of Auchenorrhyncha monophyly, either in support or in refutation.

Review: morphological evidence

Traditionally, Auchenorrhyncha is thought to be supported by the autapomorphic presence of a complex tymbal acoustic system on abdominal segment I (this may be the most significant morphological synapomorphy supporting Auchenorrhyncha) and of an aristoid antennal flagellum (Kristensen, 1975). Kristensen (1975) discussed the possibility that jumping ability may also be a synapomorphic feature of Auchenorrhyncha; furthermore, Auchenorrhyncha is unique within Hemiptera as having the labium originating from the posterior region of the ventral head surface, close to the occiput, and having no intervening sclerotic gula present (Carver *et al.*, 1991).

Goodchild (1966) presented a detailed, comparative anatomical examination of the hemipteran alimentary system, contrasting the internal gut anatomy of the major lineages of Hemiptera/Homoptera. Of particular importance to

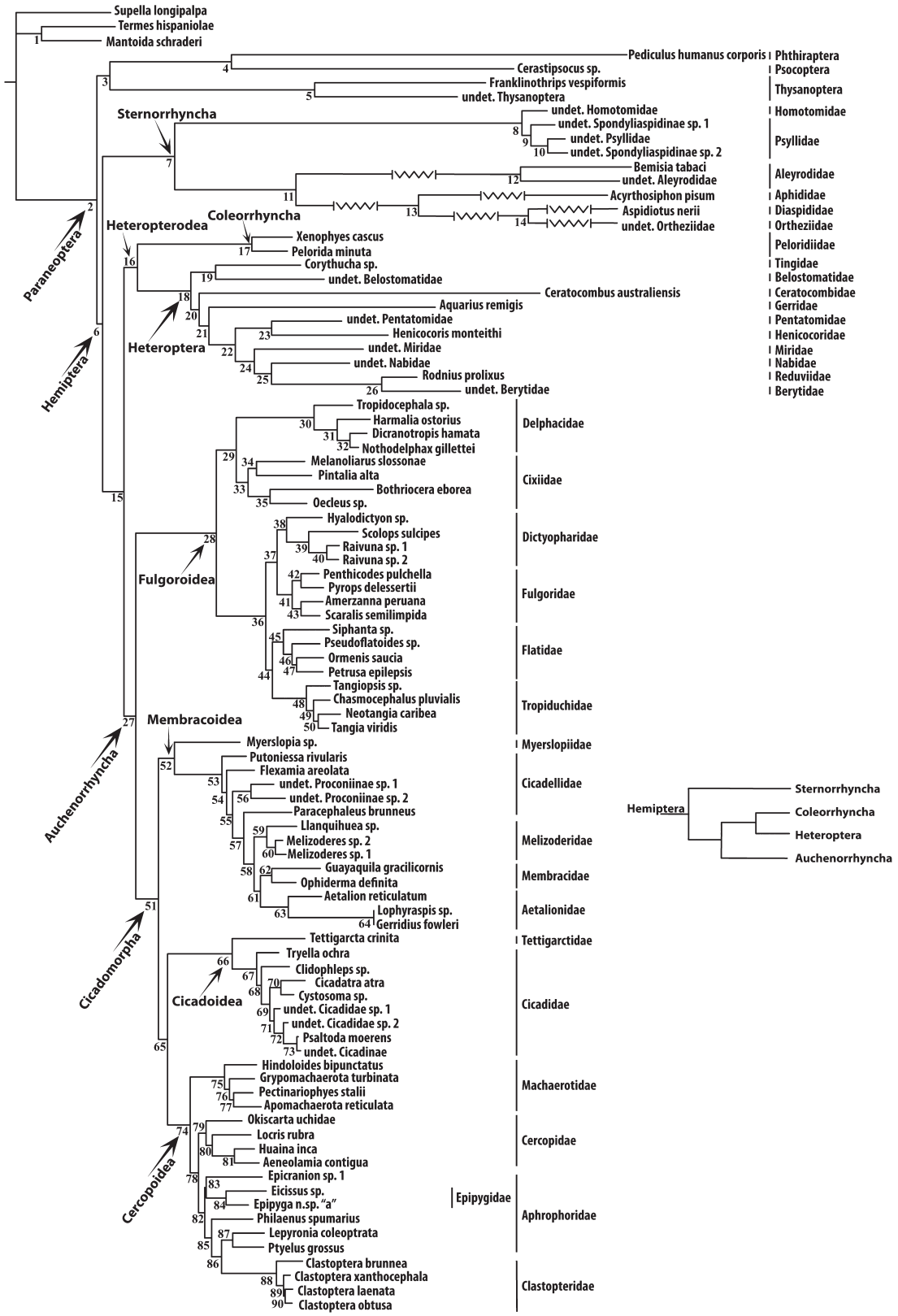

Fig. 2. Maximum-likelihood phylogram recovered in 20 independent GARLI analyses. Nodes are numbered and correspond with branch support values presented in Table 1. Several lineages subtended by node 11 are depicted as broken because of their exceedingly long branch lengths.

Auchenorrhyncha was the relative location of the hindgut junction: Cicadomorpha (referred to as 'Cicadoidea' by Goodchild) and Sternorrhyncha apparently share the condition wherein the hindgut junction is more anterior in the alimentary canal, whereas Fulgoroidea and Heteroptera share the condition wherein the junction is located in a more posterior position. Goodchild concluded, therefore, that Cicadomorpha is the sister group to Sternorrhyncha and Fulgoroidea is the sister group to Heteroptera; interestingly, Coleorrhyncha was judged to occupy an intermediate position between these two groupings.

Hennig (1981) discussed several morphological features that had been proposed previously as potential synapomorphies for Auchenorrhyncha, including the pronotal coverage of the mesonotal fore margin, stridulatory and auditory organs, jumping ability, antennal structure, and various features of the wings. Despite listing some divergent features between Fulgoroidea (as 'Fulgoriformes') and Cicadomorpha (as 'Cicadiformes'), including the hind coxal structure, antennal position and sensilla arrangement, the constituency of bacterial endosymbionts found to occur within Fulgoroidea versus Cicadomorpha, and the presumed age of these lineages from the fossil record, Hennig nevertheless concluded that there was significant morphological evidence in support of the monophyly of Auchenorrhyncha.

Hamilton's (1981) examination of Hemiptera/Homoptera head capsule morphology included detailed observations and illustrations of both hard (chitinous) and soft (musculature) structures. In comparing the anatomy of Fulgoromorpha and Cicadomorpha, Hamilton observed fundamental differences that, in his estimation, provided evidence against the monophyly of Auchenorrhyncha. Specifically citing the presence of a developed 'filter chamber' (this term was neither defined nor labelled in any of the illustrations, and therefore it is unknown if this term refers to the 'cibarial chamber' of the head or the 'filter chamber' typically associated with the midgut), Hamilton hypothesized that Cicadomorpha and Sternorrhyncha (as 'Aphidomorpha') formed a monophyletic group, the sister to which was Fulgoromorpha (thus, Hamilton recognized support for 'Homoptera'). Based on the presence of a developed gula, Coleorrhyncha was grouped with Heteroptera (as 'Hemiptera').

Zrzavý (1992) briefly reviewed a number of morphological and ecological traits of Hemiptera, including antennal structure, alimentary system morphology and feeding ecology, and asserted that Homoptera 'should be regarded as [an] artificial non-monophyletic' assemblage. Zrzavý seemed only to consider Auchenorrhyncha as a monophyletic unit, however, and therefore did not comment on the possibility of Fulgoromorpha and Cicadomorpha being independent lineages on the hemipteran phylogeny.

Bourgoin (1988, 1993) performed anatomical studies of the male and female genitalia of Hemiptera, comparing structures observed in certain Fulgoromorpha with homologous structures observed in Heteroptera and Cicadomorpha. Citing several potential morphological synapomorphies supporting (Fulgoromorpha + Heteroptera) (i.e. loss of accessory glands, spermathecal structure, male genitalic 'conformation' and tentorial 'construction'), Bourgoin interpreted the general anatomy of the fulgoromorph female and male genitalic assemblies as more similar to that of Heteroptera than to that of Cicadomorpha, concluding that these structures offered no evidence to support the monophyly of Auchenorrhyncha.

Several publications focused on the forewing–hindwing coupling structures in Hemiptera, and the potential phylogenetic implications of those features. D'Urso (1993) observed that the wing-coupling structure of Coleorrhyncha was most similar to that in Heteroptera, therefore concluding that a monophyletic Heteropterodea was sister to a monophyletic Homoptera (Auchenorrhyncha + Sternorrhyncha). D'Urso & Ippolito (1994), described differences in the wing-coupling structures between Fulgoromorpha and Cicadomorpha, although those differences were interpreted as insufficient to refute the monophyly of Auchenorrhyncha. These observations were reiterated by D'Urso (2002), who concluded that the wing coupling morphology provides evidence supporting the monophyly of Auchenorrhyncha.

Yoshizawa & Saigusa (2001) conducted a phylogenetic analysis of Paraneoptera based on coded characters of the forewing base structure. Their results supported the monophyly of Auchenorrhyncha based primarily on the potential synapomorphic condition of a reduced or entirely membranous proximal median plate (in non-auchenorrhynchous Hemiptera, the proximal median plate is a triangular sclerite of the forewing base, articulating with the second axillary sclerite). Whereas the authors acknowledged that reduction/loss characters are not as compelling phylogenetically as synapomorphic novelties, nevertheless they assert that the reduction/loss of the proximal median plate is not observed in other groups, and therefore represents a reliable autapomorphy for Auchenorrhyncha (Yoshizawa & Saigusa, 2001).

D'Urso *et al*. (2005) discussed characters of the male and female internal reproductive structures of Auchenorrhyncha, reporting the first observation of male lateral ejaculatory ducts in a species of Fulgoromorpha (these structures were known previously only from Cicadomorpha). The authors were circumspect with regard to the phylogenetic importance of internal genitalic characters, and did not comment on the direct impact of their study on the question of Auchenorrhyncha monophyly; nevertheless, these male lateral ejaculatory ducts represent a potentially synapomorphic condition for Auchenorrhyncha.

Dmitriev (2010) treated Auchenorrhyncha as a monophyletic group in a recent morphological investigation of adult and nymphal head capsule features. The ground plan for auchenorrhynchan head structure was discussed, and numerous hypotheses of structural homology across Fulgoromorpha and Cicadomorpha were detailed; however, because of the apparent assumption of Auchenorrhyncha monophyly, no characters are described specifically as synapomorphies for the suborder.

Review: molecular evidence

The first application of DNA nucleotide sequence data to questions of higher-level hemipteran phylogeny (Wheeler

et al., 1993) focused on relationships within Heteroptera, and thus included few representatives of nonheteropteran taxa: their out-group sampling included one exemplar of Coleorrhyncha, one exemplar of Sternorrhyncha and three exemplars of Cicadomorpha (two leafhoppers and one cicada). Their MP analyses, based on a data matrix combining coded morphological characters and partial nucleotide sequences from *18S* rDNA, resulted in support for the relationships (Cicadomorpha + (Coleorrhyncha + Heteroptera)). Although the authors listed their sampling of Cicadomorpha as 'Auchenorrhyncha' (see Wheeler *et al*., 1993: table 1), Fulgoroidea was not represented and therefore the monophyly of Auchenorrhyncha was not tested.

Campbell *et al*. (1994) published a phylogenetic analysis based on *18S* rDNA sequences in a study designed to reconstruct relationships within Sternorrhyncha; their out-group sampling included one exemplar of Flatidae (Fulgoromorpha) and one exemplar each of Cercopoidea, Cicadoidea and Membracoidea (Cicadomorpha). Although not specifically discussed, their results indicated the nonmonophyly of Auchenorrhyncha. Subsequently, Sorensen *et al*. (1995) published a re-analysis of the Campbell *et al*. (1994) *18S* dataset, but with data from an exemplar of Delphacidae substituted for the flatid originally used, and also with the addition of data from an out-group representing Coleoptera: as with the previous analysis, Sorensen *et al.*'s (1995) results indicated no support for the monophyly of Auchenorrhyncha. Later that year, the same authors (Campbell *et al*., 1995) further expanded their *18S* rDNA dataset to include several additional taxa (five exemplars of Fulgoroidea, three of Membracoidea, one of Cicadoidea and two of Cercopoidea): phylogenetic results were equivocal with regard to the monophyly of Auchenorrhyncha, although the authors interpreted this result as evidence of auchenorrhynchan paraphyly.

Von Dohlen & Moran's (1995) higher-level phylogenetic analysis of Hemiptera/Homoptera, based on *18S* rDNA nucleotide sequences, yielded strong evidence for the paraphyly of the order Homoptera (i.e. Sternorrhyncha + Auchenorrhyncha; Coleorrhyncha was not represented in their sampling), with Auchenorrhyncha being placed as the sister group of Heteroptera. However, their results were equivocal regarding the monophyly of Auchenorrhyncha (represented in that study by three exemplars of Membracoidea, one of Cercopoidea, two of Cicadoidea, and three of Fulgoroidea); the authors concluded that their *18S* rDNA sequence data were insufficient to fully resolve phylogenetic relationships among Fulgoromorpha, Cicadomorpha and Heteroptera.

Adding new data generated from exemplars of the Fulgoroidea families Tettigometridae and Tropiduchidae, Bourgoin *et al*. (1997) re-analysed the *18S* rDNA dataset from Campbell *et al*. (1995). Their results consistently indicated the nonmonophyly of Auchenorrhyncha. In another re-analysis of Campbell *et al*.'s (1995) *18S* rDNA dataset, Ouvrard *et al*. (2000) added two new sequences generated from exemplars of Coleorrhyncha and re-aligned the data according to *18S* secondary structure; however, their results were ambiguous with regard to the monophyly and placement of Auchenorrhyncha. Xie *et al*. (2008) assembled an *18S* rDNA dataset, largely from GenBank-archived nucleotide sequences (indeed, four of their five Auchenorrhyncha sequences were from Campbell *et al*., 1995); unsurprisingly, results of that analysis also found no support for the monophyly of Auchenorrhyncha.

Urban & Cryan (2007) included four exemplars of Cicadomorpha in a phylogenetic analysis of the planthopper superfamily Fulgoroidea based on nucleotide sequence data from four genes (*18S* rDNA, *28S* rDNA, *H3* and *Wg*). The results of their MP and Bayesian analyses recovered the monophyly of Auchenorrhyncha. However, statistical support was moderate to weak (Bayesian posterior probability = 91%, MP bootstrap <50%) for the nodes corresponding to Auchenorrhyncha on the resulting topologies, as the study was not designed to test the monophyly of Auchenorrhyncha and thus the taxonomic sampling included in that study was insufficient to investigate that question.

Song & Liang (2009) documented the complete mitochondrial genomic sequence of the delphacid planthopper, *Laodelphax striatellus* (Fallén), in a study that also included a phylogenetic analysis of relationships within Hemiptera based on a dataset comprising 13 aligned mitochondrial protein coding genes generated from 29 hemipteran taxa (of which only four are representatives of Auchenorrhyncha). Their results recovered Heteroptera as sister to the remaining hemipteran lineages (Coleorrhyncha was not represented in that analysis); (Fulgoromorpha + Sternorrhyncha) was recovered as a monophyletic group, the sister of which was (Cercopoidea + Membracoidea) (i.e. Cicadomorpha). Thus, although that analysis did not recover a monophyletic Auchenorrhyncha, their results interestingly suggested support for a monophyletic Homoptera.

Review: bacterial endosymbiont evidence

Buchner (1965) summarized the results of light microscopy surveys of endosymbiotic bacteria observed within a diverse assemblage of host organisms, including several hemipteran lineages. Based on those observations, Auchenorrhyncha exhibit an apparent 'hunger for symbionts' (Buchner, 1965: 346), as certain included lineages house several species of endosymbiotic bacteria. Müller (1962) surveyed more than 400 species of Cicadomorpha and Fulgoromorpha, as well as one species of Coleorrhyncha, and observed three morphologically distinct 'primary' endosymbionts. A 'primary' endosymbiont is defined as a bacterial species hypothesized to have a relatively long-term, mutually obligatory evolutionary association with its host insect, based on observations that the endosymbionts are localized within bacteriomes (specialized organs), and that the development of the bacterial 'infection' is integrated with the development of the host insect (i.e. nymph to adult stages) (Müller, 1962; Buchner, 1965). Whereas species of Heteroptera are known to harbour bacteria, the bacteria are not housed in bacteriomes and are therefore not considered primary endosymbiotic associations.

Müller (1962) observed that the same primary endosymbiont (which he called the a-symbiont) occurred in Fulgoromorpha,

Cicadomorpha and Coleorrhyncha. In Fulgoromorpha and Cicadomorpha, this primary endosymbiont occurred with additional companion symbionts, whereas in Coleorrhyncha the primary endosymbiont occurs alone; however, Müller noted that this primary endosymbiont was housed differently in Fulgoromorpha than in Cicadomorpha (i.e. housed in different locations, and in nonhomologous bacteriomes). Based on his observations, Müller hypothesized that Coleorrhyncha was sister to (Fulgoromorpha + Cicadomorpha), and that a single bacterial infection occurred in the common ancestor of this lineage (Müller, 1962; Buchner, 1965).

Moran *et al*. (2005) used a PCR assay to detect Müller's a-endosymbiont based on bacterial *16S* rDNA nucleotide sequences generated from a selection of potential insect host species representing Auchenorrhyncha and Coleorrhyncha. This endosymbiont, which they named *Sulcia muelleri*, was detected in most (21 of 23) Cicadomorpha species examined and some (two of six) Fulgoroidea species examined. Although *S. muelleri* was not found in the two species of Coleorrhyncha examined, based on light microscopy and PCR assay Moran *et al.* detected another bacterial endosymbiont in Coleorrhyncha that belongs to a different phylum (*Betaproteobacteria*) to that of *S. muelleri* (phylum *Bacteroidetes*); they concluded that Müller's (1962) identification of the Coleorrhyncha endosymbiont was erroneous because Müller relied on observed morphological similarity, which is thought to be insufficient evidence (as quite distantly related bacteria can appear morphologically similar; Moran *et al*., 2005). Although Moran *et al*. conceded that their results could be explained either by independent bacterial infections of Cicadomorpha and Fulgoromorpha or a single infection of the common ancestor of (Cicadomorpha + Fulgoromorpha + Heteroptera + Coleorrhyncha), with subsequent losses of the bacterium in Heteroptera and Coleorrhyncha, the authors concluded that their results were more consistent evolutionarily with a monophyletic Auchenorrhyncha, requiring only a single bacterial infection of the common ancestor of (Cicadomorpha + Fulgoromorpha).

Summary: previous evidence for and against Auchenorrhyncha monophyly

As reviewed above, evidence seemingly supporting the monophyly of Auchenorrhyncha includes structures of the head (aristoid antennal flagellum; labium originating from the posterior region of the ventral head surface; absence of a sclerotized gula), structures of the wings (wing-coupling structure; sclerite structure of forewing base) and structures of the abdomen (complex tymbal acoustic system; presence of male lateral ejaculatory ducts); in addition, potentially supporting evidence comes from the presence of the bacterial endosymbiont *Sulcia* in many Auchenorrhyncha species and from a multigene phylogenetic analysis (albeit one not designed to address the question of Auchenorrhyncha monophyly). Conversely, evidence cited as refuting the monophyly of Auchenorrhyncha includes the internal morphology of the alimentary canal, head capsule morphology, features of the female reproductive system and single-gene analyses of *18S* rDNA nucleotide sequence data.

Bourgoin & Campbell (2002) proposed an evolutionary framework for Hemiptera, attempting to nonquantitatively synthesize previous phylogenetic hypotheses generated in published and unpublished analyses of *18S* rDNA sequence data, as suggested in morphological studies and by palaeontological evidence. This 'summary hypothesis' did not include Auchenorrhyncha as a monophyletic lineage, but rather placed Fulgoromorpha and Cicadomorpha as separately occurring lineages within Hemiptera with the following arrangement: (Sternorrhyncha + (Fulgoromorpha + (Cicadomorpha + (Coleorrhyncha + Heteroptera)))). Bourgoin & Campbell (2002) suggested that new sources of evidence, both morphological and molecular, should be explored to provide additional information on relationships among the major hemipteran lineages.

Phylogenetic reconstruction of Hemipteran relationships

Despite the numerous previous attempts to reconstruct evolutionary relationships among the major lineages within Hemiptera, limitations in methodology, data sampling and/or taxonomic sampling in those studies have rendered their results unconvincing. For example, most morphological studies relevant to questions of higher-level hemipteran relationships resulted in non-analytically derived hypotheses, based on comparisons of 'single-character' systems (e.g. Goodchild, 1966; Bourgoin, 1988, 1993). Most relevant molecular phylogenetic studies (e.g., Campbell *et al*., 1994, 1995; Sorensen *et al*., 1995), although incorporating quantitative analyses, were based on relatively small datasets from only a single genomic locus (*18S* rDNA, which seems to hold insufficient information to resolve these relationships). With particular regard to the question of Auchenorrhyncha monophyly, most previous studies included an insufficient taxonomic sample with which to test this hypothesis (e.g. Wheeler *et al*., 1993; Urban & Cryan, 2007; Song & Liang, 2009).

Furthermore, previous studies predominantly employed MP-based methods of phylogenetic reconstruction. In this present investigation, phylograms resulting from ML (Fig. 2) and Bayesian (not shown) analyses show the general pattern of terminal taxa exhibiting relatively long branch lengths (especially within Sternorrhyncha), whereas internode branch lengths (particularly in the backbone of the topology) are relatively short. This pattern can be problematic under any method of phylogenetic reconstruction, but is especially challenging for MP-based reconstructions (making topological results subject to 'long branch attraction' artifacts; Whitfield & Kjer, 2008). With the results of the present study, the placement of Sternorrhyncha outside of an otherwise monophyletic Hemiptera under MP only is probably such an artifact. Reconstruction methodologies that use evolutionary models (ML and Bayesian methods) are regarded as more reliable in that they serve to increase the ratio of phylogenetic signal (at short internodes) relative to homoplastic noise (subsequent

changes in long branches) in reconstruction (Whitfield & Lockhart, 2007; Whitfield & Kjer, 2008).

The present study sought to alleviate the limitations of previous studies in order to reconstruct the higher-level phylogeny within Hemiptera, and to determine specifically whether or not Auchenorrhyncha is a monophyletic clade, by: (i) including a sufficient taxonomic representation of each major hemipteran lineage; (ii) basing quantitative, analytical results on a dataset incorporating data generated from multiple, independent genetic loci; and (iii) using multiple methods of phylogenetic reconstruction (MP, ML and Bayesian).

Results obtained with all three phylogenetic reconstruction methods supported the monophyly of the hemipteran suborders Sternorrhyncha (Fig. 2, node 7), Coleorrhyncha (Fig. 2, node 17), Heteroptera (Fig. 2, node 18) and Auchenorrhyncha (Fig. 2, node 27). These results also supported the monophyly of the superfamilies Fulgoroidea (Fig. 2, node 28), Membracoidea (Fig. 2, node 52), Cicadoidea (Fig. 2, node 66) and Cercopoidea (Fig. 2, node 74). Furthermore, these analyses recovered the monophyly of the lineages Cicadomorpha [i.e. (Membracoidea + (Cicadoidea + Cercopoidea)); Fig. 2, node 51] and Heteropterodea [i.e. (Coleorrhyncha + Heteroptera); Fig. 2, node 16]. All of these clades were recovered with 100% Bayesian posterior probability and moderate to high levels of ML and MP support (Table 1).

We caution that relationships recovered here within Sternorrhyncha (Fig. 2, nodes 7–14) should not be regarded as particularly compelling. This suborder is commonly split into four superfamilies (Psylloidea, Aleyrodoidea, Aphidoidea and Coccoidea); our taxonomic sampling within Sternorrhyncha is clearly insufficient to reconstruct these internal relationships, especially when one considers the evidence for extremely long branches apparent for several included representatives (i.e. as indicated by broken branches in Fig. 2).

Heteropterodea is defined as (Coleorrhyncha + Heteroptera) (Schlee, 1969; Zrzavý, 1992): this clade is defined by several morphological synapomorphies, including characters of the antennae, forewing venation, wing coupling mechanism and abdominal structures (as reviewed by Forero, 2008). Additionally, the monophyly of Heteropterodea was supported by Wheeler *et al.*'s (1993) analysis based on *18S* rDNA sequence data, and in the present investigation, Heteropterodea was recovered as a monophyletic lineage (Fig. 2, node 16). Within Heteropterodea, the monophyly of Coleorrhyncha (Fig. 2, node 17) was strongly supported, although only two exemplars were included. Coleorrhyncha is a taxonomically small group with 25 extant species in the family Peloridiidae and a relictual, Southern Hemisphere distribution. Notably, this is the first study to report DNA sequence data from the two Peloridiidae exemplars included here (*Peloridora minuta* China and *Xenophyes cascus* Bergroth from Chile and New Zealand, respectively). Recovered as sister group to Coleorrhyncha was Heteroptera (Fig. 2, node 18), for which monophyly was also strongly supported (Bayesian posterior probability = 100%, ML bootstrap = 100%, LS = 52.0, MP bootstrap = 99%). Heteroptera is a large and diverse suborder, also supported by several strong morphological synapomorphies (reviewed by Forero, 2008). Although this lineage was recovered as monophyletic in every analysis conducted here, we do not regard relationships reconstructed within Heteroptera as necessarily reflecting the actual heteropteran phylogeny, because the taxonomic sampling was insufficient to recover those internal relationships.

Auchenorrhyncha (Fulgoromorpha + Cicadomorpha; Fig. 2, node 27) was recovered as a monophyletic lineage in all analyses of the combined dataset, receiving strong (Bayesian posterior probability = 100%) to moderate (ML bootstrap = 73%; MP bootstrap = 66%) support across reconstruction methods. The monophyly of Fulgoromorpha (i.e. Fulgoroidea; Fig. 2, node 28) was strongly supported (Bayesian posterior probability, ML bootstrap and MP bootstrap all = 100%); relationships recovered among the six planthopper families sampled here were consistent with the phylogeny of Fulgoroidea as reconstructed by Urban & Cryan (2007). The monophyly of Cicadomorpha (Fig. 2, node 51) was well supported, as was the monophyly of the included superfamilies Membracoidea (Fig. 2, node 52), Cicadoidea (Fig. 2, node 66) and Cercopoidea (Fig. 2, node 74), with strong statistical support under all reconstruction methods (Table 1). The topological arrangement of these superfamilies, (Membracoidea + (Cicadoidea + Cercopoidea)), was concordant with the results of a previous molecular phylogenetic study of Cicadomorpha (Cryan, 2005).

The included exemplar of Myerslopiidae was recovered with strong support (Bayesian posterior probability = 100%, ML bootstrap = 97%, MP bootstrap = 86%) as the sister group to the rest of the superfamily Membracoidea (Fig. 2, node 52), in agreement with previous studies based on morphology (Hamilton, 1999) and multi-locus DNA sequence data (Cryan, 2005). Myerslopiidae grouped with Cicadoidea in Dietrich *et al.*'s (2001) analysis of partial *28S* rDNA sequences; however, this placement received weak statistical support. Other relationships recovered within Membracoidea provide support for the hypothesis that the treehoppers (Fig. 2, node 58; Membracidae, Melizoderidae and Aetalionidae) represent a specialized, monophyletic lineage arising from within the paraphyletic leafhopper family Cicadellidae.

Relationships recovered within the spittlebug superfamily Cercopoidea (Fig. 2, node 74) agreed largely with the phylogenetic hypotheses presented by Cryan & Svenson (2010), although we regard the recovery of the family Clastopteridae (Fig. 2, node 88) as originating from within (Aphrophoridae + Epipygidae) as erroneous. This result was also obtained in Cryan & Svenson's (2010) mixed-model Bayesian analysis, whereas in their MP and ML analyses, Clastopteridae was placed as sister group to (Cercopidae + (Aphrophoridae + Epipygidae)). The latter placement seems more likely based on trends in some morphological features (discussed in Cryan & Svenson, 2010); however, the position of Clastopteridae seems to be another inherently difficult phylogenetic issue within Auchenorrhyncha, and is being investigated in a separate analysis, which is now in progress.

Examination of data conflict among genes

Separate ML analyses of the seven individual genes and computation of PLS scores for 12 selected deeper nodes of interest in the ML topology were examined in order to identify potential conflict among genes. The only gene that showed no evidence of conflict was *28S*. That is, the topology obtained from the ML reconstruction of *28S* recovered the same major relationships as were recovered in the complete dataset. In the ML analysis of the combined dataset, *28S* contributed no negative PLS values for the selected nodes. Although none of the remaining genes recovered a monophyletic Hemiptera when analysed alone, they did vary to the extent in which they recovered the superfamilies Fulgoroidea, Membracoidea, Cicadoidea and Cercopoidea as monophyletic, and to the extent in which families within these groups were recovered as monophyletic. Along these lines, *18S* performed the 'best' (after *28S*), followed by *COI*; the remaining genes (*H3*, *H2A*, *Wg* and *ND4*) performed poorly. These findings are consistent with PLS scores, particularly the number of nodes (of the 12 selected nodes for which PLS scores were computed) to which each gene showed conflict: *18S* showed conflict with two nodes; *COI* showed conflict with three nodes; *H2A* showed conflict with five nodes; *ND4* showed conflict with six nodes; *H3* showed conflict with seven nodes; and Wg showed conflict with eight nodes.

Although any single gene might exhibit conflict for a particular node in the combined data ML topology, the combination of all genes was necessary to provide sufficient phylogenetic signal for the reconstruction of the complete topology. We hypothesize that the better performance (as indicated by single gene trees and PLS scores) of *28S*, *18S* and *COI*, relative to the other genes, may result in part from the length/number of phylogenetically informative sites in each of these single gene datasets. Of particular interest was the potential conflict introduced by *18S*, because previous analyses based solely on *18S* (Campbell *et al.*, 1995; von Dohlen & Moran, 1995; Sorensen *et al*., 1995) either did not support the monophyly of Auchenorrhyncha or did so only equivocally, whereas the results of our analyses indicated that *18S* did not introduce appreciable conflict to that node. We believe this contrast is correlated with the number of informative characters included: the aligned *18S* dataset in our analyses included 2188 bp (of which 544 were parsimony-informative characters), whereas previous *18S* datasets contained significantly fewer informative characters (Campbell *et al.*, 1995; von Dohlen & Moran, 1995; Sorensen *et al*., 1995).

Conclusion

The ML (Fig. 2) and Bayesian phylograms of relationships within Hemiptera sensu lato show a pattern of short internodes (including the node corresponding to Auchenorrhyncha diversification) and longer branch lengths leading to the terminals. Such a pattern is characteristic of rapid diversification events, as the comparatively large extent of recent evolutionary change tends to obscure earlier changes that are critical to resolving the radiation event (Whitfield & Kjer, 2008). Sorensen *et al.* (1995) observed a similar pattern in their *18S* data and, interpreting their results with evidence from the fossil record, concluded that the diversification of the major hemipteran lineages occurred quickly. Whereas we do not necessarily assert that the diversification of Auchenorrhyncha was a true 'rapid radiation' event (we did not test for it; branching patterns in some lineages appear to be better candidates for rapid diversifications, such as in the planthopper tribe Delphacini; Urban *et al.*, 2010), it is important to note that this branching pattern is known to be problematic for phylogenetic reconstruction.

Unlike previous studies, the present investigation was able to reconstruct stable relationships among the major hemipteran lineages because, we: (i) combined data from seven molecular loci; (ii) employed model-based (ML and Bayesian) methods of reconstruction; and (iii) had stronger taxonomic sampling, representing the major lineages within Auchenorrhyncha. By combining data from multiple loci, we were able to increase the number of phylogenetically informative sites (beyond those present in any single gene), which is needed to resolve deeper relationships exhibiting short internodes. Model-based methods perform better than parsimony-based methods when there are extreme differences in branch lengths. Finally, increased taxonomic sampling probably serves to 'break up' potentially problematic long branches.

The monophyly of Auchenorrhyncha was recovered consistently under all three reconstruction methodologies, with strong to moderate statistical support. Our topological tests of alternative hypotheses of hemipteran phylogeny significantly rejected previous hypotheses based on morphology (Hennig, 1981) and *18S* (Campbell *et al.*, 1995; von Dohlen & Moran, 1995; Sorensen *et al*., 1995), although not those based on *18S* and fossil evidence (Bourgoin & Campbell, 2002) and endosymbiont evidence (Müller, 1962; Buchner, 1965). In light of our statistically defensible results, and considering all available evidence from past studies, we assert that the compelling majority of evidence, both morphological and molecular, now supports Auchenorrhyncha (Fulgoromorpha + Cicadomorpha) as a united lineage. We anticipate that the results of our study will inspire future research to contribute additional suites of phylogenetically informative characters (molecular and morphological) that can explore the diversification of early hemipteran lineages with even greater rigour.

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/j.1365-3113.2011.00611.x

Table S1. Taxa included in *18S*, *28S*, *H2A*, *H3*, *Wg*, *COI* and *ND4* nucleotide sequence datasets.

Table S2. Oligonucleotide primer sequences.

Table S3. Descriptive statistics for data partitions.

Table S4. Partitioned likelihood scores. Likelihood difference (between optimal ML tree score and score of reverse constrained trees) partitioned by gene for selected nodes in ML tree (Fig. 2). Note: computation of PLS scores required the use of nonpartitioned ML searches of optimal and reverse-constrained trees. Therefore, the total likelihood scores of these trees differ from those underlying Table 1, but do reflect the relative contribution of each gene partition to likelihood support for that branch.

Table S5. Significance tests. Results of approximately unbiased (AU) significance tests comparing the ML topology (recovering a monophyletic Auchenorrhyncha) with the alternative hypotheses of Hemipteran phylogeny. Note: computation of AU tests was based on nonpartitioned ML searches of optimal and reverse-constrained trees.

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References

Akaike, H. (1974) A new look at the statistical model identification. *IEEE Transactions on Automatic Control*, **19**, 716–723.

Bourgoin, T. (1986a) Morphologie imaginable du tentorium des Hemiptera Fulgoromorpha. *International Journal of Insect Morphology and Embryology*, **15**, 237–252.

Bourgoin, T. (1986b) Valeur morphologique de la lame maxillaire chez les Hemiptera. Remarques phylogénétiques. *Annales de la Société Entomologique de France (N.S.)*, **22**, 413–422.

Bourgoin, T. (1988) A new interpretation of the homologies in the Hemiptera male genitalia, illustrated by the Tettigometridae (Hemiptera Fulgoromorpha). *Proceedings 6th Auchenorrhyncha Meeting*. Turin, 1987, pp. 113–120.

Bourgoin, T. (1993) Female genitalia in Hemiptera Fulgoromorpha, morphological and phylogenetic data. *Annales de la Société Entomologique de France*, **29**, 225–244.

Bourgoin, T. & Campbell, B.C. (2002) Inferring a phylogeny for Hemiptera: falling into the 'autapomorphic trap'. *Denisia*, **4**, 67–82.

Bourgoin, T., Steffen-Campbell, J.D. & Campbell, B.C. (1997) Molecular phylogeny of Fulgoromorpha (Insecta, Hemiptera, Archaeorrhyncha). The enigmatic Tettigometridae: evolutionary affiliations and historical biogeography. *Cladistics*, **13**, 207–224.

Buchner, P. (1965) *Endosymbiosis of Animals with Plant Microorganisms.* Interscience, New York, New York.

Campbell, B.C., Steffen-Campbell, J.D. & Gill, R.J. (1994) Evolutionary origin of whiteflies (Hemiptera: Sternorrhyncha: Aleyrodidae) inferred from 18S rDNA sequences. *Insect Molecular Biology*, **3**, 73–89.

Campbell, B.C., Steffen-Campbell, J.D., Sorensen, J.T. & Gill, R.J. (1995) Paraphyly of Homoptera and Auchenorrhyncha inferred from 18S rDNA nucleotide sequences. *Systematic Entomology*, **20**, 175–194.

Carver, M., Gross, G.F. & Woodward, T.E. (1991) Hemiptera (Bugs, leafhoppers, cicadas, aphids, scale insects, etc.). *The Insects of Australia. A Textbook for Students and Research Workers*, 2nd edn, Vol. 1, Chapter 30 (ed. by I.D. Naumann, P.B. Carne, J.F. Lawrence, E.S. Nielsen and J.P. Spradbery), pp. 429–509. Melbourne University Press, Carlton.

Cryan, J.R. (2005) Molecular phylogeny of Cicadomorpha (Insecta: Hemiptera: Cicadoidea, Cercopoidea, and Membracoidea): adding evidence to the controversy. *Systematic Entomology*, **30**, 563–574.

Cryan, J.R. & Svenson, G.J. (2010) Family-level relationships of the spittlebugs and froghoppers (Hemiptera: Cicadomorpha: Cercopoidea). *Systematic Entomology*, **35**, 393–415.

Cryan, J.R., Wiegmann, B.M., Deitz, L.L. & Dietrich, C.H. (2000) Phylogeny of the treehoppers (Insecta: Hemiptera: Membracidae): evidence from two nuclear genes. *Molecular Phylogenetics and Evolution*, **17**, 317–334.

Cryan, J.R., Liebherr, J.K., Fetzner, J.W. Jr & Whiting, M.F. (2001) Evaluation of relationships within the endemic Hawaiian Platynini

(Coleoptera: Carabidae) based on molecular and morphological evidence. *Molecular Phylogenetics and Evolution*, **21**, 72–85.

Cryan, J.R., Wiegmann, B.M., Deitz, L.L., Dietrich, C.H. & Whiting, M.F. (2004) Treehopper trees: phylogeny of Membracidae (Hemiptera: Cicadomorpha: Membracoidea) based on molecules and morphology. *Systematic Entomology*, **29**, 441–454.

Dietrich, C.H., Rakitov, R.A., Holmes, J.L. & Black, W.C. IV (2001) Phylogeny of the major lineages of Membracoidea (Insecta: Hemiptera: Cicadomorpha) based on 28S rDNA sequences. *Molecular Phylogenetics and Evolution*, **18**, 293–305.

Dmitriev, D.A. (2010) Homologies of the head of Membracoidea based on nymphal morphology with notes on other groups of Auchenorrhyncha (Hemiptera). *European Journal of Entomology*, **107**, 597–613.

von Dohlen, C.D. & Moran, N.A. (1995) Molecular phylogeny of the Homoptera: a paraphyletic taxon. *Journal of Molecular Evolution*, **41**, 211–223.

D'Urso, V. (1993) The wing-coupling apparatus in *Peloridium hammomiorum* Breddin, 1897 (Insecta, Rhynchota). *Spixiana*, **16**, 133–139.

D'Urso, V. (2002) The wing-coupling apparatus of Hemiptera Auchenorrhyncha: structure, function, and systematic value. *Denisia*, **176**, 401–410.

D'Urso, V. & Ippolito, S. (1994) Wing-coupling apparatus of Auchenorrhyncha (Insecta: Homoptera). *International Journal of Insect Morphology and Embryology*, **23**, 221–224.

D'Urso, V., Guglielmino, A. & Mauceri, A. (2005) The internal male and female reproductive apparatus in *Cixia sikaniae* D'Urso & Guglielmino, 1995 (Fulgoromorpha, Achilidae). *Zootaxa*, **1077**, 25–36.

Forero, D. (2008) The systematics of the Hemiptera. *Revista Colombiana de Entomología*, **34**, 1–21.

GeneCodes (2010) *Sequencher Version 4.10.1.* Genecodes Co., Ann Arbor, Michigan.

Goodchild, A.J.P. (1966) Evolution of the alimentary canal in the Hemiptera. *Biological Reviews of the Cambridge Philosophical Society*, **41**, 97–140.

Hamilton, K.G.A. (1981) Morphology and evolution of the rhynchotan head (Insecta: Hemiptera, Homoptera). *The Canadian Entomologist*, **113**, 953–974.

Hamilton, K.G.A. (1999) The ground-dwelling leafhoppers Myerslopiidae, new family, and Sagmatiini, new tribe (Homoptera: Membracoidea). *Invertebrate Taxonomy*, **13**, 207–235.

Hennig, W. (1981) *Insect Phylogeny.* John Wiley & Sons, New York, New York.

Katoh, K., Kuma, K., Toh, H. & Miyata, T. (2005) MAFFT version 5: improvement in accuracy of multiple sequence alignment. *Nucleic Acids Research*, **33**, 511–518.

Kristensen, N.P. (1975) The phylogeny of the hexapod "orders". A critical review of recent accounts. *Zeitschrift für Zoologische Systematik und Evolutionsforschung*, **13**, 1–44.

Latreille, P.A. (1810) *Considérations générales sur l'ordre naturel des animaux composant les classes des crustacés, des arachnides, et des insectes; avec un tableau méthodique de leur genres diposés en familles.* Schoell, Paris.

Lee, M.S.Y. & Hugall, A.F. (2003) Partitioned likelihood support and the evaluation of data set conflict. *Systematic Biology*, **52**, 15–22.

Linné, C. (1758) II. Hemiptera. Systema Naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis. *Editio Decima, Reformata*, **1**, 1–824.

Miller, M.A., Pfeiffer, W. & Schwartz, T. (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. *Proceedings of the Gateway Computing Environments Workshop (GCE).* New Orleans, Louisiana, 14 November 2010, pp. 1–8.

Moran, N.A., Tran, P. & Gerardo, N.M. (2005) Symbiosis and insect diversification: an ancient symbiont of sap-feeding insects from the bacterial phylum Bacteroidetes. *Applied and Environmental Microbiology*, **71**, 8802–8810.

Müller, H.J. (1962) Neuere vorstellungen über verbreitung und phylogenie der endosymbiosen der zikaden. *Zeitschrift für Morphologie und Ökologie der Tiere*, **51**, 190–210.

Ouvrard, D., Campbell, B.C., Bourgoin, T. & Chan, K.L. (2000) 18S rRNA secondary structure and phylogenetic position of Peloridiidae (Insecta, Hemiptera). *Molecular Phylogenetics and Evolution*, **16**, 403–417.

Posada, D. & Crandall, K.A. (1998) Modeltest: testing the model of DNA substitution. *Bioinformatics*, **14**, 817–818.

Ronquist, F. & Huelsenbeck, J.P. (2003) MrBayes 3: bayesian phylogenetic inference under mixed models. *Bioinformatics*, **19**, 1572–1574.

Schlee, D. (1969) Bau und funktion des aedeagus bei Psyllina und deren bedeutung für systematische und phylogenetische untersuchungen (Insecta, Hemiptera). Phylogenetische studien an Hemiptera. III. Entkräftung eines argument gegen die monophylie der Sternorrhyncha. *Zoomorphology*, **64**, 139–150.

Shimodaira, H. (2002) An approximately unbiased test of phylogenetic tree selection. *Systematic Biology*, **51**, 492–508.

Shimodaira, H. & Hasegawa, M. (2001) CONSEL: for assessing the confidence of phylogenetic tree selection. *Bioinformatics*, **17**, 1246–1247.

Song, N. & Liang, A.-P. (2009) Complete mitochondrial genome of the small brown planthopper, *Laodelphax striatellus* (Delphacidae: Hemiptera), with a novel gene order. *Zoological Science*, **26**, 851–860.

Sorensen, J.T., Campbell, B.C., Gill, R.J. & Steffen-Campbell, J.D. (1995) Non-monophyly of Auchenorrhyncha ("Homoptera"), based upon 18S rDNA phylogeny: eco-evolutionary and cladistic implications within pre-Heteropterodea Hemiptera (s.l.) and a proposal for new monophyletic suborders. *Pan-Pacific Entomologist*, **71**, 31–60.

Svenson, G.J. & Whiting, M.F. (2009) Reconstructing the origins of praying mantises (Dictyoptera, Mantodea): the roles of Gondwanan vicariance and morphological convergence. *Cladistics*, **25**, 468–514.

Sweet, M.H. (1996) Comparative external morphology of the pregenital abdomen of the Hemiptera. *Studies on Hemipteran Phylogeny, Proceedings, Thomas Say Publications in Entomology* (ed. by C.W. Schaefer), pp. 119–158, 244 p. Entomological Society of America, Lanham, MarylandD.

Swofford, D.L. (2001) *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods), Version 4.0b10.* Sinauer Associates, Sunderland, Massachusetts.

Urban, J.M. & Cryan, J.R. (2007) Evolution of the planthoppers (Insecta: Hemiptera: Fulgoroidea). *Molecular Phylogenetics and Evolution*, **42**, 556–572.

Urban, J.M. & Cryan, J.R. (2009) Entomologically famous, evolutionarily unexplored: the first phylogeny of the lanternfly family Fulgoridae (Insecta: Hemiptera: Fulgoroidea). *Molecular Phylogenetics and Evolution*, **50**, 471–484.

Urban, J.M., Bartlett, C.R. & Cryan, J.R. (2010) Evolution of Delphacidae (Hemiptera: Fulgoroidea): combined-evidence phylogenetics reveals importance of grass host shifts. *Systematic Entomology*, **35**, 678–691.

Wheeler, W.C., Schuh, R.T. & Bang, R. (1993) Cladistic relationships among higher groups of Heteroptera: congruence between morphological and molecular datasets. *Entomologica Scandinavica*, **24**, 121–137.

Whitfield, J.B. & Kjer, K.M. (2008) Ancient rapid radiations of insects: challenges for phylogenetic analysis. *Annual Review of Entomology*, **53**, 449–472.

Whitfield, J.B. & Lockhart, P.J. (2007) Deciphering ancient rapid radiations. *Trends in Ecology and Evolution*, **22**, 258–265.

Xie, Q., Tian, Y., Zheng, L. & Bu, W. (2008) 18S rDNA hyperelongation and the phylogeny of Euhemiptera (Insecta: Hemiptera). *Molecular Phylogenetics and Evolution*, **47**, 463–471.

Yoshizawa, K. & Saigusa, T. (2001) Phylogenetic analysis of paraneopteran orders (Insecta: Neoptera) based on forewing base structure, with comments on monophyly of Auchenorrhyncha (Hemiptera). *Systematic Entomology*, **26**, 1–13.

Zrzavý, J. (1992) Evolution of antennae and historical ecology of the hemipteran insects (Paraneoptera). *Acta Entomologica Bohemoslovica*, **89**, 77–86.

Zwickl, D.J. (2006) *Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion*. PhD Dissertation, The University of Texas, Austin, Texas.

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