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

JASON R. CRYAN and JULIE M. URBAN

Laboratory for Conservation and Evolutionary Genetics, New York State Museum, Albany, NY, U.S.A.

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 (18S rDNA, 28S rDNA, histone H3, histone 2A, 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 nonholometabolous 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...
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 auchenorrhynch 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); Cicadoidae (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
Higher-Level Phylogeny of Hemiptera

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 SEQUENCER 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 SEQUENCER, 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 g arl i 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 in the ML topology were computed with g arl i 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 g arl i. 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 m rbay es 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 m rbay es.

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 m ode ltest. 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 G ARL I 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 g arl i, 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 cons e l (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
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.

**Table 1.** Nodal support for Fig. 2.

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

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.

**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). Heteropteroidea was recovered as monophyletic, with Coleorrhyncha placed as the sister group of Heteroptera; Heteropteroidea 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; Heteropteroidea 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 Henning, 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 (1981) examination of Hemiptera/Homoptera head capsule morphology included detailed observations and illustrations of both hard (chitinuous) 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 ‘Homiptera’).

Zrzavý (1992) briefly reviewed a number of morphological and ecological traits of Hemiptera, including antennal structures, alimentary system morphology and feeding ecology, and asserted that Hemiptera ‘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 genitalia 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 genital 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 re-construct relationships within Sternorrhyncha; their out-group sampling included one exemplar of Flatidae (Fulgoromorpha) and one exemplar each of Cercopoidea, Cicadoida 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 Cicadoida 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 Cicadoida, 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 (Cicadoidea 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 Heteroptera, 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 Heteropteroidea [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).

Heteropteroidea is defined as (Coleorrhyncha + Heteroptera) (Schlee, 1969; Zrzavy, 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 Heteropteroidea was supported by Wheeler et al.’s (1993) analysis based on 18S rDNA sequence data, and in the present investigation, Heteropteroidea was recovered as a monophyletic lineage (Fig. 2, node 16). Within Heteropteroidea, 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 (i.e. Cicadoidea; 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; Membracoidea, Melizoderidae and Aetalionidae) represent a specialized, monophyletic lineage arising from within the paraphyletic leaffhopper family Cicadellidae.

Relationships recovered within the spittlebug superfam-ily Cicadopoidea (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 (Cercopiidae + (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, Membraecoidea, 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 plant hopper 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.

Please note: Neither the Editors nor Wiley-Blackwell are responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

Acknowledgements

We are indebted to G. Svenson for significant assistance with optimizing the PCR protocols used in this investigation. For generously providing specimens, we thank M. Adams, C. Bartlett, C. Dietrich, the Field Museum (M. Thayer and J. Boone), G. Gibbs, K. Hill, K. Kinser, D. Marshall, T. McCabe, S. McKamey, K. Miller, K. Morishima, M. Moulds, N. Nazdrowicz, A. Newton, B. Normark, L. O’Brien, H. Ogden, R. Rakitov, W. Shepard, G. Svenson, M. Thayer, V. Thompson, M. Whiting, M. Wilson and I. Winkler. Thanks to P.J. Gullan and A. Sanborn for assistance with specimen identification. Specimens collected by the authors were obtained in several countries, and we wish to thank the many contacts and officials who assisted our research through the permitting process; we therefore express our gratitude to (in alphabetical order by country): Australia, Queensland Government, Environmental Protection Agency (S. Sullivan), permit no. WITK08882211; Belize, Ministry of Natural Resources, the Environment and Industry, Forest Department, Conservation Division and the Belize Agricultural Health Authority (M. Windsor, O. Ulloa and K. Witty), permit nos CD/60/3/03 and CD/72/2/03 and Baha certificate no. 08981; Costa Rica, Ministry of Environment and Energy and the National Institute of Biodiversity (J.J. Guevara Sequeira and H. Ramirez Murillo), permit nos 128-2003-OFAU and 2529201; Ghana, Wildlife Division, Forestry Commission (V. Attaah), permit nos WD/A.185/ Vol.6/22 and 005833; Malaysia, Economic Planning Unit (Munirah Abd. Manan), permit no. 40/2000/19/1476; Sarawak, Forests Department and Sarawak Forestry Corp. (H. Ali Bin Yusop and L. Chong), permit nos 30/2006 and 08521, and Gunung Mulu World Heritage Area (B. Clark Park Manager); Zambia, Ministry of Tourism, Environment and Natural Resources, Forest Department (F. Malaya and L. Mulongwe), permit no. FDHQ/101/3/25. This material is based upon work supported by the National Science Foundation, under grant nos DEB-0342538, DEB-0813897, DEB-0529679 and DEB-0949082, and by the New York State Museum. Any opinions, findings and conclusions or recommendations expressed in this material are those of the authors, and do not necessarily reflect the views of the National Science Foundation or the New York State Museum. The authors, who contributed equally, declare no conflict of interest.

References


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


Accepted 16 September 2011