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Phylogenomics of Auchenorrhyncha (Insecta: Hemiptera) using transcriptomes: examining controversial relationships via degeneracy coding and interrogation of gene conflict

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Abstract. The hemipteran suborder Auchenorrhyncha is a highly diverse, ecologically and agriculturally important group of primarily phytophagous insects which has been a source of phylogenetic contention for many years. Here, we have used transcriptome sequencing to assemble 2139 orthologues from 84 auchenorrhynchan species representing 27 families; this is the largest and most taxonomically comprehensive phylogenetic dataset for this group to date. We used both maximum likelihood and multispecies coalescent analyses to reconstruct the evolutionary history in this group using amino acid, nucleotide, and degeneracy-coded nucleotide orthologue data. Although many relationships at the superfamily level were consistent between analyses, several differing, highly supported topologies were recovered using different datasets and reconstruction methods, most notably the differential placement of Cercopoidea as sister to either Cicadoidea or Membracoidea. To further interrogate the recovered topologies, we explored the contribution of genes as partitioned by third-codon-position guanine-cytosine (GC) content and heterogeneity. We found consistent support for several relationships, including Cercopoidea + Cicadoidea, most often in genes that would be expected to be enriched for the true species tree if recombination-based dynamics in GC content have contributed to the observed GC heterogeneity. Our results provide a generally well-supported framework for future studies of auchenorrhynchan phylogeny and suggest that transcriptome sequencing is likely to be a fruitful source of phylogenetic data for resolving its clades. However, we caution that future work should account for the potential effects of GC content heterogeneity on relationships recovered in this group.

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Introduction

Insects of the order Hemiptera Linné, 1758 are of considerable scientific interest due to their abundance, ecological significance, economic importance in agricultural systems, and relevance to human health. The earliest fossils of this group are from the Carboniferous (Nel *et al.*, 2013), although molecular divergence time estimates place its origin in the Devonian (Johnson *et al.*, 2018). Hemiptera became one of the dominant insect groups in the Permian (Shcherbakov, 1996) and remains one of the most diverse and abundant groups with over 100 000 extant species (Zhang, 2011). Additionally, the diversity of ecological roles and interactions, novel morphological characteristics, and behavioural patterns exhibited by hemipterans make them ideal model systems for studies of comparative trait evolution (e.g. Lin *et al.*, 2004; Weirauch & Schuh, 2011; Evangelista *et al.*, 2017; Li *et al.*, 2017). Phylogenetic relationships within Hemiptera, however, have frequently been contentious, with Hemiptera, Homoptera Latreille, 1810, and Heteroptera Latreille, 1810 assigned different taxonomic ranks over time. Some authors have considered Heteroptera and Homoptera as separate orders (e.g. Comstock, 1924), whereas others have used Hemiptera in a broad sense to include both groups (e.g. Tillyard, 1919; Hennig, 1981). Current evidence supports a monophyletic Hemiptera (sensu lato) including a paraphyletic 'Homoptera' and the monophyletic groups Heteroptera, Sternorrhyncha, Coleorrhyncha, Fulgoromorpha, and Cicadomorpha (Bourgoin & Campbell, 2002). However, many aspects of the classification within Hemiptera remain unstable, and the lack of comprehensive, well-supported hypotheses of relationships among and within these groups hinders the advancement of comparative evolutionary studies.

Some of the most prominent relationships in question have been those among the superfamilies and families of the infraorders Fulgoromorpha and Cicadomorpha, which together make up the suborder Auchenorrhyncha. This suborder is an exceptionally speciose group (> 43 000 described species) whose members display diverse morphological, behavioural and ecological characteristics (Bartlett *et al.*, 2018). Additionally, many members of the Auchenorrhyncha are important or emerging agricultural pests in both their native and introduced ranges, such as the spotted lanternfly (Dara *et al.*, 2015), glassy-winged sharpshooter (Hoddle, 2004) and brown planthopper (Bottrell & Schoenly, 2012). Auchenorrhynchan pests infest a wide variety of crops and ornamentals and can cause substantial economic damage through direct harm to plants (e.g. hopper-burn) or by serving as vectors of agents of plant disease, including viruses (Ammar & Nault, 2002) and various bacteria such as phytoplasmas (Wilson & Weintraub, 2007), spiroplasmas (Golino & Oldfield, 1990) and *Xylella fastidiosa* (Redak *et al.*, 2004).

The monophyly of Auchenorrhyncha has been questioned in the past on the basis of morphological (Goodchild, 1966; Bourgoin, 1987, 1993) and molecular studies (Campbell *et al.*, 1995; Sorensen *et al.*, 1995; von Dohlen & Moran, 1995; Xie *et al.*, 2008; Song *et al.*, 2012, 2016), but recent molecular evidence suggests that Auchenorrhyncha is a monophyletic group (Cryan & Urban, 2012; Misof *et al.*, 2014; Johnson *et al.*, 2018). Morphological synapomorphies include the presence of a tymbal acoustic system on the first abdominal segment and an aristoid antennal flagellum (Kristensen, 1975), as well as by the insertion of the labium posteroventrally on the head surface (Carver *et al.*, 1991). Emelyanov (1987) also lists a further four exclusive synapomorphies of the Auchenorrhyncha and five apomorphies that are widely distributed throughout Auchenorrhyncha but lost in some lineages. Presence of *Candidatus* Sulcia muelleri, a primary intracellular bacterial endosymbiont apparently restricted to Auchenorrhyncha and present in nearly all tested species, also constitutes evidence for the monophyly of the suborder (Moran *et al.*, 2005; Takiya *et al.*, 2006). As currently constructed, Auchenorrhyncha consists of two infraorders comprising four superfamilies. The infraorder Fulgoromorpha contains one superfamily, Fulgoroidea (planthoppers), and 20 recognized families, and the infraorder Cicadomorpha includes the superfamilies Cicadoidea (cicadas, two families), Cercopoidea [spittlebugs, five families recognized here, but three in Hamilton (2001)], and Membracoidea (leafhoppers and treehoppers, five families).

Morphological, fossil and molecular evidence has been used previously to propose hypotheses of relationships within Auchenorrhyncha. Evans (1963) presented an intuitive morphology-based hypothesis in which Auchenorrhyncha, Fulgoroidea, Cercopoidea, Cicadoidea and Membracoidea were regarded as monophyletic groups. He suggested that Cicadoidea and Membracoidea are sister clades, with Cercopoidea sister to Cicadoidea + Membracoidea (Fig. 1A). He later regarded Membracoidea as sister to Cercopoidea + Cicadoidea (Evans, 1977), but noted that there was uncertainty regarding these relationships. Hennig (1981) regarded Fulgoromorpha and Cicadomorpha as sister groups, but remarked that the relationships between Membracoidea, Cicadoidea and Cercopoidea were not yet confirmed.

Using head characters, Hamilton (1981) placed Cicadoidea as sister to Membracoidea + Cercopoidea (Fig. 1B), but later suggested, based on more extensive character sampling and fossil evidence (Hamilton, 1996, 1999), that Cercopoidea was sister to Membracoidea + Cicadoidea and Fulgoroidea was sister to Cicadomorpha + Sternorrhyncha (Fig. 1C). At the family level, Hamilton (1999) used characters primarily of the head, legs and genitalia to place Myerslopiidae (previously considered a tribe of Cicadellidae) as sister to the remaining extant membracoids, and regarded Membracidae and Aetalionidae as derived from within Cicadellidae, rendering Cicadellidae paraphyletic. Blocker (1996) synthesized several previous morphologically based phylogenies to propose a monophyletic Auchenorrhyncha, with Fulgoroidea sister to [Cicadidae (Cercopidae + Membracoidea)] (Fig. 1B). Blocker (1996) also noted that there was disagreement as to whether Tettigometridae should be placed in a basal or derived position within Fulgoroidea. Emelyanov (1987) examined an extensive suite of external and internal morphological characters, as well as symbiont relationships, to propose Cicadoidea as sister to Cercopoidea, with Membracoidea sister to Fulgoroidea (Fig. 1D). Szwedo (2002) used fossil evidence to propose that Cercopoidea was sister to

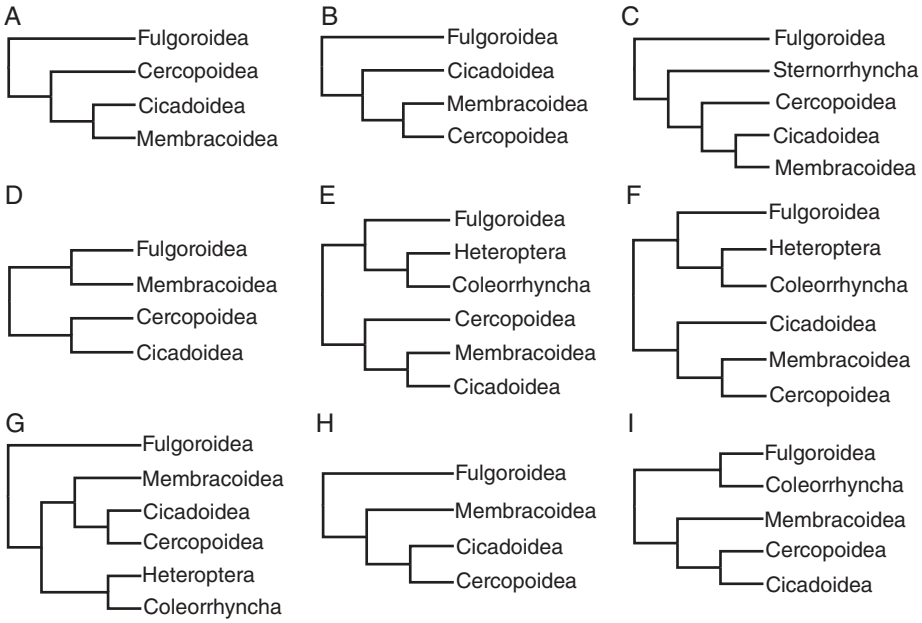

Fig. 1. Previous hypotheses of relationship proposed for the auchenorrhynchan superfamilies. (A) Topology consistent with Evans (1963). (B) Topology consistent with Hamilton (1981) and Blocker (1996). (C) Topology consistent with Hamilton (1996) (fossil evidence) and Hamilton (1999). (D) Topology consistent with Emelyanov (1987) and Song *et al.* (2017). (E) Topology consistent with Szwedo (2002). (F) Topology consistent with Campbell *et al.* (1995), von Dohlen & Moran (1995), Sorensen *et al.* (1995) and Ouvrard *et al.* (2000). (G) Consistent with Bourgoin & Campbell (2002). (H) Consistent with Cryan & Urban (2012) and Johnson *et al.* (2018). (I) Consistent with Li *et al.* (2017).

[(Cicadoidea + Myerslopiidae) Membracoidea], while placing Fulgoroidea sister to Heteroptera + Coleorrhyncha (Fig. 1E).

Characters of the Malpighian tubules were used by Rakitov (2002) to support Cercopoidea as sister to Cicadoidea. This relationship was also supported by Liang & Fletcher (2002) on the basis of antennal characteristics. However, both studies had highly limited taxon sampling. Parsimony analysis of wing base characters from 13 auchenorrhynchan families by Yoshizawa & Wagatsuma (2012) also recovered Cercopoidea and Cicadoidea as sister groups. However, the relationships of the cercopoid families were not resolved, because the placement of either Aphrophoridae or Cercopidae as sister to the remaining cercopoids was equally parsimonious.

Early molecular phylogenies of Hemiptera based on partial 18S rDNA sequences (Campbell *et al.*, 1995; Sorensen *et al.*, 1995; von Dohlen & Moran, 1995) recovered Fulgoromorpha and Cicadomorpha as monophyletic groups, but placed Fulgoroidea sister to Heteroptera, thus rendering Auchenorrhyncha paraphyletic (Fig. 1F). These analyses were not able to confidently reconstruct relationships among the cicadomorphan superfamilies in relation to one another. Ouvrard *et al.* (2000) incorporated secondary structural folding data into their 18S analysis and recovered a monophyletic Cicadomorpha and Fulgoromorpha (Fig. 1F). They also found low support for a sister relationship between Cicadoidea and Cercopoidea, but were unable to resolve relationships of members of Auchenorrhyncha with Heteroptera and Coleorrhyncha. In the Ouvrard *et al.* (2000) study, Membracoidea was recovered as monophyletic, but three families (Melizoderidae, Myerslopiidae and Aetalionidae) were not represented and the relationship between membracids and cicadellids was unresolved. Bourgoin & Campbell (2002) proposed a hypothesis that placed Cercopoidea + Cicadoidea as sister to Membracoidea and Cicadomorpha sister to Heteroptera + Coleorrhyncha, with Fulgoroidea sister to that entire clade (Fig. 1G). However, they did not perform a formal phylogenetic analysis, basing their hypothesis on the existing morphological and molecular evidence, the latter of which was then limited to 18S sequence data. Cryan & Urban's (2012) analysis using seven nuclear and mitochondrial genes recovered Fulgoroidea sister to [Membracoidea (Cercopoidea + Cicadoidea)] (Fig. 1H), although maximum likelihood bootstrap support for the monophyly of Cercopoidea + Cicadoidea was only moderate.

Several other molecular phylogenetic studies have examined relationships among major lineages (e.g. families, subfamilies) within the auchenorrhynchan superfamilies Membracoidea (Dietrich *et al.*, 2001, 2017), Fulgoroidea (Urban & Cryan, 2007), Cercopoidea (Cryan & Svenson, 2010) and Cicadoidea (Marshall *et al.*, 2018). Although most of these studies used only a few genes and often recovered low branch support for deep relationships, one study using data from hundreds of genes (Dietrich *et al.*, 2017) was still unable to resolve several deep internal branches with confidence.

The advent of next-generation sequencing has recently facilitated analyses incorporating many more genes. Song *et al.* (2012) used complete mitochondrial genomes to infer hemipteran phylogeny and placed Membracoidea as sister to Cercopoidea and Fulgoroidea as sister to Sternorrhyncha.

However, the analysis did not include representatives of Cicadoidea, so their placement relative to Membracoidea and Cercopoidea remained ambiguous. Another analysis of a broader sample of hemipterans using mitochondrial genomes, including representatives of all auchenorrhynchan superfamilies (Li *et al.*, 2017), recovered Fulgoromorpha as sister to Coleorrhyncha and Membracoidea as sister to Cicadoidea + Cercopoidea (Fig. 1I). A later analysis of Cicadomorpha using mitochondrial genomes (Song *et al.*, 2017) included representatives from all auchnorrhynchan superfamilies and recovered Fulgoroidea as sister to [Membracoidea + (Cicadoidea + Cercopoidea)] (Fig. 1H).

Misof *et al.*'s (2014) analysis of all insect orders using transcriptomes recovered a monophyletic Auchenorrhyncha as sister to Coleorrhyncha. Within Auchenorrhyncha, Fulgoroidea was placed sister to a Cercopoidea + Cicadoidea, but the analysis included only three auchenorrhynchan representatives and no membracoids. Most recently, Johnson *et al.*'s (2018) transcriptome-based analysis of Paraneoptera found high support for {Fulgoroidea [Membracoidea (Cicadoidea + Cercopoidea)]} (Fig. 1H), but included only 34 auchenorrhynchan specimens, representing approximately half of the recognized auchenorrhynchan families. Because previous analyses have not yet achieved consensus, denser taxonomic sampling is needed to adequately resolve relationships between families within each superfamily and test the stability of the placements of Cicadoidea and Cercopoidea.

In addition to increased access to genetic data, the phylogenomic era has also highlighted the possibility of potential biases that may be exacerbated by inclusion of numerous genes in a single dataset. Notably, guanine-cytosine (GC) content heterogeneity across taxa and/or across genes included in a phylogenomic analysis can introduce conflicting signal due to biological mechanisms tied to overall GC content. When GC content varies among taxa in a study, phylogenetic analyses may group taxa together because they have similar GC content, and not because they are phylogenetically related (Weisburg *et al.*, 1989; Lockhart *et al.*, 1992; Hasegawa & Hashimoto, 1993; Collins *et al.*, 2005; Simon *et al.*, 2006). Unfortunately, there are few methods to account for variation across taxa in GC composition, and most of the methods that exist are computationally extremely intensive, especially for large datasets. However, some of these difficulties can be ameliorated using codon-based degeneracy coding (Simmons, 2017).

In the case of variation among genes, regions of the genome with high GC content have been shown to possess greater conflict in signal and longer branch lengths than genes with low GC content in vertebrates (Romiguier *et al.*, 2013; Jarvis *et al.*, 2014) and in Hymenoptera (Romiguier *et al.*, 2013; Bossert *et al.*, 2017). The widely accepted explanation is linked to a process that occurs during meiotic recombination called GC-biased gene conversion. This process acts to increase GC content in regions of the genome with high recombination rates by preferentially overwriting GC-poor alleles with GC-rich alleles. Although first described in mammals (Galtier *et al.*, 2001) and birds (Weber *et al.*, 2014), and for the first time in invertebrates in a eusocial hymenopteran (Kent *et al.*, 2012), GC-biased gene conversion is now thought to be widespread and responsible for recombination-based dynamics in genomic GC content across most metazoans, including invertebrates such as crustaceans, lepidopterans and isopterans (Pessia *et al.*, 2012; Robinson *et al.*, 2013; Galtier *et al.*, 2018). In species where GC-biased gene conversion occurs, GC content, and especially third-codon-position GC content (GC3), is correlated with relative recombination rate in that region of the genome (Romiguier *et al.*, 2010; Mugal *et al.*, 2015) and overall variance in GC3 content and average GC3 content are correlated; thus high recombination rates tend to affect a given locus in a subset of taxa rather than universally (Weber *et al.*, 2014; Figuet *et al.*, 2015).

The GC-biased gene conversion itself could cause difficulties in adequately modelling sequence evolution because it may promote the accumulation of multiple substitutions at the same site due to AT compensatory mutations after recombination hotspots have shifted, as is known to occur commonly, especially across evolutionary time in some taxa (Coop *et al.*, 2008; Comeron *et al.*, 2012; Romiguier & Roux, 2017). These regions could also contain genes that do not reflect the true species topology simply due to their frequent recombination. Areas of the genome with high recombination are also the most likely to have introgressed alleles (Martin *et al.*, 2019) and remnant alleles surviving from incomplete lineage sorting (Hobolth *et al.*, 2011) because of their higher effective population sizes. By contrast, areas of the genome with low recombination rates tend to resolve the most accurate species tree as they possess smaller effective population sizes due to hitchhiking from linked loci under selection. These regions tend to exhibit lower rates of incomplete lineage sorting, as well as having fewer introgressed alleles (Li *et al.*, 2018). Even in species without direct evidence of GC-biased gene conversion, high GC regions of the genome are still associated with high rates of recombination, e.g. in *Drosophila* autosomes (Singh *et al.*, 2005) and yeast (Kiktev *et al.*, 2018). It is possible that this recombination-linked property of more heterogeneous and higher GC content genes is of greater concern in recovering the correct species topology than it is in proper modelling of heterogeneous nucleotide content (Conant & Lewis, 2001; Romiguier & Roux, 2017). In either case, the effect of GC content heterogeneity has not been investigated in auchenorrhynchan phylogenomic datasets and, if unexplored, this potential source of systematic bias could undermine confidence in the relationships recovered from a phylogenomic analysis of Auchenorrhyncha.

Bearing in mind the previous studies and limitations of auchenorrhynchan phylogeny and potential biases introduced by GC content heterogeneity, we used transcriptome sequencing to acquire sequences of 2139 orthologous genes from 84 auchenorrhynchan species representing 27 of the 32 currently recognized families in the superorder, which represents the most comprehensive sampling of Auchenorrhyncha to date in a phylogenomic analysis. Using this dataset, we set out to construct a well-resolved, highly supported phylogeny of the Auchenorrhyncha that would allow us to address the following questions: (i) what is the relationship among the cicadomorphan superfamilies; (ii) what are the relationships among families

in Fulgoromorpha and Cicadomorpha; (iii) how do the relationships we observe compare with previous hypotheses; and (iv) does nucleotide compositional heterogeneity in terms of GC content have an effect on the topologies recovered by our dataset? Construction of a comprehensive phylogeny of Auchenorrhyncha, while addressing dataset heterogeneity, will also enhance our understanding of hemipteran evolution, facilitate further studies of comparative evolution in this group, and identify phylogenetic relationships in need of further investigation due to low support or gene tree conflict.

Materials and methods

Transcriptome sampling

We included transcriptomes from 94 taxa, including 84 auchenorrhynchans, 50 of which were newly sequenced for this study to increase taxonomic representation of auchenorrhynchan family and subfamily lineages. Four moss bugs (suborder Coleorrhyncha) and six true bugs (suborder Heteroptera) were included as outgroups based on the recent comprehensive phylogenomic analysis of Johnson *et al.* (2018). The ingroup sample includes representatives of 27 auchenorrhynchan families and is the most comprehensive sampling within this suborder to date. Raw transcriptome data were obtained from previously sequenced individuals (Misof *et al.*, 2014; Johnson *et al.*, 2018) or from individuals newly sequenced for this study. A complete list of specimens included in this study is shown in Table 1. For newly sequenced individuals, fresh specimens were ground and preserved in RNAlater® (Ambion; Austin, TX, U.S.A.) and stored at +4 or −80°C or directly frozen at −80°C until the time of extraction. RNA was extracted from the entire body of adult specimens for most species, whereas in a few cases, nymphs or individuals of unknown life stages were used. Library preparation and transcriptome sequencing of 100-bp paired-end reads of new specimens was performed at the W.M. Keck Center for Comparative and Functional Genomics at the University of Illinois at Urbana-Champaign using the Illumina TruSeq Stranded mRNAseq Sample Prep Kit and an Illumina HiSeq 4000 (Illumina, San Diego, California). We multiplexed 24 individuals per lane, to achieve at least 2.5 Gbp of raw data for the sample. Voucher specimens of different individuals, but from the same collecting event, were deposited in the Illinois Natural History Survey insect collection.

De novo transcriptome assembly

Following sequencing, FASTQ files were generated and demultiplexed using BCL2FASTQ v.2.17.1.14 conversion software (Illumina). Raw paired-end reads were assessed for quality using FASTQC (Andrews, 2010) and then trimmed using TRIMMOMATICPE (Bolger *et al.*, 2014) with a headcrop setting of 9, leading and trailing settings of 3, sliding window size of 4 with a minimum quality 15, minimum length of 50, and an Illuminaclip setting of 2:30:10. Trimmed reads were assembled using SOAPDENOVO-TRANS with $k = 49$ (Xie *et al.*, 2014). Decontamination procedures of trimmed reads followed Peters *et al.* (2017) (for details, see Johnson *et al.*, 2018, supplemental information). Assembled, decontaminated contigs were screened for vector contamination and duplicates and contigs < 200 nt long were removed before NCBI submission. Raw reads and filtered assemblies were submitted to the NCBI SRA (Sequence Read Archive) and TSA (Transcriptome Shotgun Assembly) archives. Specimen information, including species names, localities and accession numbers, are shown in Table 1.

Identification of orthologues

ORTHOGRAPH (Petersen *et al.*, 2017) was used to identify protein-coding genes corresponding to 2395 genes in the ORTHDB v.7 database (Kriventseva *et al.*, 2008) predicted to be single-copy orthologues across the arthropod species *Acromyrmex echinatior*, *Daphnia pulex*, *Pediculus humanus*, *Rhodnius prolixus*, *Tribolium castaneum* and *Zootermopsis nevadensis*. ORTHOGRAPH utilizes a graph-based approach that avoids orthology assignment of redundant transcripts and is able to account for the presence of paralogues and alternative splicing in transcripts which can mislead phylogenetic inference (Petersen *et al.*, 2017). See Johnson *et al.* (2018) for further details of gene selection.

Orthologue alignment and filtering

Orthologous amino acid sequences were aligned individually by gene using PASTA (Mirarab *et al.*, 2015) under default settings. Nucleotide sequences were then aligned with reference to the amino acid sequence alignments to preserve reading frames using a custom perl script. Amino acid and nucleotide alignments were then trimmed using TRIMAL v.1.4 (Capella-Gutierrez *et al.*, 2009) with a gap threshold of 0.25. After trimming, the dataset was filtered to reduce missing data by identifying alignments that included at least half of all ingroup taxa and at least one outgroup taxon using custom python scripts. A cutoff point of 50% ingroup taxa was chosen to maximize the number of included loci while excluding genes for which the majority of the data were missing. An additional four genes were removed due to discrepancies between the number of amino acid alignment positions and the expected number of corresponding nucleotide alignment positions. Following alignment filtering, a total of 2139 orthologues were retained for further analyses.

Maximum likelihood analyses and bootstrapping

SEQUENCE MATRIX v.100 (Vaidya *et al.*, 2011) was used to concatenate amino acid and nucleotide alignments from the 2139 retained orthologues. RAXML v.8.2.11 (Stamatakis, 2014) was used to remove completely ambiguous positions in the alignment and then to perform 100 rapid bootstrap replicates of

Table 1. National Center for Biotechnology Information accession numbers, collecting localities, and percentage missing data for Auchenorrhyncha and outgroup specimens used in this study.

Family	Species	BioSample no.	Run no.	TSA project no.	Locality	Missing data in AA and complete nt alignment (%)	Missing data in degeneracy-coded nt alignment (%)
Auchenorrhyncha							
Aphrophoridae[b]	*Aphrophora alni*	SAMN03331949	SRR2051468	GDEN00000000	Germany: Thuringia	43.5	49.0
Aphrophoridae[b]	*Philaenus spumarius*	SAMN03341992	SRR1821955	GCZA00000000	United States: Kentucky	24.2	31.7
Cercopidae[a]	*Cercopis vulnurata*	SAMN02047155	SRR921578	GAUN00000000	Germany: North Rhine-Westphalia	27.2	34.4
Cercopidae[b]	*Prosapia bicincta*	SAMN03341999	SRR1821958	GCYJ00000000	United States: Illinois	26.5	33.7
Clastopteridae	*Clastoptera obtusa*	SAMN04101382	SRR2496621	GEJT00000000	United States: Illinois	16.9	24.9
Epipygidae	*Epipyga* sp.	SAMN04101414	SRR2496653	GEJW00000000	Brazil: Minas Gerais	35.7	42.0
Machaerotidae	*Pectinariophyes stalii*	SAMN04101409	SRR2496648	GEKO00000000	Australia: Queensland	29.1	36.0
Cicadidae[a]	*Okanagana villosa*	SAMN02047193	SRR921625	GAWQ00000000	United States: California	30.1	37.1
Cicadidae	*Chilecicada* sp.	SAMN04101397	SRR2496636	GEKH00000000	Chile: Santiago Metropolitan Region	31.4	38.3
Cicadidae	*Tettigades auropilosa*	SAMN04101401	SRR2496640	GEIM00000000	Chile: O'Higgins Region	50.5	55.3
Cicadidae	*Megatibicen dorsatus*	SAMN03342009	SRR1821973	GCYV01000000	United States: Illinois	30.4	37.3
Cicadidae	*Guineapsaltria flava*	SAMN04101375	SRR2496614	GEJK00000000	Australia: Queensland	89.6	90.6
Cicadidae	*Tamasa doddi*	SAMN04101376	SRR2496615	GEIK00000000	Australia: Queensland	28.4	35.6
Cicadidae	*Burbunga queenslandica*	SAMN04101374	SRR2496613	GEJZ00000000	Australia: New South Wales	47.3	52.5
Cicadidae	*Kikihia scutellaris*	SAMN04101390	SRR2496629	GEJF00000000	New Zealand: Wellington District	41.2	47.0
Cicadidae	*Maoricicada tenuis*	SAMN04101392	SRR2496631	GEJB00000000	New Zealand: Marlborough District	40.9	46.7
Tettigarctidae	*Tettigarcta crinita*	SAMN04101386	SRR2496625	GEHU00000000	Australia: New South Wales	43.2	48.8
Aetalionidae	*Aetalion reticulatum*	SAMN06145360	SRR5134719	GFDW00000000	Brazil: Rio de Janeiro	20.0	28.1
Aetalionidae	*Lophyraspis* sp.	SAMN06145352	SRR5134711	GFEQ00000000	French Guiana	24.7	32.1
Cicadellidae[b]	*Graphocelphala fennahi*	SAMN03331970	SRR2051489	GDEF00000000	Germany: Hamburg	37.5	43.9
Cicadellidae[b]	*Dalbulus maidis*	SAMN03341953	SRR1821981	GCWP00000000	United States: Lab stock	62.2	65.7
Cicadellidae[b]	*Ponana quadralaba*	SAMN03341997	SRR1821957	GCZF00000000	United States: Illinois	18.8	27.0
Cicadellidae[b]	*Agallia constricta*	SAMN03341926	SRR1821894	GCWT00000000	United States: Illinois	28.1	35.4
Cicadellidae[b]	*Hespenedra chilensis*	SAMN03341961	SRR1821922	GCXG00000000	Chile: Los Lagos	35.4	41.9
Cicadellidae[b]	*Empoasca fabae*	SAMN03341955	SRR1821917	GCVV00000000	United States: Illinois	39.2	45.2
Cicadellidae[b]	*Vidanoana flavomaculata*	SAMN03342016	SRR1821980	GCZD00000000	Chile: Los Lagos	32.0	38.9
Cicadellidae	*Nionia palmeri*	SAMN04101395	SRR2496634	GEKN00000000	United States: Illinois	30.5	37.6
Cicadellidae	*Macropsis decisa*	SAMN04101399	SRR2496638	GEJE00000000	United States: Illinois	32.1	39.1
Cicadellidae	*Neocoelidia tumidifrons*	SAMN04101400	SRR2496639	GEJC00000000	United States: Illinois	21.2	29.1
Cicadellidae	*Xestocephalus desertorum*	SAMN04101404	SRR2496643	GELC00000000	United States: Illinois	38.9	45.0
Cicadellidae	*Idiocerus rotundens*	SAMN04101398	SRR2496637	GEJG00000000	United States: Illinois	30.2	37.3
Cicadellidae	*Tinobregmus viridescens*	SAMN04101402	SRR2496641	GEIL00000000	United States: Illinois	18.8	27.0
Cicadellidae	*Eucanthella palustris*	SAMN04101405	SRR2496644	GEJL00000000	Australia: Queensland	33.3	40.0
Cicadellidae	*Stenocotis depressa*	SAMN04101413	SRR2496652	GEIN00000000	Australia: Queensland	25.0	32.5
Cicadellidae	*Tituria* sp.	SAMN06145374	SRR5134733	GFEI00000000	China: Shaanxi	26.5	33.8
Cicadellidae	*Penthimia* sp.	SAMN06145376	SRR5134735	GFFF00000000	United States: Illinois	18.8	27.1
Cicadellidae	*Penestragania robusta*	SAMN06145377	SRR5134736	GFFC00000000	United States: Illinois	32.2	39.0
Cicadellidae	*Aphrodes bicincta*	SAMN06145363	SRR5134722	GFDZ00000000	United States: Illinois	25.3	32.8
Cicadellidae	*Agudus* sp.	SAMN06145361	SRR5134720	GFDX00000000	Brazil: Minas Gerais	39.3	45.4

Table 1. Continued

Family	Species	BioSample no.	Run no.	TSA project no.	Locality	Missing data in AA and complete nt alignment (%)	Missing data in degeneracy-coded nt alignment (%)
Cicadellidae	*Haldorus* sp.	SAMN06145381	SRR5134740	GFEX00000000	Brazil: Minas Gerais	52.7	57.5
Cicadellidae[b]	*Ulopa reticulata*	SAMN03331994	SRR2051513	GDEO00000000	Germany: Lower Saxony	58.9	63.0
Melizoderidae[b]	*Llanquihuea pilosa*	SAMN03341973	SRR1821930	GCWX00000000	Chile: Los Lagos	38.8	45.0
Membracidae[b]	*Centrotus cornutus*	SAMN03331956	SRR2051475	GDFF00000000	Croatia: Zadar	35.6	42.0
Membracidae[b]	*Stictocephala bisonia*	SAMN03342008	SRR1821970	GCZK00000000	United States: Illinois	21.7	29.6
Membracidae[b]	*Enchenopa latipes*	SAMN03341938	SRR1821904	GCWI00000000	United States: Illinois	22.3	30.1
Membracidae[b]	*Nessorhinus gibberulus*	SAMN03341984	SRR1821942	GCYF00000000	United States: Puerto Rico	38.2	44.5
Membracidae	*Microcentrus caryae*	SAMN04101394	SRR2496633	GEIX00000000	United States: Illinois	20.9	28.9
Membracidae[b]	*Holdgatiella chepuensis*	SAMN03341963	SRR1821924	GCXW00000000	Chile: Los Lagos	30.7	37.8
Membracidae	*Notocera* sp.	SAMN06145375	SRR5134734	GFFB00000000	Brazil: Roraima	28.1	35.3
Membracidae	*Lycoderes burmeisteri*	SAMN06145370	SRR5134729	GFER00000000	Brazil: Rio de Janeiro	24.2	31.9
Membracidae	*Umbonia crassicornis*	SAMN04101403	SRR2496642	GEIZ00000000	United States: Puerto Rico	91.6	92.4
Membracidae	*Heteronotus* sp.	SAMN04101416	SRR2496655	GEJQ00000000	Brazil: Rio de Janeiro	43.4	49.1
Membracidae	*Amastris* sp.	SAMN06145362	SRR5134721	GFDY00000000	Brazil: Roraima	28.5	35.6
Membracidae	*Cyphonia clavata*	SAMN06145366	SRR5134725	GFEC00000000	Brazil: Rio de Janeiro	28.6	35.7
Membracidae	*Membracis tectigera*	SAMN06145371	SRR5134730	GFET00000000	Brazil: Rio de Janeiro	29.4	36.5
Membracidae	*Tolania* sp.	SAMN06145383	SRR5134742	GFEO00000000	Brazil: Roraima	55.4	59.8
Membracidae	*Chelyoidea* sp.	SAMN06145365	SRR5134724	GFEB00000000	Brazil: Rio de Janeiro	37.3	43.6
Membracidae	*Procyrta* sp.	SAMN06145379	SRR5134738	GFFD00000000	Brazil: Roraima	27.2	34.6
Myerslopiidae[b]	*Mapuchea* sp.	SAMN03341978	SRR1821937	GCXN00000000	Chile: Los Lagos	23.0	30.9
Acanaloniidae[b]	*Acanalonia conica*	SAMN03341923	SRR1821891	GCXC00000000	United States: Kentucky	24.9	32.3
Achilidae	*Catonia nava*	SAMN04101381	SRR2496620	GEHT00000000	United States: Illinois	26.8	34.0
Caliscelidae[b]	*Caliscelis bonelii*	SAMN03331955	SRR2051474	GDFE00000000	Italy: Umbria	33.3	39.5
Caliscelidae[b]	*Bruchomorpha oculata*	SAMN03341935	SRR1821902	GCWF00000000	United States: Illinois	23.4	30.8
Cixiidae[b]	*Tachycixius pilosus*	SAMN03331993	SRR2051512	GDEX00000000	Germany: Thuringia	63.2	66.8
Cixiidae[b]	*Melanoliarus placitus*	SAMN03341980	SRR1821943	GCZE00000000	United States: Illinois	30.5	37.5
Delphacidae[b]	*Nilaparvata lugens*	SAMN02047185	SRR921622	GAYF00000000	Switzerland: Lab stock	23.8	31.4
Delphacidae[b]	*Idiosystatus acutiusculus*	SAMN03341965	SRR1821950	GCXZ00000000	Chile: Los Lagos	27.9	35.1
Derbidae	*Omolicna uhleri*	SAMN04101384	SRR2496623	GEIW00000000	United States: Illinois	24.2	31.6
Dictyopharidae[b]	*Dictyophara europaea*	SAMN03331963	SRR2051482	GDEP00000000	Germany: Thuringia	54.3	58.7
Dictyopharidae[b]	*Phylloscelis atra*	SAMN03341994	SRR1821952	GCYH00000000	United States: Illinois	23.8	31.2
Dictyopharidae[b]	*Chondrophana gayi*	SAMN03341944	SRR1821915	GCYG00000000	Chile: Los Lagos	31.2	37.9
Dictyopharidae	*Yucanda albida*	SAMN06145384	SRR2496623	GEIW00000000	United States: Nevada	48.8	53.8
Eurybrachidae	*Platybrachys* sp.	SAMN04101411	SRR2496650	GEIQ00000000	Australia: Queensland	35.9	42.1
Flatidae[b]	*Metcalfa pruinosa*	SAMN03331985	SRR2051504	GDFH00000000	Italy: Umbria	35.9	42.2
Flatidae	*Jamella australiae*	SAMN04101418	SRR2496657	GEJH00000000	United States: Queensland	23.4	30.9

Table 1. Continued

Family	Species	BioSample no.	Run no.	TSA project no.	Locality	Missing data in AA and complete nt alignment (%)	Missing data in degeneracy-coded nt alignment (%)
Fulgoridae[b]	*Cyrpoptus belfragei*	SAMN03341951	SRR1821913	GCWQ00000000	United States: Illinois	27.7	34.8
Fulgoridae	*Lycorma delicatula*	SAMN06145353	SRR5134712	GFES00000000	China: Shaanxi	48.1	53.0
Issidae	*Thionia simplex*	SAMN04101396	SRR2496635	GEIJ00000000	United States: Illinois	21.4	29.2
Nogodinidae	*Lipocallia australensis*	SAMN04101407	SRR2496646	GEIY00000000	Australia: Queensland	40.3	46.1
Nogodinidae	*Bladina* sp.	SAMN06145364	SRR5134723	GFEA00000000	Brazil: Roraima	72.6	75.2
Ricaniidae	*Scolypopa* sp.	SAMN04101412	SRR2496651	GEIO00000000	Australia: Queensland	37.6	43.7
Ricaniidae	*Ricania speculum*	SAMN06145380	SRR5134739	GFEY00000000	China: Shaanxi	26.8	34.0
Tettigometridae	*Tettigometra bipunctata*	SAMN06145359	SRR5134718	GFEJ00000000	China: Shaanxi	23.3	30.6
Tropiduchidae[b]	*Ladella* sp.	SAMN03341969	SRR1821928	GCXO00000000	United States: Puerto Rico	39.8	45.7
Coleorrhyncha							
Peloridiidae	*Hackeriella veitchi*	SAMN06145349	SRR5134708	GFEH00000000	Australia: Queensland	29.6	37.0
Peloridiidae	*Peloridium pomponorum*	SAMN03341991	SRR1821949	GCZG00000000	Chile: Los Lagos	31.3	38.4
Peloridiidae[b]	*Xenophyes metoponcus*	SAMN03331996	SRR2051515	GDEM01000000	New Zealand: Westland District	40.2	46.4
Peloridiidae[a]	*Xenophysella greensladeae*	SAMN02047181	SRR921658	GAYI02000000	New Zealand: Westland District	44.2	50.0
Heteroptera							
Ceratocombidae[b]	*Ceratocombus* sp.	SAMN03341939	SRR1821906	GCWS01000000	Argentina: Salta	30.8	37.7
Hydrometridae[b]	*Hydrometra stagnorum*	SAMN03331975	SRR2051494	GDEG01000000	Germany: North Rhine-Westphalia	52.1	57.0
Veliidae[a]	*Velia caprai*	SAMN02047131	SRR921656	GAUO02000000	Germany: Lower Saxony	49.6	54.6
Corixidae[b]	*Corixa punctata*	SAMN03331959	SRR2051478	GDDR01000000	Germany: Lower Saxony	52.4	57.1
Gelastocoridae[b]	*Gelastocoris oculatus*	SAMN03341957	SRR1821920	GCXI01000000	United States: Illinois	29.9	36.8
Notonectidae[b]	*Notonecta glauca*	SAMN03331987	SRR2051506	GDEH01000000	Germany: North Rhine-Westphalia	50.7	55.8

[a]From Misof *et al.* (2014).
[b]From Johnson *et al.* (2018).
AA, amino acid; nt, nucleotide.

maximum likelihood searches for the concatenated amino acid and nucleotide alignments. DECISIVATOR v.0.591 (Brown, 2017; available at: https://github.com/josephwb/Decisivator, accessed 24 May 2019) was used to determine gene occupancy of the alignment matrices and complete decisiveness of the concatenated datasets (Sanderson *et al.*, 2010). DECISIVATOR indicated that gene occupancy across the concatenated alignments was the same, so SUMAC (Freyman, 2015) was used to calculate the percentage of missing genes for each taxon using the complete concatenated nucleotide alignment. For maximum likelihood searches, five non-identical randomized stepwise addition order parsimony trees and five completely random starting trees were created using RAXML for both the concatenated amino acid and nucleotide alignments. These starting trees were used in separate EXAML v.3 (Kozlov *et al.*, 2015) maximum likelihood searches. For the amino acids, the RAXML option PROTGAMMAAUTO was used during the bootstrapping phase to select the best amino acid substitution matrix for maximum likelihood analysis; the JTT model was selected and EXAML runs were conducted under JTT + Γ. For nucleotide analyses, EXAML runs were conducted under GTR + Γ. The preferred trees were those with the best maximum likelihood scores.

Additionally, PARTITIONFINDER2 (Lanfear *et al.*, 2016) with rcluster_max = 100 (Lanfear *et al.*, 2014) was used to partition the nucleotide dataset by gene. Alignments and partition schemes were submitted to the University of Illinois at Urbana-Champaign Illinois Data Bank (UIUC IDB) (https://doi.org/10.13012/B2IDB-1461292_V1). Bootstrapping and maximum likelihood searches on the partitioned nucleotide dataset were performed as described earlier for the concatenated dataset. Resulting trees were visualized using ITOL, with bootstrap values displayed as text and 'position on branch' set to 50% (Letunic & Bork, 2016). Trees were rooted using Heteroptera as the outgroup. Newick files for each tree were deposited in the UIUC IDB.

GC content, degeneracy coding and third-codon GC content analyses

Heterogeneity in GC content could occur either at the level of species or at the level of genes, and we explored both of these possibilities. As GC content has been shown to be a source of systematic bias that can affect phylogenetic inference (Romiguier *et al.*, 2013; Jarvis *et al.*, 2014; Bossert *et al.*, 2017), we performed several analyses to examine the role of GC content in our dataset. At the level of species, the GC content of each codon position across all genes from each species was measured using a custom script and the results were plotted using the GGPLOT2 package in R (Wickham, 2016). A chi-squared test of compositional heterogeneity was performed on the concatenated nucleotide alignment using PAUP* v.4.0a (build 165) (Swofford, 2002) to statistically investigate compositional heterogeneity, a known source of potential systematic bias in phylogenomic datasets (Weisburg *et al.*, 1989; Lockhart *et al.*, 1992; Hasegawa & Hashimoto, 1993; Phillips *et al.*, 2004; Collins *et al.*, 2005; Jeffroy *et al.*, 2006; Regier *et al.*, 2010). We also employed a codon degeneracy coding strategy (Regier *et al.*, 2010) to investigate the effect of compositional heterogeneity on the recovered maximum likelihood concatenated and partitioned topologies. Degeneracy coding is an extension of R-Y coding that reduces nucleotide compositional heterogeneity by fully degenerating nucleotides at sites that could potentially undergo synonymous change, while leaving sites undergoing nonsynonymous change mostly unaltered. The DEGEN_V1_4 script (Zwick & Hussey, 2008) was used to recode codons in trimmed nucleotide alignments as degenerated codons using IUPAC ambiguity codes. The alignments were then concatenated and columns consisting solely of missing data were removed using RAXML. PARTITIONFINDER2 was used to estimate optimal gene partitioning schemes for the concatenated dataset. The concatenated and partitioned degeneracy-coded datasets were used for maximum likelihood analyses in EXAML, and 100 rapid bootstraps for each dataset were obtained in RAXML following the protocols described for the nondegeneracy-coded nucleotides.

We also investigated GC compositional heterogeneity at the level of the gene. In particular, we wanted to determine whether we could partition support for alternative topologies observed in different analyses into gene bins that are predicted to be more and less reliable, based on GC3 content, in recovering the underlying species tree. We calculated the average GC3 content and GC3 variance for all of the 2139 genes and then ranked genes by average GC3 and GC3 variance. We then placed the 2139 genes into 21 100-gene bins (discarding the 39 genes with GC3 variance and GC3 content closest to the median). For each GC3 and GC3 variance bin, we collapsed nodes in gene trees with < 10% bootstrap support using nw-ed in newick_utils (Junier & Zdobnov, 2010) and recorded how many nodes were collapsed in each bin. A multispecies coalescent analysis in ASTRAL was used to obtain the species tree and normalized quartet score for each bin in order to determine if there was greater conflict in higher GC3 and GC3 variance genes. We also measured average branch length of input gene trees for both sets of 21 bins. Plots were constructed using R (R Core Team, 2018).

Next, we binned the 2139 genes ranked by GC3 heterogeneity and average GC3 content into quintiles containing a roughly equivalent number of sites (*c.* 53 000 nucleotides). We then analysed the partitioned top, middle, and bottom quintiles with RAXML for each of three forms of the data: (i) nucleotide; (ii) first and second codon positions only (hereafter nt12); and (iii) amino acids. Nucleotide datasets were partitioned by gene and codon position and amino acids datasets were partitioned by gene and assigned a JTT model. We assessed congruence among these 18 datasets by UPGMA clustering of resulting trees based on normalized Robinson–Foulds distances and calculated average bootstrap support across trees to assess relative total confidence. We assessed saturation of each of nucleotide quintile using the Xie *et al.* (2008) test implemented in DAMBE7 on fully resolved sites (Xia, 2018) and by plotting model-uncorrected transversions and transitions against JC69 distances (Fig. S9).

In an effort to determine whether low conflict in low-GC3-variance and low-GC3-content gene quintiles that contained many of the same genes was due primarily to one

property or another, we also calculated normalized quartet distance scores of the intersection and unique elements of these sets for the bottom (368 loci shared out of a total 578 or 585, respectively) and top quintiles (146 loci shared out of a total 358 and 378, respectively).

Multispecies coalescent analysis

To explore the effects of possible conflict among gene trees on the phylogeny, individual gene trees and 100 bootstrap replicates for each gene were obtained from trimmed nucleotide alignments using RAXML under a GTR + Γ model. These gene trees were used as input for a multispecies coalescent analysis using ASTRAL v.4.10.5 under default settings (Mirarab & Warnow, 2015). ASTRAL analysis was performed using the -t 2 full annotation option, which provides several metrics including branch support values in the form of local posterior probabilities (Sayyari & Mirarab, 2016), quartet support as the percentage of quartets in the gene trees that agree with the branch for each of the three possible quartets at each branch, and effective number, which indicates the number of gene trees that contributed information to a branch. The resulting multispecies coalescent tree and local posterior clade probabilities were visualized in FIGTREE v.1.4.4 (Rambaut, 2018), and ETE TOOLKIT (Huerta-Cepas *et al.*, 2016) was used to visualize quartet support and effective number.

Results

A final total of 2139 orthologue genes were included in this study. Across alignments, the average number of taxa per locus was 84.8, and the average number of loci per taxon was 1930.2. No taxon had sequence data for all orthologues, and 245 orthologue alignments included all taxa. Gene occupancy data are shown in Tables S1 and S2. The final amino acid alignment contained 882 806 positions, including 615 838 variable and 485 378 parsimony-informative sites. The concatenated and partitioned nucleotide alignment contained 2 648 418 sites, including 1 932 685 variable and 1 695 924 parsimony-informative sites. Both the amino acid and nucleotide alignments contained 35.34% of sites for which no data were available (missing data). DECISIVATOR indicated that matrix occupancy for the complete nucleotide and amino acid datasets was 90.2% and the datasets were decisive for all possible trees.

As degeneracy coding can result in conversion of sites from data present to data absent [e.g. conversion of GGG → GGN (glycine)], the degeneracy-coded alignment contained an increased amount of missing data compared with the nondegeneracy-coded alignment. Additionally, degeneracy coding resulted in more columns that contained only missing data which were removed by RAXML before analyses, leading to a decreased number of alignment positions compared with the nondegeneracy-coded alignment, but matrix occupancy remained at 90.2% and the matrix was decisive for all possible trees. The final degeneracy-coded nucleotide alignment contained 2 464 828 positions, including 964 502 variable sites, 723 696 parsimony-informative sites, and 41.78% missing data.

Across taxa, GC content at the first codon position ranged from 47.3% in *Tettigometra bipunctata* to 54.7% in *Hackierella veitchi*. At the second codon position, GC content ranged from 35.6% in *Dalbulus maidis* to 38.4% in *Hackeriella veitchi*. The GC content at the third codon position was more variable and ranged from 19.8% in *Phylloscelis atra* to 63.0% in *Graphocephala fennahi*. Results of the chi-squared analysis of compositional heterogeneity for the concatenated complete nucleotide alignment were highly significant ($\chi^2 = 1\,256\,318.35$, df = 279, $P < 1 \times 10^{-8)}$) A box plot showing the distribution of GC content for each codon across taxa is shown in Fig. 2.

Maximum likelihood analyses

Maximum likelihood scores of the best trees from each analysis performed are summarized in Table 2. The best trees from the partitioned degeneracy-coded and complete nucleotide datasets were highly resolved and generally well supported by bootstrapping (Fig. 3). The partitioned degeneracy-coded and complete nucleotide trees were generally similar with high bootstrap support (Figs S1 and S2, respectively). The partitioned degeneracy-coded nucleotide dataset recovered Cercopoidea as sister to Membracoidea with less than maximum bootstrap support (Fig. 3A), whereas the amino acid analysis recovered this relationship with full bootstrap support (Fig. S3). The partitioned complete nucleotide dataset recovered Cercopoidea as sister to Cicadoidea with maximum support (Fig. 3B), and the multispecies coalescent analysis also recovered the Cercopoidea + Cicadoidea relationship with maximum posterior probability support (Figs 4, S4). The quartet scores for this branch indicated that slightly more than half of the contributing gene trees supported this relationship (Fig. 4). Concatenated analysis of the degeneracy-coded nucleotide dataset recovered Cercopoidea as sister to Membracoidea (Fig. S5), whereas concatenated analysis of the complete nucleotide data recovered Cercopoidea as sister to Cicadoidea (Fig. S6).

Within the Fulgoroidea, all analyses consistently placed a clade containing Delphacidae and Cixiidae as sister to the remaining fulgoroids. The placement of other fulgoroids was mostly consistent between analyses, with the exception the genus *Bladina* (Nogodinidae). The degeneracy-coded nucleotide and amino acid analyses placed *Bladina* sister to *Platybrachus* (Eurybrachidae) with high support (Figs 3A, S1, S3), whereas the partitioned and concatenated complete nucleotide analyses placed *Bladina* as sister to *Thionia simplex* (Issidae) with moderate support (Figs 3B, S6). Both placements render Nogodinidae paraphyletic because the other representative of this family (*Lipocallia australensis*) was consistently placed as sister to Ricaniidae with high support. Additionally, although the majority of the family-level relationships within Fulgoroidea were highly supported in the maximum likelihood

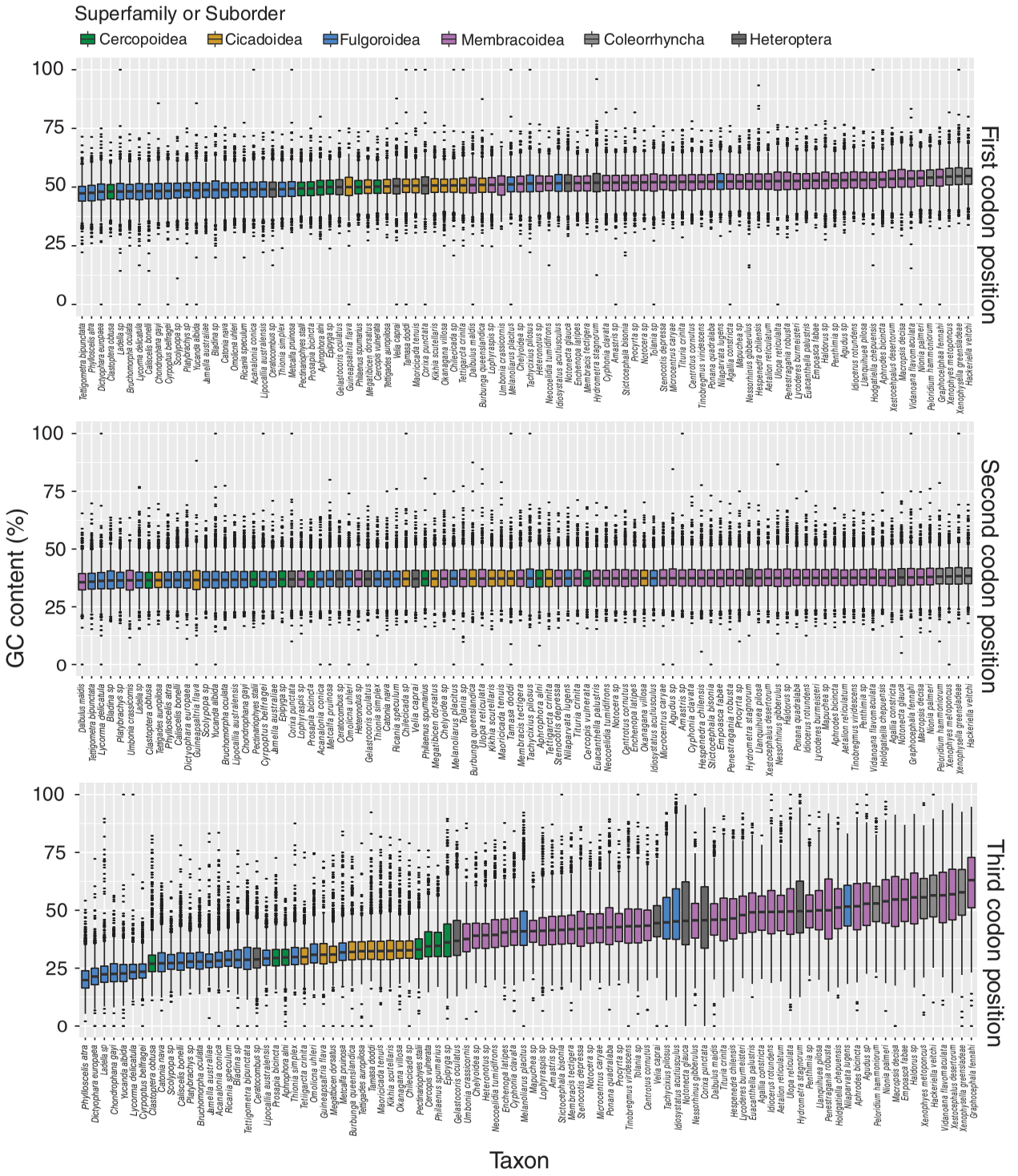

Fig. 2. Variation in guanine-cytosine (GC) content across auchenorrhynchan and outgroup taxa at the first, second and third codon positions of individual orthologues. The lower and upper box boundaries correspond to the 25th and 75th quartiles, respectively. The upper whisker corresponds to the largest value $\leq 1.5 \times$ interquartile range, and the lower whisker corresponds to the smallest value in the $1.5 \times$ interquartile range. Outlier genes beyond these boundaries are represented by individual points. Boxes are coloured according to superfamily (for ingroups) or suborder (for outgroups). [Colour figure can be viewed at wileyonlinelibrary.com].

Table 2. Likelihood scores of the best trees obtained from maximum likelihood analysis in EXAML.

Dataset	Likelihood score of best tree
Concatenated amino acid	−28 523 711.9
Complete concatenated nucleotide	−82 805 459.9
Complete partitioned nucleotide	−82 532 202.4
Degeneracy-coded concatenated nucleotide	−30 658 511.6
Degeneracy-coded partitioned nucleotide	−30 489 206.1

analyses and multispecies coalescent analyses, the clade containing Eurybrachidae, Acanaloniidae, Flatidae, Nogodinidae, Issidae and Ricaniidae included several branches with low to moderate bootstrap or local posterior probability support in each analysis.

Degeneracy-coded nucleotide and amino acid analyses likewise differed from the concatenated and partitioned nucleotide analyses in their placement of *Clastoptera obtusa* (Clastopteridae) and *Pectinariophyes stalii* (Machaerotidae) within Cercopoidea. Degeneracy-coded and amino acid analyses place *Clastoptera* as sister to the remaining cercopoids, whereas the nucleotide analyses placed *Pectinariophyes* in this position. However, support for the placement of *Pectinariophyes* was low [bootstrap support (BS) = 53] in the partitioned complete nucleotide analysis (Figs 3B, S2) and moderate (BS = 73) in the concatenated complete nucleotide analysis (Fig. S6). By contrast, in the degeneracy-coded and amino acid analyses, there is high support for all branches within Cercopoidea (Figs 3A, S1, S3, S5). The remaining relationships within the Cercopoidea were consistent across analyses, with Aphrophoridae recovered as a polyphyletic group.

In the Cicadoidea, the family Tettigarctidae, represented by *Tettigarcta crinita*, was recovered with maximum support as sister to Cicadidae in all analyses. Within the family Cicadidae, three highly supported monophyletic lineages corresponding to the three sampled cicada subfamilies were recovered in all analyses.

In Membracoidea, concatenated analyses consistently yielded high support for the placement of Myerslopiidae, represented by *Mapuchea*, as sister to the remaining taxa in the Membracoidea. The family Cicadellidae was always recovered as paraphyletic with respect to Membracidae and Aetalionidae. However, the placement of the clade containing *Llanquihuea pilosa* and *Holdgatiella chepuensis* differed between analyses. Although degenerate nucleotide and amino acid analyses placed this clade sister to the remaining treehoppers with high support (Figs 3A, S1, S3 S5), thus rendering Membracidae nonmonophyletic, the partitioned and concatenated complete nucleotide analyses placed this clade in more derived position within Membracidae (Figs 3B, S2, S6), also with high support (BS = 100 for both). Additionally, the placement of Aetalionidae differed between analyses; it was placed sister to the Stegaspidinae + Centrotinae with high support in the degeneracy-coded nucleotide and amino acid analyses (Figs 3A, S1, S3, S5), whereas it was sister to the remaining treehoppers in the complete nucleotide analyses (Figs 3B, S2, S6). Within the membracids, the subfamily Stegaspidinae was paraphyletic with respect to Centrotinae, and Membracinae and Smiliinae were recovered as monophyletic groups with high support. The placement of *Heteronotus* differed between analyses, with all but the amino acid analysis placing *Heteronotus* as sister to Membracinae + Smiliinae. Amino acids placed *Heteronotus* as sister to *Procyrta*, but with 88% bootstrap support.

The concatenated analyses also differed in their placement of *Empoasca*. In the concatenated amino acid analysis, *Empoasca* was recovered as sister to the remaining cicadellids with moderate support (BS = 89); however, this relationship received low support in the concatenated (BS = 26) and partitioned (BS = 70) degenerate nucleotide analyses. In both the concatenated and partitioned nucleotide analyses, the clade *Empoasca* + *Macropsis* was recovered as sister to the remaining cicadellids with high support (BS = 98 for concatenated, BS = 99 for partitioned). This placement of *Macropsis* renders the cicadellid subfamily Eurymelinae polyphyletic, as the other eurymeline included in this analysis, *Idiocerus rotundens*, was nested within Cicadellidae.

The cicadellid subfamily Deltocephalinae was recovered as monophyletic with maximum bootstrap support in all maximum likelihood trees, as were Cicadellinae and Ledrinae. Other subfamilies were represented by single individuals or received < 100% bootstrap support. Additionally, relatively lower support among branches was observed in all trees in the clade comprising Aphrodinae, Iassinae, Coelidiinae, Eucanthallinae, Tartressinae and Ledrinae, and the placements of these clades relative to one another was not consistent between analyses.

Multispecies coalescent analysis

The multispecies coalescent analysis also recovered each of the four superfamilies within Auchenorrhyncha as monophyletic with high support (Figs 4, S4) and recovered many of the relationships observed in the maximum likelihood nucleotide analyses, including unaltered third codon positions. This analysis found strong support for the relationship of Cercopoidea + Cicadoidea, but only 51.6% of the 2134 quartets contributing to this branch recovered this relationship (Fig. 4). As in the maximum likelihood analyses, many of the relationships across the species tree received high support as measured by local posterior probabilities, but the placement of *Bladina* and several other relationships within the Fulgoroidea received low or submaximal support. In the Membracoidea, a clade containing *Empoasca* + *Macropsis* received less than maximal support (local pP = 0.9767, quartet score = 36.0%), as did several other relationships among the remaining Cicadellidae. The clade including *Llanquihuea* + *Holdgatiella* was placed within Membracidae, as was observed in the complete nucleotide analyses, although the monophyly of this clade plus the remaining membracids received less than maximum support (local posterior probability (pP) = 0.9921, quartet score = 38.0%). Additionally, the quartet scores (Fig. 4) indicate that many

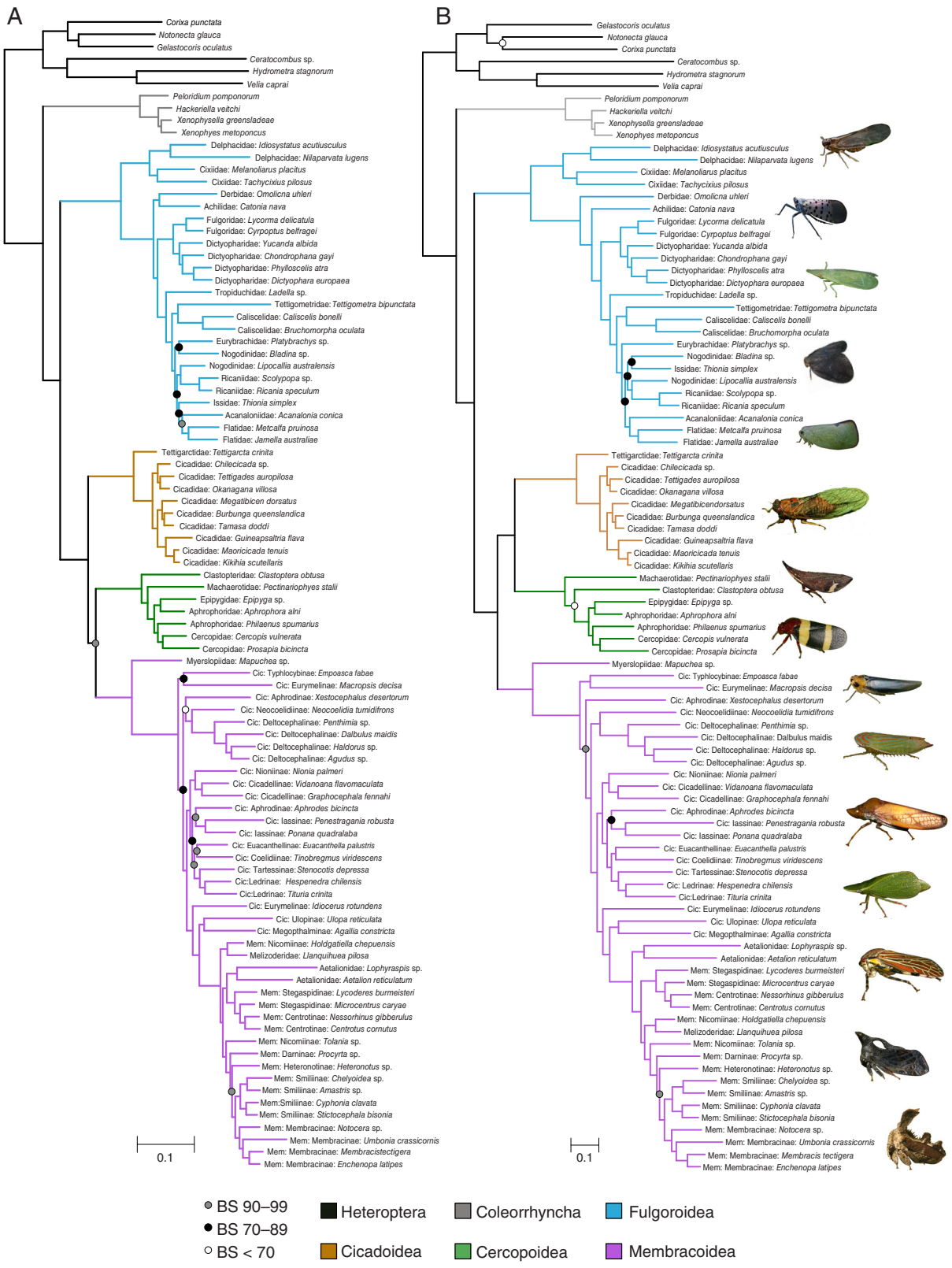

Fig. 3. Legend on next page. [Colour figure can be viewed at wileyonlinelibrary.com].

branches of the main topology were recovered only by a slim majority or a minority of gene trees, and the monophyly of Auchenorrhyncha was supported by only 44.5% of quartets in the species tree obtained from gene trees produced using alignments including the third codon position and by 46.3% of quartets in the degeneracy-coded species tree.

GC3 analyses

The total GC3 variance of individual genes ranged from 0.000 31 to 0.011 40, and the GC3 content ranged from 21.2% to 74.4% (Table S3). We found a strong correlation between both GC3 variance and GC3 content with intra-bin gene tree conflict (Table S4; Fig. S7A,B; variance: $R = 0.928$, $P = 1.403\text{E}{-}09$; content: $R = 0.883$, $P = 1.188\text{E}{-}07$) and no significant relationship of either measure with the number of nodes collapsed in input gene trees ($P = 0.145$ for variance and 0.120 for content). We also found that the average branch length of input gene trees was correlated with both measures (Fig. S7C,D; variance: $R = 0.956$, $P = 1.307\text{E}{-}11$; content: $R = 0.863$, $P = 4.648\text{E}{-}07$). The GC3 heterogeneity and GC3 content themselves were correlated across variance-ranked gene bins of 100 (Fig. S7E; $R = 0.945$, $P = 1.155\text{E}{-}10$) and vice versa for content-ranked gene bins (Fig. S7F; $R = 0.972$, $P = 2.607\text{E}{-}13$) as is found in other species with demonstrated GC biased gene conversion (Figuet *et al.*, 2015).

Normalized quartet scores as a measure of concordance were slightly greater in the lowest quintile bin of low GC3 variance compared with low GC3 content (0.903 vs 0.902) and lower in the highest quintile of variance versus content (0.865 vs 0.873; Table S5). In our investigation to disentangle the relative contributions of GC3 variance and GC3 content (Table S8), we found that overall concordance was greatest in the 368 genes that were lowest in both GC3 content and GC3 heterogeneity (0.905), with the unique low-variance genes displaying a higher concordance among themselves than the unique low-GC3-content genes (0.899 vs 0.895). Similarly, the common set of 146 high-GC3-variance and high-GC3-content genes displayed the highest incongruence (0.859) with the unique high-variance genes less congruent with each other than the unique high-content genes (0.869 vs 0.881). This is consistent with both GC3 variance and GC3 content playing a role in incongruence in gene tree resolution, but heterogeneity playing a relatively larger role than GC3 content alone.

Across the 18 datasets of bottom, middle and high GC3 heterogeneity and content-ranked quintiles of amino acid, codon position 1 and 2, and nucleotide datasets (Table S6), we found that the four most topologically congruent trees included both the lowest GC3 variance and the lowest GC content nucleotide quintiles as well as the middle GC3 nucleotide content and lowest GC3 variance nt12 datasets (Fig. S8; Table S7). We summarized support values from these four trees on the low-GC3-content nucleotide tree (Fig. 5) which possessed the highest average bootstrap support of all the quintile trees analysed (97.98%; Table S6), indicating lower levels of conflict in this dataset than in other concatenated quintile datasets. This tree was almost completely congruent with the total nucleotide tree (Fig. S2), but with higher support values on several branches mainly within Fulgoroidea and for deep splits within Membracoidea, possibly indicating successful partitioning of genes supporting different topologies. Indeed, in the middle-GC3-content nucleotide quintile, the topology was largely the same as for the low-GC3 (and low-GC3-variance) nucleotide quintiles, but the support was reduced for many branches (15 branches in both trees had lower support than the 100% bootstrap support in the low-GC3-content tree). The topology of this tree differed from the total nucleotide tree only among interrelationships in the clade of Cicadellidae, including Aphrodinae, Iassinae, Coelidiinae, Eucanthallinae, Tartressinae and Ledrinae, and with respect to the sister group to the rest of the Cercopoidea being *Clastoptera* rather than *Pectanariophyes*. Two relationships were recovered in the three shown nucleotide analyses but not in the low-heterogeneity nt12 analyses, presumably because recovery of that topology was driven primarily by third nucleotide sites. The relationships supported in the nt12 analyses but not in any of the nucleotide trees described were *Catonia* + *Omolicna* and Melizoderidae + (Aetalionidae + Membracidae). Underestimation of transitions and especially transversions at third nucleotide positions was apparent at high phylogenetic distances in all datasets, but was less severe in the low quintiles than in the middle and top quintiles (Fig. S9). Whereas the first two codon positions never showed signs of saturation, third codon positions did show signs of saturation according to the Xia et al. (2003) test across all quintiles, except for the top heterogeneity quintile, when the true tree was completely asymmetrical (Table S6).

Discussion

Although recent phylogenetic results converge on a monophyletic Auchenorrhyncha, the relationships among major lineages of Auchenorrhyncha remain contentious. Phylogenies using morphological or molecular data from small numbers of loci have failed to confidently resolve relationships within this insect group. Here, we have assembled a phylogenomic dataset

Fig. 3. Maximum likelihood trees of Auchenorrhyncha and outgroup taxa from partitioned nucleotide analyses. (A) Best tree recovered from partitioned maximum likelihood analysis of the degeneracy-coded nucleotide dataset. (B) Best tree recovered from partitioned maximum likelihood analysis of the nucleotide dataset containing unaltered third codon positions. Coloured circles are shown at nodes that received less than maximum support in their respective analyses. Branches are coloured according to superfamily (ingroups) or suborder (outgroups). Within Membracoidea, subfamily names are given in addition to family designation(Cic, Cicadellidae; Mem, Membracidae). Photographs shown are representatives of the corresponding higher-level groups but are not necessarily species represented in the analysis. Complete bootstrap support values for the partitioned degeneracy-coded nucleotide and partitioned unaltered third-codon-position nucleotide analysis are shown in Figures S1 and S2, respectively. (Photographs by C. Dietrich and R. Skinner.) . [Colour figure can be viewed at wileyonlinelibrary.com].

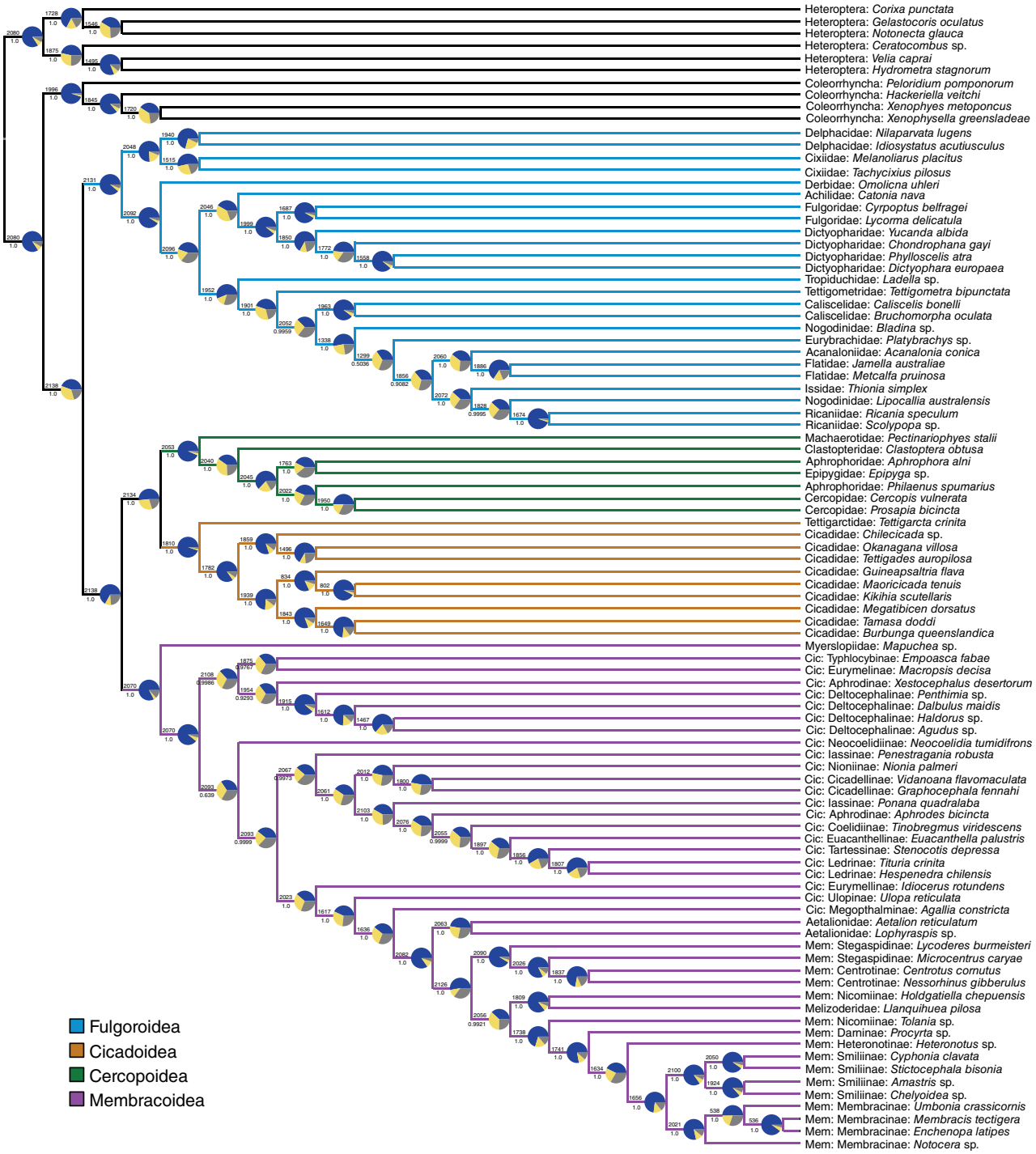

Fig. 4. Cladogram of the tree resulting from the multispecies coalescent analysis in ASTRAL showing quartet support from 2139 gene trees of Auchenorrhyncha and outgroup taxa. Each pie chart represents the proportion of gene trees supporting the main topology (= the topology shown) (navy), the proportion supporting the first alternative quartet topology (yellow), and the proportion supporting the second alternative quartet topology (grey) at each branch. Local posterior probability support values are shown below each branch. Effective number, indicating how many gene trees contributed information to a branch and thus could be used to derive quartet scores, is shown above each branch. [Colour figure can be viewed at wileyonlinelibrary.com].

using the most comprehensive taxon and gene sampling within Auchenorrhyncha to date. In this analysis, based on over 2.6 million aligned nucleotides, each of the four currently recognized superfamiles was recovered as monophyletic with strong support (Fig. 3) and all analyses recovered Fulgoroidea as sister to the remaining auchenorrhynchans with 100% branch support. This placement has been observed in previous multigene molecular studies (Cryan & Urban, 2012), including a recent transcriptome analysis of all Paraneoptera (Johnson *et al.*, 2018).

Most previous analyses have recovered Cicadomorpha as monophyletic with strong support, but relationships among the three cicadomorphan superfamilies (Cicadoidea, Cercopoidea and Membracoidea) have not been consistent in previous studies. This was also the case in our results. The degeneracy-coded nucleotide and amino acid datasets recovered the relationship (Cicadoidea + (Cercopoidea + Membracoidea)). This was maximally supported in the amino acid analysis but not in the degeneracy-coded analyses (Figs 3A, S1, S3, S5). This relationship was also observed in some early molecular studies of the Auchenorrhyncha using 18S sequences (Sorensen *et al.*, 1995; von Dohlen & Moran, 1995); however, this molecule is problematic in that it is notoriously difficult to align and model due to strong among-site rate variation (Shull *et al.*, 2001) and the authors of these early studies did not have sophisticated modelling available to them. This arrangement was also supported by morphological evidence including the presence of a subgenital plate on the male genital capsule and absence of the median ocellus in all modern cercopoids and membracoids (Hamilton, 1981; Blocker, 1996), although both superfamilies include extinct lineages that retain the medial ocellus (Shcherbakov, 1992; Wang *et al.*, 2012).

By contrast, we obtained the relationship [Membracoidea (Cicadoidea + Cercopoidea)] with 100% bootstrap support in both concatenated and partitioned analyses of the nucleotide dataset with third codon positions included. This result was also recovered with maximum local posterior probability support in the multispecies coalescent analysis, and in our analyses when excluding the third position entirely. The sister relationship of Cicadoidea and Cercopoidea has been recovered from single-gene, multigene, and mitochondrial genome analyses, although it has not always received high support in previous studies (Campbell *et al.*, 1995; Ouvrard *et al.*, 2000; Cryan, 2005; Cryan & Urban, 2012; Song *et al.*, 2017). Several authors have supported this relationship based on morphological evidence, such as similarities of cicadoid and cercopoid antennae (Liang & Fletcher, 2002), secretory adaptations of the

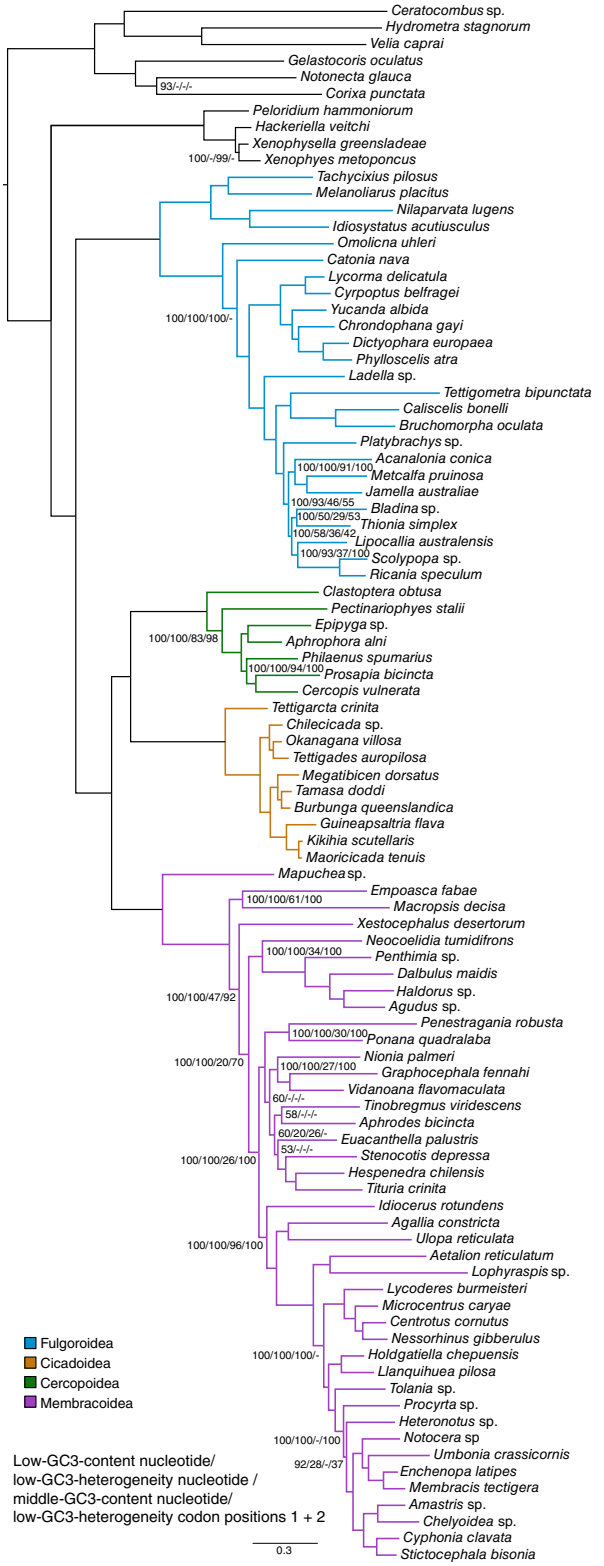

Fig. 5. Maximum likelihood (RAXML) phylogeny of Auchenorrhyncha and outgroup taxa for the concatenated quintile tree with the highest average bootstrap value [low third-codon-position GC (GC3) content nucleotide quintile; 97.98%] partitioned by codon position and gene with bootstrap support values for the four most congruent phylogenies as measured by normalized Robinson–Foulds distance (low-GC3-content nucleotide, low-GC3-variance nucleotide, middle-GC3-content nucleotide, low-GC3-heterogeneity nucleotide (first and second codon positions only)]. [Colour figure can be viewed at wileyonlinelibrary.com].

Fig. 5. Legend on next column. [Colour figure can be viewed at wileyonlinelibrary.com].

nymphal Malpighian tubules (Rakitov, 2002), and structure of the nymphal abdominal spiracles (Emelyanov, 1987). Bourgoin & Campbell (2002) also proposed this relationship in their phylogenetic hypothesis incorporating genetic, morphological and fossil evidence.

The discrepancy between the position of Cercopoidea in the amino acid and degeneracy-coded nucleotide versus the complete nucleotide alignments might suggest that biases in the third codon position could be contributing to instability in this relationship, but results from our GC3 analyses suggest an alternate interpretation. In our GC3 quintile analyses, we recovered (Cicadoidea + (Cercopoidea + Membracoidea)) only in amino acid datasets where the number of informative sites was relatively small, but this relationship was never recovered in the nucleotide datasets even when the third position was excluded. It may be possible that this relationship recovered in degeneracy-coded and amino acid analyses is driven by some other mechanism, such as convergence. For example, the same amino acids could be coded by entirely different codons, or, in the case of degeneracy coding, codons that code for different amino acids are being binned together, e.g. for some sixfold degenerate amino acids, arginine and leucine (Simmons, 2017).

Because of the difficulties of obtaining well-resolved trees from single genes, the alignments used to construct individual gene trees for ASTRAL also included third codon positions. Amino acid sequences frequently lack resolving power within the individual gene trees required for ASTRAL (Townsend *et al.*, 2008); thus, we used individual gene nucleotide alignments and retained all codon positions for these analyses. This necessity may have contributed to recovery of the Cercopoidea + Cicadoidea relationship in the coalescent analyses, which was also obtained in the complete concatenated nucleotide analyses, although there was a high degree of conflict at this node. Quartet scores from the ASTRAL analysis indicated that there was some gene tree conflict regarding the sister relationship between Cicadoidea and Cercopoidea, but the posterior probability score for this branch was 100% and we found that all analysed nucleotide datasets, including only codon positions 1 and 2, also support this relationship when partitioned by GC3 variance or GC3 content (Table S8). Taken together, the majority of our results support the sister relationship of Cercopoidea and Cicadoidea.

Fulgoroidea

The placement of the Fulgoroidea among the Hemiptera was contentious for many years and several prior placements of this superfamily rendered Auchenorrhyncha paraphyletic (Goodchild, 1966; Bourgoin, 1987, 1993; Campbell *et al.*, 1995; Sorensen *et al.*, 1995; von Dohlen & Moran, 1995; Bourgoin & Campbell, 2002; Xie *et al.*, 2008; Song *et al.*, 2012, 2016). However, most of these studies used data from limited morphological characteristics or sampled from few genes or taxa. We refer to the results of Cryan & Urban (2012) and Johnson *et al.* (2018), two molecular studies that achieved a high degree of taxonomic coverage and which recover a monophyletic Auchenorrhyncha with Fulgoroidea sister to Cicadomorpha in subsequent discussion.

The fulgoroids (planthoppers) have been of scientific interest for many years and include many morphologically distinctive and agriculturally important species. They are a relatively ancient lineage, dating at least to the early Jurassic (Zhang *et al.*, 2003; Szwedo *et al.*, 2004; Martin, 2008; Szwedo *et al.*, 2011). In early studies of this group, all species were considered as belonging to a single family, Fulgoridae (Hansen, 1903; Metcalf, 1913), but considerable variation in wing morphology, even among members of the same species, led to recognition of Metcalf's (1913) nine subfamilies as full families by Muir (1923). In total, Muir (1923) recognized 14 families within Fulgoroidea, but split the family Cixiidae into two nonmonophyletic subfamilies while still maintaining the family name for both constituents. Currently, 21 families are recognized (Bartlett *et al.*, 2018).

Our results recovered a monophyletic Fulgoroidea that is sister to Cicadomorpha with high support across analyses, although conflict among gene trees was observed in this placement (Fig. 4). High support was found for a sister relationship between Cixiidae and Delphacidae, a relationship that was proposed early in the studies of Fulgoromorpha and has been recovered in both morphological and molecular studies (Asche, 1987; Bourgoin *et al.*, 1997; Yeh *et al.*, 2005; Urban & Cryan, 2007; Song & Liang, 2013; Johnson *et al.*, 2018), although it has been questioned (Emelyanov, 1991). Some previous analyses have reported possible paraphyly between Cixiidae and Delphacidae (Yeh *et al.*, 2005; Urban & Cryan, 2007), but we did not observe this in our results, and a much larger taxon sample of both families will be needed to confirm their status. We also observed high support for the close relationship of Dictyopharidae and Fulgoridae, which has also been observed in previous studies (Emelyanov, 1991; Yang & Fang, 1993; Chen & Yang, 1995;Yeh *et al.*, 2005; Urban & Cryan, 2007; Song & Liang, 2013).

Among the remaining Fulgoroidea, Tropiduchidae was recovered as sister to the remaining taxa with high support. Tettigometridae was placed sister to Caliscelidae, also with high support, except in the multispecies coalescent analysis. However, these results may have been influenced by conflict between gene trees, as the placement of Tettigometridae and Caliscelidae were both recovered by a minority of gene trees. Our results do support the recognition of Caliscelidae as a family independent of Issidae, as proposed by Emelyanov (1999). The family Ricaniidae was recovered as monophyletic with high support, although its sister lineage differed between analyses. Placement of Issidae also differed between analyses and was poorly supported. However, support for the placement of this family seemed to be successfully partitioned by our quintile analyses, with almost all low quintiles (except the low variance amino acid quintile) supporting a relationship with *Bladina*, whereas almost all middle and high quintiles supported Issidae as sister to *Lipocallia* or *Platybrachys*.

Acanaloniidae was placed sister to Flatidae with high support in the concatenated and partitioned nucleotide analyses and in the multispecies coalescent analysis, but this relationship

received low support in the amino acid and degeneracy-coded nucleotide analyses and has not been observed in previous molecular studies. A recent morphological study using antennal characters suggested a close alliance between these clades (Hamilton, 2011), but further indicated that sinking of both families into Issidae would be morphologically supportable. Acanaloniidae has been included in Issidae (Fennah, 1954), but our results support the assessment of Urban & Cryan (2007) that Acanaloniidae should be treated separately from Issidae. However, our limited taxonomic sampling within these groups necessitates that further studies be performed prior to taxonomic revision of these groups.

The placement of Tettigometridae has been a source of disagreement in the literature. The earliest phylogenetic hypotheses placed Tettigometridae sister to the remaining fulgoroids (Muir, 1923, 1930), citing its possession of characters from both the Cicadoidea and the Fulgoroidea. Later morphological studies also placed Tettigometridae at the base of Fulgoroidea (Asche, 1987; Emelyanov, 1991), although some authors suggested that a more derived placement was supported (Bourgoin, 1986, 1993; Bourgoin & Campbell, 1996). Yang & Fang (1993), using characters of nymphs, placed Tettigometridae sister to Cicadelloidea, rendering Fulgoroidea paraphyletic, but other authors suggested that this result was a methodological artifact (Bourgoin *et al.*, 1997). Most recently, a phylogeny based on characters of the antennae suggested that Tettigometridae and Caliscelidae are sister lineages (Hamilton, 2011), as recovered in our study.

All previous analyses of molecular data have failed to support the basal placement of Tettigometridae, but have differed in its placement among the Fulgoroidea. Bourgoin *et al.* (1997) recovered a sister relationship between Tettigometridae and Tropiduchidae using 18S rDNA sequences, but their taxon sample did not include Caliscelidae. Yeh *et al.*'s (2005) 16S phylogeny of the Fulgoroidea indicated an affiliation of Tettigometridae with a paraphyletic Issidae, while the analysis of Urban & Cryan (2009) placed Tettigometridae sister to the genus *Bladina* (Nogodinidae). However, this relationship received conflicting levels of support between their parsimony and Bayesian analyses. Our analysis included a representative of *Bladina*, but failed to recover a sister relationship to Tettigometridae. Song & Liang's (2013) phylogeny using nuclear and mitochondrial genes recovered Tettigometridae sister to Caliscelidae with weak bootstrap support in their maximum likelihood analysis and did not resolve tettigometrid placement in their Bayesian analysis.

The status and composition of Nogodinidae have long been controversial. The family was recovered as paraphyletic in our analyses, as has been seen in several previous molecular studies (Yeh *et al.*, 2005; Urban & Cryan, 2007; Song & Liang, 2013). The nogodinid *Lipocallia australensis* was consistently placed with high support as the sister to Ricaniidae in the concatenated analyses, while lowered support for this relationship and high gene tree conflict was observed in the multispecies coalescent analysis (Figs 4, S4). The placement of the second nogodinid included in our study (*Bladina*) was inconsistent between datasets and poorly supported except in the concatenated amino acid analysis, where it was recovered with 100% bootstrap support as sister to *Platybrachus* (Eurybrachidae). Because Nogodinidae was originally described as a subfamily of Ricaniidae, the close association between these families recovered in our concatenated analyses is not without precedent, although a close relationship of Nogodinidae and Eurybrachidae appears to be a novel hypothesis. However, hypotheses of fulgoroidean relationships (Muir, 1930; Emelyanov, 1991; Chen & Yang, 1995; Urban & Cryan, 2007; Song & Liang, 2013) have not been consistent in their placement of Nogodinidae and it remains a problematic and likely paraphyletic taxon.

Several interfamilial relationships among the Fulgoroidea received poor support across analyses and were characterized by short branch lengths (Fig. 3). Short branch lengths may complicate phylogenetic reconstruction due to evolutionary processes such as rapid radiations (Whitfield & Lockhart, 2007). Relationships among many fulgoroidean families have been unstable over morphological and molecular analyses, although no comprehensive analysis has focused on this superfamily since Urban & Cryan (2007). Further study and taxonomic revision of the Fulgoroidea will probably be necessary to achieve a stable and highly supported phylogenetic hypothesis of this charismatic group.

Cicadoidea

Despite significant interest in the evolution of cicadas, due in part to their utility as models of speciation (Simon *et al.*, 2000; Cooley *et al.*, 2001; Marshall *et al.*, 2011; Hertach *et al.*, 2016; Fujisawa *et al.*, 2018), phylogenetic analysis of this group has been lacking until very recently. Different authors have proposed classification schemes recognizing anywhere from one to five families (Distant, 1906; Myers, 1929; Kato, 1954; Metcalf, 1963; Boulard, 1976; Hayashi, 1984; Moulds, 2005), but few authors have performed formal phylogenetic analyses of the superfamily to investigate the phylogenetic status of the families. Moulds' (2005) morphological analysis of Cicadidae supported the recognition of two families, Tettigarctidae and Cicadidae, and three subfamilies within Cicadidae. Molecular phylogenetic analyses of the Cicadomorpha (Cryan, 2005) and Auchenorrhyncha (Cryan & Urban, 2012) also supported a sister relationship between the Cicadidae and Tettigarctidae, but taxon sampling was not sufficient to allow assessment of subfamily validity. A recently completed molecular phylogeny of the Cicadidae based on five gene regions (Marshall *et al.*, 2018) recovered the sister relationship of Tettigarctidae to Cicadidae and found support for the three cicadid subfamilies proposed by Moulds (2005). This analysis also led to the proposal of a novel, paraphyletic subfamily comprising African taxa, of which no representatives were included in our dataset. Within Cicadoidea, all of our concatenated analyses and the multispecies coalescent analysis recovered Tettigarctidae and Cicadidae as sister groups with full support, and the phylogeny among the genera that were included in our analyses was consistent with the three-subfamily system of Moulds (2005) and Marshall *et al.* (2018). Our phylogenetically consistent and highly supported results within

this superfamily indicate that use of transcriptomic data could be especially fruitful for future phylogenetic studies of cicadas.

Cercopoidea

The superfamily Cercopoidea includes insects commonly known as spittlebugs and froghoppers and is relatively small compared with the highly diverse Membracoidea and Fulgoroidea. Cercopoids are perhaps best known for their production of frothy, saliva-like secretions that protect the nymphs from desiccation and predation. Several species are important in agricultural systems (Holmann & Peck, 2002; Ravaneli *et al.*, 2011), but it is likely that the majority of species in this group remain undescribed (Kosztarab *et al.*, 1990). The taxonomy of the Cercopoidea, which has been regarded as containing anywhere from three to five families, also remains complicated and in need of revision at higher and lower levels (Hamilton, 2001, 2015; Cryan & Svenson, 2010). For convenience, we refer to the five-family system used by Cryan & Svenson (2010) in the following discussion, although Hamilton (2012, 2015) treated Machaerotidae as a junior synonym of Clastopteridae, and Aphrophoridae as a junior synonym of Cercopidae using morphological criteria.

Members of the family Machaerotidae are unique among the cercopoids for their construction of liquid-filled tubes as nymphs. Although originally likened to the calcareous shell of molluscs (Ratte, 1884), later work indicated that these tubes were constructed of 'mucofibrils' of protein and mucopolysaccharides secreted from the Malpighian tubules in a manner similar to the production of spittle in other cercopoids (Marshall, 1965, 1968). This unusual biology attracted the attention of many authors, but status and placement of the family Machaerotidae among the Cercopoidea have not been consistent among previous works. Several early authors followed the originally described classification scheme in which Machaerotidae was considered a subfamily of Cercopidae (as Machaerotinae) (Schmidt, 1907; Baker, 1919). Baker (1927) treated it as a full family in later work, but without specifying a reason for the change. Metcalf (1960) likewise treated it as a family in his extensive catalogue. Hamilton (2001) recognized only three cercopoid families and subsumed Machaerotidae as a subfamily within Clastopteridae, citing the minimal morphological distinction between the groups. However, Machaerotidae was included as a full family in a subsequent key to the Cicadomorpha (Dietrich, 2005). Multilocus molecular phylogenies including multiple representatives of Machaerotidae have supported the recognition of this clade as a full family independent of Cercopidae and Clastopteridae (Cryan & Svenson, 2010; Cryan & Urban, 2012; Bell *et al.*, 2014), and Paladini *et al.* (2018) treated it as a full family in their recent analysis.

An initial phylogenetic analysis using three nuclear markers recovered a clade containing Machaerotidae and Cercopidae as sister to the remaining cercopoids (Cryan, 2005). Cryan & Svenson (2010) recovered Machaerotidae as sister to the remaining cercopoids using nuclear and mitochondrial genetic markers and increased taxonomic sampling within the Cercopoidea, but the branches separating these families received low bootstrap support. Machaerotidae was recovered as sister to the remaining cercopoids in our concatenated and partitioned nucleotide analysis as well as in the multispecies coalescent analysis. This placement received low support in the nucleotide analyses and high support in the multispecies coalescent analysis, although it was recovered by a minority of gene trees (Fig. 4). By contrast, in the degeneracy coding nucleotide and amino acid analyses, Clastopteridae was recovered as the earliest diverging family and Machaerotidae was sister to the remaining taxa, both with maximum bootstrap support (Figs 3A, S1, S3, S5). Our GC3 quintile analyses consistently found *Clastoptera* as sister to the rest of Cercopoidea in low quintiles across the three data types with decreasing support in high quintiles, especially in the GC3 content analyses (Table S8). Thus, relationships at the base of Cercopoidea remain relatively unstable, but may be improved in future studies incorporating greater taxon sampling and recognition of the potential influence of GC content.

The family status of Clastopteridae has been more stable in its taxonomic history. Metcalf (1962b) regarded Clastopteridae as a valid family containing only two genera – *Clastoptera* Germar and *Iba* Schmidt – the former of which is a large genus distributed throughout the New World while the latter includes three species recorded from the Philippines and Borneo (Hamilton, 2015). Hamilton (2001), however, placed *Clastoptera* and *Iba* within the subfamily Clastopterinae and regarded Machaerotinae as a subfamily of Clastopteridae. Although all included representatives of *Clastoptera* were recovered as a clade by Cryan & Svenson (2010), no phylogenetic studies to date have included *Iba* or other taxa that Hamilton (2015) suggested to be morphologically intermediate between Clastopteridae and Machaerotidae.

The placement of Clastopteridae remains an open question. Using morphological criteria, Emelyanov (1987) hypothesized that Clastopteridae could be sister to or derived from Cercopidae, or sister to Aphrophoridae + Machaerotidae. Previous molecular analyses have not supported either of these hypotheses and have placed Clastopteridae sister to Cercopidae + Aphrophoridae (including Epipygidae; Cryan & Svenson, 2010), sister to a paraphyletic Aphrophoridae (Cryan & Urban, 2012), or sister to Machaerotidae alone (Bell *et al.*, 2014), although taxonomic representation across Cercopoidea was sparse in the latter two studies. Our multispecies coalescent analysis supports its sister relationship to Cercopidae + Aphrophoridae, if Epipygidae is included within Aphrophoridae.

The family Aphrophoridae was consistently recovered as polyphyletic across our analyses, although it is possible that this is an artifact of limited taxonomic sampling within the cercopoids. Metcalf's (1962a) catalogue treated Aphrophoridae as a valid family, as did Dietrich (2005). However, Hamilton's extensive revision of Neotropical species (2012, 2013) treated it as a subfamily of Cercopidae, and he moved some aphrophorid species to Clastopteridae or Epipygidae (Hamilton, 2001, 2015). Molecular studies of this group have given mixed results concerning the monophyly of Aphrophoridae. A study with extensive cercopoid sampling recovered it as monophyletic with the exception of a single species that grouped within Cercopidae (Cryan & Svenson, 2010), whereas studies with more

limited sampling have recovered it as the monophyletic sister to Epipygidae (Bell *et al.*, 2014) or paraphyletic with respect to Epipygidae and Cercopidae (Cryan & Urban, 2012). Hamilton (2015) continued to treat Aphrophoridae as a subfamily but Bartlett *et al.* (2018) treated it as a full family. Our analyses consistently placed one included aphrophorid (*Aphrophora*) as sister to Epipygidae and the other (*Philaenus*) as sister to Cercopidae (sensu stricto).

Hamilton (2001) erected Epipygidae to include three froghopper genera from Central and South America thought to display unusual biology. This family was separated from other Cercopoidea by the presence of extensive fat body in the abdomen and a small cibarial pump, features which led Hamilton (2001) to hypothesize that adults of this family rarely fed or do not feed at all and survive solely from fat reserves. Nymphs of this family are unknown, but are not believed to produce spittle (Hamilton, 2001). Since its inception, little further information has become available about the biology or diversity of this group. However, it is not clear that Epipygidae should be considered an independent lineage, and it appears likely that they are a specialized lineage of Aphrophoridae. While the family was recognized as valid by Dietrich (2005), molecular phylogenies have consistently recovered epipygid species within Aphrophoridae (Cryan, 2005; Cryan & Svenson, 2010; Cryan & Urban, 2012). We consistently recovered Epipygidae as sister to *Aphrophora* with high support, but our current taxonomic sampling within Cercopoidea is not sufficient to test the derivation hypothesis proposed in previous studies.

Our results strongly support Cercopoidea as a valid, monophyletic superfamily of the Auchenorrhyncha, but questions concerning the monophyly and placement of the families included within this clade remain. Our recovered topologies are not consistent with the three-family system of Hamilton (2001, 2015), and are ambiguous concerning the inclusion of Epipygidae within Aphrophoridae as recovered by Cryan & Svenson (2010). Although our sample includes representatives from every putative family within Cercopoidea, it is not sufficient to test the monophyly of these families. Given the remaining uncertainty in relationships among major cercopoid lineages, a comprehensive, taxonomically inclusive phylogenomic study of this group is needed and would facilitate comparative studies of their remarkable physiological adaptations.

Membracoidea

Membracoidea, including the leafhoppers and treehoppers, is the most diverse superfamily within Auchenorrhyncha, comprising nearly 25 000 extant species (Bartlett *et al.*, 2018). Both morphological and molecular phylogenies have supported the monophyly of Membracoidea (Hamilton, 1983; Dietrich & Dietz, 1993; Dietrich *et al.*, 2001; Cryan, 2005; Cryan & Urban, 2012), but have failed to establish a consensus concerning the relationships of its included lineages. Currently, five families are recognized within Membracoidea – Aetalionidae, Cicadellidae, Melizoderidae, Membracidae and Myerslopiidae – but previous authors have recognized as many as 22 (Evans, 1938). An early phylogenetic hypothesis of the group placed treehoppers (Membracidae+Aetalionidae) sister to Cicadellidae (Evans, 1938), but later morphological phylogenies have not been consistent in their placement of these groups. Some have recovered treehoppers as a derived lineage of Cicadellidae (Hamilton, 1983; Rakitov, 1997; Dietrich, 1999), while other analyses agree with the cicadellid−treehopper sister hypothesis (Dietrich & Dietz, 1993; Dietrich, 2002).

Molecular phylogenetic studies have largely supported the hypothesis that treehoppers are derived from leafhoppers. An initial study using 28S rDNA sequences (Dietrich *et al.*, 2001) recovered treehoppers sister to a clade containing the cicadellid subfamilies Ulopinae and Megopthalminae, a result that was also obtained in our amino acid analysis (Fig. S3). A later analysis using additional molecular data also recovered leafhoppers paraphyletic with respect to treehoppers, with Melizoderidae sister to Aetalionidae + Membracidae (Cryan & Urban, 2012). Most recently, phylogenomic studies using transcriptomes or anchored hybrid enrichment have also supported the hypothesis that treehoppers are derived from a paraphyletic Cicadellidae (Dietrich *et al.*, 2017; Johnson *et al.*, 2018), and the results of our analyses strongly support this hypothesis as well.

The placement of the family Myerslopiidae as sister to the remaining membracoids was highly supported across all of our analyses in agreement with previous studies (Hamilton, 1999; Dietrich, 2002; Cryan & Urban, 2012; Dietrich *et al.*, 2017; Johnson *et al.*, 2018). These small, flightless insects are restricted to New Zealand and temperate Chile where they inhabit leaf litter and soil debris (Szwedo, 2004; Rakitov, 2015). Although various authors speculated that myerslopiids were fungivores or even predators (Nielson, 1996), Rakitov (2015) presented evidence that they feed on the phloem of low-growing angiosperm vines. Myerslopiids were originally placed in the cicadellid subfamily Ulopinae (Evans, 1947, 1957; Linnavuori, 1972; Oman *et al.*, 1990), treated by some recent authors as a family distinct from Cicadellidae (Emelyanov, 1996; Szwedo, 2002). Hamilton (1999) provided morphological evidence distinguishing myerslopiids from both Cicadellidae and Ulopinae, and suggested that they represent a distinct, early-diverging lineage of Membracoidea. Our results agree with other recent phylogenomic analyses in placing Myerslopiidae as sister to the remaining Membracoidea, while Ulopinae is recovered in the clade of leafhoppers that is sister to the treehoppers (Dietrich *et al.*, 2017; Johnson *et al.*, 2018).

Previous molecular and morphology-based phylogenies have provided strong support for several large lineages of Membracoidea currently recognized as subfamilies, but relationships among these major lineages have been particularly difficult to resolve, especially among the Cicadellidae. The cicadellids are by far the most diverse family within the Membracoidea, comprising more than 21 000 extant species (Bartlett *et al.*, 2018). Cicadellidae was recovered as paraphyletic in all analyses, and our results support previous studies that have recovered treehoppers as a lineage derived from within leafhoppers (Hamilton, 1983; Rakitov, 1997; Dietrich *et al.*, 2001, 2017). Relationships among many of the early-diverging leafhopper lineages in our study received suboptimal support across analyses.

Bootstrapping highly supported a sister relationship between *Empoasca* + *Macropsis* in the complete concatenated nucleotide analysis and in the phylogeny most supported by the GC3 quintile analyses, but placements of *Empoasca fabae* (Typhlocybinae), *Macropis decisa* (Macropsinae) and *Xestocephalus desertorum* (Xestocephalinae) were essentially unresolved in the other analyses. Typhlocybine leafhoppers, unusual in their lacerate-flush feeding on mesophyll rather than vascular fluids, and macropsine leafhoppers have been closely allied in previous molecular studies (Dietrich *et al.*, 2001, 2017), but their relatively basal placement in our analysis is unexpected, as they have previously been closely affiliated with the more derived lineages (Hamilton, 1983; Dietrich *et al.*, 2001, 2017). Suboptimal local posterior probability support and quartet scores from the multispecies coalescent analysis also indicate substantial conflict among gene trees in these placements (Fig. 4).

The topology within Cicadellidae was characterized by deep internal nodes with short branches and longer external branches, a pattern that can result from rapid diversification within a group and which has been known to complicate phylogenetic reconstruction in other ancient lineages such as birds, corbiculate bees and black flies, among others (Whitfield & Lockhart, 2007). Previous divergence estimates for this group suggest that many of the major cicadellid lineages arose within an *c*. 30 Ma time frame during the early or mid-Cretaceous (Dietrich *et al.*, 2017; Johnson *et al.*, 2018), a relatively short time frame compared with the following 80–100 Ma of diversification. Multispecies coalescent analyses may overcome conflicts due to incomplete lineage sorting, a known issue that can contribute to inaccurate relationships in concatenated datasets and which may be a common phenomenon in ancient, rapid diversifications (Kubatko & Degan, 2007; Degnan & Rosenberg, 2009). However, we still observed suboptimal support and potentially spurious taxon placement within Cicadellidae in our multispecies coalescent analysis. Indeed, the clade containing Aphrodinae, Iassinae, Coelidiinae, Eucanthallinae, Tartressinae and Ledrinae was the only group with low support values in the low-GC3-content nucleotide tree. Although not expected to be the true species tree based on studies in other taxa, relationships within this clade were recovered with high support in the high-GC3-content and high-heterogeneity nt12 datasets (Table S6; File S1) though with different topologies probably reflecting high gene conflict in this group, as can also be seen on the ASTRAL tree (Fig. 4). Previous analyses including Cicadellidae have also encountered difficulty in confidently resolving the relationships among cicadellid subfamilies (Dietrich *et al.*, 2001, 2005, 2017; Cryan, 2005; Cryan & Urban, 2012), but further research will be needed to investigate if support for these relationships can be improved by inclusion of additional taxa or characters or to determine if this could be a case of a true hard polytomy.

Relationships among the treehoppers were mostly consistent and well supported between analyses, with the exception of the relationships among the three treehopper families. Although members of Melizoderidae (represented here by *Llanquihuea pilosa*) have been considered 'primitive' treehoppers and have been recovered as sister to Aetalionidae + Membracidae or Membracidae in previous studies (Dietrich & Dietz, 1993; Dietrich, 2002; Cryan & Urban, 2012), all of our analyses placed *Llanquihuea* sister to *Holdgatiella*, another endemic Chilean genus, currently placed in the membracid subfamily Nicomiinae (Albertson & Dietrich, 2005). Several of our analyses placed this clade within Membracidae with high support. However, the degeneracy-coded nucleotide analyses and low nt12 quintiles obtained high support for the placement of *Llanquihuea* + *Holdgatiella* as sister to Membracidae + Aetalionidae. Morphological analyses place melizoderids outside of Membracidae based on the plesiomorphic structure of the adult mesonotum and the pygofer of the nymph (Dietrich & Dietz, 1993; Dietrich, 2002). No morphological synapomorphies are known that would support their placement within Membracidae. The multispecies coalescent analyses recovered high but suboptimal support for this branch and only a minority of gene trees supported the relevant quartet, suggesting that this placement is unstable. Notably, this relationship seems to be one that may be driven by third codon position sites and it is possible that saturation or base composition heterogeneity at the third codon position contributed to the unexpected placement, as none of the amino acid, degeneracy-coded nucleotide or low nt12 quintile datasets recovered this result. In some cases, use of a taxon-dense concatenated nucleotide dataset such as ours can overcome the effects of saturation (Breinholt & Kawahara, 2013), but this is not true in all datasets, and concatenated analyses may also still be misled by incomplete lineage sorting (Degnan & Rosenberg, 2009).

Placement of Aetalionidae (represented by *Aetalion* and *Lophyraspis*) also varied between analyses. Complete nucleotide maximum likelihood and multispecies coalescent analyses placed this group as sister to the remaining treehoppers, consistent with the results of Dietrich *et al.* (2017), but the analyses of amino acid sequences and degeneracy-coded nucleotides placed this group within Membracidae as sister to the clade comprising Stegaspidinae and Centrotinae.

The membracid treehoppers are of great interest to evolutionary biology due to their extreme morphological modifications which may play roles in crypsis or predator defence (Wood, 1993; Evangelista *et al.*, 2017), maternal care (Morales, 2000; Billick *et al.*, 2001; Cocroft, 2002; Zink, 2003; Camacho *et al.*, 2014), and hymenopteran mutualisms (Bristow, 1984; Billick *et al.*, 2001; Moreira & Del-Claro, 2005). However, none of our analyses recovered Membracidae as a monophyletic group due to the placement of Melizoderidae and Aetalionidae, albeit several relationships within the membracids were consistent with previous analyses. In particular, we found support for a major lineage comprising all membracids that have the pronotum partly but not completely concealing the scutellum as the sister clade to the remaining membracids. In this clade, subfamily Stegaspidinae is paraphyletic with respect to Centrotinae, as previously observed (Cryan *et al.*, 2000, 2004; Dietrich *et al.*, 2001, 2017; Lin *et al.*, 2004; Evangelista *et al.*, 2017). These previous analyses generally supported the monophyly of Membracinae and Heteronotinae, but indicated that both Darninae and Smiliinae are not monophyletic. Although our analyses consistently recovered the included representatives of Membracinae and Smiliinae as monophyletic with high support,

our taxon sample included only single representatives of Darninae and Heteronotinae and so additional taxon sampling will be needed to provide adequate tests of membracid subfamily monophyly. However, based on our current results, transcriptomic analysis appears promising for this group and may help to facilitate comparative evolutionary and taxonomic studies in the future.

GC variance and content partitioning

We found variability in the topology of trees across amino acid, nt12, and nucleotide datasets for the same gene bins, probably due in part to the nt12 and amino acid datasets having fewer informative sites (on average *c*. 50% and 25% of the informative sites, respectively, of the nucleotide dataset within the same bin; Table S6). The high-heterogeneity and high-GC3-content quintiles across all three types of data were the most incongruent with respect to the low-heterogeneity and low-GC3-content nucleotide quintiles and sometimes contained nodes with very low bootstrap support. The high quintiles displayed the highest amount of third-codon-position saturation (Fig. S9). Although there is not any generally accepted topology of this group with which to compare, we can examine some uncontroversial clades for which there has been little previous support for an alternative relationship. Such nodes were sometimes not recovered in high quintile bins (e.g. an alternative topology to the uncontroversial *Tettigades* + *Okanagana* recovered 100% support in the high-content amino acid and nt12 datasets). The previously unhypothesized *Notonecta* + *Corixa* relationship seen in the low-GC3-content nucleotide quintile is probably driven by convergent changes in content in the third nucleotide position not seen it its likely true sister group *Gelastocoris* (Fig. 2), which is recovered as the sister to *Notonecta* in the low-variance; middle-GC-content; and low-variance nt12 bins. Within the clade of Cicadellidae, which includes Aphrodinae, Iassinae, Coelidiinae, Eucanthellinae, Tartessinae and Ledrinae, some nodes share the same pattern of a relationship recovered in the low-GC3-content tree but with less than 100% bootstrap support; also, they are not found in the other three trees and may thus reflect a similar pattern of nucleotide bias, albeit one that is not as obvious.

Another node that differed among these four trees was within the Coleorrhyncha. *Xenophyes* and *Xenophysella* have been shown to be more closely related to each other than *Hackeriella* based on morphological (Burckhardt, 2009) and molecular data (Kuechler *et al.*, 2013), but there are a large number of genes trees which support an alternative relationship among those three taxa (Fig. 4). We found support for *Xenophysella* + *Hackeriella* in all three low-variance datasets, although the alternative relationship was found with high support in all other datasets. The phylogeny of the bacterial symbionts of the group also contrasts with the accepted host relationships among these genera (Kuechler *et al.*, 2013). These low-heterogeneity genes may reflect the true species tree for this group, although, unusually, this is the only case where the low-GC3-content genes reflect a different relationship with high support from the low-GC3-heterogeneity genes.

As we found the same pattern of higher congruence in low-GC and heterogeneous genes as has been found in vertebrate and Hymenoptera datasets, GC-biased gene conversion may be responsible for the underlying mechanism. Heterogeneity in gene nucleotide compositions is not normally included in sequence models and can mislead phylogenetic reconstruction due to convergence. Even when highly heterogeneous genes are analysed with heterogeneous modes, they often reconstruct the wrong tree, presumably due to recombination and GC-biased gene conversion affecting these regions (Romiguier *et al.*, 2016). Indeed, we found that the unique genes with only high GC3 content that were not especially heterogeneous also gave a relatively incongruent gene tree signal, and, similarly, low-GC3-content genes that were not especially homogenous gave relatively congruent signal. Although the congruence in the low-GC3 heterogenous quintile gene trees was slightly higher than in the low-GC3-content quintile gene trees, the low-GC3-content concatenated quintile dataset recovered the strongest consistent bootstrap support by a considerable margin. These two measures of incongruence may differ due to obfuscation of underlying consistent signal in gene trees based on relatively few nucleotides.

For phylogenomic or transcriptomic datasets, we suggest that partitioning gene tree conflict by nucleotide composition and heterogeneity may be useful for validating topologies seen in the multispecies coalescent tree. Species tree methods assume that incongruences in gene trees reflect incomplete lineage sorting events and reconstruct a species tree that is most compatible with that observed pattern of gene trees. We expect low-GC3-content and low-GC3-heterogeneity trees to contain higher proportions of genes that reflect the true species tree. Indeed, in many cases, we found the same clades supported in both these trees and the ASTRAL multispecies coalescent tree (e.g. the placement of *Idiocerus* and the relationships of the families of Membracoidea), yet we also found several nodes that differed between these trees and the ASTRAL tree. The ASTRAL tree reconstructed *Catonia* + (Fulgoridae + Dictyopharidae) with high support, but this relationship is not seen in any other analysis. The placements of *Neoceolidia* and *Bladina* in the ASTRAL tree are also not found in other analyses, although support for those relationships is lower. In other cases, the relationships recovered in the species tree are consistent with other low quintile trees as is the placement of *Idiocerus* and the relationships of the families of Membracoidea. Summary methods of species tree estimation are sensitive to gene tree error, which increases when the number of sites per locus is small and is also sensitive to long branch attraction effects when the number of sites per locus is not infinite (Roch *et al.*, 2018). Thus, it may be beneficial to interrogate sets of conflicting loci to see if species tree topologies are actually supported by sets of individual genes.

Conclusions

Our study is the most data-rich, taxonomically inclusive study of the Auchenorrhyncha to date and has, in general, provided a

well-supported framework for future studies of the superfamilies and families within this highly diverse suborder. However, despite the large quantities of sequence data analysed, some deep internal nodes remain difficult to resolve with confidence and are not robust to alternative analytical approaches. Our analysis of GC content heterogeneity indicates that there are systemic biases in genomes across this group. Utilizing methods to correct for this bias yielded different topologies and, in some cases, led to maximum support for conflicting alternative topologies. In such cases, partitioning of genes by GC content and heterogeneity may provide a means for identifying the topology more likely to represent the true species tree. Additional taxon sampling may be expected to improve the accuracy of the results, but some deep internal branches with short internodes may remain exceedingly difficult to resolve with the data types and analytical methods that are currently available.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Best tree from the maximum likelihood partitioned nucleotide analysis, including degeneracy-coded third codon positions of 2139 orthologue genes of Auchenorrhyncha and related taxa. Bootstrap values from 100 bootstrap replicates are shown at the nodes.

Figure S2. Best tree from the maximum likelihood partitioned nucleotide analysis, including unaltered third codon positions of 2139 orthologue genes of Auchenorrhyncha and related taxa. Bootstrap values from 100 bootstrap replicates are shown at the nodes.

Figure S3. Best tree from the maximum likelihood concatenated amino acid analysis of 2139 orthologue genes of Auchenorrhyncha and related taxa. Bootstrap values from 100 bootstrap replicates are shown at the nodes.

Figure S4. Tree resulting from ASTRAL multispecies coalescent analysis of 2139 gene trees of Auchenorrhyncha and related taxa. Local posterior probability values are shown at the nodes.

Figure S5. Best tree from the maximum likelihood concatenated nucleotide analysis, including degeneracy-coded third codon positions of 2139 orthologue genes of Auchenorrhyncha and related taxa. Bootstrap values from 100 bootstrap replicates are shown at the nodes.

Figure S6. Best tree from the maximum likelihood concatenated nucleotide analysis, including unaltered third codon positions of 2139 orthologue genes of Auchenorrhyncha and related taxa. Bootstrap values from 100 bootstrap replicates are shown at the nodes.

Figure S7. Normalized quartet score (A, B), average branch length of input gene trees (C, D) and GC3 content (E) and GC3 heterogeneity (F) with respect to GC3 variance (A, C, E) and GC3 content (B, D, F) of 100 gene bins with R^2 values shown for regressions.

Figure S8. Dendrogram of quintile trees clustered with UPGMA using normalized Robinson–Foulds distances.

Figure S9. Uncorrected pairwise distance versus Jukes–Cantor model-corrected distances for transitions and transversions for all nucleotide quintiles.

Table S1. Summary statistics of taxon coverage by gene.

Table S2. Gene presence and absence data for all taxa.

Table S3. Statistics of individual genes.

Table S4. ASTRAL statistics of 100 gene bins.

Table S5. Intersection and unique elements of low and high quintiles of heterogeneity and GC3 content.

Table S6. Statistics of concatenated quintiles

Table S7. Weighted Robinson–Foulds distance matrix of concatenated quintile RAXML trees.

Table S8. Comparison of bootstrap support for specific nodes across quintile analyses.

File. S1. Nexus file of trees based on GC content and variance.

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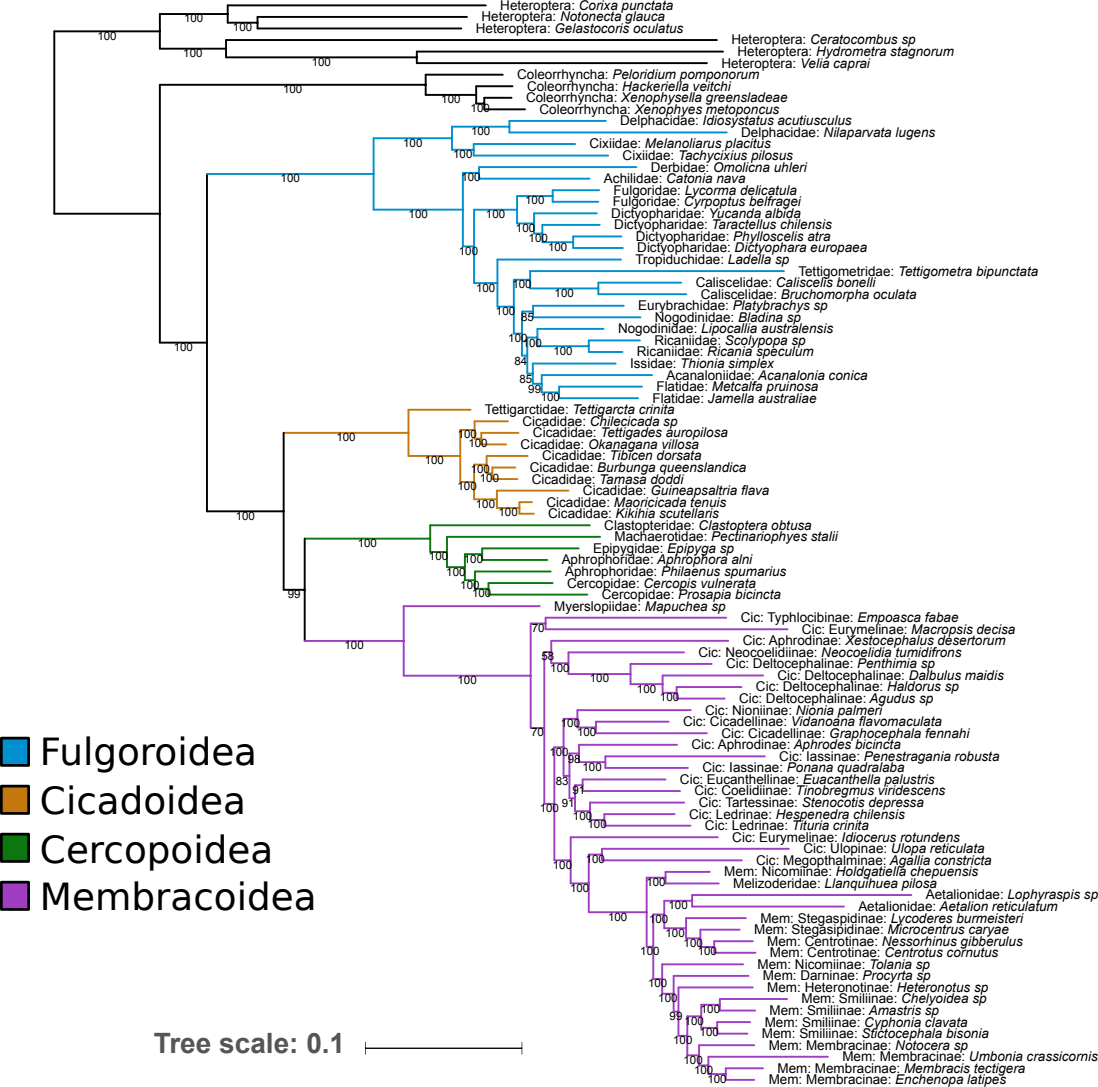
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Caliscelidae: Bruchomorpha oculata
Eurybrachidae: Platybrachys sp
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Cicadidae: Burbunga queenslandica
Cicadidae: Tamasa doddi
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Cicadidae: Kikihia scutellaris
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Machaerotidae: Pectinariophyes stalii
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Mem: Membracinae: Umbonia crassicornis
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Cicadoidea
Cercopoidea
Membracoidea
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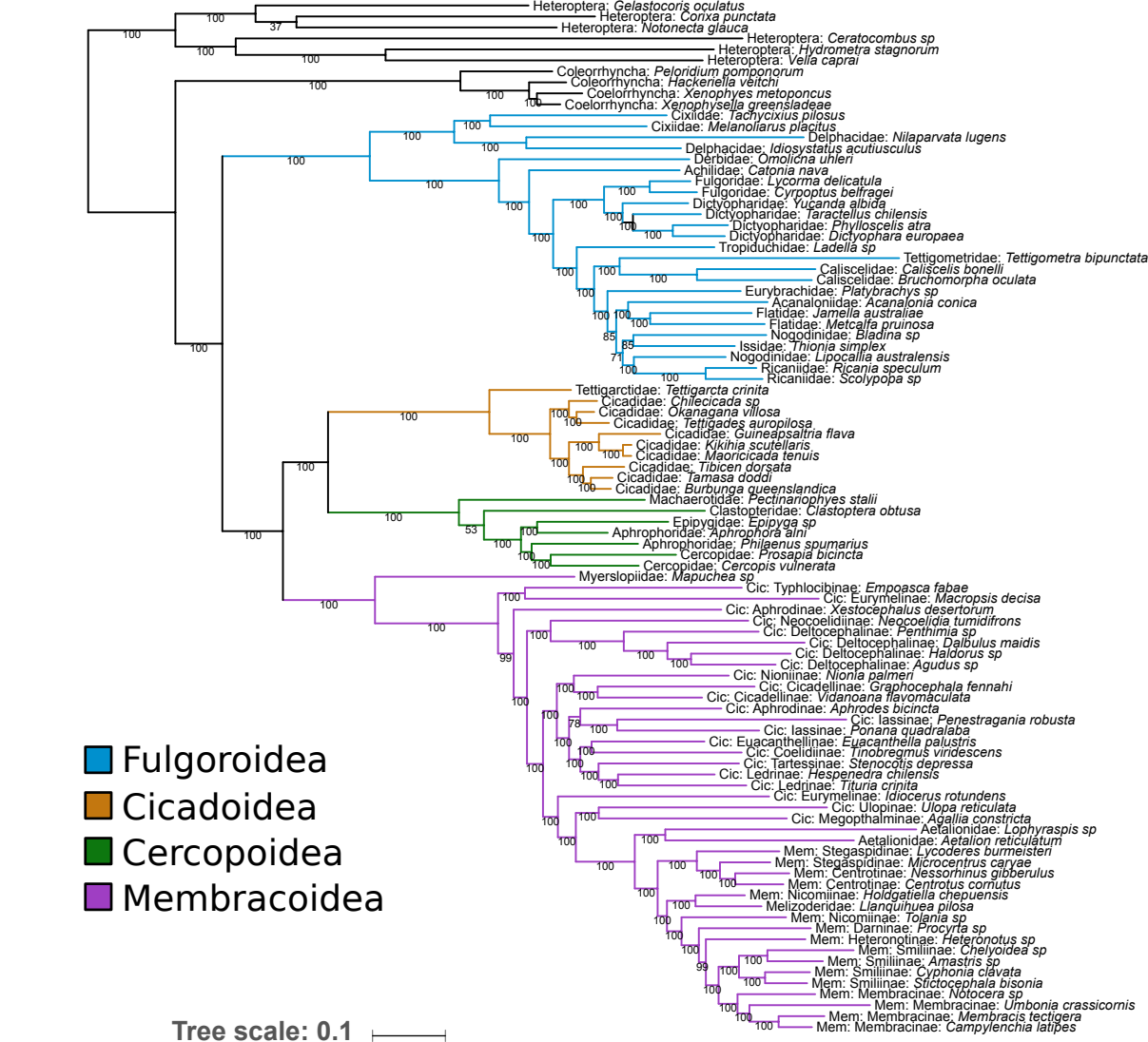
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Cicadidae: Burbunga queenslandica
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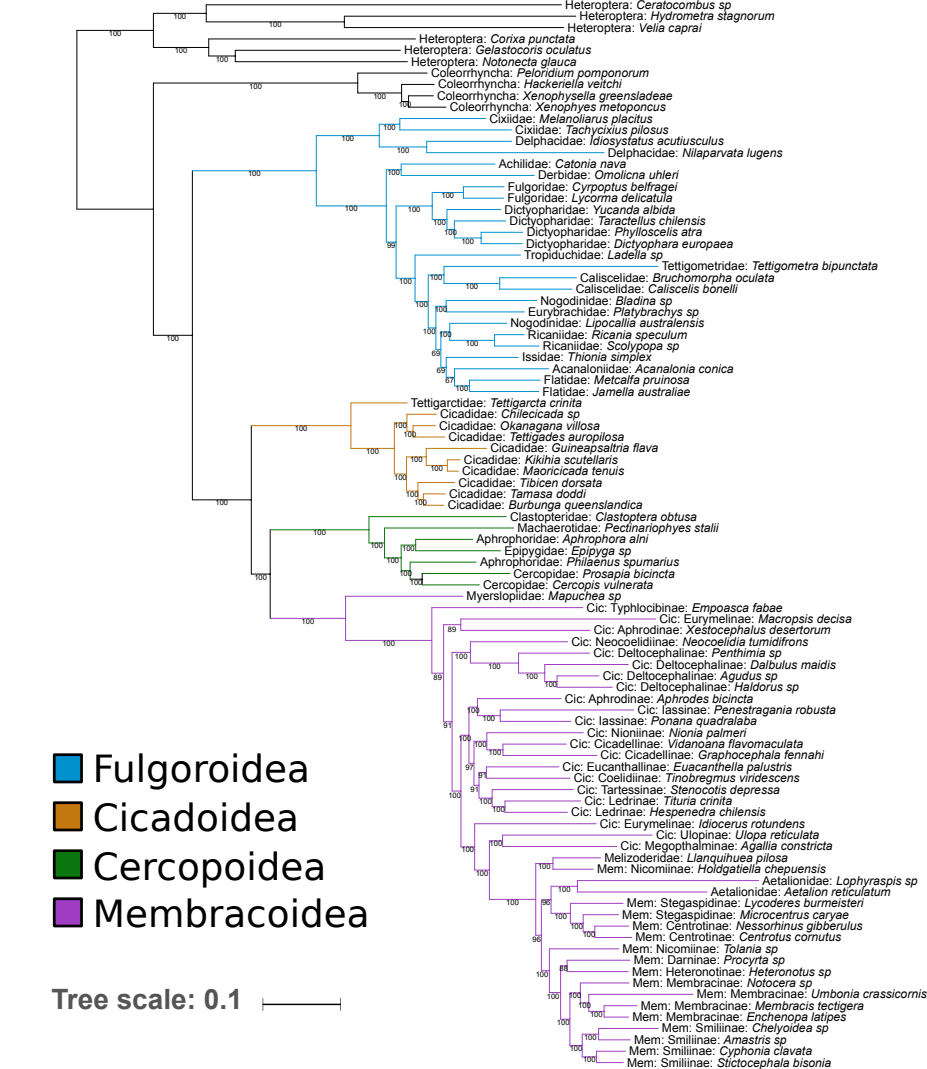
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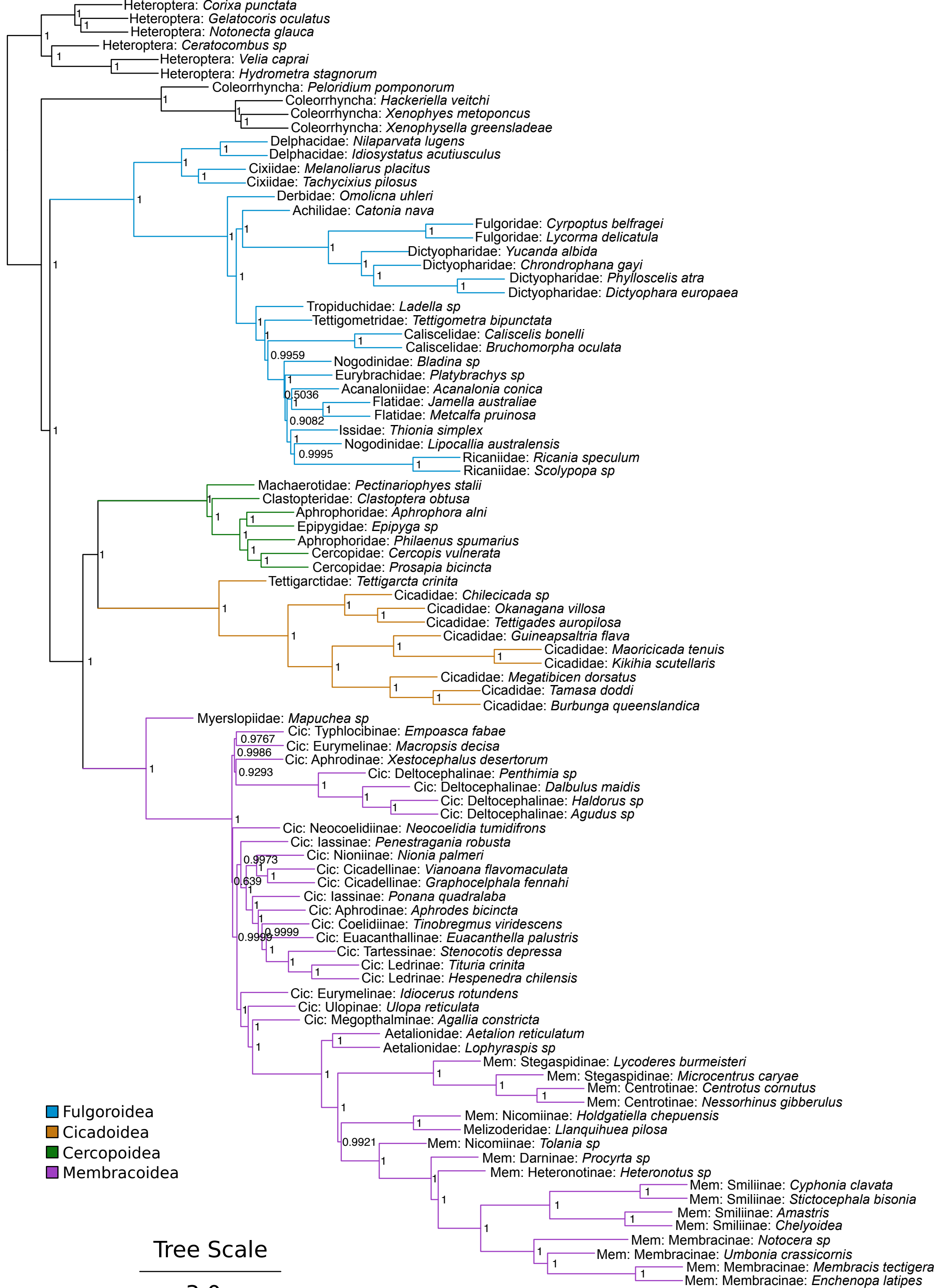
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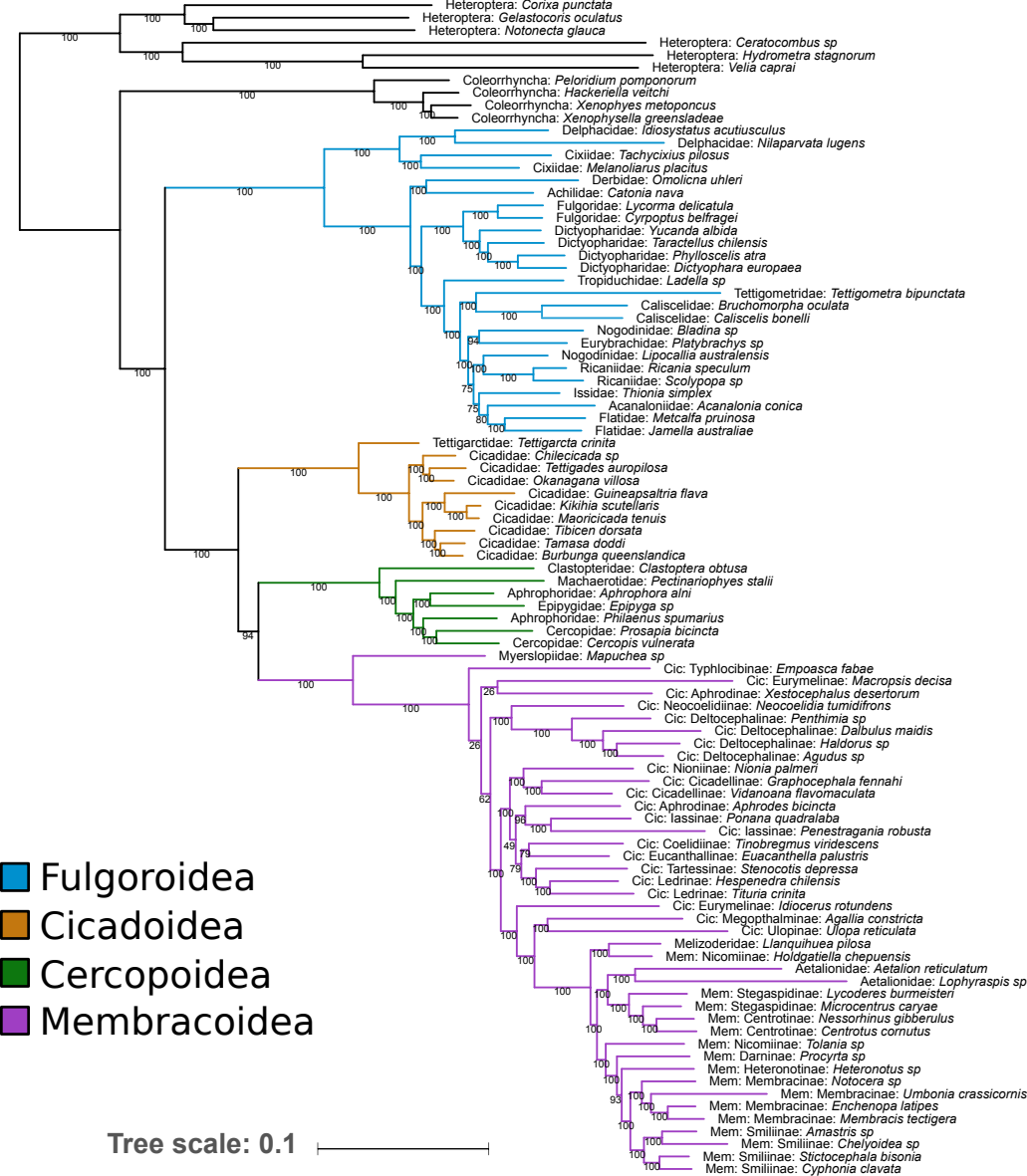
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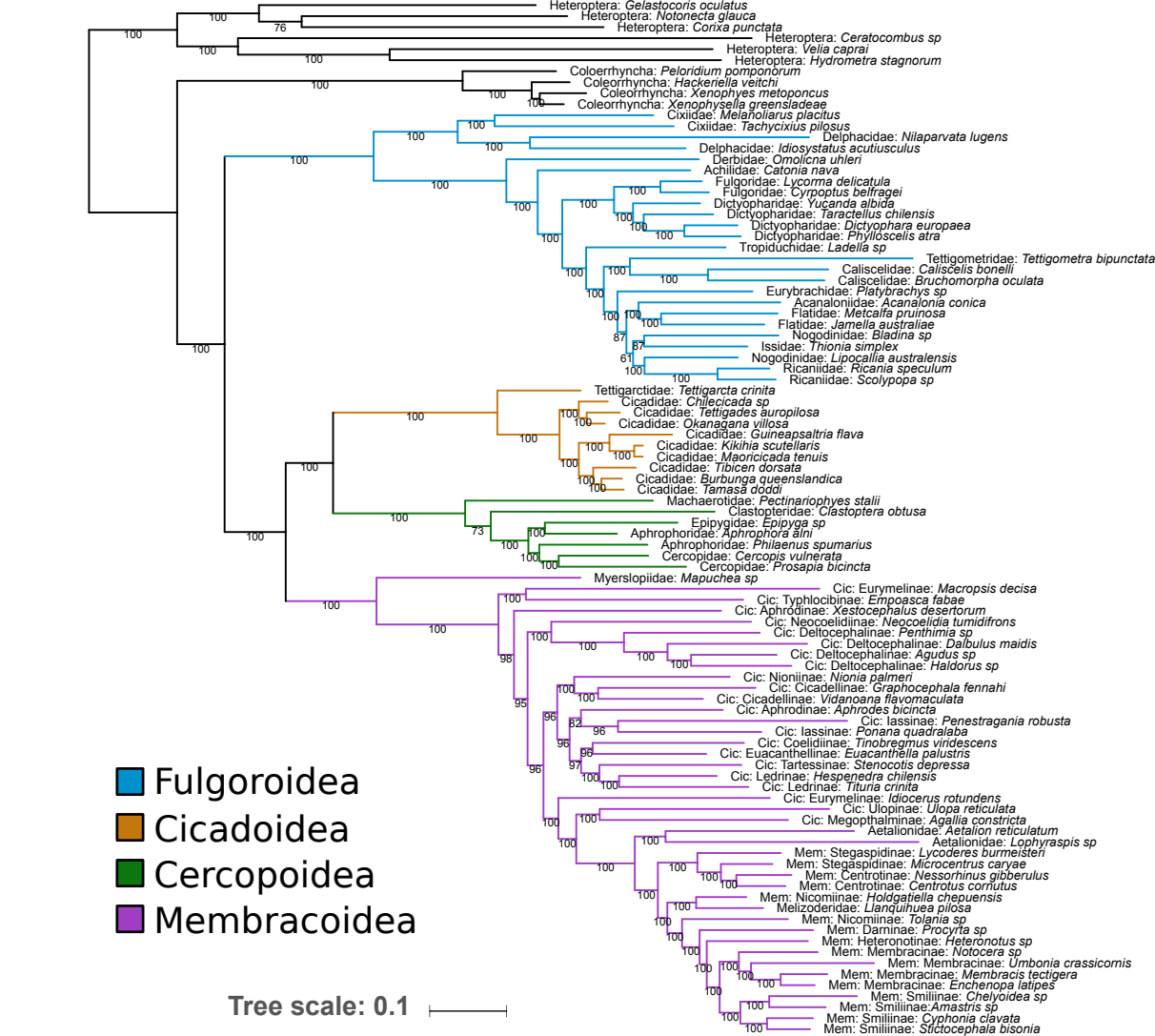
Cicadidae: Chilecicada sp
Cicadidae: Tettigades auropilosa
Cicadidae: Okanagana villosa
Cicadidae: Guineapsaltria flava
Cicadidae: Kikihia scutellaris
Cicadidae: Maoricicada tenuis
Cicadidae: Tibicen dorsata
Cicadidae: Tamasa doddi
Cicadidae: Burbunga queenslandica
Clastopteridae: Clastoptera obtusa
Machaerotidae: Pectinariophyes stalii
Aphrophoridae: Aphrophora alni
Epipygidae: Epipyga sp
Aphrophoridae: Philaenus spumarius
Cercopidae: Prosapia bicincta
Cercopidae: Cercopis vulnerata
Myerslopiidae: Mapuchea sp
Cic: Typhlocibinae: Empoasca fabae
Cic: Eurymelinae: Macropsis decisa
Cic: Aphrodinae: Xestocephalus desertorum
Cic: Neocoelidiinae: Neocoelidia tumidifrons
Cic: Deltocephalinae: Penthimia sp
Cic: Deltocephalinae: Dalbulus maidis
Cic: Deltocephalinae: Haldorus sp
Cic: Deltocephalinae: Agudus sp
Cic: Nioniinae: Nionia palmeri
Cic: Cicadellinae: Graphocephala fennahi
Cic: Cicadellinae: Vidanoana flavomaculata
Cic: Aphrodinae: Aphrodes bicincta
Cic: Iassinae: Ponana quadralaba
Cic: Iassinae: Penestragania robusta
Cic: Coelidiinae: Tinobregmus viridescens
Cic: Eucanthallinae: Euacanthella palustris
Cic: Tartessinae: Stenocotis depressa
Cic: Ledrinae: Hespenedra chilensis
Cic: Ledrinae: Tituria crinita
Cic: Eurymelinae: Idiocerus rotundens
Cic: Megopthalminae: Agallia constricta
Cic: Ulopinae: Ulopa reticulata
Melizoderidae: Llanquihuea pilosa
Mem: Nicomiinae: Holdgatiella chepuensis
Aetalionidae: Aetalion reticulatum
Aetalionidae: Lophyraspis sp
Mem: Stegaspidinae: Lycoderes burmeisteri
Mem: Stegaspidinae: Microcentrus caryae
Mem: Centrotinae: Nessorhinus gibberulus
Mem: Centrotinae: Centrotus cornutus
Mem: Nicomiinae: Tolania sp
Mem: Darninae: Procyrta sp
Mem: Heteronotinae: Heteronotus sp
Mem: Membracinae: Notocera sp
Mem: Membracinae: Umbonia crassicornis
Mem: Membracinae: Enchenopa latipes
Mem: Membracinae: Membracis tectigera
Mem: Smiliinae: Amastris sp
Mem: Smiliinae: Chelyoidea sp
Mem: Smiliinae: Stictocephala bisonia
Mem: Smiliinae: Cyphonia clavata
Fulgoroidea
Cicadoidea
Cercopoidea
Membracoidea
Tree scale: 0.1


Heteroptera: Gelastocoris oculatus
Heteroptera: Notonecta glauca
Heteroptera: Corixa punctata
Heteroptera: Ceratocombus sp
Heteroptera: Velia caprai
Heteroptera: Hydrometra stagnorum
Coleorrhyncha: Peloridium pomponorum
Coleorrhyncha: Hackeriella veitchi
Coleorrhyncha: Xenophyes metoponcus
Coleorrhyncha: Xenophysella greensladeae
Cixiidae: Melanoliarus placitus
Cixiidae: Tachycixius pilosus
Delphacidae: Nilaparvata lugens
Delphacidae: Idiosystatus acutiusculus
Derbidae: Omolicna uhleri
Achilidae: Catonia nava
Fulgoridae: Lycorma delicatula
Fulgoridae: Cyrpoptus belfragei
Dictyopharidae: Yucanda albida
Dictyopharidae: Taractellus chilensis
Dictyopharidae: Dictyophara europaea
Dictyopharidae: Phyllosceles atra
Tropiduchidae: Ladella sp
Tettigometridae: Tettigometra bipunctata
Caliscelidae: Caliscelis bonelli
Caliscelidae: Bruchomorpha oculata
Eurybrachidae: Platybrachys sp
Acanaloniidae: Acanalonia conica
Flatidae: Metcalfa pruinosa
Flatidae: Jamella australiae
Nogodinidae: Bladina sp
Issidae: Thionia simplex
Nogodinidae: Lipocalila australensis
Ricaniidae: Ricania speculum
Ricaniidae: Scolypopa sp
Tettigarctidae: Tettigarcta crinita
Cicadidae: Chilecicada sp
Cicadidae: Tettigades auropilosa
Cicadidae: Okanagana villosa
Cicadidae: Guineapsaltria flava
Cicadidae: Kikihia scutellaris
Cicadidae: Maoricicada tenuis
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Cic: Aphrodinae: Aphrodes bicincta
Cic: Iassinae: Penestragania robusta
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Cic: Tartessinae: Stenocotis depressa
Cic: Ledriinae: Hespenedra chilensis
Cic: Ledriinae: Tituria crinita
Cic: Eurymelinae: Idiocerus rotundens
Cic: Ulopinae: Ulopa reticulata
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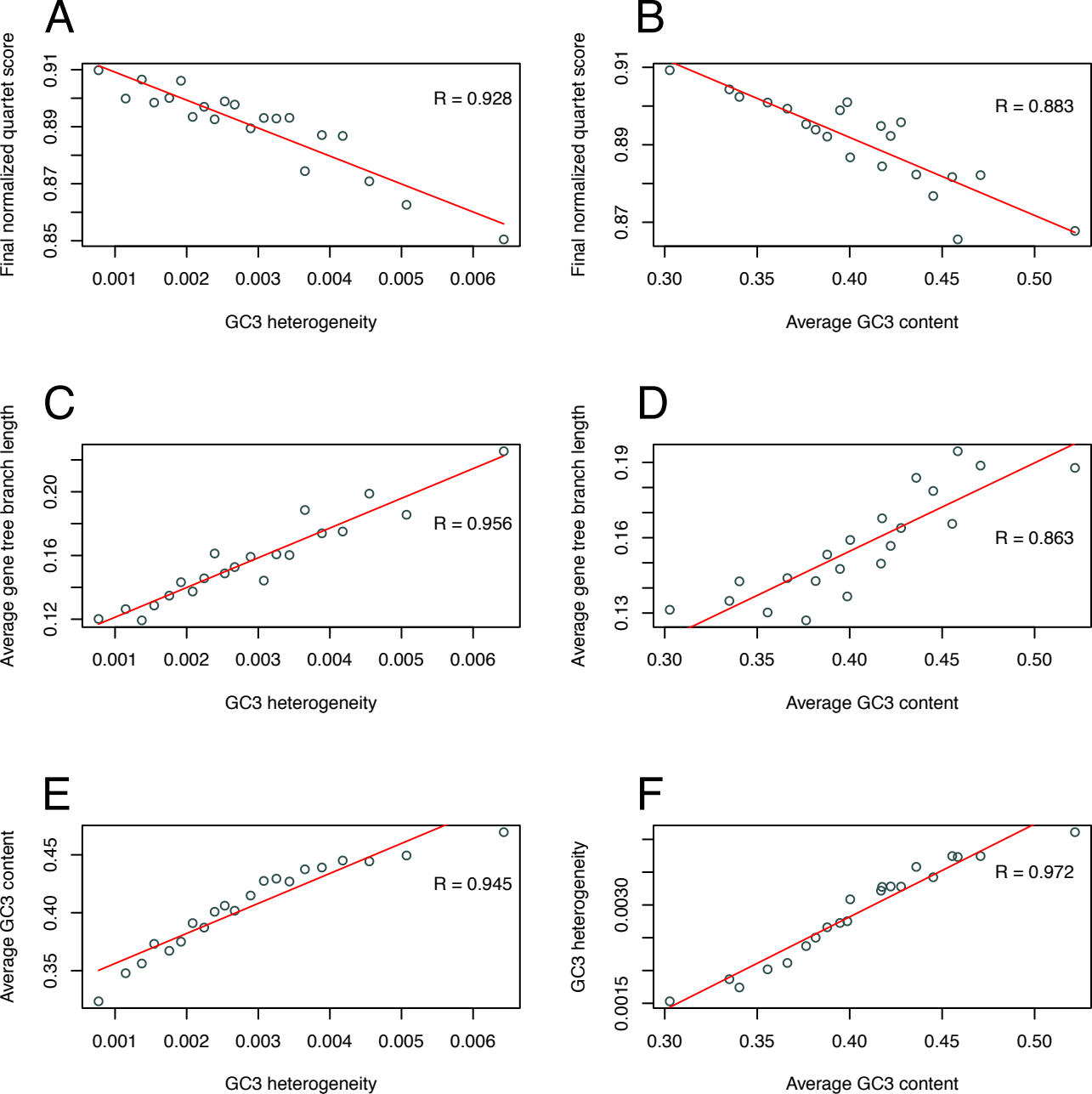
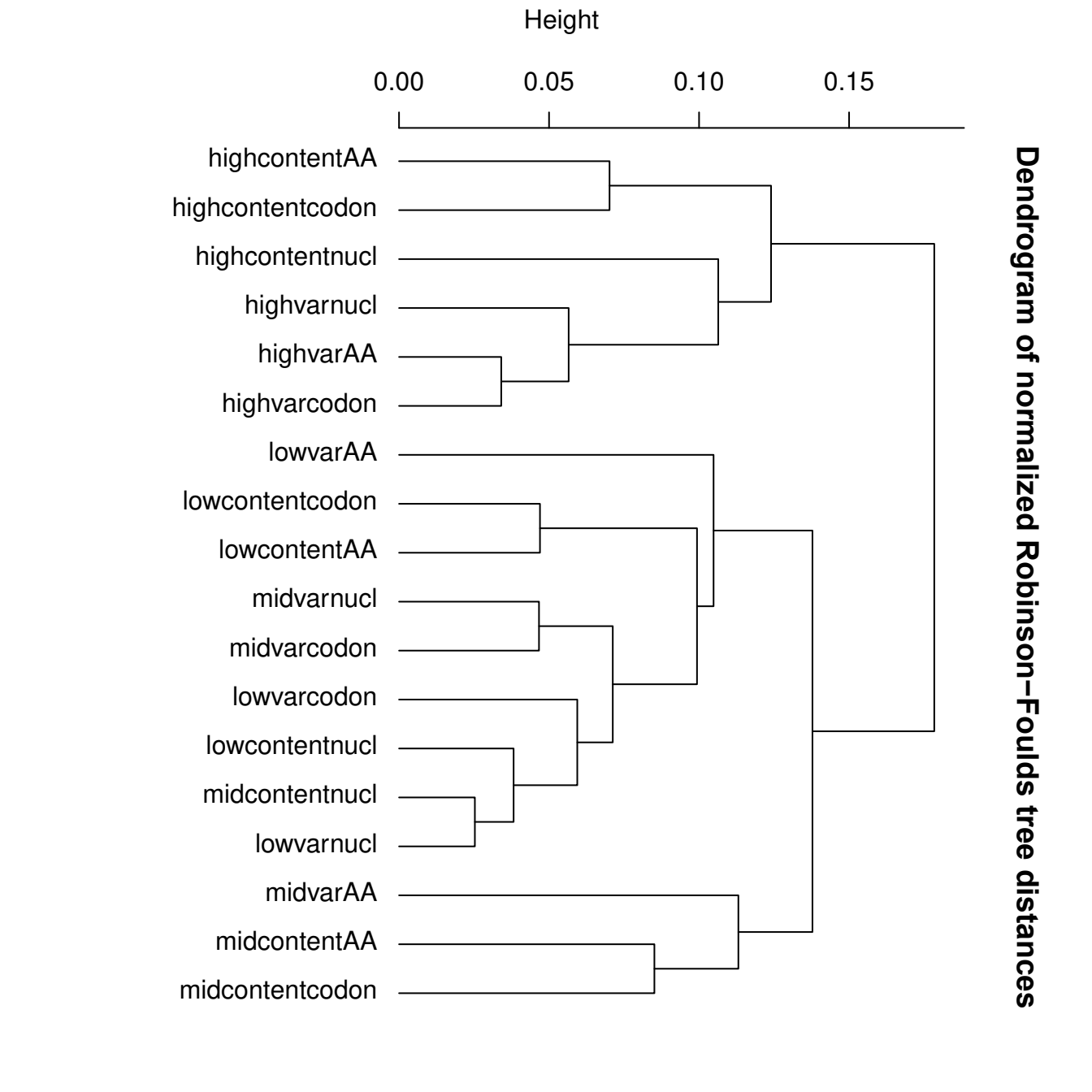
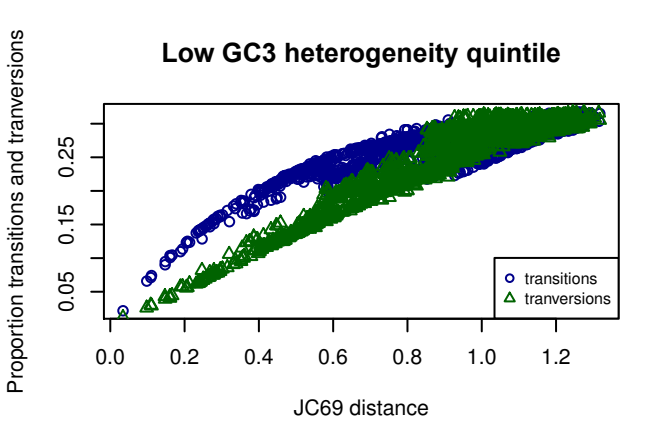
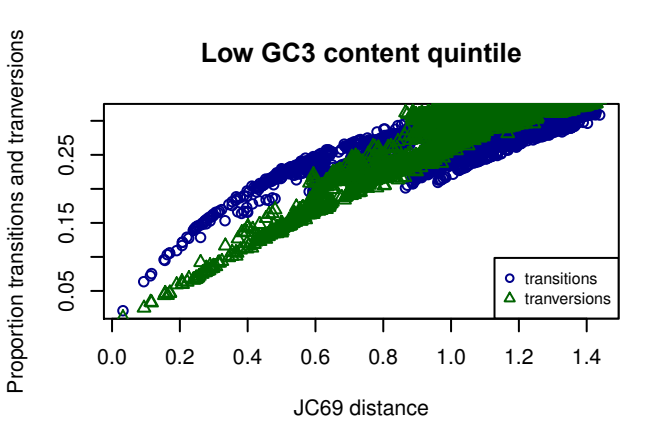
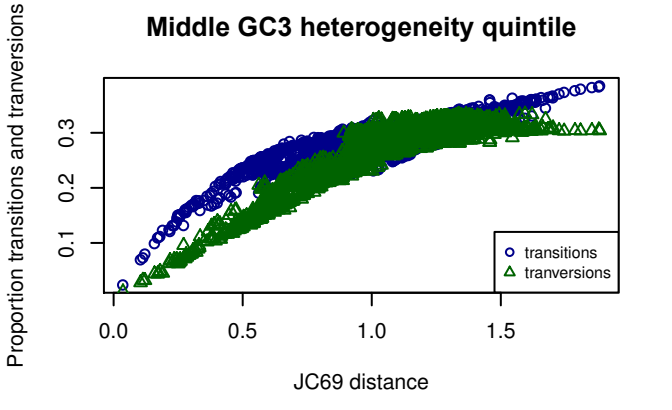
Mem: Smiliinae: Stictocephala bisonia
Fulgoroidea
Cicadoidea
Cercopoidea
Membracoidea
Tree scale: 0.1

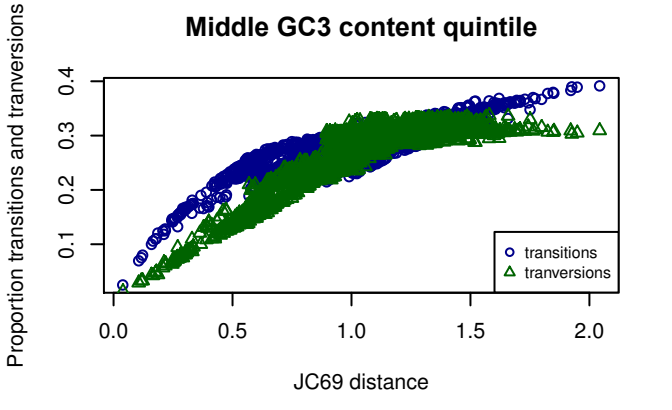

A
Final normalized quartet score
0.85
0.87
0.89
0.91
R = 0.928
0.001
0.002
0.003
0.004
0.005
0.006
GC3 heterogeneity
B
Final normalized quartet score
0.87
0.89
0.91
R = 0.883
0.30
0.35
0.40
0.45
0.50
Average GC3 content
C
Average gene tree branch length
0.12
0.16
0.20
R = 0.956
0.001
0.002
0.003
0.004
0.005
0.006
GC3 heterogeneity
D
Average gene tree branch length
0.13
0.16
0.19
R = 0.863
0.30
0.35
0.40
0.45
0.50
Average GC3 content
E
Average GC3 content
0.35
0.40
0.45
R = 0.945
0.001
0.002
0.003
0.004
0.005
0.006
GC3 heterogeneity
F
GC3 heterogeneity
0.0015
0.0030
R = 0.972
0.30
0.35
0.40
0.45
0.50
Average GC3 content

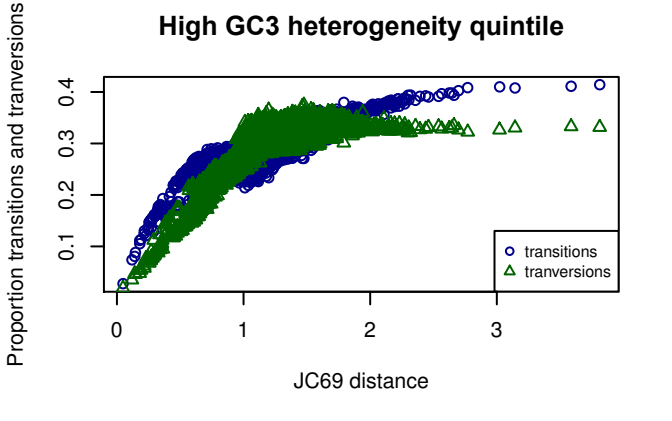

Height
0.00
0.05
0.10
0.15
highcontentAA
highcontentcodon
highcontentnucl
highvarnucl
highvarAA
highvarcodon
lowvarAA
lowcontentcodon
lowcontentAA
midvarnucl
midvarcodon
lowvarcodon
lowcontentnucl
midcontentnucl
lowvarnucl
midvarAA
midcontentAA
midcontentcodon
Dendrogram of normalized Robinson−Foulds tree distances

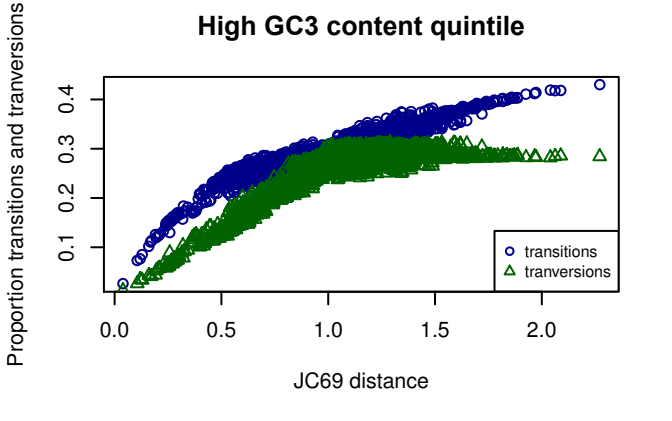

Low GC3 heterogeneity quintile
Proportion transitions and tranversions
0.05
0.15
0.25
0.0
0.2
0.4
0.6
0.8
1.0
1.2
JC69 distance
transitions
tranversions


Low GC3 content quintile
Proportion transitions and tranversions
0.05
0.15
0.25
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
JC69 distance
transitions
tranversions


Middle GC3 heterogeneity quintile
Proportion transitions and tranversions
0.1
0.2
0.3
0.0
0.5
1.0
1.5
JC69 distance
transitions
tranversions


Middle GC3 content quintile
Proportion transitions and tranversions
0.1
0.2
0.3
0.4
0.0
0.5
1.0
1.5
2.0
JC69 distance
transitions
tranversions


High GC3 heterogeneity quintile
Proportion transitions and tranversions
0.1
0.2
0.3
0.4
0
1
2
3
JC69 distance
transitions
tranversions


High GC3 content quintile
Proportion transitions and tranversions
0.1
0.2
0.3
0.4
0.0
0.5
1.0
1.5
2.0
JC69 distance
transitions
tranversions