International Journal of Biological Macromolecules 139 (2019) 1018–1027

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: http://www.elsevier.com/locate/ijbiomac

Structural features and phylogenetic implications of four new mitogenomes of Centrotinae (Hemiptera: Membracidae)

Kai Hu [a], Feng Yuan [a], Christopher H. Dietrich [b], Xiang-Qun Yuan [a,*]

[a] Key Laboratory of Plant Protection Resources and Pest Management, Ministry of Education, College of Plant Protection, Northwest A&F University, Yangling, Shaanxi, China
[b] Illinois Natural History Survey, Prairie Research Institute, University of Illinois at Urbana-Champaign, IL, United States of America

ARTICLE INFO

Article history:
Received 21 April 2019
Received in revised form 7 August 2019
Accepted 7 August 2019
Available online 08 August 2019

Keywords:
Mitochondrial genome
Treehopper
Phylogenetic analysis
Secondary structure
Cicadomorpha

ABSTRACT

To explore the variation and phylogenetic utility of mitogenomes among lineages of the diverse hemipteran superfamily Membracoidea, we sequenced four new mitogenomes of four treehopper species of the subfamily Centrotinae (Membracidae): *Hypsauchenia hardwichii*, *Leptocentrus albolineatus*, *Maurya qinlingensis*, and *Tricentrus brunneus*. The mitogenomes are 15,508 to 16,467 bp in size, and comprise the typical set of 37 mitochondrial genes and a large non-coding region (AT-rich region). Gene organization, nucleotide composition and codon usage of protein-coding genes (PCGs) are similar to those of most other sequenced Membracidae mitogenomes. All PCGs start with a typical ATN or TTG and end with TAA/G or the incomplete stop codon (a single T). All transfer RNA genes can be folded into typical clover-leaf secondary structures, except for *trnS1*. The location, length and AT content of the *rrnS* and *rrnL* genes are highly conserved in the Membracidae mitogenomes. In contrast, the AT-rich control region is highly variable in length and in numbers of tandem repeats present. Phylogenetic analyses based on the nucleotide and amino acid sequence data of 13 PCGs from 59 species of Membracoidea and four outgroups (Cercopoidea and Cicadoidea species) recovered Membracoidea as monophyletic with strong support, and Cicadellidae as paraphyletic with respect to Aetalionidae + Membracidae, in agreement with previous analyses. Relationships among membracoid subfamilies were also in general agreement with results from prior studies. The monophyly of Centrotinae is strongly supported, with relationships among tribes recovered as ((Centrotini+ (Tricentrini + Antialcidini)) + ((Leptobelini + Hypsauchenini) + Leptocentrini).

1. Introduction

The treehopper family Membracidae is one of the largest and most morphologically diverse groups of Hemiptera. The variety of bizarre external forms, diverse behaviors, and complex life histories of these sap-sucking insects have long attracted the interest of naturalists [1–6]. So far, a total of 3224 species in 412 genera of Membracidae have been recorded worldwide [7–10]. Centrotinae is the only cosmopolitan subfamily of Membracidae and is also the largest, comprising about 216 genera and 1350 species [5]. The remaining membracid subfamilies are restricted to the New World, except for the buffalo treehopper, *Stictocephala bisonia* Kopp & Yonke (Smiliinae), accidentally introduced and now widespread in Eurasia. Improved knowledge of the phylogeny of Centrotinae is needed not only to better understand their global biogeography, but also for studies of the evolution of parental care behavior and the management of plant pests [5,6,9]. Previous studies of this group have focused primarily on morphology-based taxonomy of the subfamily Centrotinae in particular geographical areas [6,11–16]. Phylogenetic studies of the Centrotinae began with Ahmad [17], and subsequent studies either included only morphological data, or incorporated only a few Centrotinae taxa into broader molecular phylogenetic studies [1,2,4,6–9,18]. As a result, even the monophyly of Centrotinae remains contentious, and the phylogeny and taxonomy of the Centrotinae tribes are unstable. Data from additional sources, such as complete mitogenomes, may help improve phylogenetic resolution within this group.

The insect mitogenome is typically a covalently closed circular double-stranded DNA molecule, usually 15–18 kb in length and encoding 37 genes, including 13 protein-coding genes (PCG), 2 ribosomal RNA genes (rRNA) and 22 transfer RNA genes (tRNA) [19,20]. The mitogenome also includes a non-coding region of variable length that plays a regulatory role in transcription and replication, namely, the mitochondrial control region [19,21]. The mitogenome, in whole or part, has been widely used as a molecular marker to study the population genetics, phylogeny and evolution of insects [20,22,23].

Currently, there are only five complete or nearly complete mitogenomes of Centrotinae in GenBank (as of 28 February 2019). To facilitate comparative studies and phylogenetic analyses of Centrotinae, we sequenced the mitogenomes of four additional Centrotinae species representing four additional tribes, *Hypsauchenia hardwichii* (Hypsauchenini), *Leptocentrus albolineatus* (Leptocentrini), *Maurya*

* Corresponding author.
E-mail address: yuanxq@nwsuaf.edu.cn (X.-Q. Yuan).

https://doi.org/10.1016/j.ijbiomac.2019.08.064
0141-8130/

qinlingensis (Antialcidini), and *Tricentrus brunneus* (Tricentrini), and analyzed their mitogenome characteristics in detail. Combining these new sequences with previously available mitogenomes of Membracoidea, we reconstructed phylogenetic relationships among major lineages of this superfamily. This enabled us to test the monophyly of Centrotinae, explore relationships within Centrotinae, and examine as broader evolutionary patterns in Membracoidea.

2. Materials and methods

2.1. Sample collection and DNA extraction

Table S1 provides detailed collection information for the adult specimens used in this study. All adult specimens were preserved in 100% ethyl alcohol and stored in a −20 °C freezer in the laboratory at the Institute of Entomology of Northwest A&F University, Yangling, Shaanxi, China. Identification of adult specimens was based on morphological characteristics [6]. Total DNA was extracted from the thoracic muscles using the Biospin Insect Genomic DNA Extraction Kit (BioFlux) following manufacturer's instructions. Voucher specimens are deposited in the entomological collection of Northwest A&F University.

2.2. Sequencing, assembly and annotation

The complete mitogenomes of four centrotine treehoppers were sequenced by next-generation sequencing (NGS) (Illumina HiSeq X10; 5.46 Gb raw data; Biomarker Technologies Corporation, Beijing, China.). For each species, raw data was trimmed with default parameters, and clean reads were preliminarily assembled using *de novo* assembly with the minimum contig length >8000 bp in the CLC Genomics Workbench v10.0.1 (CLC Bio, Aarhus, Denmark). The reads were assembled into the complete circular mitogenome in Geneious 8.1.3 (Biomatters, Auckland, New Zealand), with *Leptobelus gazella* (Membracidae: Centrotinae; GenBank: JF801955) [24] as a reference (Table S2, an example is shown in Fig. S1). Genome annotation was conducted in a similar fashion, using Geneious 8.1.3 and *Le. gazella* as a reference: 13 PCGs were predicted by finding the ORFs (employing the invertebrate mitochondrial genetic codon Table S5); two ribosomal RNA genes (*rrnS* and *rrnL*) and control (AT-rich) regions were identified based on the locations of adjacent genes and by comparison with the homologous sequences from other Centrotinae mitogenomes. tRNA genes were identified using the MITOS Web Server (http://mitos.bioinf.uni-leipzig.de/index.py) [25] and secondary structures were manually plotted with Adobe Illustrator CC2017 according to the MITOS predictions. Mitogenomic circular maps were depicted using Organellar Genome DRAW (OGDRAW) (https://chlorobox.mpimp-golm.mpg.de/OGDraw.html) [26].

2.3. Bioinformatic analyses

Analyses of the four sequenced mitogenomes, including base composition, composition skew, codon usage of PCGs, relative synonymous codon usage (RSCU), and comparative mitogenomic organization tables were conducted using PhyloSuite v1.1.15 [27]. Tandem repeat units of the AT-rich region were determined using the Tandem Repeats Finder online server [28]. Gene arrangements were investigated by comparing the newly sequenced genomes with those of five Centrotinae species available from GenBank (one species from Centrotini, two species from Tricentrini and two species from Leptobelini).

2.4. Phylogenetic analysis

A total of 63 mitogenomes of Cicadomorpha insects were used in the phylogenetic analysis, including 10 treehoppers (four newly sequenced mitogenomes and six from GenBank) and 49 leafhoppers. Two froghoppers (*Phymatostetha huangshanensis* and *Cosmoscarta bispecularis*) and two cicadas (*Meimuna opalifera* and *Tettigades auropilosa*) were selected as outgroups (Table 1). The included centrotine treehoppers represent six tribes: Centrotini, Tricentrini, Antialcidini, Leptobelini, Hypsauchenini, and Leptocentrini. Because *rrnS* and *rrnL* genes were not found in many partial mitogenomes and are also difficult to align, phylogenetic relationships were reconstructed using only the concatenated 13 PCGs (nucleotide and amino acid sequences).

Sequences of 13 PCGs of 63 species were aligned in batches with MAFFT integrated into PhyloSuite v1.1.15. Nucleotide sequences were aligned using the G-INS-i (accurate) strategy and codon alignment mode, and amino acid sequences were aligned using —auto strategy and normal alignment mode. Poorly aligned regions in the alignments were removed using Gblocks v0.91b [49]. Individual gene alignments were then concatenated using PhyloSuite v1.1.15. Bayesian inference (BI) phylogenetic analyses were conducted using amino acid sequence data and PHYLOBAYES MPI v.1.5a [50], which employs the site-heterogeneous model CAT + GTR. Two independent Markov chain Monte Carlo (MCMC) chains were run, and the analysis was stopped when the two runs had satisfactorily converged (maxdiff. fell below 0.3). A consensus tree was computed from the remaining trees combined from two runs after the initial 25% trees from each MCMC chain run were discarded as burn-in. For nucleotide sequence data, the best partitioning scheme and nucleotide substitution model for maximum likelihood (ML) and BI phylogenetic analyses were determined with PartitionFinder2 [51] using the Bayesian information criterion (BIC) and a greedy search algorithm with branch lengths linked (Tables S3–S4). Maximum likelihood phylogenies were inferred by IQ-TREE [52] using the ultrafast bootstrap (UFB) approximation approach [53] with 10,000 replicates, as well as the Shimodaira–Hasegawa–like approximate likelihood-ratio test [54] with 10,000 replicates. Bayesian inference was conducted using MrBayes 3.2.6 [55] with the following conditions: two independent runs of 10,000,000 generations were conducted with sampling every 1000 generations, four independent Markov chains, with the initial 25% of sampled data discarded as burn-in. Stationarity was assumed after the average standard deviation of split frequencies fell below 0.01.

3. Results and discussion

3.1. Mitogenome organization and nucleotide composition

The mitogenomes of *H. hardwichii* (15,618 bp), *L. albolineatus* (15,508 bp), *M. qinlingensis* (16,011 bp), and *T. brunneus* (16,467 bp) are single, covalently closed circular double-stranded DNA molecules (Tables S5–S8, Fig. 1). The mitogenomes of *H. hardwichii*, *L. albolineatus* are medium-sized compared to those of other Membracidae, which range from 15,201 bp (*Leptobelus* sp.) [45] to 16,007 bp (*Le. gazella*) [24]. The mitogenomes of *M. qinlingensis* and *T. brunneus* are larger than all other sequenced Membracidae species. Length variation of Membracidae mitogenomes occurs mainly due to variation in the size of the AT rich region. Each mitogenome includes the 37 typical animal mitochondrial genes (13 PCGs, 22 tRNAs, and 2 rRNAs) and a large non-coding control region (AT-rich region), which are usually present in bilaterian animals [20]. The gene order of the four newly sequenced centrotine treehoppers is consistent with the presumed ancestral arrangement of insects [56]. A majority of genes (9 PCGs and 14 tRNAs) are encoded on the majority strand (J-strand), while 14 genes (4 PCGs, 8 tRNAs and 2 rRNAs) are encoded on the minority strand (N-strand). There are two gene overlaps conserved among the four mitogenomes: *atp8-atp6* (7 bp: ATGATAA) and *nad4-nad4L* (7 bp: C/TATCAT) (Tables S5–S8). These overlaps are also found in other Membracidae species [24,32,44,45].

The AT nucleotide content of the four mitogenomes is similar: an average of 78.7% A + T in *H. hardwichii*, 78.1% in *L. albolineatus*, 77.6% in *M. qinlingensis*, and 78.6% in *T. brunneus* (Table 2), indicating strong AT bias, similar to that of other Membracidae insects [24,32,44,45]. The PCGs have the lowest AT content (76.1%–77.3%), and the AT-rich region has the highest (81.5%–86.5%) (Table 2), as in all previously sequenced

Table 1
Mitogenomes of the 63 Cicadomorpha insects used in this study.

Superfamily	Family	Subfamily	Species	Accession number	Reference
Membracoidea	Cicadellidae	Idiocerinae	*Populicerus populi*	NC_039427	[29]
			Idioscopus nitidulus	NC_029203	[30]
			Idiocerus laurifoliae	NC_039741	[29]
			Idioscopus clypealis	NC_039642	[31]
			Idioscopus myrica	MH492317	[29]
		Iassinae	*Trocnadella arisana*	NC_036480	Unpublished
		Evacanthinae	*Sophonia linealis*	KX437723	[32]
		Megophthalminae	*Japanagallia spinosa*	NC_035685	[33]
			Durgades nigropicta	NC_035684	[33]
		Coelidiinae	*Taharana fasciana*	NC_036015	[34]
			Olidiana sp.	KY039119	Unpublished
		Ledrinae	*Petalocephala ochracea*	KX437734	[32]
		Typhlocybinae	*Empoasca* sp.	KX437737	[32]
			Empoasca onukii	NC_037210	[35]
			Empoasca vitis	NC_024838	[36]
			Illinigina sp.	KY039129	[37]
			Typhlocyba sp.	KY039138	[37]
		Deltocephalinae	*Japananus hyalinus*	NC_036298	[38]
			Maiestas dorsalis	NC_036296	[38]
			Macrosteles quadrilineatus	NC_034781	[39]
			Macrosteles quadrimaculatus	NC_039560	[40]
			Tambocerus sp.	KT827824	[41]
			Nephotettix cincticeps	NC_026977	Unpublished
			Hishimonus phycitis	KX437727	[32]
			Psammotettix sp.1.	KX437742	[32]
			Psammotettix sp.2.	KX437725	[32]
			Cicadula sp.	KX437724	[32]
			Exitianus sp.	KX437722	[32]
			Phlogotettix sp.1.	KX437721	[32]
			Phlogotettix sp.2.	KY039135	[37]
			Dryadomorpha sp.	KX437736	[32]
			Osbornellus sp.	KX437739	[32]
			Agellus sp.	KX437738	[32]
			Scaphoideus varius	KY817245	[42]
			Scaphoideus nigrivalveus	KY817244	[42]
			Scaphoideus maai	KY817243	[42]
			Yanocephalus yanonis	NC_036131	[37]
			Alobaldia tobae	KY039116	[37]
			Exitianus indicus	KY039128	[37]
			Orosius orientalis	KY039146	[37]
			Deltocephalinae sp.	KX437726	[32]
			Norvellina sp.	KY039131	[37]
			Drabescoides nuchalis	NC_028154	[43]
		Cicadellinae	*Bothrogonia ferruginea*	KU167550	Unpublished
			Cuerna sp.	KX437741	[32]
			Graphocephala sp.	KX437740	[32]
			Cicadella viridis	KY752061	Unpublished
			Homalodisca coagulata	AY875213	Unpublished
			Cicadellinae sp.	KX437743	[32]
	Membracidae	Smiliinae	*Entylia carinata*	NC_033539	[44]
		Centrotinae	*Centrotus cornutus*	KX437728	[32]
			Tricentrus sp.	KY039118	Unpublished
			Leptobelus gazella	JF801955	[24]
			Leptobelus sp.	JQ910984	[45]
			Hypsauchenia hardwichii	MK746135	This study
			Leptocentrus albolineatus	MK746137	This study
			Maurya qinlingensis	MK746136	This study
			Tricentrus brunneus	MK746138	This study
	Aetalionidae	Aetalioninae	*Darthula hardwickii*	NC_026699	[46]
Cicadoidea	Cicadidae	Cicadinae	*Meimuna opalifera*	KY039112	[37]
		Tibicininae	*Tettigades auropilosa*	KM000129	Unpublished
Cercopoidea	Cercopidae	Cercopinae	*Cosmoscarta bispecularis*	KP064511	[47]
			Phymatostetha huangshanensis	MG878381	[48]

mitogenomes of membracid treehoppers [24,32,44,45]. The four new mitogenomes exhibit negative GC-skews (−0.207 to −0.120) and positive AT-skews (0.091–0.143), which is also common for centrotine treehoppers and the Hemiptera in general [57–59].

3.2. Protein-coding genes

The newly sequenced mitogenomes include the usual set of 13 PCGs. Most are initiated by a typical start codon ATN (ATA/T/G/C) and end with the TAA stop codon, or its incomplete form T–. Such incomplete stop codons are common in insects, and believed to be completed by posttranscriptional polyadenylation [60]. The *nad5* gene in all four mitogenomes uses TTG as the start codon (Tables S5–S8), as in the previously sequenced membracids *Entylia carinata* (NC_033539) [44] and *Le.* sp. (JQ910984) [45]. Furthermore, *nad2* and *cob* of *H. hardwichii*, *nad4L* of *L. albolineatus*, *nad4* of *M. qinlingensis*, and *cob* of *T. brunneus* use TAG as the stop codon (Tables S5–S8), as in other membracid mitogenomes [24,44,45].

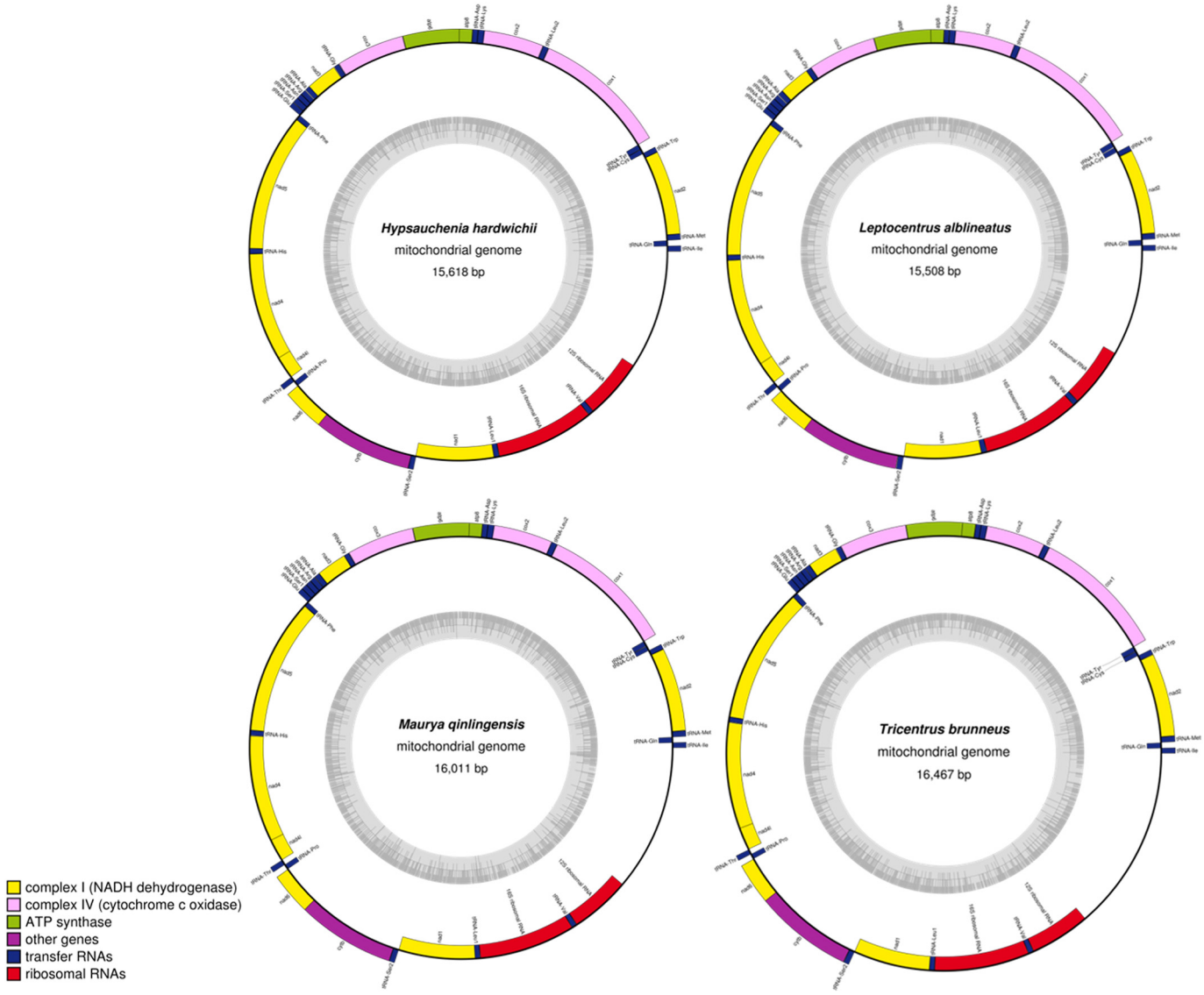

Fig. 1. Circular maps of the mitogenomes of *H. hardwichii*, *L. albolineatus*, *M. qinlingensis*, *T. brunneus*.

The AT-skews of the PCGs are similar (−0.151 to −0.135) among the four treehoppers (Table 2). Relative synonymous codon usage (RSCU) is summarized in Fig. 2, indicating that the three most frequently utilized amino acids were Leu, Ser, and Ile. Each species includes all 62 available codons (excluding TAA and TAG). In the four new mitogenomes, as well as other reported Membracidae mitogenomes [24,32,44,45], the four most frequently used codons are UUA (Leu), AUU (Ile), UUU (Phe) and AUA (Met). All of them are composed solely of A or U, reflecting the high A + T content of PCGs.

3.3. Transfer and ribosomal RNA genes

Each of the sequenced mitogenomes includes 22 tRNA genes (Tables S5–S8). The AT content of tRNA genes is slightly higher than that of the PCGs, ranging from 79.0% to 79.8% (Table 2). The arrangement of tRNAs is identical to those of previously sequenced Membracoidea, with the exception of three species of deltocephaline leafhoppers, which reported to have minor tRNA rearrangements [38–40]. Length of the 22 tRNAs ranges from 59 bp (*trnR* of *L. albolineatus*) to 72 bp (*trnKs* of *H. hardwichii*, *M. qinlingensis*, and

Table 2
Base composition and skewness of mitogenomes of *H. hardwichii*, *L. albolineatus*, *M. qinlingensis* and *T. brunneus*.

Feature	Length	A + T%	AT-skew	GC-skew
H. hardwichii, *L. albolineatus*, *M. qinlingensis* and *T. brunneus*				
Whole genome	15,618/15508/16011/16467	78.8/78.1/77.6/78.6	0.091/0.143/0.114/0.132	−0.125/−0.207/−0.12/−0.125
PCGs	10,923/10929/10929/10914	77.3/77.0/76.1/77.1	−0.15/−0.135/−0.151/−0.147	0.009/−0.033/0.003/0.002
tRNAs	1403/1419/1420/1412	79.4/79.0/79.4/79.8	0.007/0.008/0.013/0.018	0.183/0.154/0.181/0.168
rRNAs	1916/1910/1918/1904	81.4/80.1/81.1/81.4	−0.158/−0.183/−0.209/−0.215	0.298/0.279/0.262/0.266
AT-rich region	1433/1271/1789/2261	86.5/83.4/81.5/83.3	0.096/0.064/0.018/0.094	0.041/0.005/0.057/0.141

T. brunneus) (Tables S5–S8). The tRNAs can be folded into the common clover-leaf secondary structures, except for *trnS1*, in which the dihydrouridine (DHU) arm is replaced by a simple loop (Figs. S2–S5). The missing DHU arm of *trnS1* gene appeared very early in the Metazoa [61], and is common in insect mitogenomes. Based on the predicted secondary structure, we recognized a total of six types of unmatched base pairs (G-U, U-U, A-A, G-A, U-C, and A-C) in the arm structures of tRNAs of the four new mitogenomes. In some cases, there are also extra single A/U nucleotides in the stem structures.

The *rrnS* and *rrnL* genes have an AT nucleotide content ranging from 80.1% to 81.4% (Table 2). The *rrnL* gene, located between *trnL1* and *trnV*, ranges from 1163 bp to 1187 bp in length, and the *rrnS* gene, located between *trnV* and the AT-rich region, ranges from 727 bp to 741 bp (Tables S5–S8), similar to other sequenced membracids [24,32,44,45]. Therefore, the location, length and AT content of rRNAs are highly conserved in the Membracidae.

3.4. AT-rich region

The AT-rich region is believed to be involved in regulating the transcription and replication of DNA in insects [19,20]. All AT-rich regions of the four mitogenomes are located between *rrnS* and *trnI*, and their size ranges from 1271 bp to 2261 bp (Table 2). Analyses of the AT-rich regions indicate that the four taxa have different numbers of absolute tandem repeat units. Two types of absolute tandem repeats are present in *H. hardwichii* (nucleotide positions 114 to 335 and 1104 to 1364) and *T. brunneus* (positions 171 to 519 and 975 to 1189). The AT-rich regions of L. *albolineatus* and *M. qinlingensis* have only one kind of absolute tandem repeat, located at positions 84 to 363, and 218 to 586, respectively (Fig. 3). As in most insect mitogenomes, tandem repeats are common, and the size of tandem repeat regions varies depending on the number of copies of the repeating units [62]. Tandem repeats are thought to play an important role in the control of DNA methylation, gene transcription and replication [63,64].

3.5. Phylogenetic relationships

Phylogenetic analyses of 63 species of Cicadomorpha, including four outgroups, based on ML and BI analyses of nucleotide sequence data of 13 PCGs, yielded largely congruent topologies, with most branches receiving strong support (Figs. 4–5). Overall, the

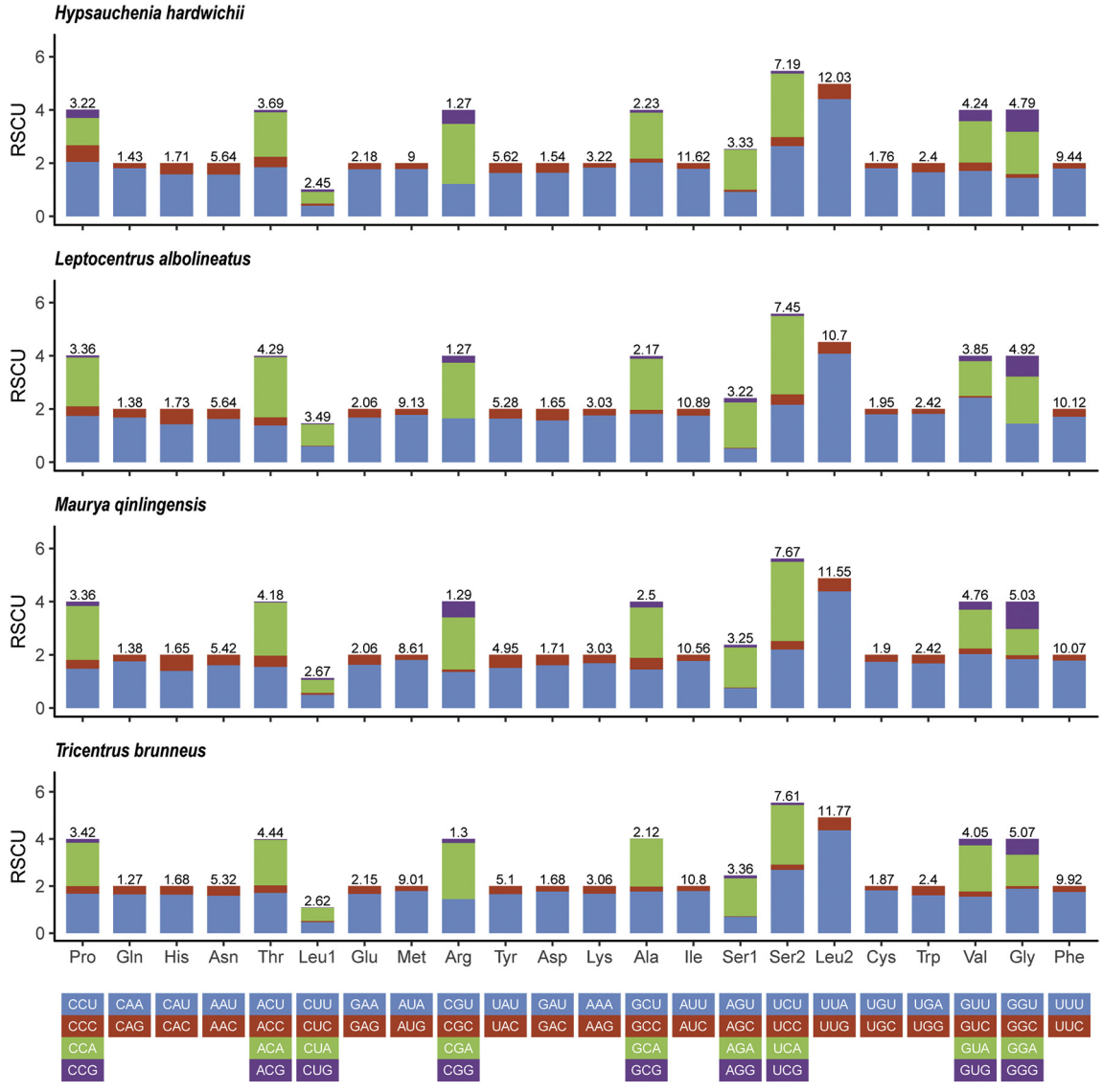

Fig. 2. Relative synonymous codon usage (RSCU) of the mitogenomes of four centrotine treehoppers. The stop codon is not shown.

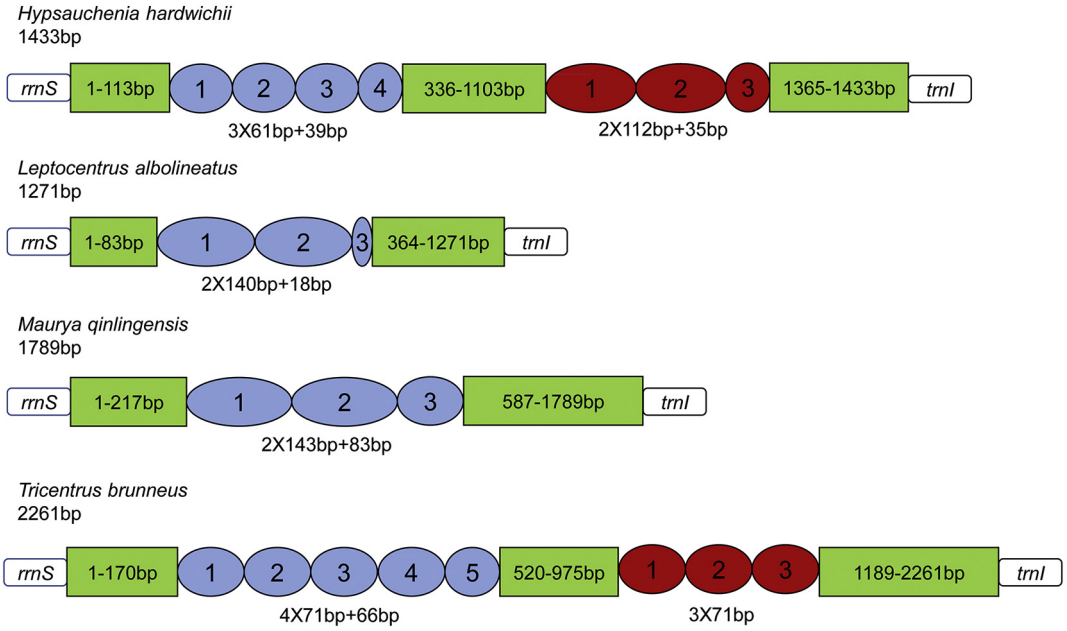

Fig. 3. Structures of AT-rich regions in the four centrotine treehopper mitogenomes. The location and copy number of absolute tandem repeat units are displayed by blue and red ovals. Green boxes indicate non-repeat regions.

relationships recovered in our analyses are similar to those found by Du et al. [40], but our taxon sample is much larger and overall branch support is higher. Nevertheless, as in other recent phylogenetic analyses of Membracoidea [*e.g.*, 18], some deep internal nodes within Cicadellidae were not consistently resolved among analyses and received less than maximal branch support. For example, in the BI tree (Fig. 5) Coelidiinae and Iassinae are sister to the remaining Membracoidea except Deltocephalinae, while in the ML tree (Fig. 4), Coelidiinae and Iassinae are sister to a clade comprising Idiocerinae, Megophthalminae and the treehoppers. The latter result was also obtained from analysis of amino acid sequences using Phylobayes (Fig. S6), but with less than maximum support (PP = 0.76). Deltocephaline leafhoppers were sister the remaining Membracoidea both in ML and BI trees (SH-aLRT = 100; BS = 100; PP = 1.00), consistent with some previous studies [18,29,40].

All analyses consistently supported the monophyly of Membracoidea and the included membracoid subfamilies represented by more than one species (Deltocephalinae, Typhlocybinae, Cicadellinae, Coelidiinae, Idiocerinae, Megophthalminae, and Centrotinae) with strong support (SH-aLRT > 96.2; BS > 93; PP > 0.97). The relationships among Idiocerinae, Megophthalminae, Smiliinae, Aetalionidae, and Centrotinae were congruent among results (Figs. 4–5; Fig. S6), but support for some branches was not very high (SH-aLRT < 70; BS < 75; PP < 0.90). The recovered relationships generally agree with previously published phylogenies based on the *28S* rRNA gene and mitogenomes [29,40,65], although placement of Smiliinae as sister to the remaining treehoppers has not been suggested previously and may be due to the absence of other non-centrotine membracids in the dataset. Treehoppers (Membracidae and Aetalionidae) are monophyletic and originate from paraphyletic Cicadellidae, as indicated by previous molecular phylogenetic analyses [18,29,32,38,40,42,65]. Monophyly of Centrotinae received strong support (SH-aLRT = 100; BS = 100; PP = 1.00), but more data, especially for representatives of Centronodinae are needed to better understand the monophyly of Centrotinae [66]. In Centrotinae, the relationships among included tribes are inferred as (Centrotini + (Tricentrini + Antialcidini)) + ((Leptobelini + Hypsauchenini) + Leptocentrini). Although the sister-group relationship of Tricentrini and Antialcidini was also recovered by previous analyses of morphological data [6,9], other aspects of the phylogeny differ from these prior results. This may be due, in part, to the very limited taxon sample in the present study. Nevertheless, our overall results suggest that sequences of mitochondrial PCGs are informative of relationships at different levels within the taxonomic hierarchy of Membracoidea. Therefore, sequencing of additional mitogenomes may help improve phylogenetic resolution of this group.

4. Conclusion

Mitogenomes of *H. hardwichii*, *L. albolineatus*, *M. qinlingensis*, and *T. brunneus* are highly conservative in overall structure and AT content and similar to those of other Membracidae. Bayesian inference and maximum likelihood analysis of concatenated alignments of all mitochondrial PCGs yielded well-resolved phylogenies largely congruent with previous studies and with most branches receiving strong bootstrap support except a few deep internal nodes within Cicadellidae. Membracoidea are recovered as monophyletic with strong support, and Cicadellidae as paraphyletic with respect to Aetalionidae + Membracidae. Within the Membracoidea, currently recognized subfamilies for which more than one representative was available were recovered as monophyletic. This suggests that further sequencing of mitogenomes can contribute to resolving phylogenetic relationships at various levels within the taxonomic hierarchy of Membracoidea.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijbiomac.2019.08.064.

Acknowledgements

We thank Dr. John Richard Schrock (Emporia State University, Emporia, KS, USA) for reviewing the manuscript. We sincerely thank Dr. Hu Li (China Agricultural University, China) for help with data analysis. This study was supported by the National Key Research and Development Program of China (2017YFD0200900, 2017YFD0201800) and the National Natural Science Foundation of China (Nos. 31772503, 31272345, 31871971 and 31420103911).

Declaration of competing interest

All authors report no conflicting interests.

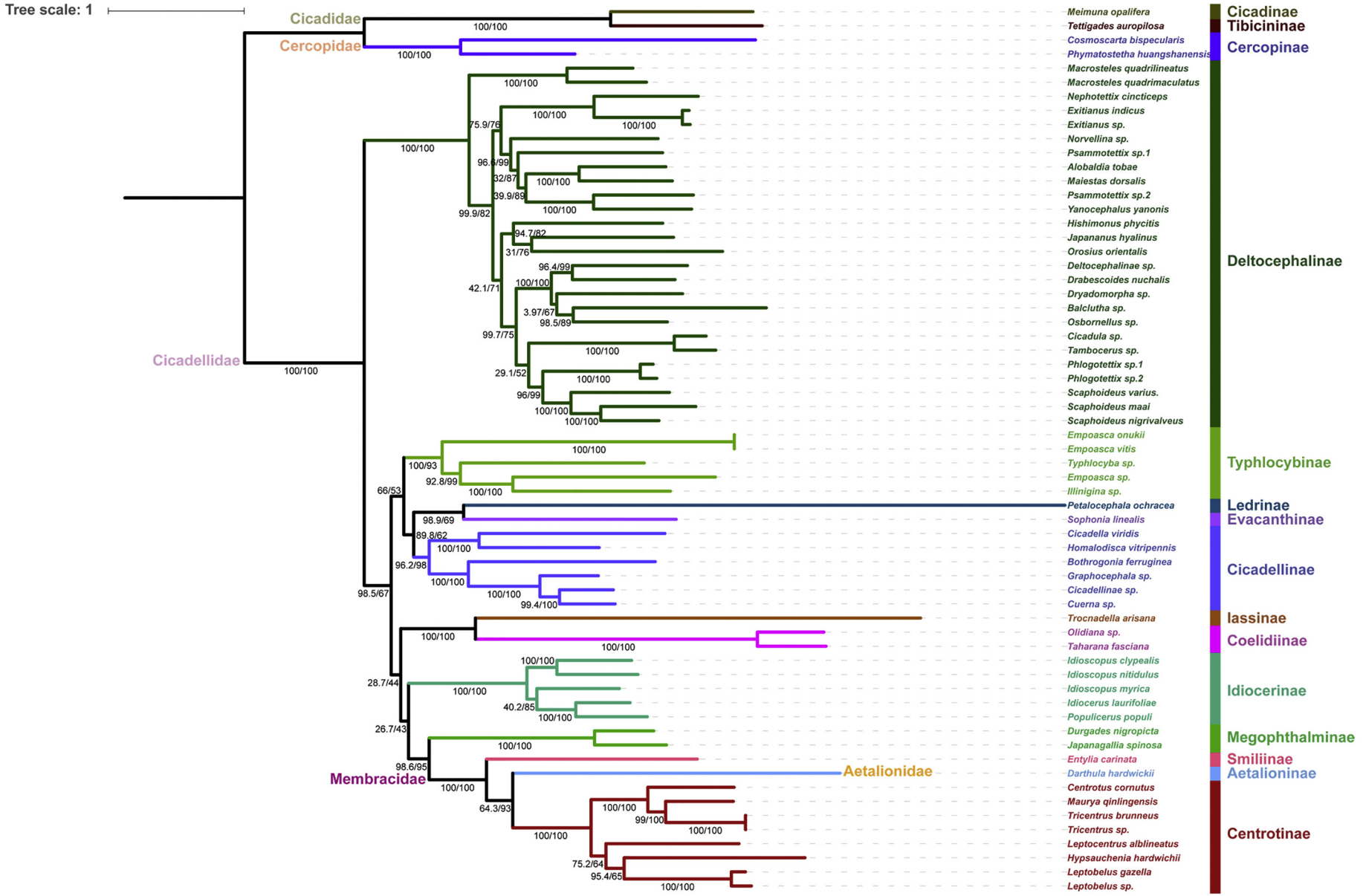

Fig. 4. ML tree inferred using IQ-TREE and the PCG123 dataset. SH-aLRT values and Bootstrap support values (BS) are indicated on branches.

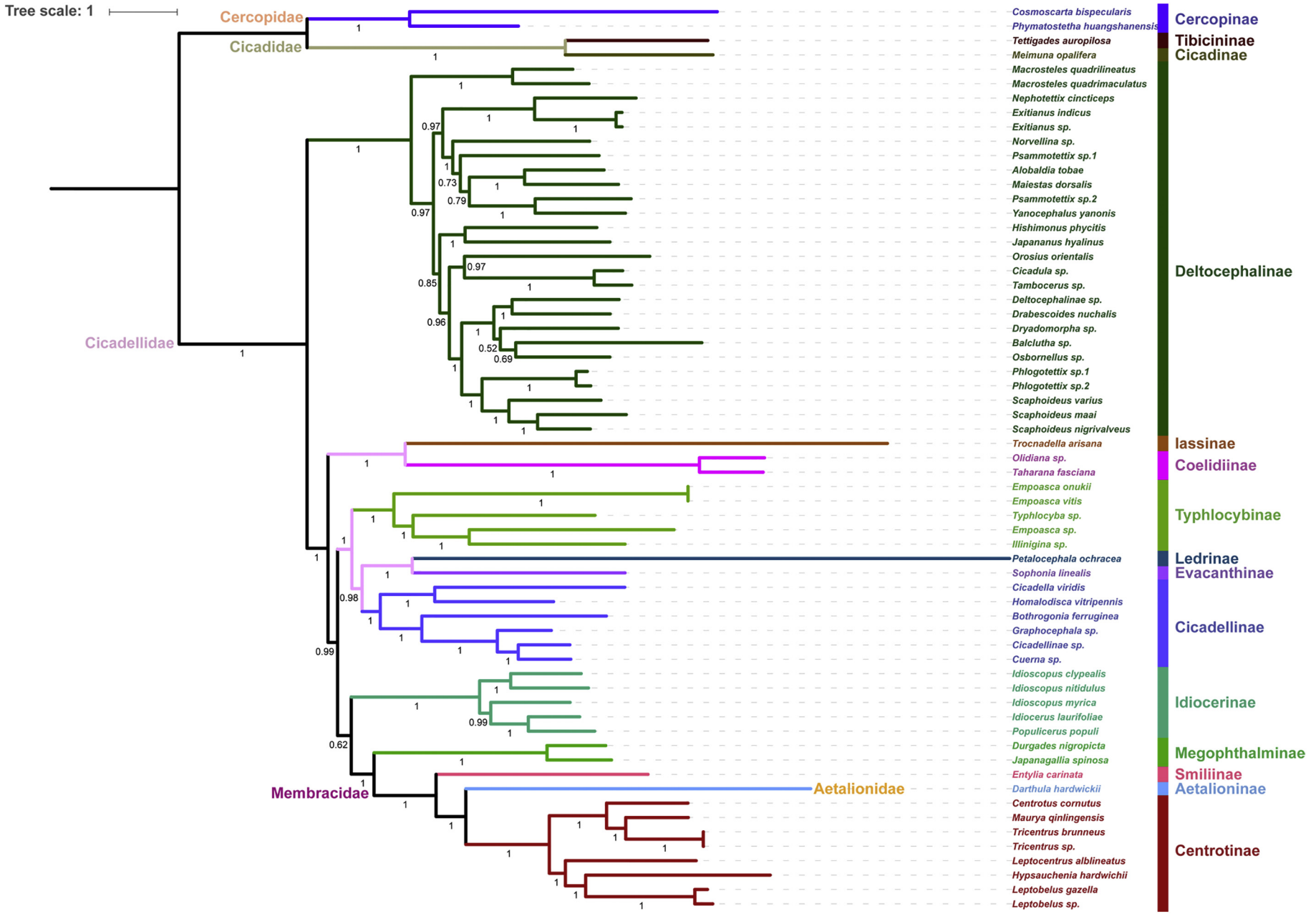

Fig. 5. Bayesian tree inferred using MrBayes and the PCG123 dataset. Bayesian posterior probabilities (PP) are indicated on branches.

References

[1] J.R. Cryan, B.M. Wiegmann, L.L. Deitz, C.H. Dietrich, Phylogeny of the treehoppers (Insecta: Hemiptera: Membracidae): evidence from two nuclear genes, Mol. Phylogenet. Evol. 17 (2) (2000) 317–334.

[2] C.H. Dietrich, S.H. Mckamey, L.L. Deitz, Morphology-based phylogeny of the treehopper family Membracidae (Hemiptera Cicadomorpha Membracoidea), Syst. Entomol. 26 (2001) 213–239.

[3] O. Evangelista, A.M. Sakakibara, J.R. Cryan, J.M. Urban, A phylogeny of the treehopper subfamily Heteronotinae reveals convergent pronotal traits (Hemiptera: Auchenorrhyncha: Membracidae), Syst. Entomol. 42 (2) (2017) 410–428.

[4] C.P. Lin, B.N. Danforth, T.K. Wood, Molecular phylogenetics and evolution of maternal care in membracine treehoppers, Syst. Biol. 53 (3) (2004) 400–421.

[5] M.S. Wallace, L.L. Deitz, Australian treehoppers (Hemiptera Membracidae Centrotinae Terentiini) phylogeny and biogeography, Invertebr. Syst. 20 (2006) 163–183.

[6] F. Yuan, I. Chou, Fauna sinica insecta, Homoptera Membracoidea Aetalionidae Membracidae, vol.28, Science Press, Beijing, China 2002, pp. 1–590.

[7] B.O. Morris, Studies of New World Treehoppers of the Subfamily Centrotinae With Emphasis on the Caribbean Fauna (Hemiptera: Membracidae), [Master's Degree] University of Illinois at Urbana-Champaign, Illinois, USA, 2017 1–150.

[8] L.L. Deitz, C.H. Dietrich, Superfamily Membracoidea (Homoptera: Auchenorrhyncha). I. Introduction and revised classification with new family-group taxa, Syst. Entomol. 18 (4) (1993) 287–296.

[9] M.S. Wallace, L.L. Deitz, Phylogeny and Systematics of the Treehopper Subfamily Centrotinae (Hemiptera: Membracidae), Associated Publishers, Florida, USA, 2004 1–377.

[10] S.H. McKamey, Taxonomic catalogue of the Membracoidea (exclusive of leafhoppers): second supplement to fascicle 1, Membracidae, of the General catalogue of the Hemiptera, Mem. American Entomol. Institute 60 (1998) 1–377.

[11] A.L. Capener, The Taxonomy of the African Membracidae. Part I. The Oxyrhachinae, Entomology Memoir, vol. 6, Department of Agricultural Technical Service, Republic of South Africa, 1962 1–164.

[12] A.L. Capener, The Taxonomy of the African Membracidae: Part 2, the Centrotinae, Entomology Memoir, vol. 17, Department of Agricultural Technical Service, Republic of South Africa, 1968 1–124.

[13] M.F. Day, The genera of Australian Membracidae (Hemiptera: Auchenorrhyncha), Invertebr. Syst. 13 (4) (1999) 629–747.

[14] W.L. Distant, Rhynchota-Homoptera, the Fauna of British India, Including Ceylon and Burma, vol. 4, 1908 1–501.

[15] J.W. Evans, The leafhoppers and froghoppers of Australia and New Zealand (Homoptera: Cicadelloidea and Cercopoidea), Australian Mus. Mem. 12 (1966) 1–347.

[16] F.W. Goding, A synopsis of the subfamilies and genera of the Membracidae of North America, T. Am. Entomol. Soc. 19 (1892) 253–260.

[17] I. Ahmad, A cladistic analysis of Tricentrini (Homoptera: Auchenorrhyncha: Membracidae) with a note on their origin, distribution and food plants, in: C. Vidano, A. Arzone (Eds.),6th Auchenorrhyncha Meeting, Turin, Italy 1987, pp. 473–484.

[18] C.H. Dietrich, J.M. Allen, A.R. Lemmon, E.M. Lemmon, D.M. Takiya, O. Evangelista, K.K.O. Walden, P.G.S. Grady, K.P. Johnson, Anchored hybrid enrichment-based phylogenomics of leafhoppers and treehoppers (Hemiptera: Cicadomorpha: Membracoidea), Insect Systematics and Diversity 1 (1) (2017) 57–72.

[19] J.L. Boore, Animal mitochondrial genomes, Nucleic Acids Res. 27 (8) (1999) 1767–1780.

[20] S.L. Cameron, Insect mitochondrial genomics: implications for evolution and phylogeny, Annu. Rev. Entomol. 59 (2014) 95–117.

[21] D.A. Clayton, Replication of animal mitochondrial DNA, Cell 28 (4) (1982) 693–705.

[22] N. Galtier, B. Nabholz, S. Glemin, G.D. Hurst, Mitochondrial DNA as a marker of molecular diversity: a reappraisal, Mol. Ecol. 18 (22) (2009) 4541–4550.

[23] J.D. Fenn, H. Song, S.L. Cameron, M.F. Whiting, A preliminary mitochondrial genome phylogeny of Orthoptera (Insecta) and approaches to maximizing phylogenetic signal found within mitochondrial genome data, Mol. Phylogenet. Evol. 49 (1) (2008) 59–68.

[24] X. Zhao, A.P. Liang, Complete DNA sequence of the mitochondrial genome of the treehopper *Leptobelus gazella* (Membracoidea: Hemiptera), Mitochondrial DNA A 27 (5) (2016) 3318–3319.

[25] M. Bernt, A. Donath, F. Juhling, F. Externbrink, C. Florentz, G. Fritzsch, J. Putz, M. Middendorf, P.F. Stadler, MITOS: improved de novo metazoan mitochondrial genome annotation, Mol. Phylogenet. Evol. 69 (2) (2013) 313–319.

[26] M. Lohse, O. Drechsel, S. Kahlau, R. Bock, OrganellarGenomeDRAW—a suite of tools for generating physical maps of plastid and mitochondrial genomes and visualizing expression data sets, Nucleic Acids Res. 41 (2013) W575–W581 (Web Server issue).

[27] D. Zhang, F.L. Gao, W.X. Li, I. Jakovlić, H. Zou, J. Zhang, G.T. Wang, PhyloSuite: an integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies, bioRxiv (2018) 489088.

[28] G. Benson, Tandem repeats finder a program to analyze DNA sequences, Nucleic Acids Res. 27 (2) (1999) 573–580.

[29] J.J. Wang, M.F. Yang, R.H. Dai, H. Li, X.Y. Wang, Characterization and phylogenetic implications of the complete mitochondrial genome of Idiocerinae (Hemiptera: Cicadellidae), Int. J. Biol. Macromol. 120 (B) (2018) 2366–2372.

[30] J.S. Choudhary, N. Naaz, B. Das, B.P. Bhatt, C.S. Prabhakar, Complete mitochondrial genome of *Idioscopus nitidulus* (Hemiptera: Cicadellidae), Mitochondrial DNA Part B 3 (1) (2018) 191–192.

[31] R.H. Dai, J.J. Wang, M.F. Yang, The complete mitochondrial genome of the leafhopper *Idioscopus clypealis* (Hemiptera: Cicadellidae: Idiocerinae), Mitochondrial DNA Part B 3 (1) (2017) 32–33.

[32] N. Song, H. Li, W.Z. Cai, Insufficient power of mitogenomic data in resolving the auchenorrhynchan monophyly, Zool. J. Linn. Sco-Lond. 183 (4) (2017) 776–790.

[33] J.J. Wang, R.H. Dai, H. Li, H.P. Zhan, Characterization of the complete mitochondrial genome of *Japanagallia spinosa* and *Durgades nigropicta* (Hemiptera: Cicadellidae: Megophthalminae), Biochem. Syst. Ecol. 74 (2017) 33–41.

[34] J.J. Wang, H. Li, R.H. Dai, Complete mitochondrial genome of *Taharana fasciana* (Insecta, Hemiptera: Cicadellidae) and comparison with other Cicadellidae insects, Genetica 145 (6) (2017) 593–602.

[35] J.H. Liu, C.Y. Sun, J. Long, J.J. Guo, Complete mitogenome of tea green leafhopper, *Empoasca onukii* (Hemiptera: Cicadellidae) from Anshun, Guizhou Province in China, Mitochondrial DNA Part B 2 (2) (2017) 808–809.

[36] N.N. Zhou, M.X. Wang, L. Cui, X.X. Chen, B.Y. Han, Complete mitochondrial genome of *Empoasca vitis* (Hemiptera: Cicadellidae), Mitochondrial DNA Part A 27 (2) (2016) 1052–1053.

[37] N. Song, W.Z. Cai, H. Li, Deep-level phylogeny of Cicadomorpha inferred from mitochondrial genomes sequenced by NGS, Sci. Rep. 7 (1) (2017) 10429.

[38] Y.M. Du, C.N. Zhang, C.H. Dietrich, Y.L. Zhang, W. Dai, Characterization of the complete mitochondrial genomes of *Maiestas dorsalis* and *Japananus hyalinus* (Hemiptera: Cicadellidae) and comparison with other Membracoidea, Sci. Rep. 7 (1) (2017), 14197.

[39] X.S. Yang, G. Bennett, M. Mao, The complete mitochondrial genome of *Macrosteles quadrilineatus* (Hemiptera: Cicadellidae), Mitochondrial DNA Part B 2 (1) (2017) 173–175.

[40] Y.M. Du, C.H. Dietrich, W. Dai, Complete mitochondrial genome of *Macrosteles quadrimaculatus* (Matsumura) (Hemiptera: Cicadellidae: Deltocephalinae) with a shared tRNA rearrangement and its phylogenetic implications, Int. J. Biol. Macromol. 122 (2019) 1027–1034.

[41] P.F. Yu, M.X. Wang, L. Cui, X.X. Chen, B.Y. Han, The complete mitochondrial genome of *Tambocerus* sp. (Hemiptera: Cicadellidae), Mitochondrial DNA A 28 (1) (2017) 133–134.

[42] Y.M. Du, W. Dai, C.H. Dietrich, Mitochondrial genomic variation and phylogenetic relationships of three groups in the genus *Scaphoideus* (Hemiptera: Cicadellidae: Deltocephalinae), Sci. Rep. 7 (1) (2017), 16908.

[43] Y.F. Wu, R.H. Dai, H.P. Zhan, L. Qu, Complete mitochondrial genome of *Drabescoides nuchalis* (Hemiptera: Cicadellidae), Mitochondrial DNA Part A 27 (5) (2015) 3626–3627.

[44] M. Mao, X.S. Yang, G. Bennett, The complete mitochondrial genome of *Entylia carinata* (Hemiptera: Membracidae), Mitochondrial DNA Part B 1 (1) (2016) 662–663.

[45] H. Li, J.L. J.M., E.G. Chapman, D. Burkhardt, F. Song, P. Jiang, J. Liu, X. Zhao, W.Z. Cai, Mitochondrial phylogenomics of Hemiptera reveals adaptive innovations driving the diversification of true bugs, Proc. Biol. Sci. 284(1862) (2017).

[46] A.P. Liang, J. Gao, X. Zhao, Characterization of the complete mitochondrial genome of the treehopper *Darthula hardwickii* (Hemiptera: Aetalionidae), Mitochondrial DNA Part A 27 (5) (2016) 3291–3292.

[47] H. Yang, J. Liu, A.P. Liang, The complete mitochondrial genome of *Cosmoscarata bispecularis* (Hemiptera, Cicadomorpha, Cercopoidea, Cercopidae), Mitochondrial DNA. Part A, DNA mapping, sequencing, and analysis 27 (6) (2016) 3957–3958.

[48] T.J. Su, A.P. Liang, Characterization of the complete mitochondrial genome of *Phymatostetha huangshanensis* (Hemiptera: Cercopidae) and phylogenetic analysis, Int. J. Biol. Macromol. 119 (2018) 60–69.

[49] J. Castresana, Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis, Mol. Biol. Evol. 17 (4) (2000) 540–552.

[50] N. Lartillot, N. Rodrigue, D. Stubbs, J. Richer, PhyloBayes MPI phylogenetic reconstruction with infinite mixtures of profiles in a parallel environment, Syst. Biol. 62 (4) (2013) 611–615.

[51] R. Lanfear, P.B. Frandsen, A.M. Wright, T. Senfeld, B. Calcott, PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses, Mol. Biol. Evol. 34 (3) (2017) 772–773.

[52] L.T. Nguyen, H.A. Schmidt, A.v. Haeseler, B.Q. Minh, IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies, Mol. Biol. Evol. 32 (1) (2015) 268–274.

[53] B.Q. Minh, M.A.T. Nguyen, A.v. Haeseler, Ultrafast approximation for phylogenetic bootstrap, Mol. Biol. Evol. 30 (5) (2013) 1188–1195.

[54] S. Guindon, J.F. Dufayard, V. Lefort, M. Anisimova, W. Hordijk, O. Gascuel, New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0, Syst. Biol. 59 (3) (2010) 307–321.

[55] F. Ronquist, M. Teslenko, P.V.D. Mark, D.L. Ayres, A. Darling, S. Hohna, B. Larget, L. Liu, M.A. Suchard, J.P. Huelsenbeck, MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space, Syst. Biol. 61 (3) (2012) 539–542.

[56] D.O. Clary, D.R. Wolstenholme, The mitochondrial DNA molecule of *Drosophila yakuba* nucleotide sequence, gene organization, and genetic code, J. Mol. Evol. 22 (1985) 252–271.

[57] J. Chen, Y. Wang, M. Qin, L.Y. Jiang, G.X. Qiao, The mitochondrial genome of *Greenidea psidii* van der Goot (Hemiptera: Aphididae: Greenideinae) and comparisons with other Aphididae aphids, Int. J. Biol. Macromol. 122 (2019) 824–832.

[58] N. Song, A.P. Liang, The complete mitochondrial genome sequence of *Geisha distinctissima* (Hemiptera: Flatidae) and comparison with other hemipteran insects, Acta. Bioch. Bioph. Sin. 41 (3) (2009) 206–216.

[59] Y. Wang, J. Chen, L.Y. Jiang, G.X. Qiao, Hemipteran mitochondrial genomes: features, structures and implications for phylogeny, Int. J. Mol. Sci. 16 (6) (2015) 12382–12404.

[60] D. Ojala, J. Montoya, G. Attardi, tRNA punctuation model of RNA processing in human mitochondria, Nature 290 (1981) 470.

[61] J.R. Garey, D.R. Wolstenholme, Platyhelminth mitochondrial DNA: evidence for early evolutionary origin of a tRNAserAGN that contains a dihydrouridine arm

replacement loop, and of serine-specifying AGA and AGG codons, J. Mol. Evol. 28 (5) (1989) 374–387.
[62] D.X. Zhang, J.M. Szymura, G.M. Hewitt, Evolution and structural conservation of the control region of insect mitochondrial DNA, J. Mol. Evol. 40 (4) (1995) 382–391.
[63] W.X. Huang, J.B. Zheng, Y. He, C. Luo, Tandem repeat modification during double-strand break repair induced by an engineered TAL effector nuclease in zebrafish genome, PloS one 8 (12) (2013), e84176.
[64] G. Levinson, G.A. Gutman, Slipped-strand mispairing: a major mechanism for DNA sequence evolution, Mol. Biol. Evol. 4 (3) (1987) 203–221.
[65] C.H. Dietrich, R.A. Rakitov, J.L. Holmes, W.C. Black, Phylogeny of the major lineages of Membracoidea (Insecta: Hemiptera: Cicadomorpha) based on *28S* rDNA sequences, Mol. Phylogenet. Evol. 18 (2) (2001) 293–305.
[66] J.R. Cryan, B.M. Wiegmann, L.L. Deitz, C.H. Dietrich, M.F. Whiting, Treehopper trees phylogeny of Membracidae(Hemiptera: Cicadomorpha: Membracoidea) based on molecules and morphology, Syst. Entomol. 29 (2004) 441–454.