Comparative Mitogenomics of the Assassin Bug Genus *Peirates* (Hemiptera: Reduviidae: Peiratinae) Reveal Conserved Mitochondrial Genome Organization of *P. atromaculatus*, *P. fulvescens* and *P. turpis*

Guangyu Zhao[1,2‡], Hu Li[1,3‡] *, Ping Zhao[4], Wanzhi Cai[1] *

1 Department of Entomology, China Agricultural University, Beijing, China, **2** Daxing No.1 Middle School, Beijing, China, **3** Department of Ornamental Horticulture, China Agricultural University, Beijing, China, **4** College of Environment and Life Sciences, Kaili University, Kaili, Guizhou, China

‡ These authors contributed equally to this work.
* caiwz@cau.edu.cn (WC); tigerleecau@hotmail.com (HL)

Abstract

In this study, we sequenced four new mitochondrial genomes and presented comparative mitogenomic analyses of five species in the genus *Peirates* (Hemiptera: Reduviidae). Mitochondrial genomes of these five assassin bugs had a typical set of 37 genes and retained the ancestral gene arrangement of insects. The A+T content, AT- and GC-skews were similar to the common base composition biases of insect mtDNA. Genomic size ranges from 15,702 bp to 16,314 bp and most of the size variation was due to length and copy number of the repeat unit in the putative control region. All of the control region sequences included large tandem repeats present in two or more copies. Our result revealed similarity in mitochondrial genomes of *P. atromaculatus*, *P. fulvescens* and *P. turpis*, as well as the highly conserved genomic-level characteristics of these three species, e.g., the same start and stop codons of protein-coding genes, conserved secondary structure of tRNAs, identical location and length of non-coding and overlapping regions, and conservation of structural elements and tandem repeat unit in control region. Phylogenetic analyses also supported a close relationship between *P. atromaculatus*, *P. fulvescens* and *P. turpis*, which might be recently diverged species. The present study indicates that mitochondrial genome has important implications on phylogenetics, population genetics and speciation in the genus *Peirates*.

Introduction

Peiratinae, a subfamily of the assassin bugs (Reduviidae), consists of over 350 described species in 30 valid genera [1]. These assassin bugs are primarily ground-dwelling and are usually

attracted to lights [2]. Some of them are common predators in the crop fields and recognized as natural enemies of pest insects. *Peirates* Serville sensu stricto is one of the largest genera of Peiratinae with about 40 valid species worldwide, mainly distributed in the Oriental, Palearctic and Ethiopian regions [3]. Seven species of the genus have been recorded in China [4]. Taxonomic, phylogenetic and biogeographical studies of this genus have been conducted [5–7]. Cladistic analysis based on 51 morphological characters showed three distinct groups in this genus: the *P. quadrinotatus* group, the *P. singularis* group and the *P. lepturoides* group [7]. However, the application of molecular data to the study of this genus is nearly negligible to date, with only one complete mitochondrial (mt) genome sequence and 13 partial DNA sequences of two mt (*16S* rDNA and *COI*) and four nuclear (*18S*, *28S* rDNA, *Wingless* and *Deformed*) genes from two species available at GenBank (October 17, 2014).

The mt genome of insect typically contains 13 protein-coding genes (PCGs), 22 transfer RNAs (tRNAs), two ribosomal RNAs (rRNAs) (the large and small ribosomal subunits), and a putative control region [8]. The whole mt genome sequences not only are more informative than individual genes, but also provide a suite of genome level characters [9], such as the gene rearrangement [10–13], strand asymmetry in nucleotide composition [14] and evolutionary patterns of the control region [15,16]. Mt genomes have been applied to a wide array of studies, including molecular systematics at both deep and shallow taxonomic scales [17–20], population genetics [21,22], diagnostics [23] and molecular evolutionary studies [24–26].

The mt genome of *P. arcuatus* has been reported previously [27]. Here, we present complete mt genomes of other four *Peirates* species, *P. lepturoides*, *P. atromaculatus*, *P. fulvescens* and *P. turpis*, and provide comparative mitogenomic analyses of five species from this genus, e.g., gene order, nucleotide composition, codon usage, tRNA secondary structure, gene overlaps and non-coding regions, to explore the sequence variability and evolutionary traits of the *Peirates* mt genomes.

Materials and Methods

Ethics statement

No specific permits were required for the insects collected for this study. The insect specimens were collected from farmland and orchard. The field collections did not involve endangered or protected species. The species sequenced in the family Reduviidae are common insects and are not included in the "List of Protected Animals in China".

Samples and DNA extraction

Four adult assassin bugs used in this study were collected from the field in China, and the collection information was provided in S1 Table. Specimens were initially preserved in 100% ethanol in the field, and then stored at -20°C. Specimens were identified by the use of taxonomic keys [5, 28] and careful comparisons using morphological characters, especially the color pattern of pronotum and hemelytron (S2 Table; S1 Fig.). For each species, genomic DNA was extracted from adult muscle tissues of the thorax using the DNeasy DNA Extraction kit (Qiagen). Samples and voucher specimens were deposited in the Entomological Museum of China Agricultural University with unique numbers (S1 Table).

PCR amplification and sequencing

For each species, whole mt genome was amplified by PCR in overlapping fragments with universal insect mt primers [29] (S3 Table), and species-specific primers were designed based on

the sequenced fragments to bridge gaps. PCR and sequencing reactions were conducted following [12,16,30]. The sequence data have been deposited in GenBank (Table 1).

Genome assembly and annotation

Contigs were assembled from forward and reverse reads using Sequencher (Gene Codes Corporation, Ann Arbor, MI, USA). Protein-coding genes and rRNA genes were identified by BLAST searches in GenBank and subsequently confirmed by alignment with homologous genes from other true bugs. tRNA genes were identified by tRNAscan-SE Search Server v.1.21 [31]. *trnR* and *trnS1*, which could not be identified by tRNAscan-SE, were determined by sequence similarity comparison with tRNA genes of other insects.

Sequence alignment and genomic analyses

Each PCG was aligned individually based on codon-based multiple alignments by using the MAFFT algorithm within the TranslatorX [32] online platform. The sequences of tRNAs, rRNAs and non-coding regions were aligned respectively using ClustalW in MEGA 5.0 [33]. The base composition, codon usage and genetic distances of PCGs were analyzed with MEGA 5.0. Sequence alignments of barcoding region, single PCG and concatenated 13 PCGs were provided in S1 Dataset.

Phylogenetic analyses

Complete mt genomes of five species in the genus *Peirates* and a outgroup species *Sirthenea flavipes* (GenBank: NC_020143) from the same subfamily were used in phylogenetic analyses. We inferred phylogenies using sequences of all 37 mt genes (13 PCGs, two rRNAs and 22 tRNAs). Alignments of individual genes were concatenated using SequenceMatrix v1.7.8 [34]. The concatenated matrix with 14,692 nucleotides was analyzed with maximum likelihood (ML), Bayesian inference (BI) and neighbor-joining (NJ) methods.

The optimal partition strategy and model of each partition was selected by PartitionFinder v1.1.1 [35]. We created an input configuration file that contained pre-define 37 gene partitions. The "greedy" algorithm with branch lengths estimated as "unlinked" and Bayesian information criterion (BIC) was used to search for the best-fit scheme.

We performed ML and BI analyses using the best-fit partitioning schemes recommended by PartitionFinder (S4 Table). ML analyses were conducted using RAxML 8.0.0 [36] with GTRGAMMA model. Node support was calculated by acquiring bootstrap values from heuristic searches of 1000 resampled datasets, using the rapid bootstrap feature (random seed value 12345) [37]. Bayesian analyses were carried out using MrBayes 3.2.2 [38]. Two simultaneous runs of 5 million generations were conducted for the dataset and trees were sampled every 1000 generations, with the first 25% discarded as burn-in. Two runs have satisfactorily converged with standard deviation of split frequency lower than 0.0001. All RAxML and MrBayes analyses were conducted in the CIPRES Science Gateway v3.3 [39]. The neighbor-joining tree was constructed using MEGA 5.0 [33] with 1000 bootstrap replicates. NEXUS and PHYLIP file for BI and ML analyses were provided in S2 and S3 Datasets.

Results and Discussion

General features of Peirates mt genomes

In this study, mt genomes of four species were newly sequenced, and five complete mt genomes representing five species in the genus *Peirates* were compared (Fig. 1, Table 1). For convenience, we assigned each mt genome an abbreviation (PF for *P. fulvescens*, PAY for *P.*

Table 1. Structural feature of *Peirates* mitochondrial genomes used in this study.

Species	GenBank [a]	AT%	AT-skew	GC-skew	Size (bp)					
					Genome	PCGs	tRNAs	srRNA	lrRNA	CR [b]
P. fulvescens *	KF913537	71.9	0.149	-0.181	15,702	11,143	1,447	784	1,253	1,044
P. atromaculatus *	KF913539	71.0	0.151	-0.195	16,151	11,143	1,451	784	1,254	1,488
P. turpis *	KF913540	72.0	0.147	-0.183	15,703	11,143	1,448	784	1,253	1,044
P. arcuatus	KF752445	71.3	0.128	-0.176	16,176	11,114	1,456	781	1,254	1,552
P. lepturoides *	KF913541	72.4	0.13	-0.158	15,932	11,146	1,461	781	1,261	1,263

* mitochondrial genome sequenced in this study

[a] GenBank accession number

[b] control region.

doi:10.1371/journal.pone.0117862.t001

atromaculatus, PT for *P. turpis*, PL for *P. lepturoides* and PA for *P. arcuatus*). These mt genomes ranged in size from 15,702 bp for PF to 16,176 bp for PA and most of the size variation was due to differences in the putative control region. All of the genomes examined here had a typical set of 37 genes and retained the so-called ancestral mt genome arrangement of insects (Fig. 1). Generally, the A+T content, AT- and GC-skews exhibited similar behaviors and were similar to the common base composition biases of insect mtDNA [14]. Some general characteristics of the genomes were given in Table 1.

Protein-coding genes

All PCGs of the analyzed mt genomes initiated with ATN start codons and mostly terminated with TAA or TAG stop codons, except for several genes ended with a single T residue adjacent to a downstream tRNA gene, e.g., *COII*, *COIII*, *ND3* and *ND5* (S5 Table). The start and stop codons were identical in three assassin bugs (PF, PAY and PT), but showed differences between them and other two species in some PCGs (S5 Table). The non-stop codon usage also showed the same pattern and the total numbers of non-stop codons were identical in these three species (3,704 codons) but exhibited different numbers in other two species (S2 Fig.)

For a better understanding of the sequence divergence of PCGs in *Peirates*, we calculated the genetic distances within five assassin bugs based on barcoding region of *COI*, single PCG and concatenated 13 PCGs. The final alignment length for barcoding region was 665 bp. Kimura-2 parameter pairwise genetic distance revealed low variation among PF, PAY and PT (averaged 3.1%, range 1.5–4.1%) and high sequence divergence between these three assassin bugs and two other species (averaged 13.8%, range 11.9–16.1%) (S6 Table). Concatenated 13 PCGs and most of the single PCGs exhibited similar genetic distances with barcoding region, while *ATP8*, *ND1*, *ND2 and ND3* showed relatively higher distances than barcoding region (S6 Table). In general, the genetic distances based on barcoding region, single PCG and concatenated 13 PCGs showed consistent result that the sequence divergence of PF, PAY and PT was very low.

Transfer and ribosomal RNAs

All of the 22 typical animal tRNA genes were found in *Peirates* mt genomes, ranging from 63 to 70 bp in length (Fig. 2). Most of the tRNAs could be folded into the classic cloverleaf secondary structure except for *trnS1*, in which its dihydrouridine (DHU) stem simply formed a loop. The loss of the DHU stem in *trnS1* is a common feature in assassin bugs [30,40,41] as well as other insects [26,42,43].
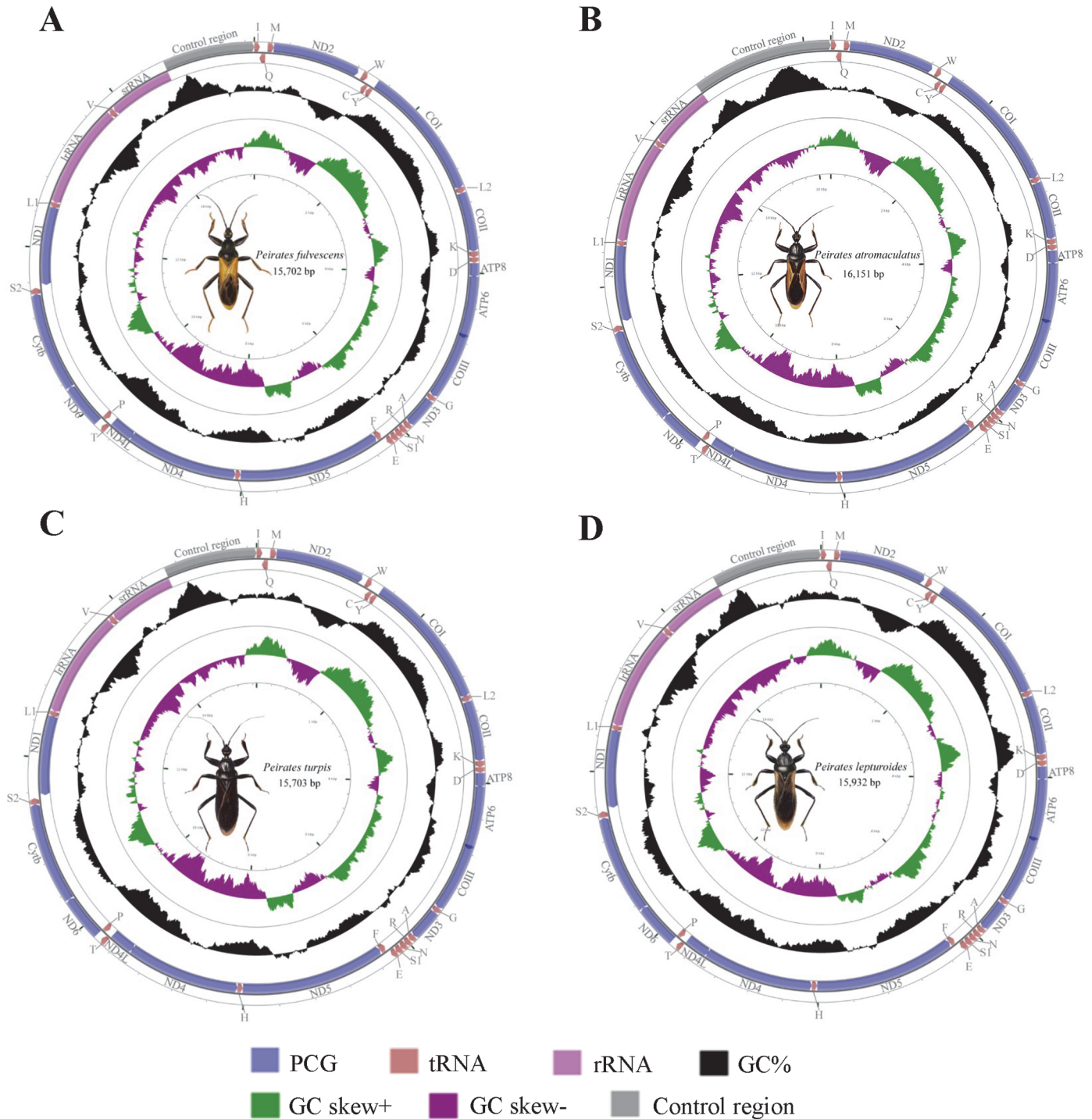
Fig 1. Mitochondrial maps of four sequenced assassin bugs. Direction of gene transcription is indicated by arrows. PCGs are shown as blue arrows, rRNAs as purple arrows, tRNAs as red arrows and large NC regions (>100 bp) as grey arrows. tRNAs are labeled according to single-letter IUPAC-IUB abbreviations (L1: UUR; L2:CUN; S1:AGN; S2:UCN). The GC content is plotted using a black sliding window, as the deviation from the average GC content of the entire sequence. GC-skew is plotted as the deviation from the average GC-skew of the entire sequence. Ticks in the inner cycle indicate the sequence length.

doi:10.1371/journal.pone.0117862.g001
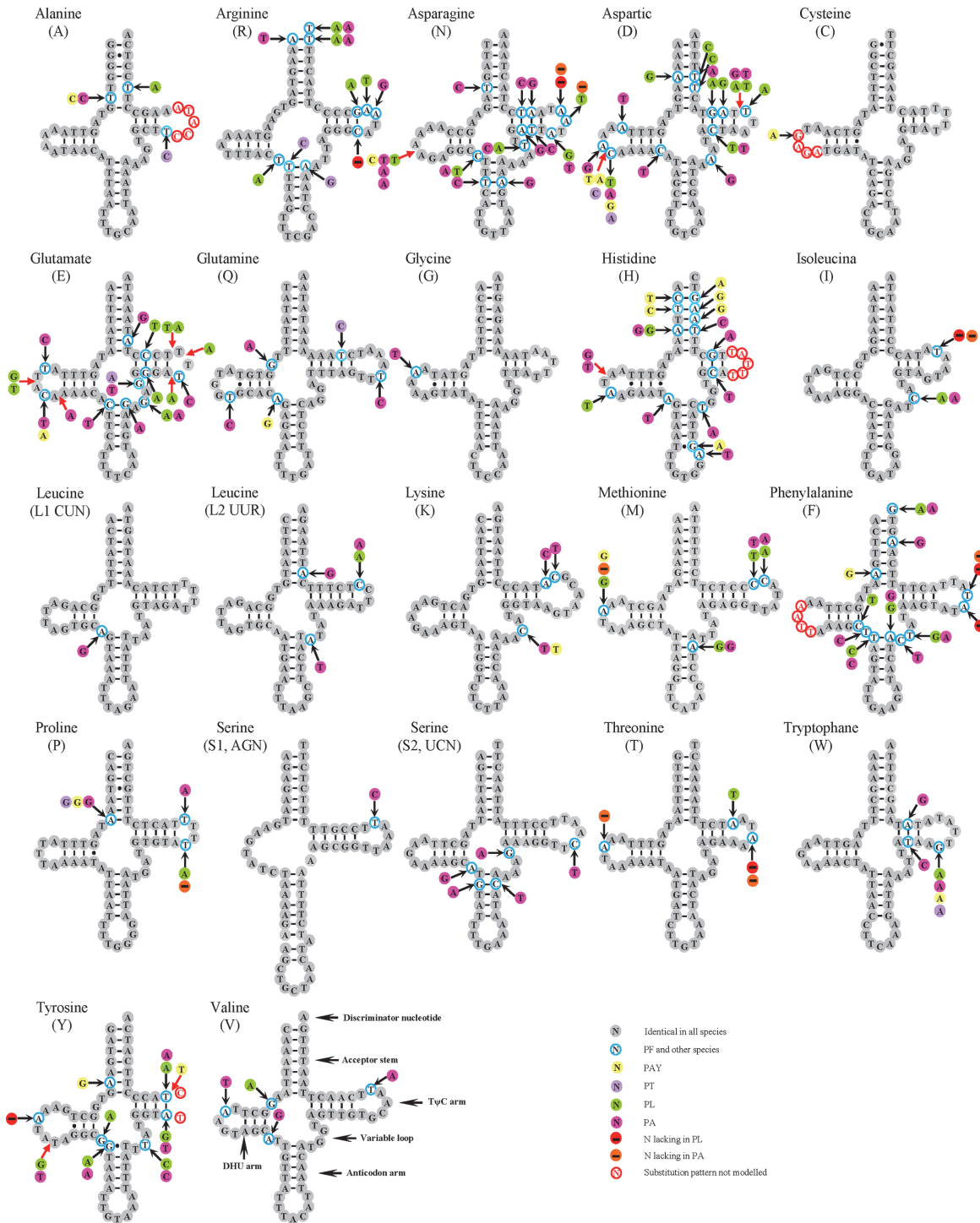
Fig 2. Secondary structure of tRNA families in *Peirates* mitochondrial genomes. The nucleotide substitution pattern for each tRNA family is modeled using as reference the structure determined for PF. Red arrows correspond to insertions. The tRNAs are labeled with the abbreviations of their corresponding amino acids. Inferred Watson-Crick bonds are illustrated by lines, whereas GU bonds are illustrated by dots.

doi:10.1371/journal.pone.0117862.g002

According to the secondary structures and sequence alignment, the most conserved tRNAs in *Peirates* mt genomes were *trnG*, *trnL1* and *trnS1* with one nucleotide substitution in each gene ([Fig. 2](#)). In the remaining tRNAs, nucleotide substitutions were mainly restricted to TΨC and DHU loops, with obvious insertion-deletion polymorphisms. In *trnA*, *trnC*, *trnH*, *trnF* and *trnY*, the substitution pattern of the TΨC or DHU loop was difficult to model due to a high level of variation among orthologous sequences. Interestingly, tRNAs of three assassin bugs (PF, PAY and PT) showed high level of sequence and structural identity, with eight identical tRNAs (*trnG*, *trnI*, *trnL1*, *trnL2*, *trnS1*, *trnS2*, *trnT* and *trnV*) ([S3](#) and [S4](#) Figs.). The fourteen remaining tRNAs only had four insertion-deletion positions in the DHU arm in total, and the nucleotide substitutions of individual gene were mostly restricted to 1–3 nucleotide sites, with the exception of *trnH* with 6 sites ([S3 Fig.](#)).

Genes for the small and large subunit ribosomal RNAs (*srRNA* and *lrRNA*) were adjacent on the same strand, interleaved by a single *trnV*. The size differences in both ribosomal subunits were not distinct among different species, especially in PF, PAY and PT ([Table 1](#)). The multiple alignments of two rRNAs displayed consistent results with tRNAs that sequences of three species (PF, PAY and PT) showed extremely higher identity ([S5 Fig.](#)).

Gene overlaps and non-coding regions

All five mt genomes had gene overlaps and the size ranged from 1 to 44 bp ([S7 Table](#)). The number of gene overlaps in PA (12 overlaps) was identical with that in three assassin bugs (PF, PAY and PT), and the fewest number was found in PL (8 overlaps). Except for the common overlap between *ATP8-ATP6* in Heteroptera [16], all five mt genomes shared three pairs of gene overlaps: *trnW-trnC* (8 bp), *COI-trnL2* (5 bp) and *ND6-CytB* (1 bp), which may be the common features in the genus *Peirates*. All gene overlaps in three assassin bugs (PF, PAY and PT) had the same locations and sequences of each overlap were almost identical, except for the overlap between *ATP6-COIII* in PAY with a nucleotide substitution.

Outside control region, there were 9 non-coding regions in the mt genomes of PF, PAY and PT, 10 in PL and 11 in PA ([S8 Table](#)). Four non-coding regions, *trnQ-trnM* (22 bp), *trnY-COI* (1 bp), *trnA-trnR* (3 bp) and *trnS2-ND1* (22 bp), were observed in all mt genomes. It is worth noting that the pattern of non-coding regions was similar to that of gene overlaps in three species (PF, PAY and PT), sharing the same number and distribution of genomic spacers, the same size and almost identical sequences of individual non-coding region.

Control region

The putative control region, which included the presumed origin of replication and promoters for transcription initiation [15,44], located between *srRNA* and *trnI*. The size of this region was relatively variable, ranging from 1,044 bp in PF to 1,552 bp in PA. The following structural elements were summarized in the control region of *Peirates* mt genomes: (1) a leading sequence adjacent to *srRNA*, (2) a string of Gs, (3) an A+T-rich sequence block, (4) a tandem repeated sequence block consisting of repeat units, and (5) the remainder of the control region ([Fig. 3](#)).

Unlike coding regions, control regions from different insect species always exhibit a very high level of divergence [15,45]. Comparison of the nucleotide sequences of structural elements (Gs and tandem repeats were not included) in control region showed the high level of sequence conservation in three assassin bugs (PF, PAY and PT), with nucleotide identity ranged from 91.13% for the remainder of control region to 98.5% for leading sequence; and extremely low sequence identity in all five species, ranging from 15.47% for AT-rich sequence to 70.37% for leading sequence ([S9 Table](#)).
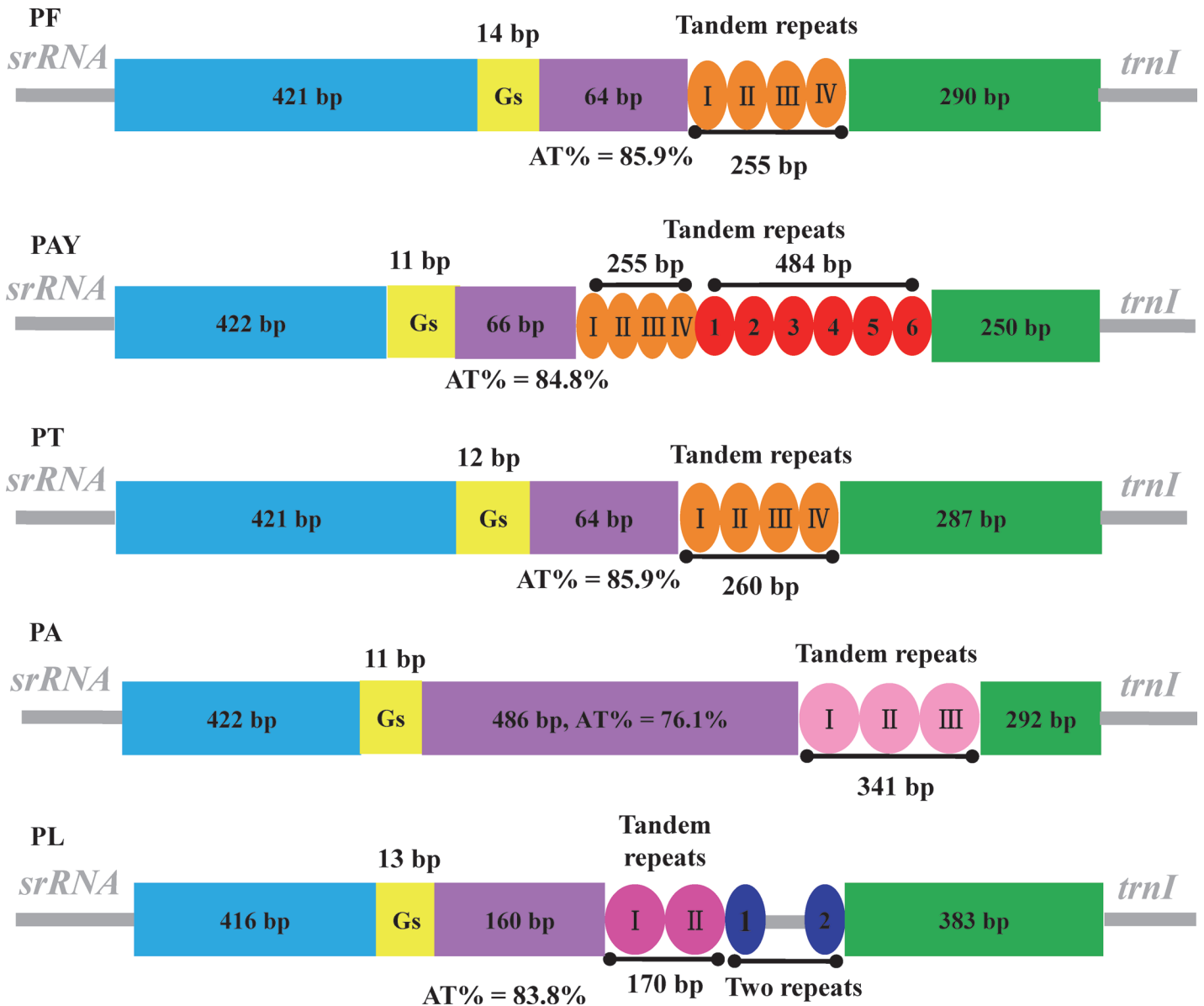
Fig 3. Organization of the control region in *Peirates* mitochondrial genomes. The colored panes indicate the structural elements in the CR, leading sequences are shown as blue panes, strings of Gs as yellow panes, A+T-rich sequences as purple panes and the remainders of control regions as green panes.

doi:10.1371/journal.pone.0117862.g003

Variation of size and copy number of the repeat unit is responsible to a large degree for the size variation of the control region [15,45,46]. All of the control region sequences examined here included large tandem repeats present in two or more copies (Fig. 3). The complete sequences of PF, PAY and PT shared a nearly identical tandem unit, a 71 bp sequence tandemly repeated three times, with a partial forth. In addition, another type of tandem unit (89 bp) with five copies and a partial sixth was found in PAY. Conversely, the other two species exhibited totally different tandem units, two 118 bp tandem repeat units and a partial third were found in PA, and PL had two 85 bp tandem repeat units and two 39 bp repeats. Collectively, mt control region of the genus *Peirates* had several distinct structural and evolutionary characteristics, including variable size, conserved structural elements, and abundant tandem repetitions. These

properties of control region were also discovered in other assassin bugs [30,40,41,47,48] as well as other true bugs [12,16,45,49–51], and made this region an ideal molecular marker for evolutionary and population genetic studies.

Phylogenetic analyses

The topologies and node support values of three phylogenetic trees (BI/ML/NJ) inferred from the nucleotide sequences of all 37 mt genes were identical (Fig. 4). In all of the phylogenetic trees constructed with different methods: 1) *P. lepturoides*, *P. atromaculatus*, *P. fulvescens* and *P. turpis* were monophyletic; and 2) *P. atromaculatus* was the sister-group to *P. fulvescens* and *P. turpis*. Previous cladistic analysis based on morphological characters showed three distinct groups in *Peirates*: *P. quadrinotatus* group, *P. singularis* group and *P. lepturoides* group [7]. *P. lepturoides* group is the largest species group (more than 20 species), including *P. lepturoides*, *P. atromaculatus* and *P. turpis*. Our results suggest that *P. fulvescens* may belong to the *P. lepturoides* species group. However, more sequences are needed to confirm this result. Although the phylogenetic analysis based on the current taxon was limited to inferring the phylogeny of *Peirates*, it still had important implications for the usefulness of mt genome sequence in the phylogenetic studies of this genus. Furthermore, next-generation sequencing (NGS) technology makes it possible to efficiently and cost-effectively obtain entire mt genome from large number of samples for resolving relationships at different taxonomic levels and population structure of species [17, 52–55].
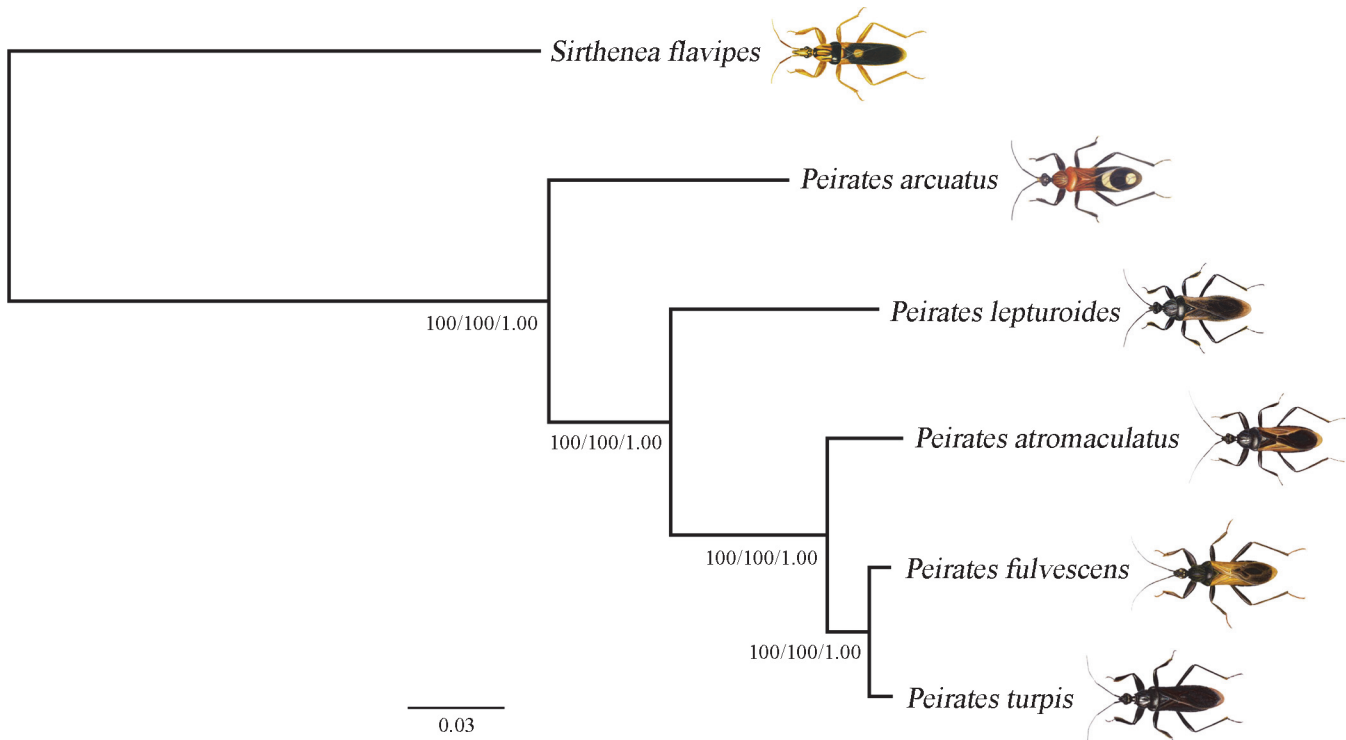
Fig 4. Phylogenetic relationships among *Peirates* assassin bugs inferred from 37 mitochondrial genes. Phylogram from the Bayesian analysis of partitioned 37 mitochondrial genes is shown. Numbers close to the branching points are bootstrap support values from the ML analysis, bootstrap support values from NJ analysis and posterior probabilities from the Bayesian analysis.

doi:10.1371/journal.pone.0117862.g004

In conclusion, with four newly sequenced mt genomes from the genus *Peirates*, we presented the first comparative mitogenomic analysis of these assassin bugs. Our results showed that gene content, gene order, base composition and tRNA secondary structures were conserved among *Peirates* mt genomes. Control region possessed several distinct characteristics, including: variable size, abundant tandem repetitions and conserved structural elements, and was useful in evolutionary and population genetic studies of this genus. Phylogenetic and comparative mitogenomic analyses revealed the close relationship of *P. atromaculatus*, *P. fulvescens* and *P. turpis*, as well as the highly conserved mitogenomic organization, e.g., 1) the overall sequence similarity and low genetic distances, 2) same start and stop codons of PCGs, 3) conserved secondary structure of tRNAs, 4) identical location and length of non-coding and overlapping regions, and 5) conservation of structural elements and tandem repeat unit in control region. All these conserved characters indicated that these three close related assassin bugs might be the recently diverged species. In summary, the present study showed the usefulness of mt genome in evolutionary and phylogenetic studies of the genus *Peirates*. More taxon sampling in the future study should help to better understanding the phylogeny and evolution of these assassin bugs.

Supporting Information

S1 Fig. The different color patterns of the hemelytron in five assassin bugs. A, *P. fulvescens* (PF); B, *P. atromaculatus* (PAY); C, *P. turpis* (PT); D, *P. lepturoides* (PL); E, *P. arcuatus* (PA).
(TIF)

S2 Fig. Codon usage of protein-coding genes in *Peirates* mitochondrial genomes. Codon families are provided on the x-axis. Numbers to the right refer to the total number of codons.
(TIF)

S3 Fig. Alignment of tRNA families (trnA-trnL2) in mitochondrial genomes of three assassin bugs (PF, PAY and PT). The loop regions are highlighted by the black pane.
(TIF)

S4 Fig. Alignment of tRNA families (trnL1-trnV) in mitochondrial genomes of three assassin bugs (PF, PAY and PT). The loop regions are highlighted by the black pane.
(TIF)

S5 Fig. Nucleotide variation of two rRNAs in *Peirates* mitochondrial genomes. Three assassin bugs indicate *P. atromaculatus*, *P. fulvescens* and *P. turpis* (PAY, PF and PT).
(TIF)

S1 Table. Collection information of *Peirates* species newly sequenced in the present study.
(DOCX)

S2 Table. Key morphological characters used to species identification in this study.
(DOCX)

S3 Table. Primer sequences used in this study.
(DOCX)

S4 Table. The best partitioning scheme selected by PartitionFinder for the BI and ML analyses.
(DOCX)

S5 Table. Start and stop codons of protein-coding genes in *Peirates* mitochondrial genomes.
(DOCX)

S6 Table. Genetic distances based on barcoding region, single PCG and concatenated 13 PCGs.
(DOCX)

S7 Table. Statistics on gene overlaps in *Peirates* mitochondrial genomes.
(DOCX)

S8 Table. Statistics on non-coding regions in *Peirates* mitochondrial genomes.
(DOCX)

S9 Table. Sequence identities of structural elements in control region.
(DOCX)

S1 Dataset. Sequence alignments of barcoding region, single PCG and concatenated 13 PCGs.
(TXT)

S2 Dataset. NEXUS file for BI analysis.
(NEX)

S3 Dataset. PHYLIP file for ML analysis.
(PHY)

Acknowledgments

Special thanks go to Dr. Dorothee Huchon and two anonymous reviewers for their helpful comments and suggestions.

Author Contributions

Conceived and designed the experiments: HL WC. Performed the experiments: GZ HL. Analyzed the data: GZ HL. Contributed reagents/materials/analysis tools: WC HL PZ. Wrote the paper: HL GZ WC.

References

1. Cai W, Taylor SJ (2006) *Lentireduvius*, a new genus of Peiratinae from Brazil, with a key to the New World genera (Hemiptera: Reduviidae). Zootaxa 1360: 51–60.

2. Schuh RT, Slater JA (1995) True bugs of the world (Hemiptera: Heteroptera): classification and natural history. Ithaca: Cornell University Press. 336 pp. PMID: 25144093

3. Maldonado Capriles J (1990) Systematic catalogue of the Reduviidae of the world (Insecta: Heteroptera). Puerto Rico: A special edition of Caribbean Journal of Science. 694 pp.

4. Liu HY, Luo ZH, Cao LM, Cai W (2011) *Peirates hybridus* (Scopoli), a new record species of the genus *Peirates* (Hemiptera: Reduviidae: Peiratinae) from China. Entomotaxonomia 33: 109–111.

5. Cascarón MC, Morrone JJ (1995) Systematics, cladistics, and biogeography of the *Peirates collarti* and *P. lepturoides* species groups (Heteroptera: Reduviidae, Peiratinae). Ent Scand 26: 191–228.

6. Cascarón MC (1996) Synonymizing *Spilodermus* Stål under *Peirates Serville*, and revision of the resulting *P. quadrinotatus* species group (Heteroptera: Reduviidae: Peiratinae). Proc Entomol Soc Wash 98: 500–508.

7. Cascarón MC (1997) Revision of the genus *Peirates Serville*, with a cladistic and biogeographic analysis (Heteroptera: Reduviidae, Peiratinae). Insect Syst Evol 28: 39–73.

8. Boore JL (1999) Animal mitochondrial genomes. Nucleic Acids Res 27: 1767–1780. PMID: 10101183

9. Boore JL (2006) The use of genome-level characters for phylogenetic reconstruction. Trends Ecol Evol 21: 439–446. PMID: 16762445

10. Boore JL, Lavrov DV, Brown WM (1998) Gene translocation links insects and crustaceans. Nature 392: 667–668. PMID: 9565028

11. Dowton M, Castro LR, Austin AD (2002) Mitochondrial gene rearrangements as phylogenetic characters in the invertebrates: the examination of genome 'morphology'. Invertebr Syst 16: 345–356.

12. Li H, Liu H, Shi AM, Štys P, Zhou XG, et al. (2012) The complete mitochondrial genome and novel gene arrangement of the unique-headed bug *Stenopirates* sp. (Hemiptera: Enicocephalidae). PLoS ONE 7: e29419. doi: 10.1371/journal.pone.0029419 PMID: 22235294

13. Timmermans MJ, Vogler AP (2012) Phylogenetically informative rearrangements in mitochondrial genomes of Coleoptera, and monophyly of aquatic elateriform beetles (Dryopoidea). Mol Phylogenet Evol 63: 299–304. doi: 10.1016/j.ympev.2011.12.021 PMID: 22245358

14. Wei SJ, Shi M, Chen XX, Sharkey MJ, van Achterberg C, et al. (2010) New views on strand asymmetry in insect mitochondrial genomes. PLoS ONE 5: e12708. doi: 10.1371/journal.pone.0012708 PMID: 20856815

15. Zhang DX, Szymura JM, Hewitt GM (1995) Evolution and structural conservation of the control region of insect mitochondrial DNA. J Mol Evol 40: 382–391. PMID: 7769615

16. Li H, Liu H, Song F, Shi A, Zhou X, et al. (2012) Comparative mitogenomic analysis of damsel bugs representing three tribes in the family Nabidae (Insecta: Hemiptera). PLoS ONE 7: e45925. doi: 10.1371/journal.pone.0045925 PMID: 23029320

17. Timmermans MJ, Dodsworth S, Culverwell CL, Bocak L, Ahrens D, et al. (2010) Why barcode? High-throughput multiplex sequencing of mitochondrial genomes for molecular systematics. Nucleic Acids Res 38: e197. doi: 10.1093/nar/gkq807 PMID: 20876691

18. Wiegmann BM, Trautwein MD, Winkler IS, Barr NB, Kim JW, et al. (2011) Episodic radiations in the fly tree of life. Proc Natl Acad Sci USA 108: 5690–5695. doi: 10.1073/pnas.1012675108 PMID: 21402926

19. Zhao J, Li H, Winterton SL, Liu Z (2013) Ancestral gene organization in the mitochondrial genome of *Thyridosmylus langii* (McLachlan, 1870) (Neuroptera: Osmylidae) and implications for lacewing evolution. PLoS ONE 8: e62943. doi: 10.1371/journal.pone.0062943 PMID: 23717397

20. Cameron SL (2014) Insect mitochondrial genomics: implications for evolution and phylogeny. Annu Rev Entomol 59: 95–117. doi: 10.1146/annurev-ento-011613-162007 PMID: 24160435

21. Morin PA, Archer FI, Foote AD, Vilstrup J, Allen EE, et al. (2010) Complete mitochondrial genome phylogeographic analysis of killer whales (*Orcinus orca*) indicates multiple species. Genome Res 20: 908–916. doi: 10.1101/gr.102954.109 PMID: 20413674

22. Ma C, Yang PC, Jiang F, Chapuis MP, Shali Y, et al. (2012) Mitochondrial genomes reveal the global phylogeography and dispersal routes of the migratory locust. Mol Ecol 21: 4344–4358. doi: 10.1111/j.1365-294X.2012.05684.x PMID: 22738353

23. Nelson LA, Lambkin CL, Batterham P, Wallman JF, Dowton M, et al. (2012) Beyond barcoding: a mitochondrial genomics approach to molecular phylogenetics and diagnostics of blowflies (Diptera: Calliphoridae). Gene 511: 131–142. doi: 10.1016/j.gene.2012.09.103 PMID: 23043935

24. Abascal F, Posada D, Knight RD, Zardoya R (2006) Parallel evolution of the genetic code in arthropod mitochondrial genomes. PLoS Biol 4: 711–718.

25. Shao R, Kirkness EF, Barker SC (2009) The single mitochondrial chromosome typical of animals has evolved into 18 minichromosomes in the human body louse, *Pediculus humanus*. Genome Res 19: 904–912. doi: 10.1101/gr.083188.108 PMID: 19336451

26. Li H, Shao R, Song F, Zhou XG, Yang QQ, et al. (2013) Mitochondrial genomes of two barklice, *Psococerastis albimaculata* and *Longivalvus hyalospilus* (Psocoptera: Psocomorpha): contrasting rates in mitochondrial gene rearrangement between major lineages of Psocodea. PLoS ONE 8: e61685. doi: 10.1371/journal.pone.0061685 PMID: 23630609

27. Zhao GY, Li H, Song F, Cai W (2014) The complete mitochondrial genome of an assassin bug *Peirates arcuatus* (Hemiptera: Reduviidae). Mitochondrial DNA (early online) doi: 10.3109/19401736.2013.867438

28. Hsiao TY, Ren SZ, Zheng LY, Jing HL, Zou HG, et al (1981) A handbook for the determination of the Chinese Hemiptera-Heteroptera, vol. 2. Beijing: Science Press. 654 pp. PMID: 10310835

29. Simon C, Buckley TR, Frati F, Stewart JB, Beckenbach AT (2006) Incorporating molecular evolution into phylogenetic analysis, and a new compilation of conserved polymerase chain reaction primers for animal mitochondrial DNA. Annu Rev Ecol Evol Syst 37: 545–579.

30. Li H, Gao JY, Liu HY, Liu H, Liang AP, et al. (2011) The architecture and complete sequence of mitochondrial genome of an assassin bug *Agriosphodrus dohrni* (Hemiptera: Reduviidae). Int J Biol Sci 7: 792–804. PMID: 21750648

31.   Lowe TM, Eddy SR (1997) tRNAscan–SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res 25: 955–964. PMID: 9023104

32.   Abascal F, Zardoya R, Telford MJ (2010) TranslatorX: multiple alignment of nucleotide sequences guided by amino acid translations. Nucleic Acids Res 38: W7–W13. doi: 10.1093/nar/gkq291 PMID: 20435676

33.   Tamura K, Peterson D, Peterson N, Stecher G, Nei M, et al. (2011) MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28: 2731–2739. doi: 10.1093/molbev/msr121 PMID: 21546353

34.   Vaidya G, Lohman DJ, Meier R (2010) SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. Cladistics 27: 171–180.

35.   Lanfear R, Calcott B, Ho SYW, Guindon S (2012) PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Mol Biol Evol 29: 1695–1701. doi: 10.1093/molbev/mss020 PMID: 22319168

36.   Stamatakis A (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22: 2688–2690. PMID: 16928733

37.   Stamatakis A, Hoover P, Rougemont J (2008) A rapid bootstrap algorithm for the RAxML web servers. Syst Biol 57: 758–771. doi: 10.1080/10635150802429642 PMID: 18853362

38.   Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574. PMID: 12912839

39.   Miller MA, Pfeiffer W, Schwartz T (2010) "Creating the CIPRES Science Gateway for inference of large phylogenetic trees" in Proceedings of the Gateway Computing Environments Workshop (GCE), 14 Nov. 2010, New Orleans, LA, pp 1–8.

40.   Dotson EM, Beard CB (2001) Sequence and organization of the mitochondrial genome of the Chagas disease vector, *Triatoma dimidiata*. Insect Mol Biol 10: 205–215. PMID: 11437912

41.   Gao JY, Li H, Truong XL, Dai X, Chang J, et al. (2013) Complete nucleotide sequence and organization of the mitochondrial genome of *Sirthenea flavipes* (Hemiptera: Reduviidae: Peiratinae) and comparison with other assassin bugs. Zootaxa 3669: 1–16.

42.   Cameron SL, Whiting MF (2007) Mitochondrial genomic comparisons of the subterranean termites from the genus *Reticulitermes* (Insecta: Isoptera: Rhinotermitidae). Genome 50: 188–202. PMID: 17546084

43.   Wan X, Kim MI, Kim MJ, Kim I (2012) Complete mitochondrial genome of the free-living earwig, *Challia fletcheri* (Dermaptera: Pygidicranidae) and phylogeny of Polyneoptera. PLoS ONE 7: e42056. doi: 10.1371/journal.pone.0042056 PMID: 22879905

44.   Clayton DA (1982) Replication of animal mitochondrial DNA. Cell 28: 693–705. PMID: 6178513

45.   Wang Y, Li H, Wang P, Song F, Cai W (2014) Comparative mitogenomics of plant bugs (Hemiptera: Miridae): identifying the AGG Codon reassignments between serine and lysine. PLoS ONE 9: e101375. doi: 10.1371/journal.pone.0101375 PMID: 24988409

46.   Zhang DX, Hewitt GM (1997) Insect mitochondrial control region: a review of its structure, evolution and usefulness in evolutionary studies. Biochem Syst Ecol 25: 99–120.

47.   Li H, Gao JY, Cai W (2013) Complete mitochondrial genome of the assassin bug *Oncocephalus breviscutum* (Hemiptera: Reduviidae). Mitochondrial DNA (early online) doi: 10.3109/19401736.2013.840602

48.   Kocher A, Kamilari M, Lhuillier E, Coissac E, Péneau J, et al. (2014) Shotgun assembly of the assassin bug *Brontostoma colossus* mitochondrial genome (Heteroptera, Reduviidae). Gene 552: 184–194. doi: 10.1016/j.gene.2014.09.033 PMID: 25240790

49.   Li H, Liu HY, Cao LM, Shi AM, Yang HL, et al. (2012) The complete mitochondrial genome of the damsel bug *Alloeorhynchus bakeri* (Hemiptera: Nabidae). Int J Bio Sci 8: 93–107.

50.   Li T, Gao C, Cui Y, Xie Q, Bu W (2013) The complete mitochondrial genome of the stalk-eyed bug *Chauliops fallax* Scott, and the monophyly of malcidae (Hemiptera: Heteroptera). PLoS ONE 8: e55381. doi: 10.1371/journal.pone.0055381 PMID: 23390534

51.   Wang Y, Huang XL, Qiao GX (2013) Comparative analysis of mitochondrial genomes of five aphid species (Hemiptera: Aphididae) and phylogenetic implications. PLoS ONE 8: e77511 doi: 10.1371/journal.pone.0077511 PMID: 24147014

52.   Hahn C, Bachmann L, Chevreux B (2013) Reconstructing mitochondrial genomes directly from genomic next-generation sequencing reads–a baiting and iterative mapping approach. Nucleic Acids Res 41: e129. doi: 10.1093/nar/gkt371 PMID: 23661685

53.   Rubinstein ND, Feldstein T, Shenkar N, Botero-Castro F, Griggio F, et al. (2013) Deep sequencing of mixed total DNA without barcodes allows efficient assembly of highly plastic ascidian mitochondrial genomes. Genome Biol Evol 5: 1185–1199. doi: 10.1093/gbe/evt081 PMID: 23709623

54. Gillett CPDT, Crampton-Platt A, Timmermans MJTN, Jordal BH, Emerson BC, et al. (2014) Bulk de novo mitogenome assembly from pooled total DNA elucidates the phylogeny of weevils (Coleoptera: Curculionoidea). Mol Biol Evol 31: 2223–2237. doi: 10.1093/molbev/msu154 PMID: 24803639

55. Sosa MX, Sivakumar IKA, Maragh S, Veeramachaneni V, Hariharan R, et al. (2012) Next-generation sequencing of human mitochondrial reference genomes uncovers high heteroplasmy frequency. PLoS Comput Biol 8: e1002737. doi: 10.1371/journal.pcbi.1002737 PMID: 23133345