The mitochondrial genomes of Panorpidae: sequence, structure and phylogenetic analysis

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Abstract

Background: Mitochondrial genomes play a significant role in reconstructing phylogenetic relationships and revealing molecular evolution in insects. However, only two species of Panorpidae have been documented for mitochondrial genomes in Mecoptera to date.

Results: We obtained complete mitochondrial genomes of 17 species of Panorpidae. The results show that the complete mitogenome sequences of Panorpidae all contain 37 genes (13 protein-coding genes (PCGs), two rRNAs, 22 tRNAs) and one control region. The mitogenomes exhibit a strong AT bias. The AT-skew can either be slightly positive or slightly negative, while the GC-skew is usually negative. The 22 tRNA genes can fold into a common cloverleaf secondary structure except *trnS1*. The sliding window and genetic distance analyses demonstrate highly variable nucleotide diversity among the 13 protein-coding genes, with comparatively low evolutionary rate of *cox1*, *cox2* and *nad1*, and high variability of *nad2* and *nad6*. The phylogeny of Panorpidae can be presented as (*Neopanorpa* + *Furcatopanorpa*) + (*Dicerapanorpa* + (*Panorpa debilis* + (*Sinopanorpa* + (*Cerapanorpa* + *Panorpa*)))).

Conclusions: Our analyses indicate that the genes *nad2* and *nad6* can be regarded as potential markers for population genetics and species delimitation in Panorpidae. *Panorpa* is reconfirmed a paraphyletic group.

Background

The mitochondrial genome (or mitogenome) of insects is a double-stranded circular molecule, varying in length from 14 to 20 kb [1]. It generally consists of 13 protein-coding genes (PCGs), 22 transfer RNA genes (tRNAs), two ribosome RNA genes (rRNAs), and one non-coding control region (CR) [2]. The mitogenome is characterized by simple genetic structure, small size, maternal inheritance, high genome copy numbers, less recombination, and fast evolutionary rate [3], thus being regarded as a valuable tool for population genetics, species delimitation, and phylogenetic analyses in numerous groups of insects [4–9]. However, only six species of Mecoptera have been documented for mitochondrial genomes to date, including one species of Boreidae (Boreus elegans Carpenter, 1935), one species of Nannochoristidae (Nannochorista philpotti (Tillyard, 1917)), two species of Bittacidae (Bittacus pilicornis Westwood, 1846 and Bittacus strigosus Hagen, 1861), and two species of Panorpidae (Neopanorpa pulchra Carpenter, 1945 and Panorpa debilis Westwood, 1846) [10–12]. The complete mitochondrial genome of Panorpidae, by and large, remains poorly studied hitherto.

Panorpidae is the most species-rich family in Mecoptera, and comprises approximately 500 species distributed mainly in the Northern Hemisphere [13–16]. The species of Panorpidae are currently assigned to eight genera [13, 17, 18]. The genus Panorpa Linnaeus, 1758 (ca. 270 spp) is distributed in the Holarctic and northern Oriental regions. Leptopanorpa MacLachlan, 1875 (15 spp) is exclusively distributed in Java and Sumatra, Indonesia [19, 20]. Neopanorpa van der Weele, 1909 (ca. 170 spp) occurs in the Oriental Region, especially abundant in southern China and Southeast Asia. Sinopanorpa

Cai & Hua, 2008 (3 spp), Furcatopanorpa Ma & Hua, 2011 (1 sp), Dicerapanorpa Zhong & Hua, 2013 (18 spp), Cerapanorpa Gao, Ma & Hua, 2016 (19 spp) and Megapanorpa Wang & Hua, 2019 (5 spp) are endemic to China, mainly in the Qinling-Baoshan and Hengduan Mountains.

Recently, the phylogeny of Panorpidae was studied based on morphological characters [21] and DNA sequences [22, 23]. Furcatopanorpa was considered to form the sister taxon with all other genera of Panorpidae based on morphological characters [21], but to some species of Panorpa based on DNA sequences [23]. Previously, Panorpa was treated as a paraphyletic group with Neopanorpa based on morphological characters and DNA sequence data [21, 24–26]. Through a recent molecular phylogenetic analysis, however, Neopanorpa was confirmed a sister taxon to all other genera of Panorpidae excluding Leptopanorpa [22]. Based on a maximum parsimony analysis, Leptopanorpa is nested with Neopanorpa [20]. This means that the phylogenetic relationships of Panorpidae have not been satisfactorily resolved to date.

In this study, we sequenced the mitochondrial genomes of 17 species of Panorpidae, aiming to reveal the mitochondrial structure, sequences, evolutionary rate, and to reconstruct the phylogeny of Panorpidae based on mitogenome sequences.

Results

Mitogenome organization and nucleotide composition

The complete mitogenomes of the 17 scorpionflies are typically circular double-strand molecules, varying in length from 16,236 bp in P. curva to 18,795 bp in C. nanwutaina TTH (Table 1 and Additional file 1). The variable length of the control region contributes to the size variation of the mitogenomes among the panorpids. All 17 newly sequenced scorpionfly mitogenomes have the same compositions with other insects, consisting of 13 PCGs, 2 rRNAs, 22 tRNAs, and one control region (Additional files 1–6). Fourteen genes (4 PCGs, 2 rRNAs, and 8 tRNAs) are from the minority strand (N-strand), and the remaining 23 genes (9 PCGs and 14 tRNAs) are from the majority strand (J-strand) in these 17 mitogenomes. The gene orders (Additional files 1–6 are identical to those of the fruit fly Drosophila yakuba [27].

Table 1

Information of the species and mitogenomes used in this study.

Family	Species	Locality	Size (bp)	Accession no.	Resource
Bittacidae	Bittacus obscurus Huang & Hua, 2005	Tongtianhe Forest Park, Shaanxi	17,388	Waiting accession no.	Unpublished
	Bittacus pilicornis Westwood, 1846	Homochitto National Forest, Mississippi	15,842	HQ696578	[10]
	Bittacus planus Cheng, 1949	Tongtianhe Forest Park, Shaanxi	16,807	Waiting accession no.	Unpublished
	Bittacus strigosus Hagen, 1861	Royal Botanical Garden Arboretum, Burlington	15,825	MK870080	[12]
Panorpidae	Cerapanorpa brevicornis (Hua & Li, 2007)	Huoshaodian, Shaanxi	16,698	Waiting accession no.	This study
	Cerapanorpa byersi (Hua & Huang, 2007)	Tongtianhe Forest Park, Shaanxi	16,714	Waiting accession no.	This study
	Cerapanorpa dubia (Chou & Wang, 1981)	Zhuque Forest Park, Shaanxi	16,450	Waiting accession no.	This study
	Cerapanorpa nanwutaina (Chou, 1981) TTH	Tongtianhe Forest Park, Shaanxi	18,795	Waiting accession no.	This study
	Cerapanorpa nanwutaina (Chou, 1981) ZQ	Zhuque Forest Park, Shaanxi	17,969	Waiting accession no.	This study
	Dicerapanorpa magna (Chou, 1981) TTH	Tongtianhe Forest Park, Shaanxi	18,053	Waiting accession no.	This study
	Dicerapanorpa magna (Chou, 1981) LP	Liping Forest Park, Shaanxi	17,667	Waiting accession no.	This study
	Dicerapanorpa magna (Chou, 1981) MCS	Micangshan, Sichuan	17,757	Waiting accession no.	This study
	Dicerapanorpa magna (Chou, 1981) WLD	Wulongdong, Shaanxi	16,376	Waiting accession no.	This study

Family	Species	Locality	Size (bp)	Accession no.	Resource
	Furcatopanorpa longihypovalva (Hua & Cai, 2009) HSD	Huoshaodian, Shaanxi	17,568	Waiting accession no.	This study
	Furcatopanorpa longihypovalva (Hua & Cai, 2009) TTH	Tongtianhe Forest Park, Shaanxi	17,744	Waiting accession no.	This study
	Furcatopanorpa longihypovalva (Hua & Cai, 2009) LP	Liping Forest Park, Shaanxi	17,199	Waiting accession no.	This study
	Neopanorpa pulchra Carpenter, 1945	Jianfengling, Hainan	16,314	JX569848	[11]
	Neopanorpa lui Chou & Ran, 1981	Wulongdong, Shaanxi	16,369	Waiting accession no.	This study
	Panorpa curva Carpenter, 1938	Wolong National Nature Reserve, Sichuan	16,236	Waiting accession no.	This study
	Panorpa debilis Westwood, 1846	cliffs Forest of rare Charitable Research Reserve, Cambridge	17,018	MK870081	[12]
	Panorpa dispergens Li &Hua, 2020	Baishuitai, Yunnan	16,683	Waiting accession no.	This study
	Panorpa fulvastra Chou, 1981	Huoditang, Shaanxi	17,843	Waiting accession no.	This study
	Sinopanorpa tincta (Navás, 1931)	Tongtianhe Forest Park, Shaanxi	18,165	Waiting accession no.	This study

The 17 whole mitogenomes exhibit a strong AT nucleotide bias ranging from 75.3% (C. nanwutaina ZQ) to 77.9% (C. brevicornis, P. dispergens, and F. longihypovalva LP) (Fig. 1A, Additional file 7). The relative numbers of A to T and G to C were measured by AT-skew and GC-skew of the base composition in nucleotide sequences. The results of the nucleotide skewness statistics show that the AT-skew can either be slightly positive or slightly negative in whole mitogenomes among panorpids (Fig. 1B), while the GC-skew is usually negative (Fig. 1C). All 17 species of Panorpidae show negative AT-skew and GC-skew on the majority strand, whereas AT-skew is negative and GC-skew is positive on the minority strand (Additional file 7).

Protein-coding genes and codon usage

Four PCGs (nad1, nad4, nad4L, and nad5) are encoded on the minority strand (N-strand), and the remaining nine PCGs on the majority strand (J-strand) in all panorpids (Additional files 1–6). The third codon positions of PCGs harbor higher A + T content than those of the first and the second positions in both strands (Fig. 1A). In all 17 species, the AT-skew is negative on both strands, while the GC-skew is negative on the J-strand and positive on the N-strand (Fig. 1B, C).

All the 17 newly sequenced mitogenomes have a variety of start codons, including standard ATN (ATA/T/G/C) and non-standard GTG and TTG (Additional files 2–6). The two non-standard start codons were found in 17 mitogenomes, with the GTG as the start codon for nad5 in four species and TTG for cox1 in all species except N. liui and for nad1 in eight species (Additional files 2–6). The most frequent start codon is ATG, which is utilized in seven PCGs across all species (Additional files 2–6). The stop codons consist of both complete TAA and TAG, and partial "T–" in all panorpids studied. TAA occurs more frequently than TAG, and "T–" is usually presented as the stop codon for cox1, cox2, cox3, nad4, and nad5 (Additional files 2–6). The relative synonymous codon usage (RSCU) is generally similar to each other in the 17 mitogenomes of Panorpidae (Fig. 2). Three most frequently used amino acids — UUA (Leu2), AUU (Ile), and UUU (Phe) — are composed solely of U or U and A (Fig. 2), and the third codon position of A/T occurs more frequently than that of G/C (Fig. 2), reflecting AT nucleotide bias in the mitochondrial PCGs among Panorpidae.

Transfer and ribosomal RNA genes

The 17 sequenced mitogenomes have 22 tRNA genes, which are scattered discontinuously over the entire mitogenome with eight transcribed from the N-strand and 14 from the J-strand ((Additional files 1–6). The A + T contents of tRNA genes are very close between the N-strand (74.5–76.7%) and the J-strand (74.9–76.7%) (Fig. 1A and (Additional files 7). The AT-skew can be either slightly positive or slightly negative in the J-strand, and is usually positive in the N-strand (Fig. 1B), whereas the GC-skew is positive in both strands in all the species (Fig. 1C). All 22 tRNAs can fold into a common cloverleaf secondary structure except trnS1 (AGN), which forms a simple loop due to lacking the DHU arm in all the 17 species (Fig. 3 and Additional file 8). Nucleotide substitutions were found in different individuals of the same species, and the number of substitutions is normally less in the same species than between different species in four stems and loops (Fig. 3 and Additional file 8). TΨC and DHU loops are more variable compared with the anticodon loop (Fig. 3 and Additional file 8). Based on the predicted secondary structure, totally six types of mismatched base pairs (A-A, A-C, A-G, G-U, U-C, and U-U) and extra single C, G, and U nucleotides were found in the 17 mitogenomes (Fig. 3 and Additional file 8).

Two rRNA genes (rrnL and rrnS) are encoded on the N-strand in the 17 mitogenomes (Additional files 1–6). The gene rrnS is located between trnV and the control region, and the gene rrnL is located between trnL1 and trnV (Additional files 1–6). The A + T content of rrnL (79.1–80.7%) is usually higher than that of rrnS (76.3–77.9%) (Fig. 1A, Additional file 7). The AT-skew of rRNAs can be either slightly negative or slightly positive (Fig. 1B), whereas the GC-skew is positive (Fig. 1C) in all 17 mitogenomes.

Control region

The control region is the largest non-coding region between the genes rrnS and trnI. The size of control region varies from 1,416 bp in P. curva to 3,975 bp in C. nanwutaina TTH (Fig. 4 and Additional files 2–6). The control region has the highest A + T content (79.20–90.35%) compared with the other three regions (PCGs, tRNAs, and rRNAs) (Fig. 1A and Additional file 7). The AT-skew of all 17 mitogenomes is greatly variable from moderately negative to moderately positive (− 0.248–0.195) (Fig. 1B and Additional file 7), whereas the GC-skew varies from moderately negative to slightly negative (− 0.289––0.075) (Fig. 1C and Additional file 7).

The poly-thymidine (T), poly-adenine (A), and $[TA(A)]_n$-like stretches were found in the control region of Mecoptera for the first time (Fig. 4). The analyses of the control regions show that the poly-A is randomly scattered in this region, while the poly-T is mostly located in the near 5'-end of rrnS except for that of F. longihypovalva TTH and C. nanwutaina ZQ at near the two-thirds of control region (Fig. 4). Each mitogenome of Panorpidae has tandem repeat units except for D. magna WLD, which has none (Fig. 4). Analyses of the control regions indicate that the length and copy number of tandem repeat units are dramatically divergent among panorpids, even among different individuals of the same species, especially C. nanwutaina TTH and C. nanwutaina ZQ (Fig. 4). Different individuals of the same species have similar tandem repeat units between F. longihypovalva HSD and F. longihypovalva LP and similar length between D. magna LP and D. magna MCS.

Nucleotide diversity and evolutionary rate analysis

The sliding window analysis demonstrates a highly variable nucleotide diversity among the 13 PCGs and two rRNAs of 19 Panorpidae mitogenomes (Fig. 5A). The values of nucleotide diversity (π values) for individual genes vary from 0.065 (rrnL) to 0.163 (nad2) (Fig. 5A). The gene nad2 exhibits the highest variability of nucleotide diversity (π = 0.163), followed by nad6 (π = 0.158), atp8 (π = 0.134), and nad3 (π = 0.134) in 13 PCGs, while cox1 (π = 0.099), cox2 (π = 0.101), and nad1 (π = 0.101) exhibit comparatively low values of nucleotide diversity (Fig. 5A). The two rRNA genes show a relatively low nucleotide diversity (π = 0.065 for rrnL and 0.084 for rrnS), thus being considered as conserved genes. Pairwise genetic distances demonstrate congruent results with high genetic distance of 0.190, 0.181, and 0.151 for nad2, nad6, and atp8, respectively, and low genetic distance of 0.108, 0.109, and 0.190 for cox1, nad1, and cox2, respectively, among 13 PCGs based on 19 Panorpidae mitogenomes (Fig. 5B). The pairwise non-synonymous/synonymous (Ka/Ks) analyses indicate that the average Ka/Ks ratios (ω) of 13 PCGs vary from 0.046 to 0.308 ($0 < \omega < 1$) (Fig. 5B), suggesting that all 13 genes are under the purifying selection [28]. The genes atp8, nad6, and nad2 exhibit relatively high Ka/Ks ratios of 0.308, 0.273, and 0.180, respectively, whereas atp6, cox2, and cytb show relatively low values of 0.046, 0.053, and 0.054, respectively (Fig. 5B).

Phylogenetic analyses

The ML and BI analyses based on four datasets (P12R, P123, P123R, P123RT) generated three trees of slightly different topology, the incongruence being restricted to the position of S. tincta (Fig. 6 and Additional files 9, 10). Most phylogenetic analyses indicate that Sinopanorpa forms a sister group to

Cerapanorpa + three species of Panorpa (P. curva, P. dispergens, and P. fulvastra) (Fig. 6), except for the ML tree based on P12R and P123, which exhibits the relationship as Cerapanorpa + (Sinopanorpa + (three species of Panorpa) (Additional file 9), and the BI tree based on P12R, which shows the relationship as Panorpa + (Sinopanorpa + Cerapanorpa) (Additional file 10). The North American species Panorpa debilis is always presented as the sister taxon of Sinopanorpa + (Cerapanorpa + other species of Panorpa) or the like with high support values (BS = 80, PP > 0.96), reconfirming that Panorpa is a paraphyletic group.

The genera Neopanorpa and Furcatopanorpa have a sister group relationship in all the phylogenetic analyses with strong supports (BS > 92, PP = 1) (Fig. 6 and Additional files 9, 10). In turn, the clade (Neopanorpa + Furcatopanorpa) forms a sister group to all the other genera of Panorpidae. The genus Dicerapanorpa is presented as a sister taxon to other genera except Neopanorpa and Furcatopanorpa (BS = 92, PP = 1) (Fig. 6). Based on our present analyses, the most consistent topologies of the phylogenetic trees of the six genera of Panorpidae could be presented as (Neopanorpa + Furcatopanorpa) + (Dicerapanorpa + (Panorpa debilis + (Sinopanorpa + (Cerapanorpa + Panorpa)))).

Discussion

Mitogenome architecture

The mitogenome sequences of Panorpidae are highly conserved in gene content, gene order, gene length, and nucleotide composition. The pattern of nucleotide skewness in whole mitogenomes of Panorpidae is coincident with that of other mecopterans and most other insects [29]. AT-skew and GC-skew are used to reflect the strand asymmetry [30]. Asymmetric patterns of mutation and selection between the two strands cause the strand asymmetry [31, 32]. All the 19 species of Panorpidae show negative AT-skew and GC-skew on the majority strand. A previous study of 120 insect mitogenomes indicates that most species show positive AT-skew and negative GC-skew on the majority strand, but a few species have negative AT-skew and positive or negative GC-skew [29]. Inversion of replication origin caused reversal of strand asymmetry (GC-skew), while that of the AT-skew value varies with replication, gene direction, and codon positions [29].

The secondary structures of trnS1 (AGN) forms a simple loop due to lacking the DHU arm in all the 17 species, as revealed in other mecopteran species [10, 12]. The gene trnS1 that lacks the dihydrouridine (DHU) stem is common in insect mitogenomes [6, 33, 34], and was found very early in a metazoan mitogenome study [35]. The mismatched pairs in stems of some tRNAs exist in all 17 newly sequenced scorpionfly mitogenomes. This mismatched pair phenomenon exists widely in the insect [36–39]. These mismatched pairs would simply represent unusual pairings [40] or be corrected by the RNA editing process [41].

The two non-standard start codons, GTG and TTG, were found in 17 mitogenomes. Besides, two non-standard codons, CCG and TCG, were also reported in Mecoptera [10]. These four types of unusual

initiation codons were usually found in cox1, nad1, nad2, and nad5 [10, 12]. The incomplete stop codons, “TA–” and “T–”, were usually used for cytochrome c oxidase genes (cox1, cox2, and cox3) and NADH dehydrogenase genes (nad1, nad4 and nad5) in Mecoptera. Only cytochrome b (cytb) gene in N. pulchra was terminated with “TA–” [11]. Incomplete stop codons are common in insects and can be converted to complete TAA stop codons by post-transcriptional polyadenylation during the mRNA maturation process [42, 43].

The control region exhibits remarkable divergence of primary nucleotide sequences, relatively high rates of nucleotide substitution, and dramatic variation in fragment length among species and individuals [44, 45]. Therefore, this non-coding region is regarded as the most variable region of the mitochondrial genome. The size of control region varies from 1,416 bp to 3,975 bp in Panorpidae, compared with that of the nannochoristid N. philpotti that contains the shortest length of control region of 898 bp in Mecoptera [10–12]. Ruspolia dubia (Orthoptera) has the smallest control region of 70 bp in size in insect mitogenomes [46], while Drosophila melanogaster (Diptera) contains the longest size of 4,601 bp [47]. Tandem repeat units are one of the most common structures in the control region [45]. Different copy number and length of tandem repeat units across various species and individuals of Panorpidae are responsible for varying sizes of the control region, which lead to different mitogenome molecule sizes. The varying mitogenome sizes among individuals of the same species are known as length heteroplasmy [44]. This phenomenon was also found in other groups of insects [44, 45, 48]. The control region is responsible for regulating the transcription and replication of mtDNA in insects [44, 45].

Nucleotide diversity analyses are useful for identifying high nucleotide divergence regions, which are crucial for designing species-specific markers [39, 49], especially in the taxa of highly variable morphological characters. Although fragment of 648 bp of the gene cox1 is often considered as a universal barcode for species identification in animals [50], it is the least variable gene in Panorpidae and has a relatively lower ratio of Ka/Ks among the PCGs in these 19 mitogenomes. Given the resolution power of cox1 is proved to be sufficiently low, other genes that have fast evolutionary rates and higher ratio of Ka/Ks and are of sufficiently large size should be proposed as potential barcode candidates [39, 51, 52]. Based on our present study, nad2 and nad6 may be chosen as potential DNA markers for future population genetics and species delimitation studies in Panorpidae.

Phylogeny

Furcatopanorpa is unique in Panorpidae in that the male adult lacks the notal organ on the posterior margin of the third tergite, and assumes an unusual O-shaped position during copulation [53]. The wings are held roof-like over the abdomen at rest and are much longer than the abdomen. The median axis of the medigynium is bifurcated distally in the female genitalia [54]. The male genitalia bear a pair of elongate hypovalves, which extend beyond the apex of gonocoxites [53, 55]. These unique morphological characters make Furcatopanorpa easily distinguished from other genera of Panorpidae, but the phylogenetic position has not been resolved satisfactorily. Based on the present phylogenetic analyses from mitogenomes, Furcatopanorpa is the sister taxon to Neopanorpa. This result is inconsistent with that of previous studies [21–23]. Based on a morphological phylogenetic analysis, Furcatopanorpa was

regarded as the basalmost taxon of Panorpidae, and forms a sister taxon relationship with all the other genera [21]. A molecular phylogenetic analysis based on two mitochondrial (cox1 and cox2) and one nuclear gene fragment (28S), however, indicate that Furcatopanorpa formed the sister group to Panorpa species from Northeastern Asia [23].

The Oriental Neopanorpa is mainly characterized by the well-developed notal organ on the posterior margin of the male tergite III, and by the vein 1A terminating at the hind margin of wing before the origin of Rs [56]. The larvae of Neopanorpa have shallow furrows and short setae on the head capsule [57]. According to a molecular phylogenetic analysis [22], Neopanorpa is the sister taxon with all other genera of Panorpidae. However, Neopanorpa is considered paraphyletic with Leptopanorpa based on another molecular phylogenetic analysis [23] and morphological phylogeny [20]. Based on our present mitogenome phylogenetic study, Neopanorpa forms a sister taxon with Furcatopanorpa.

Panorpa Linnaeus, 1758 is the first genus established in Panorpidae. According to a phylogenetic analysis form mitochondrial gene fragments, Panorpa was considered a paraphyletic group with Neopanorpa [25]. Based our recent morphological and molecular phylogenetic studies [21–23], Panorpa was also confirmed to be paraphyletic with Sinopanorpa, Dicerapanorpa, Cerapanorpa, and Furcatopanorpa. Our present phylogenetic analysis from the mitochondrial genomics proves again that Panorpa is a paraphyletic group, which need a profound taxonomic revision based on a robust phylogenetic background.

In different insect taxa, phylogenetic analyses with the third codon position of PCGs removed or not have inconsistent results [58–63]. Based on our present phylogenetic analyses, the tree topologies of Panorpidae are more reasonable based on the datasets including the third codon positions and 22 tRNAs. The inclusion of two rRNAs sequences is helpful in improving the support values. Therefore, tRNAs and rRNAs sequences should be included in phylogenetic reconstructions instead of only PCGs.

Conclusions

In the present study, we used mitochondrial genomes of Panorpidae to analyze the sequence architecture and reconstruct the phylogeny. The pattern of strand asymmetry in whole mitogenomes of Panorpidae is coincident with that of other mecopterans and most other insects, while that on the majority strand is consistent with a small part of insect taxa. The length heteroplasmy of mitogenomes, varying sizes among individuals of the same species, exists in Panorpidae. Our analyses indicate that the genes nad2 and nad6 can be regarded as potential markers for population genetics and species delimitation in Panorpidae. Furcatopanorpa is thought of as the sister taxon to Neopanorpa for the first time. Panorpa was reconfirmed a paraphyletic group. A taxonomic revision of Panorpa is needed in Panorpidae. Inclusion of the third codon positions of PCGs, 22 tRNAs, and two rRNAs can improve the accuracy and of support values of phylogenetic analyses for Panorpidae.

Materials And Methods

Taxon sampling and DNA extraction

Adults were captured in the Bashan, Qinling, and Hengduan Mountains, China from May to June in 2019 (Table 1). All specimens were preserved in 100% ethanol at − 20 °C and identified to species through morphological characters [64]. Total genomic DNA was extracted individually from the whole specimen except for abdomens using DNeasy DNA Extraction kit (Qiagen) according to the manufacturer's protocol.

Sequence analyses

The whole mitochondrial genome sequences of 17 species in Panorpidae were generated using Illumina HiSeq™2500 with paired reads of 2 × 150 bp by the Biomarker Technologies Corporation (Beijing, China). The raw paired reads quality-trimmed and assembled were conducted using Geneious 10.0.5 (Biomatters, Auckland, New Zealand) with default parameters [65]. Seventeen mitogenomes of Panorpidae were annotated using Geneious 8.1.3 with Panorpa debilis Westwood, 1846 (GenBank accession number MK870081) [12] as a reference. All 13 protein-coding genes (PCGs) were determined by the ORF Finder employing codon table 5 and compared with the homologous sequence of reference mitogenome. Two rRNA genes (rRNAs) were predicted by comparing with the homologous sequence of other mecopteran mitogenomes and the locations of adjacent genes. Twenty-two tRNA genes were identified using the MITOS Web Server employing codon table 5 (http://mitos.bioinf.uni-leipzig.de/index.py) [66]. Their secondary structures were manually plotted using Adobe Illustrator CC 2017 according to the MITOS predictions. The control region was determined by the locations of adjacent genes. Tandem repeat units of the control regions were identified by Tandem Repeats Finder server (http://tandem.bu.edu/trf/trf.html) [67]. Mitogenomic circular maps were generated using Organellar Genome DRAW (https://chlorobox.mpimp-golm.mpg.de/OGDraw.html) [68].

Analyses of the 17 newly sequenced mitogenomes of Panorpidae were calculated using PhyloSuite 1.1.16 [69], including the base composition, mitogenomic organization tables, and relative synonymous codon usage (RSCU) values. The sliding window analysis (a sliding window of 200 bp and step size of 25 bp), the nucleotide diversity (π) of 13 PCGs and two rRNAs among 19 mitogenomes of Panorpidae were conducted using DnaSP 6.0 [70]. We analyzed the genetic distances based on Kimura-2-parameter and the ratios between non-synonymous (Ka) and synonymous substitutions rates (Ks) of 13 PCGs among the 19 mitogenomes using MEGA 7.0 [71] and DnaSP 6.0, respectively [70]. The A + T content, compositional skewness (AT-skew and GC-skew), Ka/Ks ratio, and genetic distances were graphically plotted using Prism 6.01 (GraphPad Software, San Diego, USA).

Phylogenetic analyses

A total of 23 mitogenomes of Mecoptera were used in the phylogenetic analyses, including 19 species of Panorpidae (17 newly sequenced mitogenomes and two downloaded from the GenBank) as the ingroup and four species of Bittacidae (two unpublished mitogenomes and two downloaded from the GenBank) as outgroups (Table 1). The extraction of 13 PCGs, 22 tRNAs, and two rRNAs were conducted by PhyloSuite 1.1.16 [69]. The nucleotide sequences were aligned in batches with MAFFT [72] integrated into

PhyloSuite 1.1.16 [69] and the ambiguous sites were removed using Gblocks [73]. The concatenations of genes were conducted using PhyloSuite 1.1.16 [69].

Phylogenetic trees were reconstructed for six genera of Panorpidae using Bayesian inference (BI) and maximum likelihood (ML) analyses. Four datasets were generated: (1) P12R: 13 PCGs excluding the third codon position and two rRNAs (9,538 bp); (2) P123: 13 PCGs (11,169 bp); (3) P123R: 13 PCGs and two rRNAs (13,261 bp); and (4) P123RT: 13 PCGs, two rRNAs, and 22 tRNAs (14,704 bp). The nucleotide substitution models and partitioning strategies for Bayesian inference were chosen by PartitionFinder 2 [74] shown in Additional file 11. Bayesian analyses were conducted using MrBayes 3.2.6 [75] and performed for 10,000,000 generations with sampling every 1000 generations. The sampling of posterior distribution being adequate was indicated by effective sample size (ESS) > 200 in Tracer 1.7 [76] and the average standard deviation of split frequencies < 0.01 in MrBayes 3.2.6 [75]. The first 25% were discarded as burn-in, and the remaining trees were used to generate the majority consensus tree and estimate the posterior probabilities (PP). The substitution models for ML analyses were chosen using ModelFinder [77] (Additional file 12). ML analyses were performed by IQ-TREE integrated into PhyloSuite 1.1.16 with Ultrafast bootstrap [69, 78]. Bootstrap support (BS) values were calculated with 1000 replicates.

Abbreviations

BI: Bayesian inference; BP: Bootstrap probability; CR: Control region; ESS: Effective sample size; Ka: Non-synonymous mutation rate; Ks: Synonymous mutation rate; ML: Maximum likelihood; ORF: Open reading frame; PCGs: Protein-encoding genes; PP: Posterior probabilities; rRNAs: Ribosome RNA genes; RSCU: Relative synonymous codon usage; tRNAs: Transfer RNA genes.

Declarations

Ethics approval and consent to participate

All animal experiments for this project were approved by the Ethics Committee of Northwest A&F University. No vertebrate animals and human were used in this study.

Consent for publication

Not applicable.

Availability of data and materials

The datasets supporting the conclusions of this article are included within the article and its additional files. All sequence data can be available from the GenBank: waiting the accession numbers.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

HY and Li designed the study; HY, LN, and CJ analyzed the data; LN and HBZ wrote the paper; All authors contributed comments to the final version of the manuscript and gave final approval for publication.

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Additional Files

Additional file 1: Circular maps of the mitogenomes of species of Panorpidae. The J-strand is visualized on the outer circle and the N-strand on the inner circle.

Additional file 2: Mitogenomic organization of *Cerapanorpa nanwutaina* TTH, *Dicerapanorpa magna* TTH, *Neopanorpa lui*, and *Sinopanorpa tincta*.

Additional file 3: Mitogenomic organization of *Cerapanorpa dubia*, *C. nanwutaina* ZQ, *C. brevicornis*, and *C. byersi*.

Additional file 4: Mitogenomic organization of *Dicerapanorpa magna* MCS, *D. magna* LP, and *D. magna* WLD.

Additional file 5: Mitogenomic organization of *Furcatopanorpa longihypovalva* LP, *F. longihypovalva* HSD, and *F. longihypovalva* TTH.

Additional file 6: Mitogenomic organization of *Panorpa dispergens*, *P. curva*, and *P. fulvastra*.

Additional file 7: Nucleotide composition of 17 Panorpidae mitogenomes.

Additional file 8: Secondary structure for the tRNAs. (A) *Cerapanorpa brevicornis*, *C. byersi*, *C. dubia*, and *C. nanwutaina* ZQ; (B) *Dicerapanorpa magna* TTH, *D. magna* LP, *D. magna* MCS, and *D. magna* WLD; (C) *Furcatopanorpa longihypovalva* HSD, *F. longihypovalva* TTH, and *F. longihypovalva* LP; (D) *Panorpa curva*, and *P. debilis*, and *P. fulvastra*.

Additional file 9: Phylogenetic tree generated by maximum likelihood based on the dataset of P123. Numerals at nodes are bootstrap support values (BS).

Additional file 10: Phylogenetic tree generated by Bayesian inference based on the dataset of P12R. Numerals at nodes are Bayesian posterior probabilities (PP).

Additional file 11: Best partitioning scheme and models based on different datasets for Bayesian inference (BI) analysis selected by PartitionFinder.

Additional file 12: Best partitioning scheme and models based on different datasets for Maximum likelihood (ML) analysis selected by ModelFinder.

Figures

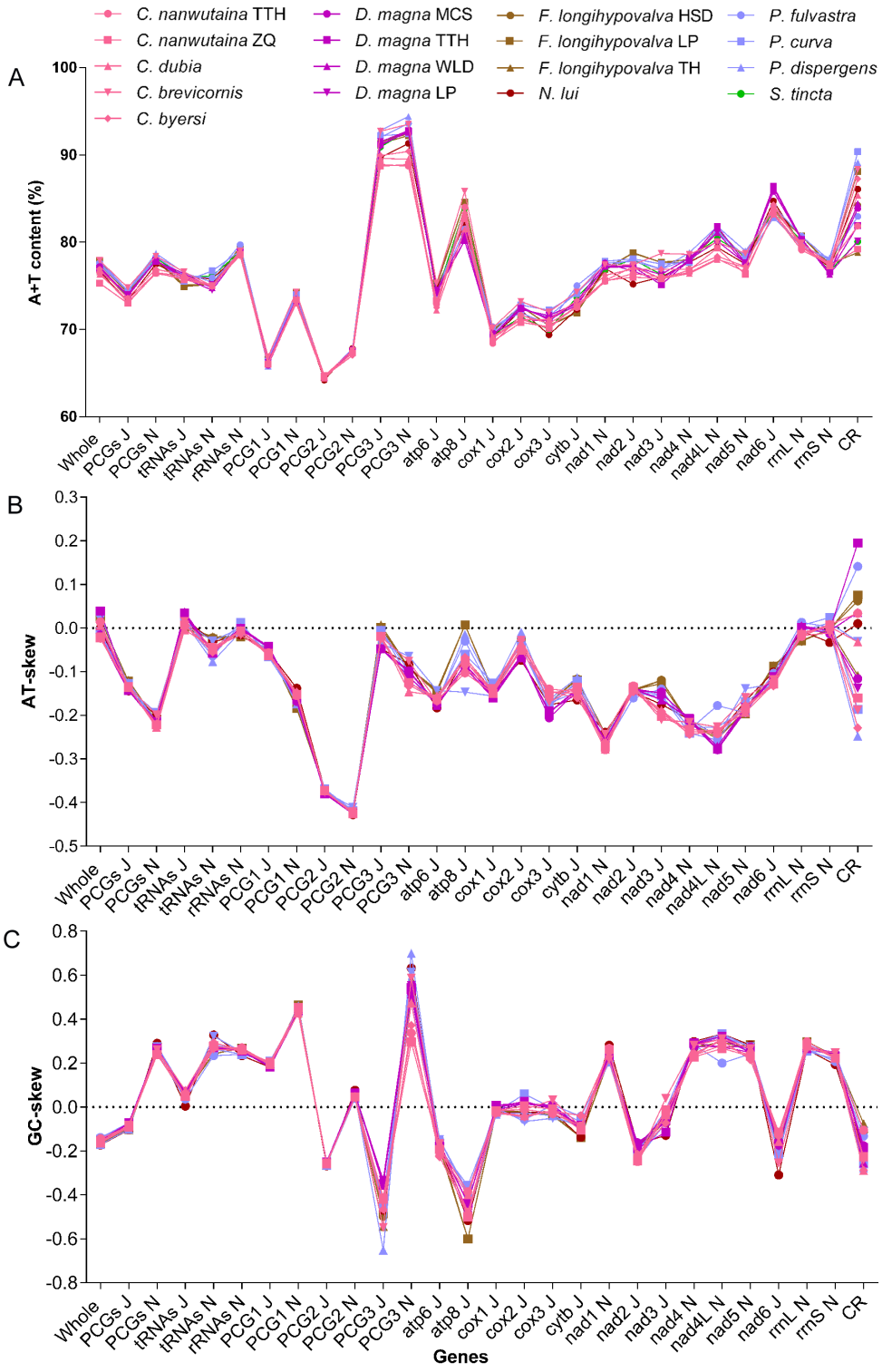

Figure 1

Comparison of the A+T contents, nucleotide skewness of 17 Panorpidae mitogenomes. (A) A+T content; (B) AT-skew; (C) GC-skew. N, N-strand; PCG1, the first codon position; PCG2, the second codon position; PCG3, the third codon position; Whole, the complete sequences of whole mitogenome; J, J-strand.

Figure 3

Relative synonymous codon usage (RSCU) of the 17 mitogenomes of Panorpidae.

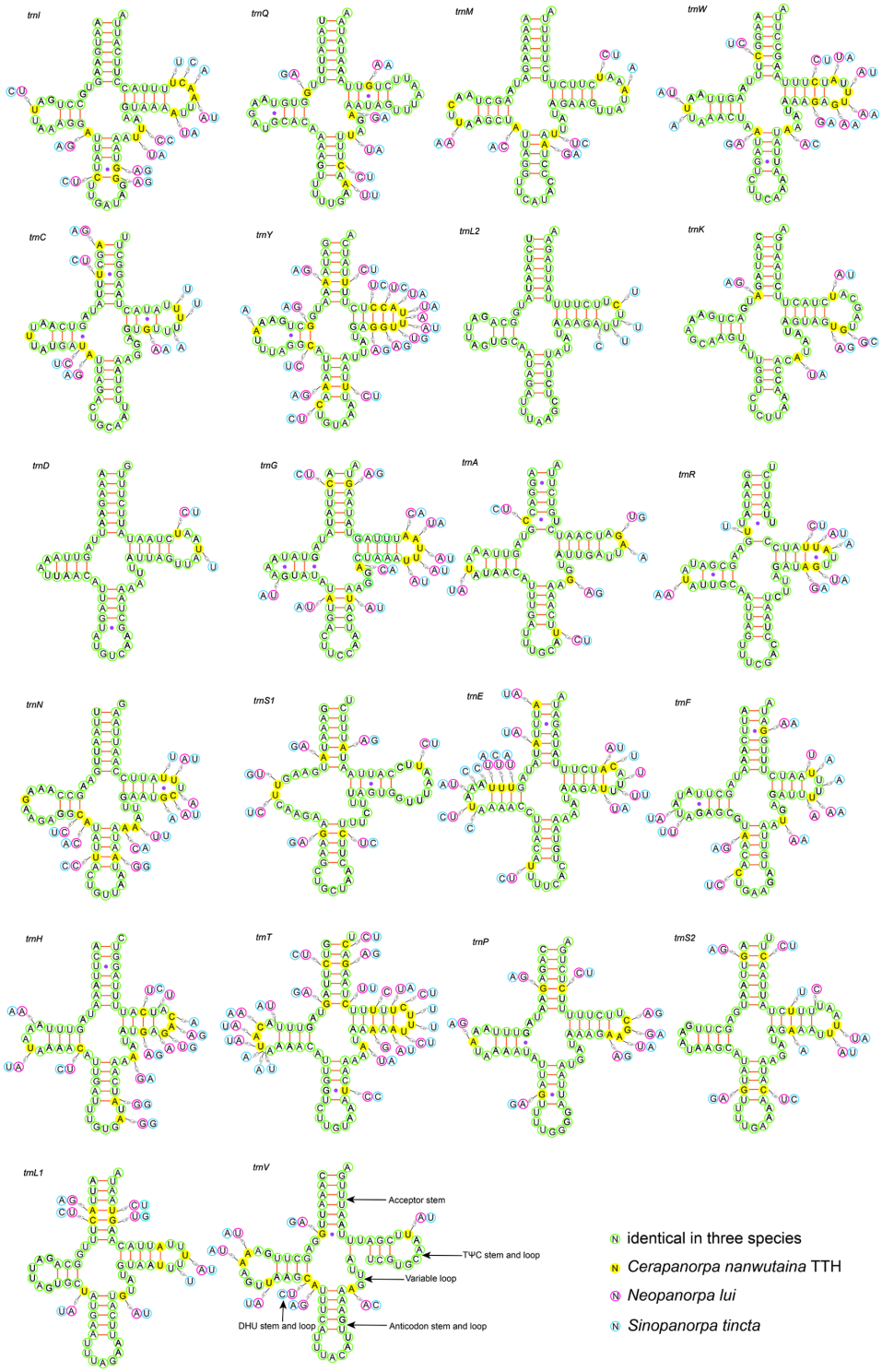

Figure 5

Secondary structure for the tRNAs of Cerapanorpa nanwutaina TTH, Neopanorpa lui, and Sinopanorpa tincta.

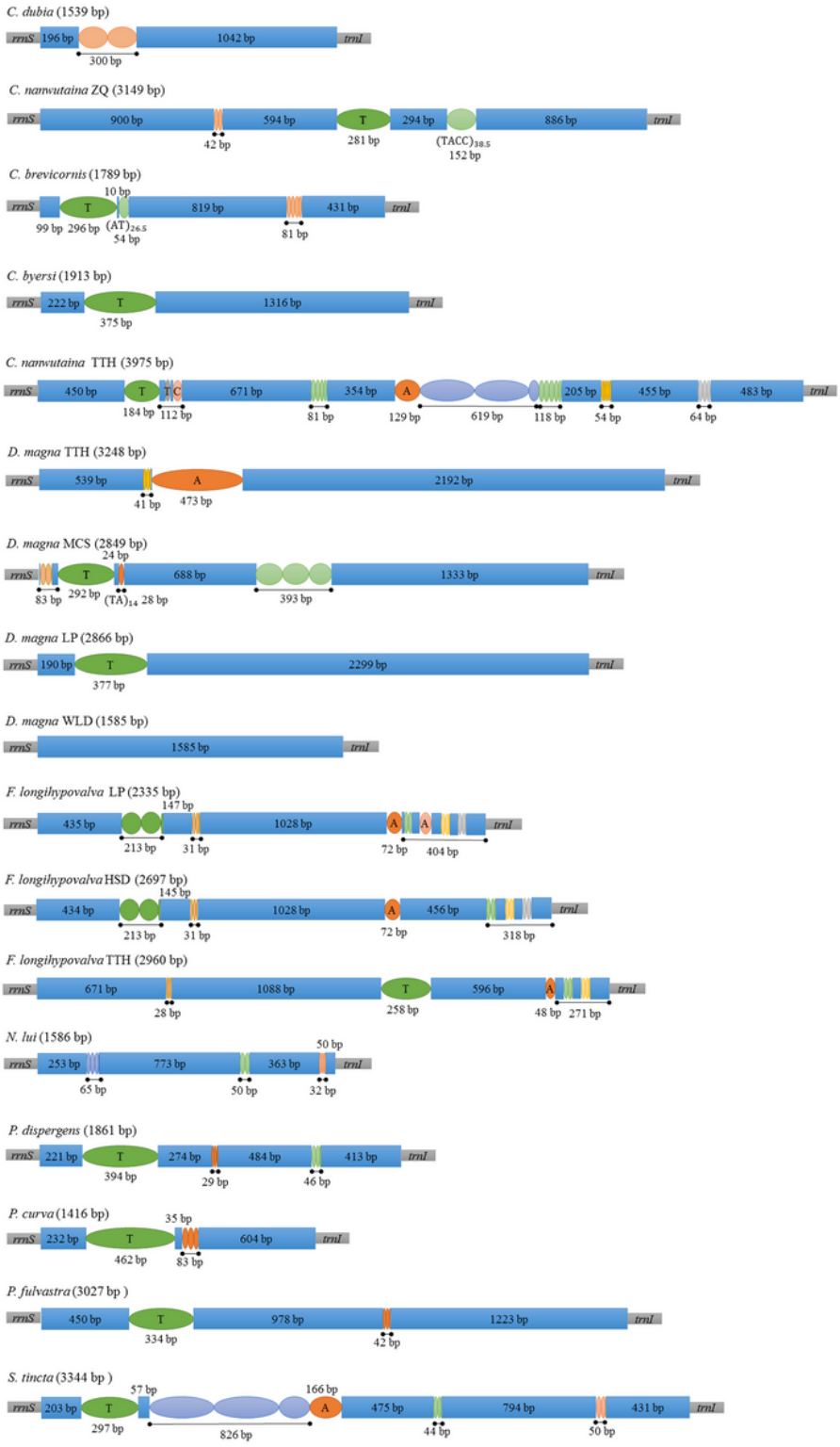

Figure 7

Organization of the control region in Panorpidae mitogenomes. The colored ovals indicate the tandem repeats; the remaining regions are shown with blue boxes.

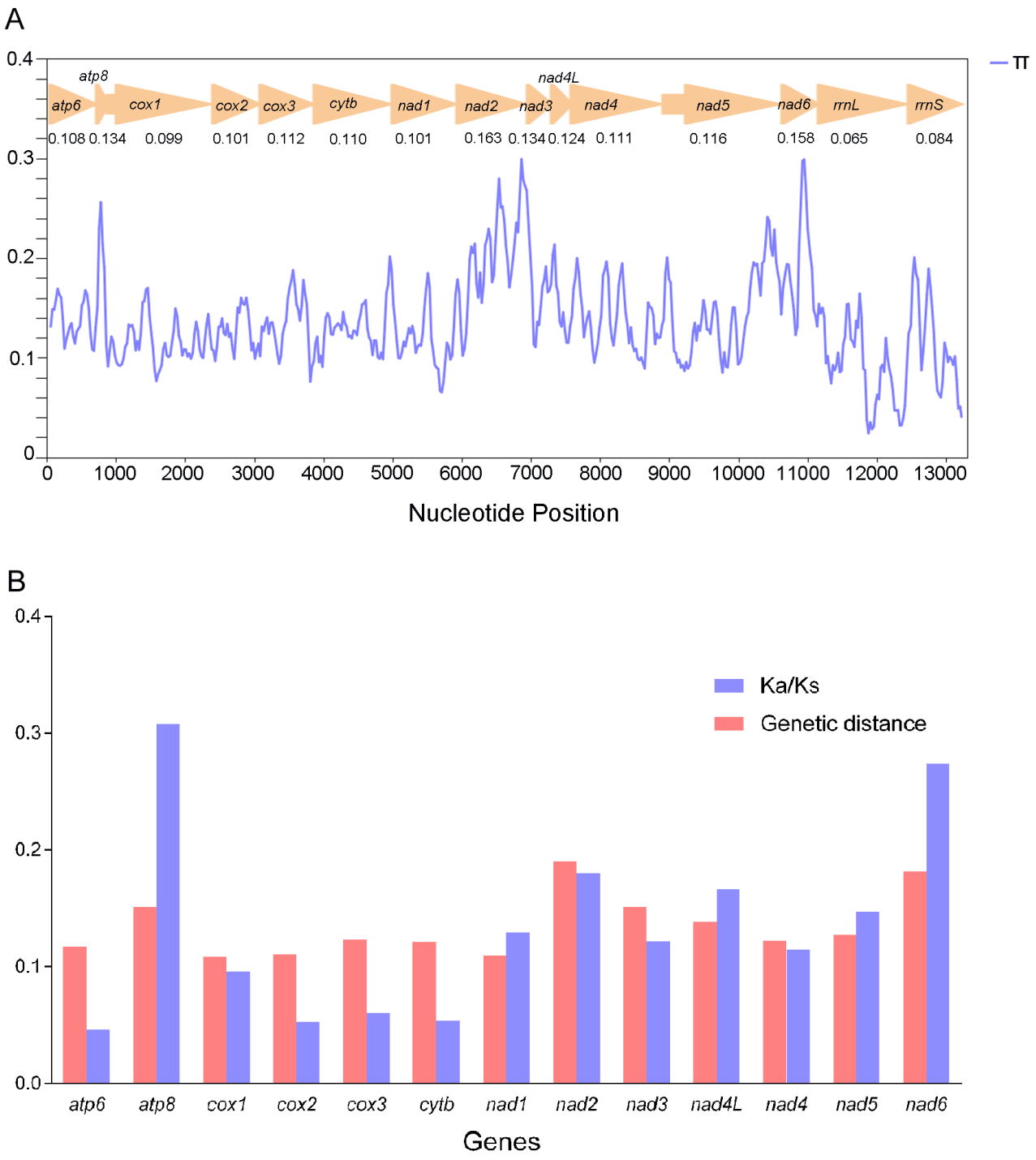

Figure 9

Evolutionary rates and selection pressures among 19 species of Panorpidae. (A) Genetic distance and ratio of non-synonymous (Ka) to synonymous (Ks) substitution rates of each protein-coding gene; (B) sliding window analysis of 13 protein-coding genes and two rRNAs. The blue curve shows the value of nucleotide diversity (π). π value of each PCG was shown above the arrows.

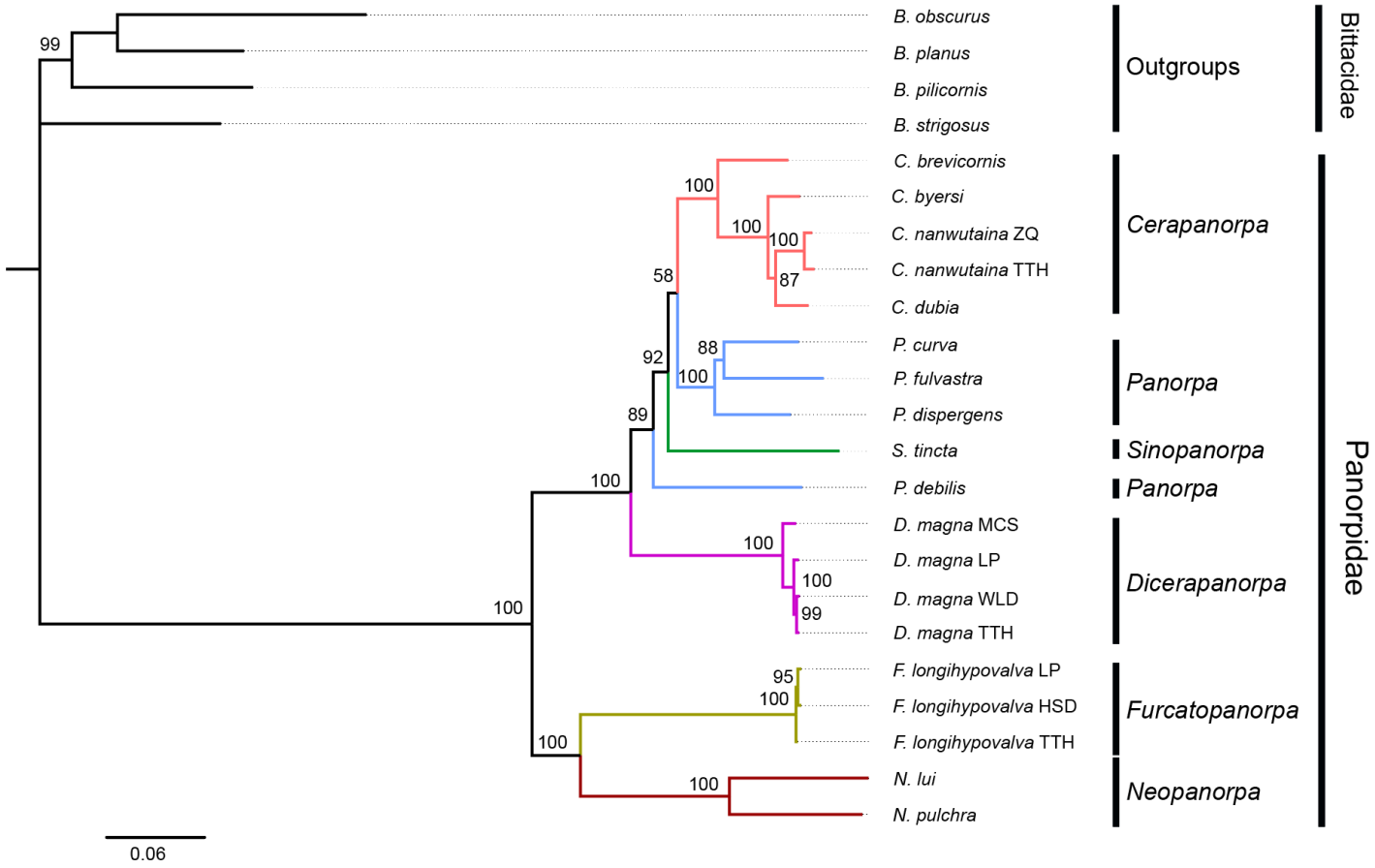

Figure 11

Phylogenetic tree generated by maximum likelihood based on the dataset of P123RT. Numerals at nodes are bootstrap support values (BS).

Supplementary Files

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