International Journal for Parasitology: Parasites and Wildlife 18 (2022) 249–259

Contents lists available at ScienceDirect

International Journal for Parasitology: Parasites and Wildlife

journal homepage: www.elsevier.com/locate/ijppaw

The complete mitochondrial genome of *Platygaster robiniae* (Hymenoptera: Platygastridae): A novel tRNA secondary structure, gene rearrangements and phylogenetic implications

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ARTICLE INFO

Keywords:
Platygaster robiniae
Mitochondrial genome
Gene rearrangement

ABSTRACT

Platygaster robiniae is economically important as a highly specific parasitoid of the invasive pest *Obolodiplosis robiniae* which was introduced into the Euro-Asia region in the last decade. Despite being a critical and specific parasitoid of the invasive pest *O. robiniae* and its use as an effective biocontrol agent, the absence of sequence information from *P. robiniae* have limited its genetic applications for pest management in forests. Mitochondrial (mt) genomes generally contain abundant nucleotide information and thus are helpful for understanding species history. Here, we sequenced the complete mt genome of *P. robiniae* using next generation sequencing, and annotated 13 protein-coding, 22 tRNA, and 2 rRNA genes and a 702 bp noncoding region. Comparative analysis indicated that this mt genome has a normal A + T content and codons use, however possessed both the expected and unique rearrangements. Ten tRNAs at four gene blocks *COII-ATP8, COIII-ND3, ND3-ND5* and the A + T-rich region-*ND2* were rearranged, including gene shuffles, transpositions and inversions. Notably, two genes $tRNA^{Ser\ (UCN)}$ and $tRNA^{Leu(CUN)}$ had undergone long-range inversions, which is the first record of this rearrangement type in the superfamily Platygastroidea. The D-loops of both $tRNA^{Ile}$ and $tRNA^{Leu(CUN)}$ were absent from the tRNA secondary structure, which has not been reported from hymenopteran previously. Phylogenetic analysis based with the maximum likelihood and Bayesian methods showed that *P. robiniae* grouped with other species of Platygastridae, and that the superfamily Platygastridea is sister to the other Proctotrupomorpha superfamilies. Our tree strongly supports the monophyly of the five superfamilies of Proctotrupomorpha. This study discovered some unique characters of *P. robiniae*, and contributes to our understanding of genome rearrangements in the order Hymenoptera.

1. Introduction

Platygastridae (Apocrita: Platygastroidea) is a diverse and speciose family of parasitic Hymenoptera, consisting of approximately 1153 species in the world (Samin and Asgari, 2012). They are generally small in size (0.5–12 mm), with most species being morphologically simple compared with other parasitic wasps (Austin et al., 2004). *Platygaster robiniae* Buhl and Duso (Hymenoptera: Platygastridae) is an egg-larvae parasitoid of the black locust gall midge *Obolodiplosis robiniae* (Diptera: Cecidomyiidae) (Kim et al., 2011; Yang et al., 2021) which is native to North America and has been considered a highly invasive pest insect in Europe and Asia in recent decades (Yao et al., 2015, 2020). It has been discovered in all places where its host has been found in both native areas and new regions, as first reported in 2010 in Qinhuangdao city, Hebei Province, China (Lu et al., 2010), and it was later found in 17 other provinces at 29 sites (Yang et al., 2019). Despite being a critical and specific parasitoid of the invasive pest *O. robiniae* and its use as an effective biocontrol agent, the absence of nucleotide information, population genetics and the phylogeny of *P. robiniae* have reduced the understanding of the history of its occurrence and its mechanism of population colonization and successful invasion, consequently, limiting genetic applications pest management in forests.

Mitochondrial (mt) genome sequences generally provide large and diverse datasets that contain abundant nucleotide information and thus are helpful for improving phylogenetic relationships at any taxon level (Cameron et al., 2006a; Fenn et al., 2008). Additionally, it has been

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https://doi.org/10.1016/j.ijppaw.2022.06.007
Received 13 May 2022; Received in revised form 14 June 2022; Accepted 20 June 2022
Available online 23 June 2022

Table 1
Sequencing sample acquisition information.

Place	Abbreviation	Time	Longitude and Latitude	Altitude
Beijing	BJ	July 2017	40°36′28″N,116°58′01″	89
Yinchuan	YC	July 2017	38°43′39″N,106°17′73″	1076
Yantai	YT	July 2017	37°56′65″N,121°24′97″	18
Shenyang	SY	July 2017	41°77′70″N,123°43′40″	55

considered a useful molecular marker for species identification and evolutionary studies because genome its features of rare recombination, maternal inheritance, conserved gene component, and high AT composition (Boore and Brown, 1999; Curole and Kocher, 1999). In insects, mt genomes are typically double-stranded circular molecules of approximately 16 kb that contain 13 protein-coding genes (PCGs), 2 ribosomal RNA (rRNA) genes, 22 transfer RNA (tRNA) genes (Boore, 1999) and contain a large noncoding region known as the A + T-rich region (in invertebrates) or control region (in vertebrates), that regulates transcription and replication (Zhang and Hewitt, 1997). With the ongoing developments of Illumina sequencing technology and bioinformatic approaches, sequence data for insect mt genomes has rapidly increased in recent years with 1000's available on NCBI databases including all orders. They are relatively conservative, most insect orders have single gene rearrangements, such as Lepidoptera and Coleoptera (Sheffield et al., 2008; Sun et al., 2020), but there are also frequent mt genome rearrangements in other orders, such as Hymenoptera (Dowton and Austin, 1999; Dowton et al., 2009b; Mao and Dowton, 2014) and Psocodea. The number of genes involved in hymenopteran mitochondrial rearrangement is large and rearrangements often independent, which has made it sometimes difficult to sequence complete hymenopteran mt genomes, resulting in fragment deletion (Mao et al., 2015).

While rearrangements of insect mt genes have now been found in 17 orders of insects (Crozier and Crozier, 1993; Flook et al., 1995; Shao and Barker, 2003; Wang et al., 2014; Wei, 2009), the frequency, types and scales of rearrangements often differ between taxa (Chen and Du, 2016). The Hymenoptera especially the suborder Apocrita, exhibit high mt rearrangement rates, and most taxa are rearranged. The rearrangements mainly occur in tRNA genes (Wei, 2009; Wei and Chen, 2011; Wei et al., 2010b). Four types of rearrangements, translocation, inversion, shuffling and remote inversion events, are primary for hymenopterans and have been found to be present at nearly equal frequencies (Dowton and Austin, 1999; Wei, 2009). However, this phenomenon needs to be explained and may be associated with a deeper mechanism, which could be possible when we have generous species nucleotide data.

Poor representation among Hymenopteran lineages, however, has limited the application of the mt genome in evolutionary analysis, especially in Proctotrupomorpha (encompassing the superfamilies Proctotrupoidea, Cynipoidea, Diaprioidea, Mymarommatoidea, Platygastroidea, and Chalcidoidea) (Mao et al., 2015; Shen et al., 2019). In total, 79 mt genomes from species of Proctotrupomorpha have been reported through NCBI database search statistics, including Chalcidoidea, Proctotrupoidea, Diaprioidea, Cynipoidea and Platygastroidea which have 52, 5, 4, 7 and 11 mt genomes, respectively, yet, no complete mt genome sequences were available from Platygastridae.

In the present study, we sequenced and annotated the mt genomes of *P. robiniae* using Illumina TruSeq and bioinformatics approaches, compared the structure of the new mt genomes with that of closely related groups, and explored rearrangement genes of Platygastridae. Additionally, we conducted phylogenetic analyses of mt genomes within the Proctotrupomorpha.

2. Materials and methods

2.1. Sampling and DNA extraction

Individuals of *P. robiniae* were collected from *Robinia pseudoacacia* L. forests in four cities in China (BJ, YC, YT, SY) in July 2017 (Table 1). Samples were preserved in 100% ethanol at −20 °C for long-term storage at the Chinese Academy of Forest (CAF). Total genomic DNA was extracted from individuals using a DNeasy tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

2.2. Sequencing and genome assembly of mitochondrial DNA

Illumina TruSeq libraries with an average insect size of 450 bp were constructed using a TruSeq™ DNA LT Sample Prep Kit (Illumina, Inc., San Diego, CA, USA) following the manufacturer's manuals. Clustering of the index-coded samples was performed in a cBot Cluster Generation System using a TruSeq PE Cluster Kit v3-cBot-HS (Illumina). Sequencing of the clustered flow cell was performed using the Illumina HiSeq 2500 platform (Erik et al., 2011). Prior to assembly, Illumina raw reads were filtered firstly. For each library, 6 Gb of clean data was obtained after trimming adapters and low-quality bases (quality score <20) using Adapter Removal v2 (Schubert et al., 2016). The genome was assembled using Geneious Prime 2020 (https://www.geneious.com/prime) and IDBA-UD assembler software (Peng et al., 2012). Geneious prime 2020 was used *de nova* assembly, preprocessed the NGS reads properly, and pruned low quality data using BBDuk, then paired the trimmed data to read lists and reassemble them. IDBA-UD iterated the value of k from kmin to kmax, and gradually increased the threshold of low-depth cutting, then remove some low-depth overlapping groups and obtain longer H_k confidence overlapping groups (C_k). The missing k-mers are reconstructed by locally assembling, and the information of these missing k-mer will be transmitted to the next iteration through these overlapping groups (LC_k). Finally, the overlapping groups of all outputs are used to form scaffolds by pairing the terminal read length information.

2.3. Mitochondrial genome annotation and analysis

The positions and direction of 13 PCGs, 2 rRNA genes, and 22 tRNA genes were predicted using MITOS WebServer (Matthias et al., 2013) with the following parameters: Reference = "RefSeq 89 Metazoa" and Genetic Code = "5 Invertebrate". The secondary structures of the tRNA genes were also determined using the MITOS WebServer using default settings (Tang et al., 2017). When they were not detected by this approach, we confirmed the tRNA positions by aligning with their homologous sequences from related species (GenBank:MG923507, MG923510, KF696669, KF696670, JN903532) (Table S1). The protein-coding and rRNA genes were initially annotated with MITOS WebServer and edited in Geneious 9.0.2 (http://www.geneious.com) through comparison to other Platygastroidea mitochondrial genomes (Table S1). The start and stop codons and length of each PCG were manually confirmed and modified. All reference mt genomes were downloaded from GenBank.

Nucleotide composition and relative synonymous codon usage (RSCU) were determined using MEGA 7.0 software (Sudhir et al., 2016). AT and GC skews were measured for the major (J) strand of each genome, using the formulae AT-skew = (A-T)/(A + T) and GC-skew = (G-C)/(G + C) (Perna and Kocher, 1995). A circular map of the complete mt genome was made using CGView (Grant and Stothard, 2008).

DnaSP6 (Rozas et al., 2003) was used to calculate the ratio of the nonsynonymous substitution rate to the synonymous substitution rate (Ka/Ks), and evolution rates for the 13 PCGs in *P. robiniae*, the evolution rates within major groups in Platygastroidea and the evolutionary rates of each mitochondrial gene.

2.4. Phylogenetic analysis

Data from the newly sequenced mt genome of *P. robiniae* and those of 48 other Proctotrupomorpha were used for phylogenetic analysis with one species from the family Ichneumonoidea (Insecta, Hymenoptera) as an outgroup (Table S1). Nucleotide sequences from each PCG and rRNA

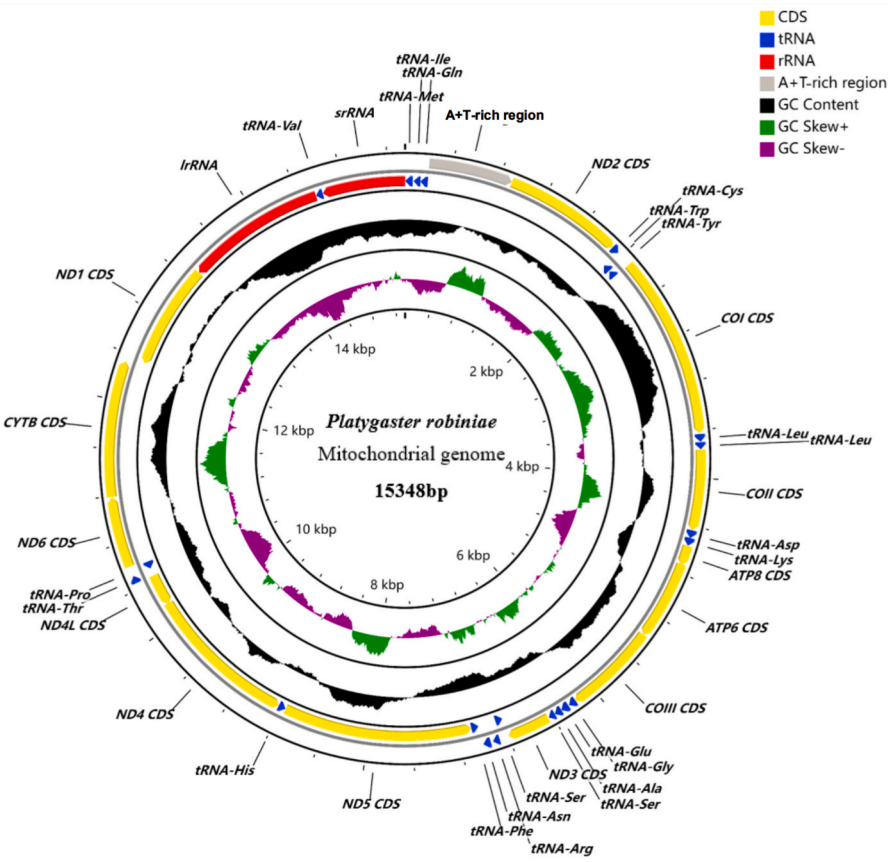

Fig. 1. Genetic map of the complete mitochondrial genome of *Platygaster robiniae*.
Notes: the blue arrow represents the direction of gene transcription; the black peak represents the deviation of GC%; the purple and green peaks represent the deviation in GC skew; green refers to positive skew, and purple indicates negative skew. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

gene were aligned individually using the MAFFT Web Server (Katoh et al., 2005), and ambiguous sites deleted manually after alignment. The dataset of 13 PCGs was used to construct phylogenetic trees. Maximum likelihood and Bayesian approaches were employed to infer phylogenetic trees. Analyses were performed using MrBayes v.3.2.5 (Ronquist and Huelsenbeck, 2003) and PhyML 3.0 (Gascuel, 2010). MrBayes v.3.2.5 was used to analyze the dataset for nucleotide substitutions with the GTR + I + G model, which was selected using jModelTest 2.1.7 (David, 2008). For maximum likelihood analyses, 1000 bootstrap replicates were performed and the GTRGAMMA substitution model applied to all partitions. For Bayesian analysis, two simultaneous runs of 10,000, 000 generations were conducted, sampled every 200 generations with a burn-in of 25%. Phylogenetic trees were viewed and edited in FigTree v1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/).

3. Results and discussion

3.1. Structure of the mitochondrial genome

3.1.1. Mitochondrial genome size

Two identical complete mt genomes for each sample were obtained using both Geneious Prime 2020 and IDBA-UD assembler software, and no different structures were caused by the different assembling methods. By comparing mt genome sequences from the four sites, it was found that mt genomes of the samples from YC, YT and SY were completely consistent in sequence, while the BJ sample has 1 bp more in $tRNA^{Trp}$ gene and showed 42 base substitutions in 14 genes, control region and intergenic spacers comparing to the other three samples (Table S2). Since three of the four samples shared the same mt genomes sequence with a length of 15,348 bp, it was used to subsequently analysis and has been assigned the GenBank accession number (GenBank:**OM372674**).

Currently, for the available mt genomes of the Proctotrupomorpha, the length ranged from 10044 bp to18217 bp. The length of the new sequence is within the range and contains all fragments, which is a complete mitochondrial sequence (Table S3). Altogether, the genome comprises 37 genes [22 tRNA genes, 13 PCGs (*COI–III*, *ND1–6*, *ND4L*, *CytB*, *ATP6* and *ATP8*), 2 rRNA genes (*lrRNA* and *srRNA*)] and a control region (Fig. 1). Some mt genome data lacked tRNAs and protein coding genes, because in the process of gene sequencing, some tRNAs ($tRNA^{Ile}$, $tRNA^{Arg}$, etc.) were not sequenced or annotated successfully due to serious rearrangement, and their positions could not be determined, leading to gene fragment deletion (Samin and Asgari, 2012; Shen et al., 2019). As a result, some mt genomes had sufficient length, but the fragment number was less than 37, which is a common problem in sequencing hymenopteran mt genomes (Castro et al., 2006; Dowton, 1999; Wei et al., 2010b). Sixteen of these genes are encoded on the

Table 2
Base composition in the mitochondrial genomes of *Platygaster robiniae*.

Gene	Length (bp)	A%	T%	AT%	AT-skew	C%	G%	CG%	GC-skew
All gene	15348/15349	44.0/43.9	38.0	81.9	0.0732	11.5	6.6	18.1	−0.2744/-0.2710
13-PCG	11151	43.1	36.4	79.5	0.0847/0.0850	12.9	7.5/7.6	20.5	−0.2641/-0.2630
rRNA	1983	47.3	41.2	88.5	0.0689	8.1/8.0	3.4/3.5	11.5	−0.4035/-0.3860
tRNA	1418/1419	45.8/45.7	43.7	89.4	0.0237/0.0229	6.6	4.0	10.6	−0.2400
Control region	702	42.9/42.6	41.5/41.3	84.3/83.0	0.0169/0.0154	9.8/10.0	5.8/6.1	15.7/16.1	−0.2545/-0.2389

Table 3
Characteristics of PCGs of mitochondrial genomes of 12 species of Platygastroidea.

Species	Length (bp)		A%	T%	AT%	AT-skew	C%	G%	CG%	GC-skew
	All	13PCGs								
Platygaster robiniae	15348/15349	11151	43.1	36.4	79.5	0.0847/0.0850	12.9	7.5/7.6	20.5	−0.2641/-0.2630
Platygaster sp. ZJUH_2016026	16098	11211	42.3	40.0	82.3	0.0279	11.7	5.9	17.7	−0.3310
Platygaster sp. ZJUH_2016029	16605	11100	43.0	39.2	82.2	0.0468	11.4	6.4	17.8	−0.2787
Ceratobaeus sp. MM-2013	15851	11107	40.6	34.9	75.6	0.0757	15.6	8.9	24.4	−0.2751
Habroteleia persimilis	17186	11182	43.0	41.3	84.2	0.0197	9.8	6.0	15.8	−0.2440
Idris sp. MM-2013	15137	11079	41.3	37.8	79.1	0.0443	13.6	7.4	20.9	−0.2951
Scelio sp. ZJUH_2016028	16851	11052	40.8	37.9	78.7	0.0368	14.4	6.8	21.3	−0.3586
Telenomus dignus	14304	11120	43.2	39.5	82.7	0.0449	10.2	7.0	17.3	−0.1839
Telenomus remus	16014	11148	43.8	39.1	83.0	0.0570	10.0	7.1	17.0	−0.1711
Telenomus sp. ZCS-2018	17023	11131	43.1	38.3	81.4	0.0586	11.5	7.1	18.6	−0.2367
Trissolcus basalis	15768	11132	43.1	39.2	82.2	0.0472	11.1	6.7	17.8	−0.2500
Trissolcus japonicus strain CREATJ	16264	11111	42.6	38.2	80.8	0.0548	12.1	7.1	19.2	−0.2616

Table 4
Mitochondrial genome structure of *Platygaster robiniae*.

Gene	Direction	Location	Length (bp)	Condon Start	Condon Stop	Intergenic Nucleotides[a]
$tRNA^{Met}$	R	1–68	68			
$tRNA^{Ile}$	R	75–136	62			6
$tRNA^{Gln}$	R	135–201	67			−2
Control region	F	202–903	702			0
ND2	F	904–1900	997	ATT	T-	0
$tRNA^{Trp}$	F	1901-1965/1901-1966	65/66			0
$tRNA^{Cys}$	R	1958-2022/1959-2023	65			−8
$tRNA^{Tyr}$	R	2029–2091	63			6
COI	F	2096–3628	1533	ATG	TAA	4
$tRNA^{Leu\ (UUR)}$	F	3635–3699	65			6
$tRNA^{Leu\ (CUN)}$	F	3702–3762	61			2
COII	F	3763–4435	673	ATA	T-	0
$tRNA^{Asp}$	F	4436–4502	67			0
$tRNA^{Lys}$	F	4501–4567	67			−2
ATP8	F	4568–4735	168	ATA	TAA	0
ATP6	F	4729–5391	663	ATG	TAA	−7
COIII	F	5391–6176	786	ATG	TAA	−1
$tRNA^{Gly}$	F	6175–6239	65			−2
$tRNA^{Glu}$	F	6246–6314	69			6
$tRNA^{Ala}$	F	6315–6377	63			0
$tRNA^{Ser\ (AGN)}$	F	6378–6435	58			0
ND3	F	6442–6801	360	ATT	TAA	6
$tRNA^{Ser\ (UCN)}$	R	6800–6864	65			−2
$tRNA^{Arg}$	F	6879–6937	59			14
$tRNA^{Asn}$	F	6956–7020	65			18
$tRNA^{Phe}$	R	7020–7084	65			−1
ND5	R	7085–8764	1680	ATT	TAG	0
$tRNA^{His}$	R	8765–8830	66			0
ND4	R	8831–10172	1342	ATG	T-	0
ND4L	R	10166–10450	285	ATA	TAG	−7
$tRNA^{Thr}$	F	10453–10516	64			2
$tRNA^{Pro}$	R	10517–10582	66			0
ND6	F	10599–11189	591	ATA	TAA	16
CytB	F	11193–12329	1137	ATG	TAA	3
ND1	R	12367–13302	936	ATA	TAA	37
lrRNA	R	13303–14548	1246			0
$tRNA^{Val}$	R	14549–14611	63			0
srRNA	R	14612–15348	737			0

[a] Represents the gene interval, and the negative number represents the number of nucleotides overlapped between adjacent genes.

minor strand (N-strand), including four PCGs (*ND1*, *ND4*, *ND4L*, and *ND5*), ten tRNA genes ($tRNA^{Gln}$, $tRNA^{Ile}$, $tRNA^{Met}$, $tRNA^{Val}$, $tRNA^{Pro}$, $tRNA^{His}$, $tRNA^{Phe}$, $tRNA^{Ser\ (UCN)}$, $tRNA^{Tyr}$, $tRNA^{Trp}$), and two rRNA genes (*lrRNA* and *srRNA*), whereas the remaining 21 genes are encoded on the major strand (J-strand) in *P. robiniae*.

3.1.2. Nucleotide composition

Mitochondrial genomes are generally characterized by significant nucleotide compositional bias (Cameron, 2014; Timmermans and Vogler, 2012), and two measures of bias, non-strand specific (A + T and G + C contents) and strand specific (AT-skew and GC-skew), are used to examine its extent (Hassanin, 2006; Wei et al., 2010a). We found the *P. robiniae* mt genome to be characterized by very high A + T content (Table 2), accounting for 81.9% of the genome. High A + T content is due to the increased A content in Apocrita mt genomes (Dowton and Austin, 1997) but is common in other Hymenoptera mt genomes (Dowton, 1999; Mao and Dowton, 2014; Samin and Asgari, 2012; Shen et al., 2019). The A + T content of the control region was higher (84.3%/83%) than that of the coding regions (79.5%), which is the general pattern in insect mt genomes (Clary and Wolstenholme, 1985; Zhang and Hewitt, 1997). The *P. robiniae* mt genome had an overall positive AT-skew and negative GC-skew (Table 2), indicating no reversal

Table 5
Statistics on codon usage of protein gene in *Platygaster robiniae* mitochondrial genome.

Amino acid	Codon	Count	RSCU	Amino acid	Codon	Count	RSCU
Phe (F)	**UUU**	**290**	**1.53**	Tyr (Y)	**UAU**	**252**	**1.57**
	UUC	90	0.47		UAC	70	0.43
Leu (L2)	**UUA**	**413**	**3.76**	Stop (*)	**UAA**	**378**	**1.77**
	UUG	47	0.43		UAG	48	0.23
Leu (L1)	CUU	48	0.44	His (H)	**CAU**	**99**	**1.56**
	CUC	32	0.29		CAC	28	0.44
	CUA	**106**	**0.97**	Gln (Q)	**CAA**	**108**	**1.6**
	CUG	13	0.12		CAG	27	0.4
Ile (I)	**AUU**	**390**	**1.55**	Asn (N)	**AAU**	**373**	**1.62**
	AUC	114	0.45		AAC	87	0.38
Met (M)	**AUA**	**382**	**1.78**	Lys (K)	**AAA**	**446**	**1.75**
	AUG	47	0.22		AAG	63	0.25
Val (V)	GUU	32	1.41	Asp (D)	**GAU**	**48**	**1.5**
	GUC	12	0.53		GAC	16	0.5
	GUA	**43**	**1.89**	Glu (E)	**GAA**	**74**	**1.49**
	GUG	4	0.18		GAG	25	0.51
Ser (S2)	UCU	75	1.54	Cys (C)	**UGU**	**28**	**1.6**
	UCC	42	0.86		UGC	7	0.4
	UCA	**111**	**2.28**	Trp (W)	**UGA**	**59**	**1.59**
	UCG	10	0.21		UGG	15	0.41
Pro (P)	CCU	28	1.05	Arg (R)	**CGU**	**11**	**1.57**
	CCC	19	0.71		CGC	4	0.57
	CCA	**57**	**2.13**		CGA	10	1.43
	CCG	3	0.11		CGG	3	0.43
Thr (T)	**ACU**	**88**	**1.68**	Ser (S1)	AGU	44	0.9
	ACC	42	0.8		AGC	20	0.41
	ACA	67	1.28		**AGA**	**52**	**1.07**
	ACG	12	0.23		AGG	35	0.72
Ala (A)	GCU	11	1.47	Gly (G)	GGU	12	1.23
	GCC	2	0.27		GGC	2	0.21
	GCA	**16**	**2.13**		**GGA**	**22**	**2.26**
	GCG	1	0.13		GGG	3	0.31

Note: the codons underlined are those that strictly match the tRNA anticodon, and the codons in bold are those used most frequently for each amino acid.

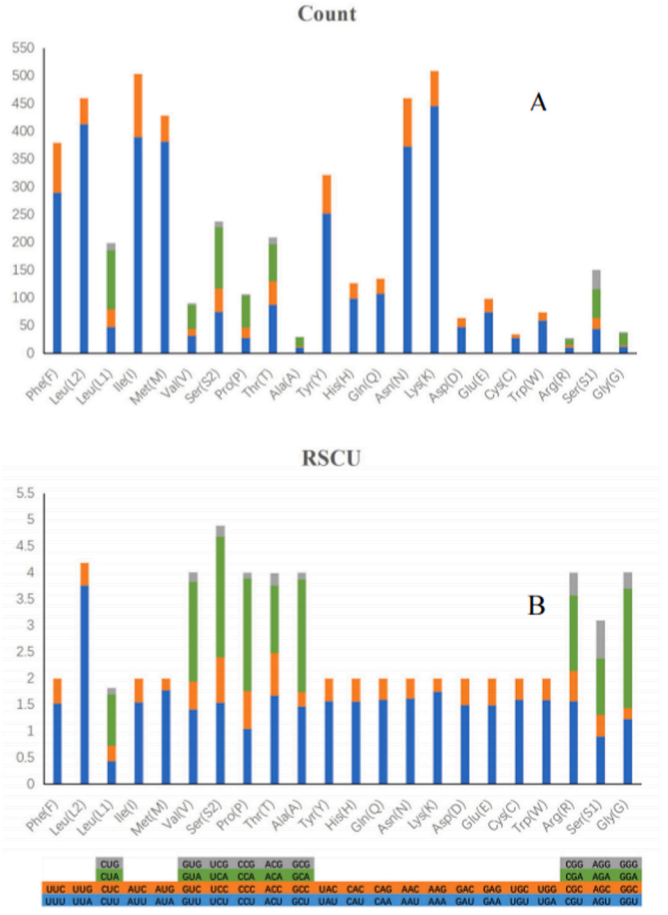

Fig. 2. Amino acids (A) and relative synonymous codons (B) of protein-coding genes of the mitochondrial genome of *Platygaster robiniae*.

of strand asymmetry within this species although reversals have been shown in other hymenopterans (Wei et al., 2010a). Comparison of coding regions in *P. robiniae* to the other 11 species in Platygastroidea, finds that base composition of the coding region has strong AT bias (75.6%–84.2%), and each of the 12 coding regions exhibit positive AT skews (ranging from 0.0197 to 0.0847/0.0850), and negative GC-skews (ranging from −0.1711 to −0.3586) (Table 3).

3.1.3. Control region, intergenic spacer and overlap

The control region of insect mt genomes can show considerable variation in length (Mao and Dowton, 2014; Shen et al., 2019). Due to rearrangement of the *P. robiniae* mt genome, the control region is located between $tRNA^{Gln}$ and *ND2*, and full length is 702 bp. The A + T content (84.3%/83.0%) of the region is higher than that of the coding region (Table 2), which is a pattern typically observed in insect mt genomes (Clary and Wolstenholme, 1985; Zhang and Hewitt, 1997). We also identified overlaps between 9 pairs of adjacent genes, ranging from 1 to 8 bp, with the largest overlap 8 bp located at the junction of $tRNA^{Trp}$-$tRNA^{Cys}$, and overlaps of 7 bp occurred at the junctions *ATP8-ATP6 and ND4-ND4L*. Overlap between *ATP8-ATP6* coding genes is a common feature of metazoan mt genomes (Campbell and Barker, 1999) and has been reported in other hymenopteran taxa (Castro and Dowton, 2005; Crozier and Crozier, 1993). In addition, a total of 13 intergenic spacers were found, with lengths ranging from 2 to 37 bp. The smallest spacer was located between two pairs of genes, $tRNA^{Leu(UUR)}$-$tRNA^{Leu(CUN)}$ and *ND4L*-$tRNA^{Thr}$, and the longest between *Cyt-B*-and *ND1* (Table 4).

3.1.4. Codon usage

The RSCU in the mt genome of *P. robiniae* shows a strong bias toward the usage of A and T, particularly at the third codon position. The most frequently used codon for each amino acid is NNA or NNU (Table 5). For some amino acids, the most frequently used codon is not the set that corresponds strictly to the corresponding tRNA anticodon (Table 5). The four most commonly encoded amino acids in the *P. robiniae* mt genome (with their corresponding codons), listed in order of decreasing frequency, are as follows: AAA (*Lys*), AUU (*Ile*), UUA (Peng et al.) and AAU (*Asn*) (Fig. 2).

3.1.5. Protein-coding genes

The location and size of 13 PCGs were determined by comparing the mt genome of *P. robiniae* with its related species. The 13 PCGs accounted for 72.65% (11151 bp total) of the whole genome, and the AT content was 79.5% in *P. robiniae* (Table 2). Nine of the 13 PCGs are encoded by the J-strand and four by the N-strand, and the start codons of all PCGs are "ATN" in these genomes (ATG, ATA, ATT) (Table 4). The three PCGs, *ND2*, *ND3* and *ND5*, use ATT as start codons, and the remaining 10 PCGs use conventional start codons, ATA or ATG. In many metazoans, numerous mitochondrial genes have incomplete termination codons (Miya et al., 2001). In the *P. robiniae* mt genome, except for *ND2*, *ND4* and *COII*, which use the incomplete stop codon "T", the remaining 10 PCGs have complete stop codons TAA and TAG. The RSCU values in the mt genomes of *P. robiniae* reflect a significant bias toward A and T nucleotides which is commonly found in other species of hymenopterans (Chen et al., 2016) (Table 5).

3.1.6. tRNA and rRNA genes

The 22 tRNA genes in the *P. robiniae* mt genome, which is 1418 bp long, ranged from 58 bp ($tRNA^{Ser(AGN)}$) to 69 bp ($tRNA^{Glu}$) in length. The A + T content and skew of the tRNAs were 89.4% and 0.0237,

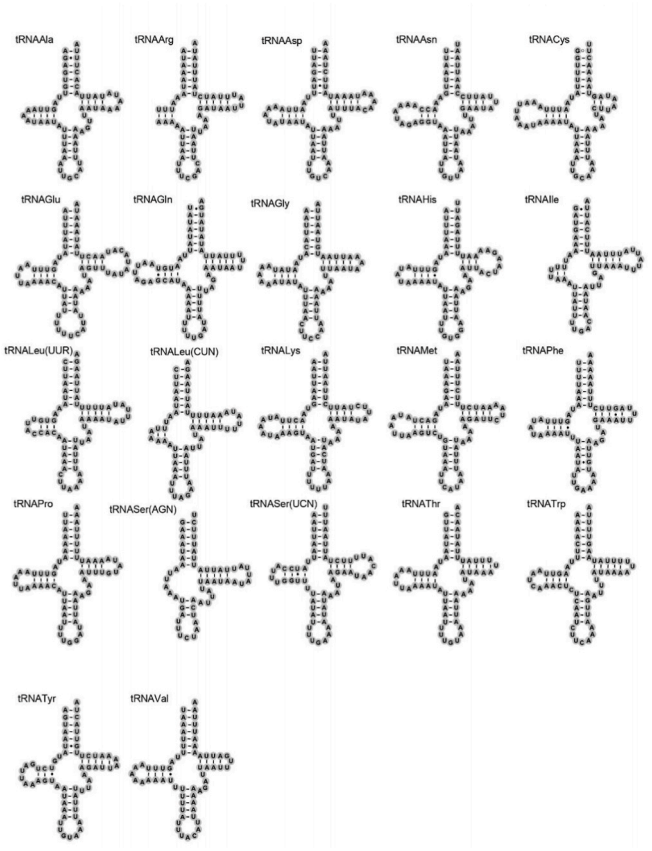

Fig. 3. The secondary structure of 22 tRNA in *Platygaster robiniae*.

respectively (Table 2). Most tRNA genes fold into a typical cloverleaf structure except $tRNA^{Arg}$ and $tRNA^{Ser\ (AGN)}$, and the two genes with D-stems are absent (Fig. 3). A missing D-stem for $tRNA^{Ser(AGN)}$ has been reported in many insect species (Castro et al., 2006; Sheffield et al., 2008; Yang et al., 2013), however in $tRNA^{Arg}$, missing D-stems have only been identified in Scelionidae (*Ceratobaeus* sp. and *Idris* sp.) (Mao and Dowton, 2014), which has been proposed as a shared derived character for the Scelionidae (Mao and Dowton, 2014). The missing D-stem in $tRNA^{Arg}$ from *P. robiniae* indicates the close evolutionary relationship with Scelionidae, and is probably shared by the superfamily Platygastroidea, (Mao et al., 2015; Tang et al., 2019). Additionally, D-loops of both $tRNA^{Ile}$ and $tRNA^{Leu(CUN)}$ are absent, this is rare genomics changes. To our knowledge, this has not been reported before in hymenopterans, consistent with the highly variable mt genomes of Hymenoptera (Chen and Du, 2016; Mao and Dowton, 2014).

Due to gene rearrangement, we verified rRNA gene boundaries by comparing sequences with other hymenopteran mt genomes. The *lrRNA* (1246 bp) is located between *ND1* and $tRNA^{Val}$, whereas the *srRNA* (737 bp) is located between $tRNA^{Val}$ and the control region. The AT content of these two genes was 88.5%, and AT skew was 0.0689 (Table 2).

3.1.7. Rate of mitochondrial gene evolution

The ratio of the nonsynonymous to synonymous substitutions (Ka/Ks), showed that *ND4*, *ND4L* and *ND5* were the most rapidly evolving, and *COI* and *CytB* the most conserved genes among the three subfamilies of Platygastroidea. Evolutionary rates of different protein-coding genes is usually different (Wei and Chen, 2011). Between subfamilies, *ND4L* in Platygastrinae and ND4 and ND5 in Telenominae evolved fastest and were subject to positive selection (Ka/Ks > 1), while the *COI* in Scelioninae was the most conserved (Fig. S1A). This result was similar to the evolution rate of *P. robiniae* mitochondrial protein-coding genes, and *ND4*, *ND4L* and *ND5* evolve the fastest, while *COI* is the most conserved (Fig. S1B), which is consistent with the characteristics of the insect mt genome (Wei, 2009). Among them, *ND4L* was positively selected (Ka/Ks > 1), while other genes were purified (Ka/Ks < 1). There were differences in the rate of mitochondrial gene evolution among the different groups. The evolution rate of hymenopteran Symphyta was similar to that of other holomorphic insects, while the evolution rate of hymenopteran Apocrita was 2–3 times as fast as that of Symphyta (Dowton et al., 2009a; Wei and Chen, 2011). The comparative analysis of the evolution rate of three subfamilies of Platygastroidea showed that the evolution rate of Telenominae was the fastest, while the evolution rate of Scelioninae was the slowest (Fig. S1A).

3.2. Genome rearrangement

Mitochondrial gene rearrangement was also found in *P. robiniae* with its specific style, as observed in most hymenopterans (Mao and Dowton, 2014; Mao et al., 2015; Tang et al., 2019). The protein-coding and rRNA genes are conserved in positions and orientations relative to the organization of the ancestral pancrustacean (Cook, 2005). However, the relative positions of tRNA genes are highly variable; each sequenced mt genome of hymenopterans has at least one tRNA translocated, and the *ND2-COI*, *COII-ATP8*, *ND3-ND5* and A + T-rich region-*ND2* junctions have been considered "hot spots'' for gene rearrangements in Hymenopter (Dowton, 1999; Dowton and Austin, 1999; Dowton et al., 2003). In *P. robiniae*, 10 tRNA genes are rearranged compared to their ancestral positions (Fig. 4), with rearrangement events mainly occurred at four junctions: *COII-ATP8*, *COIII-ND3*, *ND3-ND5* and A + T-rich region-*ND2*.

The rearrangement types of mitochondrial genes found in hymenopterans include gene shuffling, transposition and inversion (Dowton and Austin, 1999). *P. robiniae* has all three types of gene rearrangements. The genes $tRNA^{Ile}$, $tRNA^{Gln}$ and $tRNA^{Met}$ moved out of the A + T-rich region-*ND2* junction to between *srRNA* and the A + T-rich region. Additionally, $tRNA^{Met}$, $tRNA^{Ile}$ and $tRNA^{Gln}$ have translocated. $tRNA^{Ala}$, $tRNA^{Ser(AGN)}$ and $tRNA^{Glu}$ from between *ND3* and *ND5* to between *COIII* and *ND3* (Fig. 4). Additionally, $tRNA^{Lys}$ and $tRNA^{Asp}$ shuffled (switched) positions. These different types of rearrangements may occur in combination, and it is generally assumed that short-distance rearrangements are more frequent than long-distance rearrangements (Chen and Du, 2016; Mao and Dowton, 2014; Shen et al., 2019), and each of these three sets of rearrangements are short range (<1000 bp moves).

In addition, by comparing across all species of Platygastroidea (Fig. 4), we found that the *srRNA*-A + T-rich region is the commonest rearrangement location but that only in *P. robiniae* was the A + T-rich adjacent to *ND2*. In the other 11 species, there was one or more tRNA genes between the A + T-rich region and *ND2*. This may be due to the recombination, leading to the inversion of the $tRNA^{Ile}$ and $tRNA^{Met}$ genes, as well as the translocation adjacent exchange of $tRNA^{Met}$, $tRNA^{Ile}$ and $tRNA^{Gln}$ (Dowton and Campbell, 2001; Poulton et al., 1993). Furthermore, by comparing the mt genome sequences, we found some unique rearrangement characteristics in the mt genome of the family Platygastridae. The positions between *CytB-ND1* and *ND1-lrRNA* ($tRNA^{Ser(UCN)}$, $tRNA^{Leu(CUN)}$) were rearranged, but $tRNA^{Val}$ was not rearranged, which has not been discovered in other species of Proctotrupomorpha, validating that mitochondrial gene rearrangement in hymenopterans is highly diverse (Dowton et al., 2009b; Wei, 2009).

In addition, inversion is the least common type of rearrangement in insects, including local inversion and remote inversion. Remote inversion is caused by two rearrangements (Chen and Du, 2016; Wei, 2009), but it has been recorded in hymenopterans, and local inversion accounts for one-third of hymenopteran genome rearrangements (Dowton and Austin, 1999; Dowton et al., 2009b). In the *P. robiniae* mt genome, $tRNA^{Ser(UCN)}$ and $tRNA^{Leu(CUN)}$ have moved into the junction between *ND3-ND5* and *COI–COII*, and $tRNA^{Ser(UCN)}$ and $tRNA^{Leu(CUN)}$ are inverted simultaneously. This remote inversion of $tRNA^{Ser(UCN)}$ and $tRNA^{Leu(CUN)}$ was first reported in Platygastroidea. The $tRNA^{Ser(UCN)}$ inversion was found in only a few species in other superfamilies, but $tRNA^{Leu(CUN)}$

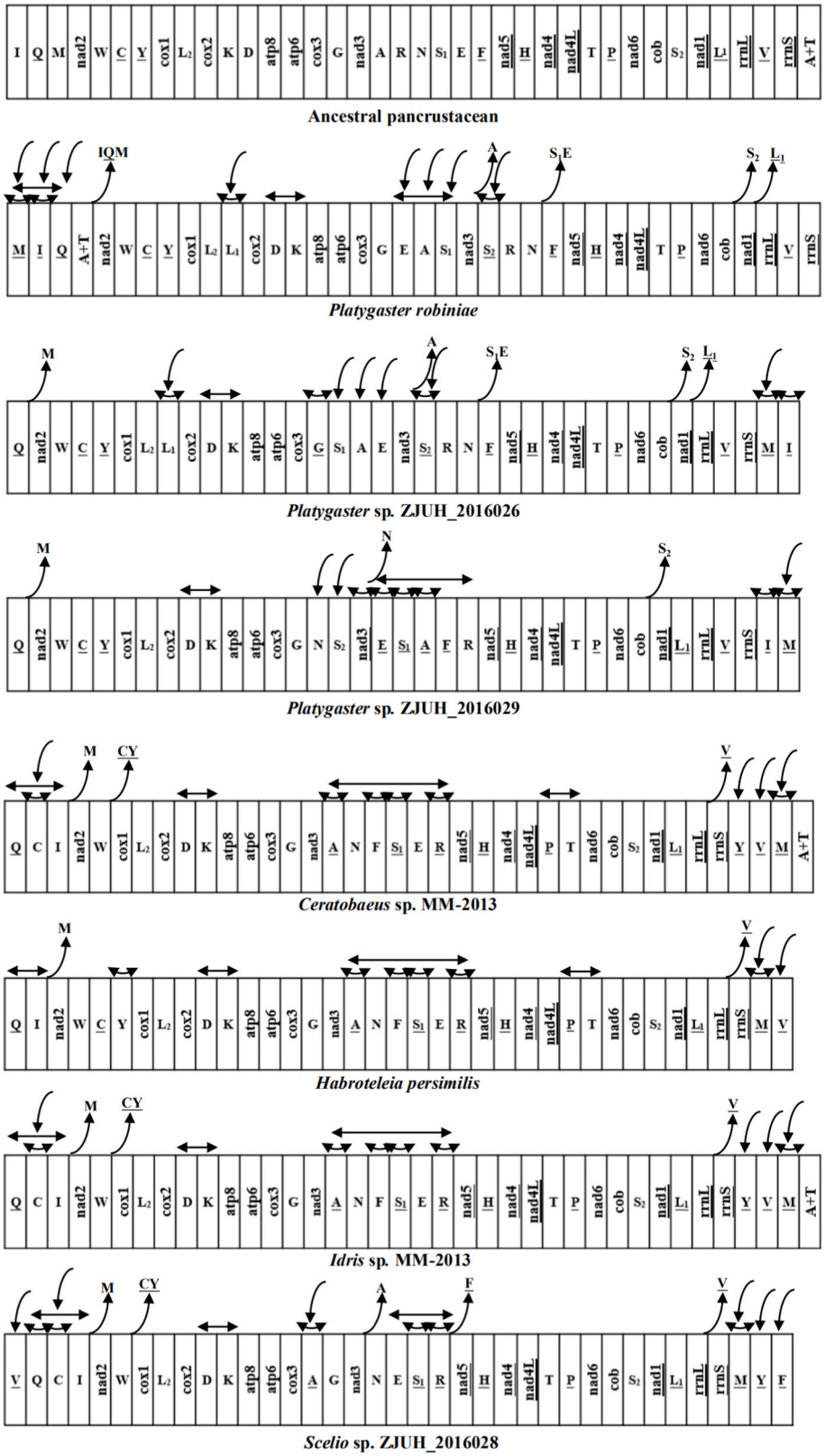

Fig. 4. Mitochondrial genome organization of *Platygaster robiniae* and 11 species of Platygastroidea, compared with the ancestral pancrustacean mt genome organization.

Note: tRNA genes are indicated by single letter amino acid codes, L1, L2, S1 and S2 denote $tRNA^{Leu(CUN)}$, $tRNA^{Leu(UUR)}$, $tRNA^{Ser(AGN)}$ and $tRNA^{Ser(UCN)}$, respectively. Genes are transcribed from left to right except those indicated by underlining. Gene movements, relative to the ancestral organization, are indicated with arrows.

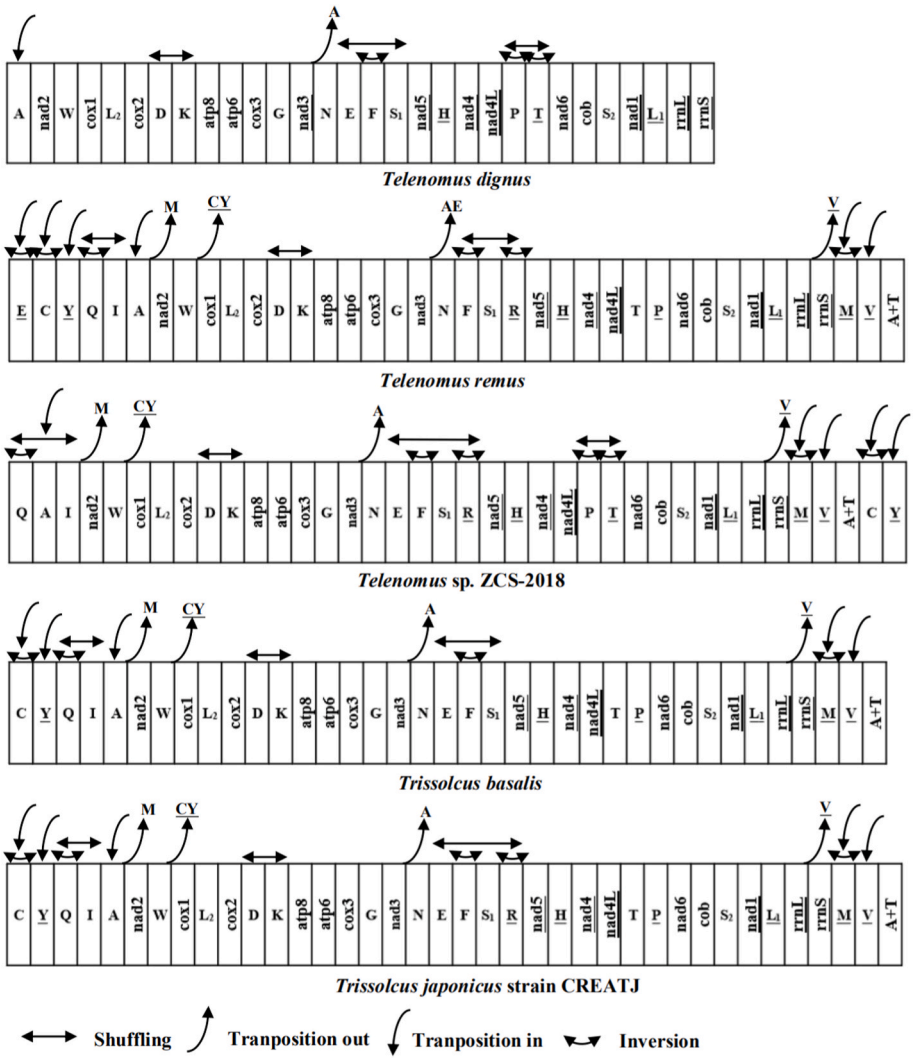

Fig. 4. (*continued*).

inversion did not occur, suggesting that $tRNA^{Leu(CUN)}$ inversion was probably the special rearrangement in Platygastroidea. For three species sequenced in Platygastridae (Table S1), $tRNA^{Ser(UCN)}$ and $tRNA^{Leu(CUN)}$ rearrangements were found in *P. robiniae* and *P.* sp. ZJUH_2016026 (Tang et al., 2019), while *P.* sp. ZJUH_2016029 only has a rearrangement of the $tRNA^{Ser(UCN)}$ gene. The rearrangement of $tRNA^{Ser(UCN)}$ may be due to the large gene space between *CytB* and *ND1*, which a rearrangement hotspot (Wei, 2009). Parasitic habits have been considered an inducing factor for rearrangement (Dowton et al., 2002; Shao et al., 2001); however, it was later found that the frequency of accelerated rearrangement in hymenopterans is not consistent with the evolution of parasitic habits.

Shared gene rearrangements are considered a valuable source for deducing phylogenetic relationships (Dowton et al., 2002), and some of the rearrangements seen here have been found in other hymenopteran linages. For example, the shuffling $tRNA^{Asp}$ and $tRNA^{Lys}$ reported here was identical to three known Scelionidae mt genomes (Mao and Dowton, 2014). One study found that unique rearrangements in hymenopterans are so common that only five of the 67 rearrangements identified are shared by two or more species, and only two of the five rearrangements are truly homologous (Dowton et al., 2009b). Our data compared 12 closely related species (Fig. 4), and all 12 species shared rearrangements of $tRNA^{Asp}$ and $tRNA^{Lys}$, suggesting that the shuffling of $tRNA^{Asp}$ and $tRNA^{Lys}$ is ancestral in the Platygastroidea.

3.3. Phylogenetic analyses

Phylogenetic analyses were performed using 50 mt genomes, 12 of which were from Platygastroidea (Table S1). *Diadegma semiclausum* (Ichneumonoidea: Ichneumonidae) was chosen as the outgroup. The topologies of the trees generated using the two phylogenetic approaches were identical with strong support at most nodes (posterior probabilities >95% and bootstrap values > 70%) (Hillis and Bull, 1993) (Fig. 5). In contrast to previous studies using hymenopteran mt genomes, the inclusion of third codon positions did not have much effect on the topology and nodal support in the current analysis, indicating that inclusion of third codon positions does not appear to be problematic when constructing the phylogeny of closely related taxa (Mao and Dowton, 2014). The three species of Platygastridae (*P. robiniae* and two species of *Platygaster* sp.) clustered together with strong support (pp = 1). Diaprioidea was supported as the sister group of Chalcidoidea, and this result was identical to that of previous analyses (Castro et al., 2006; Heraty et al., 2011; Mao et al., 2015). However, the placement of Cynipoidea, varied between previous analyses, suggesting Cynipoidea as a sister group to Proctotrupidae plus Diaprioidea plus Chalcidoidea (Heraty et al., 2011; Klopfstein et al., 2013; Tang et al., 2019; Vilhelmsen et al., 2010). Our results indicated that Cynipoidea is sister to Diaprioidea plus Chalcidoidea, in agreement with the results of Mao et al. (2015). Platygastroidea was well supported as monophyletic and sister to the remaining Proctotrupomorpha. Additionally, the relationship between Scelioninae

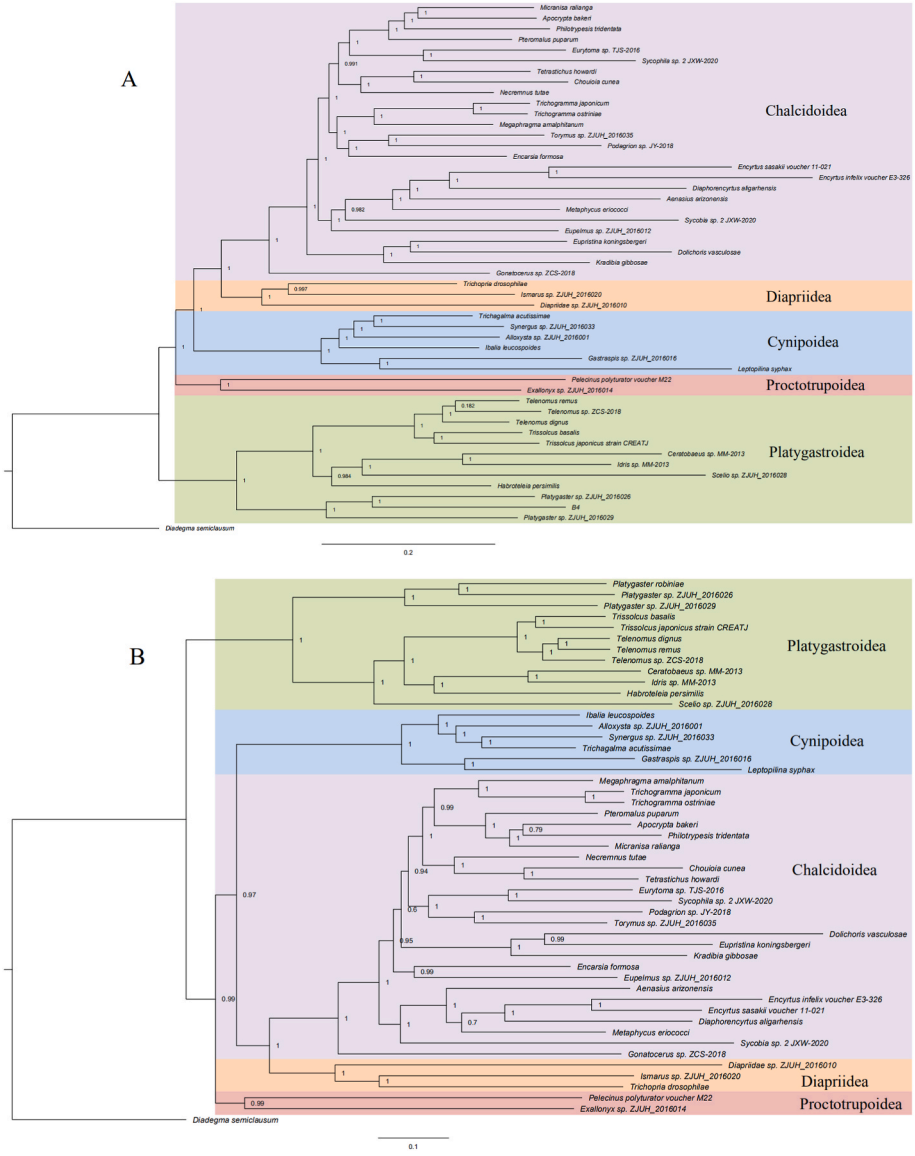

Fig. 5. Phylogenetic tree
Note: (A): Maximum likelihood (ML) phylogenetic tree inferred from the mitochondrial genome based on the 13 PCGs dataset; (B): Bayesian inference (BI) phylogenetic tree inferred from the mitochondrial genome based on the 13 PCGs dataset.

and Telenominae was shown to be obviously closer than that between Platygastrinae and Scelioninae.

As a tool for examining phylogenetics, mt gene order and tRNA secondary structures can resolve some contentious evolutionary questions (Boore and Brown, 1999; Dowton et al., 2002; Weigert et al., 2015). The gene rearrangements in Scelionidae and Platygastridae were largely different; in addition, each of the three species of *Platygaster* had different gene arrangements. This pattern likely occurs because hymenopterans exhibit a high frequency of gene rearrangement, with the gene order of each family significantly different from that of others (Chen and Du, 2016; Shen et al., 2019). Gene rearrangement events in insects contribute little to the study of phylogenetic relationships between insect orders but may be beneficial to the study of phylogenetic relationships among groups within insect orders (Cameron, 2014; Cameron et al., 2006b). In Platygastroidea, the absence of $tRNA^{Val}$ rearrangement and $tRNA^{Ser(UCN)}$ rearrangement are differences found between Scelionidea and Platygastridea, so we believe that this rearrangement feature can distinguish the two families (Fig. 4). Incomplete mitochondrial genomes of Hymenoptera can provide important sequence information for phylogenetic studies, but in comparison, complete mt genome sequences have more utility. Different branches may share the same or different rearrangements, and research provides more comprehensive information on system development (Chen and Du, 2016; Massimiliano et al., 2014; Shen et al., 2019).

Supplementary materials

Fig. S1: (A): Rate of evolution of three subfamilies of Platygastroidea; (B): Evolution rate of 13 protein-coding genes in the *Platygaster robiniae* mt genome. Table S1: GenBank accession numbers of published Proctotrupomorpha members and outgroup. Table S2: Comparison of mt genomes of BJ, YC, YT and SY samples. Table S3: 79 mitochondrial genomes and new sequence lengths of Proctotrupomorpha.

Author contributions

H.L.: Conceptualization, Methodology, Validation, Formal analysis, Data curation, Software, Visualization, Writing-original draft, Writing-

review & editing. H.Q.: Software, Visualization. C.J.: Resources. W.X.: Project administration, Funding acquisition. Y.X.: Conceptualization, Methodology, Investigation, Resources, Project administration, Funding acquisition, Writing-review & editing.

Declaration of competing interest

The authors have no conflicts of interests to declare.

Acknowledgments

We thank Mr Jun Yang (United Pesticide Industry Cropscience Co. Ltd., Jinan) for the help in samplings. This work was supported by the National Key Research and Development Program of China [2016YFC1201200].

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijppaw.2022.06.007.

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