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ORIGINAL ARTICLE

Mitochondrial gene rearrangement within genus *Gasteruption* (Hymenoptera: Evanioidea: Gasteruptiidae)

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Abstract The complete mitochondrial genome of the *Gasteruption parvicollarium* Enderlein (GenBank accession number: KR270643) was sequenced in the study. Totally 17 009 bp sequence was determined with an A+T content of 83.81%, including full set of typical animal mitochondrial genes. Two protein-coding and 10 tRNA genes as well as the A+T-rich region were rearranged compared with the putative ancestral arrangement of insects. Most of the rearranged genes were located in the ancestral region of *trnI-trnQ-trnM-nad2-trnW-trnC-trnY-cox1-trnL2*. The other rearranged genes are *trnN* and *trnS1* located in the tRNA cluster *trnA-trnR-trnN-trnS1-trnE-trnF* and *trnS2* located between *cob* and *nad1*. Remote inversion is dominant rearrangement event in *G. parvicollarium* mitochondrial genome, involving two protein-coding and 8 tRNA genes. Compared with the other mitochondrial genome reported in the same genus of *Gasteruption*, the inverted *trnN* was translocated to the tRNA cluster between *cox1* and *nad2* in *G. parvicollarium*. This is the first report of mitochondrial gene rearrangement occurred within genus of Hymenoptera. Our study points to a recently occurred gene rearrangement event in the *Gasteruption* species.

Key words Apocrita, wasp, mitochondrial genome, gene rearrangement, phylogeny.

1 Introduction

Animal mitochondrial genomes, characterized by material inherence (Barr *et al.*, 2005), rare recombination (Boore, 1999), stable gene content and proper size for sequencing (Wolstenholme, 1992; Boore, 1999; Curole & Kocher, 1999), are widely used in phylogenetics, comparative genomics, biodiversity and population genetics (Wei *et al.*, 2010b; Ma *et al.*, 2012; Cameron, 2014; Tang *et al.*, 2015). Besides gene sequences, pattern of gene arrangement has been frequently explored in mitochondrial genomes (Curole & Kocher, 1999; Dowton *et al.*, 2002). Gene arrangements are usually conserved within major lineages (Boore, 1999), but accelerated rate of rearrangement was identified in some groups (Cameron *et al.*, 2006; Cameron, 2014). Most of gene rearrangement events occurred in higher taxonomic levels, such as a common tRNA translocation in insects and crustaceans within arthropods (Boore *et al.*, 1998), extraordinary gene rearrangement in lice (Phthiraptera: Insecta) and tRNA shuffling after the divergence of Hepialoidea in Lepidoptera (Cao *et al.*, 2012). Closely related species usually share same gene arrangement pattern, even when extensive gene rearrangements occurred compared to their higher-level sister groups (Covacin *et al.*, 2006; Oliveira *et al.*, 2008; Korkmaz *et al.*, 2015).

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The Hymenoptera is one of the groups with accelerated gene rearrangement in the mitochondrial genomes. All species in Symphyta and many species in the other suborder Apocrita exhibit low level of tRAN gene rearrangement (Dowton & Austin, 1999; Dowton *et al.*, 2003; Wei *et al.*, 2015; Song *et al.*, 2016). However, several lineages sporadically distributed in the Apocrita showed extensive gene rearrangement involving both tRNA and protein-coding genes, such as the Chalcidoidea (Oliveira *et al.*, 2008; Xiao *et al.*, 2011; Nedoluzhko *et al.*, 2015), Bethylidae (Wei *et al.*, 2014), Megaspilidae (Mao *et al.*, 2014b) and *Cotesia vestalis* (Braconidae: Microgastrinae) (Wei *et al.*, 2010a). Understanding gene arrangement patterns within those rearranged groups might provide potential signals for phylogenetic inference (Mao *et al.*, 2014a; Wei *et al.*, 2014).

In this study, we sequenced the complete mitochondrial genome of *Gasteruption parvicollarium* Enderlein (Hymenoptera: Evanioidea: Gasteruptiidae) and report a genus-level mitochondrial gene rearrangement in the *Gasteruption*.

2 Materials and methods

2.1 Specimens and DNA extraction

An adult female of *G. parvicollarium* collected from China was used for DNA extraction. The species was identified according to a key to species of the genus *Gasteruption* Latreille from China (Zhao *et al.*, 2012) and confirmed by C. van Achterberg. Total genomic DNA was extracted using the DNeasy tissue kit (Qiagen, Hilden, Germany) following the manufacturer's protocols, from the single adult. The remnant voucher specimens (Code: GP01) was kept in the Integrated Pest Management Laboratory, Beijing Academy of Agriculture and Forestry Sciences.

2.2 PCR amplification and sequencing

We used a PCR-based method to sequence the mitochondrial genome of *G. parvicollarium*. Initially, universal primers modified from Simon *et al.* (2006) for Hymenoptera was used to amplify and sequence partial genomic sequences. Next, species-specific primers were designed from sequenced regions to bridge the gaps. Totally eight fragments of 1857–3585 base pairs (bp) were amplified, covering the entire mitochondrial genome. Polymerase chain reactions (PCRs) were done using Takara LA *Taq* (Takara Biomedical, Japan) under the following conditions: initial denaturation for 2 min at 94°C followed by 35 cycles of 10 s at 96°C, 15 s at 45–55°C, and 1–4 min at 60°C and a subsequent final extension for 8 min at 60°C. PCR components were added as recommended by Takara LA *Taq*, the manufacturer. All the PCR products were sequenced from both strands by TSINGKE Company (Beijing, China) using primer walking strategy.

2.3 Genome annotation and analysis

The sequenced segments were assembled into a single contig. One of the overlapped boundary was identified by alignment using ClustalW version 2.0 (Larkin *et al.*, 2007) and then removed manually. The entire mitochondrial genome sequence was then deposited into tRNAscan-SE search server (Lowe & Eddy, 1997) for transfer RNA (tRNA) identification, setting the parameters so that the source was Mito/Chloromast, and the genetic code was the Invertebrate Mito genetic code. Protein-coding genes and ribosomal RNA (rRNA) genes were identified from the large intergenetic sequences between tRNA genes using BLAST searches in GenBank, and subsequently by alignment with genes of other hymenopteran insects for the initiation and termination codons. The base composition and Relative Synonymous Codon Usage (RSCU) of all protein-coding genes was calculated by MEGA5 (Tamura *et al.*, 2011).

3 Results and discussion

3.1 General features of the *G. parvicollarium* mitochondrial genome

The complete mitochondrial genome of *G. parvicollarium* is 17 009 bp long, including full set of typical animal mitochondrial genes (GenBank accession number: KR270643) (Table 1). The A+T content of the entire genome is relative high with a value of 83.81%, typical to most sequenced ones from Apocrita of Hymenoptera (Wei *et al.*, 2009, 2010a; Mao

et al., 2012; Wu *et al.*, 2014). Nine pairs of genes are directly adjacent without intergenetic or overlapping nucleotides. There are totally 1 959 bp intergenic nucleotides in 25 locations, including two large regions locating between *cox1* and *trnL2* (924 bp), *trnC* and *trnY* (398 bp). Two pairs of genes, i.e. *atp8* and *atp6*, *trnA* and *trnR*, overlapd each other with a length of 7 and 3 bp, respectively, both of which are in their ancestral arrangement pattern. High number of intergenetic regions might be related to frequent gene rearrangement (Wei *et al.*, 2009).

Table 1. Annotation of the *Gasteruption parvicollarium* mitochondrial genome.

Gene	Strand	Position	Length (bp)	Anti/Start codon	Stop codon	Intergenic nucleotides (bp)
trnY	+	1-73	73	GTA	—	1
trnQ	-	75-144	70	TTG	—	-12
cox2	+	198-872	675	ATA	TAA	45
trnK	+	918-1000	83	TTT	—	8
trnD	+	1009-1081	73	GTC	—	0
atp8	+	1082-1252	171	ATC	TAA	-7
atp6	+	1246-1923	678	ATG	TAA	19
cox3	+	1943-2728	786	ATG	TAA	24
trnG	+	2753-2824	72	TCC	—	0
nad3	+	2825-3178	354	ATT	TAA	16
trnA	+	3253-3321	69	TGC	—	-3
trnR	+	3319-3397	79	TCG	—	43
trnS1	-	3441-3509	69	TCT	—	13
trnE	+	3523-3593	71	TTC	—	5
trnF	-	3599-3666	68	GAA	—	21
nad5	-	3688-5400	1713	ATA	TAA	0
trnH	-	5401-5471	71	GTG	—	37
nad4	-	5509-6881	1373	ATT	TA	6
nad4l	-	6888-7169	282	ATT	TAA	1
trnT	+	7171-7240	70	TGT	—	0
trnP	-	7241-7313	73	TGG	—	82
nad6	+	7396-7971	576	ATA	TAA	9
cob	+	7981-9150	1170	ATT	TAA	0
trnM	-	9151-9221	71	CAT	—	18
nad1	-	9240-10193	954	ATT	TAA	22
trnL1	-	10216-10284	69	TAG	—	-2
rrnL	-	10283-11694	1412	—	—	0
trnV	-	11695-11761	67	TAC	—	0
rrnS	-	11762-12669	908	—	—	0
cox1	-	12670-14223	1554	ATT	TAA	924
trnL2	-	15148-15221	74	TAA	—	35
trnN	-	15257-15325	69	GTT	—	25
trnW	-	15351-15421	71	TCA	—	41
nad2	-	15463-16497	1035	ATA	TAA	38
trnS2	-	16536-16604	69	TGA	—	9
trnI	-	16614-16682	69	GAT	—	8
trnC	-	16691-16761	71	GCA	—	0
AT*		16605-17002	398	—	—	0

*AT—A+T-rich region.

3.2 Protein-coding, tRNA and rRNA genes

In protein-coding genes, the lowest A+T content is 77% in *cox1*, while the highest is 89% in *atp8* (Table 2). All protein-coding genes start with ATN codons (4 with ATA, 1 with ATC, 2 with ATG and 6 with ATT). Twelve protein-coding genes stop with termination codon TAA, while one uses incomplete stop codon TA (Table 1). The incomplete stop codon was commonly reported in other invertebrates (Masta & Boore, 2004). The RSCU reflects a biased usage of A and T nucleotides in the genome (Table 3). UUA(Leu), UUU(Phe), AUU(Ile) were the most frequently used codons as in other insects. The protein-coding genes coded on the majority strand show more C than G and genes coded on

the minority strand show less C than G (Table 2). This is congruent with the observation of skew values in insect mitochondrial genomes (Wei *et al.*, 2010b).

Table 2. Base composition of protein-coding and rRNA genes in the *Gasteruption parvicollarium*. *

Gene	T%	C%	A%	G%	(A+T) %	AT skew	GC skew
nad2	52.6	4.0	36.1	7.3	88.7	-0.1860	0.2920
cox1	42.9	10.6	31.1	15.4	74.1	-0.1595	0.1846
cox2	39.0	12.9	39.6	8.6	78.5	0.0076	-0.2000
atp8	39.8	8.8	49.7	1.8	89.5	0.1106	-0.6604
atp6	43.2	13.0	36.9	6.9	80.1	-0.0787	-0.3065
cox3	42.1	13.9	35.1	8.9	77.2	-0.0907	-0.2193
nad3	40.4	12.4	41.0	6.2	81.4	0.0074	-0.3333
nad5	50.3	4.4	34.0	11.3	84.2	-0.1934	0.4395
nad4	50.7	4.9	32.2	12.2	82.9	-0.2232	0.4269
nad4l	52.8	1.4	33.0	12.8	85.8	-0.2308	0.8028
nad6	45.8	11.6	39.4	3.1	85.2	-0.0751	-0.5782
cob	41.5	13.5	36.8	8.2	78.3	-0.0600	-0.2442
nad1	48.3	6.4	32.4	12.9	80.7	-0.1970	0.3368
rrnL	41.5	4.5	44.5	9.4	86.0	0.0349	0.3525
rrnS	41.9	4.6	42.1	11.3	84	0.0024	0.4214

*Base compositions were calculated based on the sense strand of each gene.

All of the 22 tRNA genes, ranging from 67 to 83 bp, have a typical cloverleaf structure predicted in tRNAscan-SE search server. The *rrnL* is 1410 bp long with an A+T content of 86% while the *rrnS* is 908 bp longh with an A+T content of 84% (Table 2).

Table 3. Codon usage in the *Gasteruption parvicollarium* mitochondrial genome.

AA	Codon	No.	RSCU	AA	Codon	No.	RSCU	AA	Codon	No.	RSCU
Phe	UUU	385	1.83	Gly	GGU	45	1.32	Tyr	UAU	207	1.74
	UUC	35	0.17		GGC	1	0.03		UAC	31	0.26
Leu	UUA	442	4.46		GGA	66	1.94	Trp	UGA	64	1.66
	UUG	56	0.56		GGG	24	0.71		UGG	13	0.34
	CUU	38	0.38	Pro	CCU	53	2.41	His	CAU	70	1.73
	CUC	8	0.08		CCC	11	0.5		CAC	11	0.27
	CUA	40	0.4		CCA	24	1.09	Gln	CAA	60	1.64
	CUG	11	0.11	Cys	UGU	32	2		CAG	13	0.36
Ile	AUU	358	1.86	Thr	ACU	46	1.72	Asn	AAU	195	1.86
	AUC	26	0.14		ACC	10	0.37		AAC	15	0.14
Ser	AGU	27	0.73		ACA	50	1.87	Lys	AAA	130	1.7
	AGC	3	0.08		ACG	1	0.04		AAG	23	0.3
	AGA	59	1.61	Ala	GCU	32	2.61	Asp	GAU	76	1.92
	AGG	11	0.3		GCC	4	0.33		GAC	3	0.08
	UCU	85	2.31		GCA	11	0.9	Glu	GAA	79	1.7
	UCC	9	0.24		GCG	2	0.16		GAG	14	0.3
	UCA	98	2.67	Val	GUU	73	1.99	Arg	CGU	12	1.55
	UCG	2	0.05		GUC	5	0.14		CGC	1	0.13
Met	AUA	297	1.68		GUA	55	1.5		CGA	17	2.19
	AUG	57	0.32		GUG	14	0.38		CGG	1	0.13

3.3 A+T-rich region

The A+T-rich region is believed to be involved in the regulation of transcription and control of DNA replication, characterized by five elements: (1). a polyT stretch at the 5'end of the A+T-rich region; (2). a [TA(A)]n-like stretch following the polyT stretch; (3). the second strand-replication origin; (4). a TATA motif and a G (A)nT motif flanking the stem and loop structure and (5). a G+A rich sequence downstream of the stem and loop structure a stem and loop structure

(Zhang & Hewitt, 1997). This region is usually located between the *rrnS* and tRNA cluster *trnI-trnQ-trnM* with varied length among species (Zhang & Hewitt, 1997). However, rearrangement and duplication of this region has been reported (Wei *et al.*, 2010a, b). In the mitochondrial genome of G. *parvicollarium*, two large nocoding regions between *cox1* and *trnL2*, *trnC* and *trnY* are candidates of A+T-rich region. We predicted that the short one between *trnC* and *trnY* as the A+T-rich region, because of the high A+T content (94.47%) and presence of repeat element (TAATATAATTTATAATATA ATTTA) at downstream.

In the mitochondrial genome of *Gasteruption* sp., the A+T-rich region was assigned to a region between *cox1* and *trnL2* (Mao *et al.*, 2014a), which is longer (1033 bp) than the region between *trnC* and *trnY* (190 bp), but lower in A+T content (94.47% vs 89.50%). However, repeat sequences were present in both regions in this genome. Validation of the A+T-rich region is necessary by biological experiments.

3.4 Gene rearrangement

Totally two protein-coding and 10 tRNA genes and the A+T-rich region were rearranged in the mitochondrial genome of *G. parvicollarium*, compared with the putative ancestral arrangement of insects (Fig. 1). Most of the rearranged genes were located in the ancestral region of *trnI-trnQ-trnM-nad2-trnW-trnC-trnY-cox1-trnL2*. Other rearranged genes are *trnN* and *trnS1* located in the tRNA cluster *trnA-trnR-trnN-trnS1-trnE-trnF* and *trnS2* located between *cob* and *nad1*. All of those regions are rearrangement hot spots in the mitochondrial genomes of Hymenoptera (Dowton& Austin, 1999; Dowton *et al.*, 2003; Wei *et al.*, 2014) except for *cox1-trnL2* and *trnS2* (Oliveira *et al.*, 2008; Xiao *et al.*, 2011). Compared with the extensively rearranged mitochondrial genomes in species of Chalcidoidea (Oliveira *et al.*, 2008; Xiao *et al.*, 2011), Bethylidae (Wei *et al.*, 2014), Megaspilidae (Mao *et al.*, 2014b) and *Cotesia vestalis* (Braconidae: Microgastrinae) (Wei *et al.*, 2010a), and the less rearranged ones in species of Symphyta, the gene order in the mitochondrial genome of *G.* parvicollarium was intermediately rearranged, as reported in other species of Evaniomorpha (Mao *et al.*, 2014a; Wu *et al.*, 2014).

Gene rearrangement event could be classified into translocations, local inversions (inverted in the local position), gene shuffling (local translocation) and remote inversions (translocated and inverted) (Dowton *et al.*, 2003). Local inversion has been reported to be a major type of gene rearrangement in Hymenoptera (Dowton & Austin, 1999). However, we found that remote inversion is dominant in the *G. parvicollarium*, involving all of the two protein-coding and 8 of 10 tRNA genes that were rearranged. Among the rearranged genes, *nad2* and *trnW* might be remotely inverted simultaneously for parsimony. Translocation event occurred in two tRNA genes of *trnQ* and *trnC* as well as the predicted A+T-rich region.

Compared with the other mitochondrial genome reported in the same genus of *Gasteruption* (Mao *et al.*, 2014a), the inverted *trnN* was translocated to the tRNA cluster between *cox1* and *nad2* in *G. parvicollarium* (Fig. 1). This is the first report of mitochondrial gene rearrangement occurred within genus in Hymenoptera. Our study points to a recent gene rearrangement event in the *Gasteruption*.

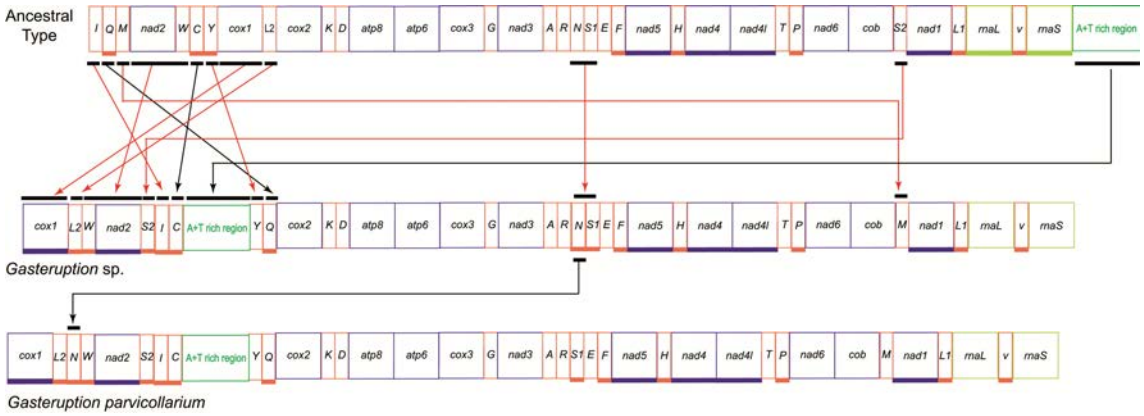

Figure 1. Gene rearrangement in the *Gasteruption* spp. mitochondrial genomes. The red line shows reversal of gene. The black line shows translocation of gene. Translocation of *trnN* occurred between *Gasteruption* sp. and *G. parvicollarium*.

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