The Complete Mitochondrial Genome of *Parasitus Fimetorum* (Parasitiformes: Parasitidae) and Phylogenetic Relationship of Parasitiformes

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Abstract

Background

The family Parasitidae (Arachnida: Acari) includes 426 species. Up to now, the complete mitochondrial genomes of only one Parasitidae species (*Parasitus wangdunqingi*) has been sequenced, and it was discovered that the mitochondrial genome of this species have retained the ancestral mitochondrial genome organization of arthropods. The scarcity of available sequence data has greatly impeded evolutionary studies in Acari (mites and ticks). Here, we report the complete mitochondrial genome of *Parasitus fimetorum* (Family Parasitidae) and provide a comparison to *Parasitus wangdunqingi*.

Methods

We use a PCR-based strategy to amplify the mitochondrial genome of *Parasitus fimetorum*, and these amplicons are sequenced using the Illumina Hiseq X-10 platform. Phylogeny of Parasitiformes mites based on *cox1* gene of 51 species using maximum likelihood (ML) methods.

Result

The complete mt genome of *P. fimetorum* is a typical circular, double-stranded DNA molecule 14,619 bp in size that contains 13 protein-coding genes, 22 transfer RNA genes, 2 ribosomal RNA genes, and 1 control region. The mitochondrial gene order of *P. fimetorum* was similar to that of the arthropods hypothesized ancestor. Mitochondrial genomes of *P. fimetorum* and *P. wangdunqingi* were compared and analyzed with the following characteristics: (1) both parasitus species evolved independently rather than synergically; (2) the preference of the two mites for codons is different, which may be due to the mutation pressure of certain mites. Phylogenetic analyses based on sequences of the *cox1* genes showed that Parasitidae was monophyletic.

Conclusion

The mitochondrial genomes of Parasitidae mites likely remained the ancestral mt genome organization of arthropods and phylogeny of Parasitiformes mites based on *cox1* gene sequences indicates that different lineages are basically monophyletic, and strong supports the current morphological taxonomy. Among others, this new resource will provided thoroughly and systematically information for further study on the mitogenomes, phylogenetics and taxonomic revision of the mites.

Background

Parasitidae belong to Acari, Arachnida, Parasitiformes [1]. Literary statistics have shown that 426 species have been registered with 35 genera in the world [2]. Parasitidae mites are common and widely distributed predators in soil ecosystems, prey on mites, eggs and other small arthropods and nematodes in the untimely stage of the soil, most of them live freely or with insects, on rotting hay, composts and feces, and are less parasitic on the surface of mammals [3–5]. Some of the Parasitidae mites are significant for soil vitality, aeration and water retention, decomposition of plant materials, control of microbial biomass and nutrient cycling; other species of the parasitidae mites are important groups of natural enemies for biological control. For instance, gamasid mites can prey on nematodes and bark beetle mites in tunnels, *Parasitus coleoptratorum* can prey on housefly larvae, *Parasitus fimetorum* can prey on edible fungus pests, but a few parasitic mites can cause harm to crops, such as *Parasitus beta*, which can reduce the yield of tobacco seedlings [6–8].

Extensive extranuclear DNA exists in mitochondrial genomes, which exhibits a number of remarkable traits, such like matrilineal inheritance, strong conservation, minimal recombination, rapid gene evolution, among others. These traits distinguish them from other closely comparable prokaryote genomes as well as from eukaryotic genomes [9–11]. Most animal mitochondrial genomes have 14–18kb sized closed twin-stranded loop molecules with 37 genes: two ribosomal RNA (*rRNA*) genes, *rns* and *rnl*, have slow evolution rates and are highly conserved genes; 22 transfer RNA (*tRNA*) genes, are relatively conservative, with a slow evolution rate and low base substitution rate in mtDNA, but the functional regions of tRNA genes are different and conserved, and the

regions with relatively high variation are T arm and TΨC loop; 13 protein-coding genes (PCGs), which protein products are very important for the process of mitochondrial oxidative phosphorylation, and a non-coding region that is mainly thought to play a role in the initiation of DNA transcription or replication (or both) [11–15].

The emergence and maturation of high-throughput sequencing technology in recent years has opened up exciting possibilities for pedigree phylogenetic analysis of lineages that have previously been difficult to study using conventional molecular and morphological approaches. As a consequence, more mitochondrial genomes of arthropods have been sequenced. To date, the mitochondrial genomes of 27 species of Acariformes and 50 species of Parasitiformes have been investigated at home and abroad, and it has been found that the mitochondrial genomes of all species of Acariformes and some species of Parasitiformes have undergone a drastic rearrangement with the mitochondrial genomes of arthropod hypothetical ancestor. A tough task is to appropriately identify the mitochondrial genome due to the presence of gene deletions and rearrangements, and also the existence of contrary base composition, atypical tRNA secondary structure, and a short rRNA sequence [16]. So far, only one complete mitochondrial genome of parasitidae mites (*Parasitus wangdunqingi*) and the partial mt gene of other species have been submitted to GenBank. The scarcity of available sequence data has greatly impeded evolutionary studies in Acari (mites and ticks). Here, we sequenced the complete mitochondrial genome of *Parasitus fimetorum* (Parasitiformes: Parasitidae) collected from *Apodemus chevrieri*. We use the 5' terminal sequence of *cox1* gene to analyse Phylogeny of Parasitiformes mites, which offers integrated scientific data from genetic aspects for the classification and study of the mitochondrial genome of Parasitiformes mites in the future.

Materials And Methods

Collection of mites

P. fimetorum were collected from *Apodemus chevrieri* in Lijiang City, Yunnan Province, China in July, 2019. Mite samples were either used immediately for DNA extraction or were preserved in 100% ethanol at – 20°C prior to DNA extraction. Samples of *P. fimetorum* were also mounted to slides as vouchers, using Hoyer's medium for morphological check with a Zeiss A2 (microphoto camera AxioCam MRc) microscope. The collected specimens and vouchers were deposited at the Institute of Pathogens and Vectors, Dali University, China. Capture of *Apodemus chevrieri* was approved by health authorities in Yunnan Province, China. Animal capture protocols and procedures were approved by the animal ethics committees at Dali University.

DNA extraction, mt genome amplification and sequencing

Genomic DNA was extracted from individual mite with DNeasy Blood and Tissue Kit (QIAGEN), following the modified protocol [17]. A 330-bp fragment of *rns* were initially amplified by polymerase chain reaction (PCR) with the primer pairs 12SA and 12SB (see Additional file 1). The pairs of primer target conserved gene sequence motifs that are highly conserved among arthropods. PCR products were directly purified and were sequenced directly using Sanger method at the Thermo Fisher Scientific Genome Sequencing Facility (Guangzhou). Specific primer for *P. fimetorum*, m12SF-m12SR, were designed from *rns* sequence obtained (see Additional file 1). PCR with the specific primer produced about 15 kb amplicon, which were sequenced from both ends (i.e. paired-end) with Illumina Miseq platform: insert size 400 bp, read length 250 bp.

Takara Ex Taq was used in the initial short PCRs with the following cycling conditions: 94°C for 3 min; 37 cycles of 94°C for 1 min, 54°C for 1 min, 72°C for 1 min; and a final extension of 72°C for 10 min. Takara LA Taq was used in the long PCRs with the cycling conditions: 96°C for 1 min; 40 cycles of 96°C for 10 sec, 56-60°C for 40 sec, 68°C for 9 min; and a final extension of 68°C for 15 min. Positive and negative controls were executed with each PCR experiment. PCR products were checked by agarose gel(1%) electrophoresis; the sizes of PCR amplicons were estimated by comparing with DNA markers. PCR products were purified with Wizard SV Gel/PCR clean-up system (Promega). Illumina sequencing was done at the Majorbio Genome Sequencing Facility (Shanghai).

Assembly of Illumina sequence reads and gene identification

Illumina sequence reads obtained (250 bp each) were assembled into contigs with Geneious 11.1.5 [18]. The assembled parameters were as follows: minimum overlap 150 bp, maximum mismatches per read 3%, minimum overlap identity 98%. The

tRNA genes were identified using tRNAscan-SE [19] and ARWEN [20] or verified by comparison of secondary structures and conserved nucleotide sequences with those of Acari species reported in published literature. Protein-coding genes (PCGs) and were identified by open reading frame (ORF) searches in Geneious using the invertebrate mt genetic code and then by BLAST searches conducted in NCBI [21]. The two rRNA genes, *rrnS* and *rrnL*, were also identified by BLAST searches of GenBank based on sequence similarity and conserved sequence motifs. The 5' and 3' ends of *rrnS* and *rrnL* cannot be determined with accuracy. We tentatively annotated the 5' end of these genes to be immediately after the 3' end of the upstream gene, and the 3' end of these genes to be immediately before the downstream gene, leaving no gaps in between. The nucleotide sequences of mt genomes of *P. fimetorum* have been deposited in GenBank under accession numbers OK572962. Codon usage and Relative Synonymous Codon Usage (RSCU) values were analyzed with MEGA-X [22] and codon W1.4.

Phylogenetic analysis

Cox1 gene is used for phylogenetic analysis of mitochondrial genome sequence because it contains enough information for phylogenetic comparison [23]. The MEGA-X software was used and the maximum likelihood method was used for Bootstrap analysis. The number of bootstrap replications was 1000. The nucleotide substitution model was JTT+F+G. Two published *Carcinoscorpius rotundicauda* and *Limulus polyphemus* were selected as outgroups in phylogenetic analysis.

Result And Discussion

General features of *P. fimetorum* mitogenomes

The circular mitochondrial genome of *P. fimetorum* (GenBank accession OK572962) is 14,619 base pairs in size, and contains 37 genes, which include 13 protein-coding genes (*nad1-nad6*, *nad4L*, *cox1-cox3*, *cob*, *atp6*, *atp8*), 2 rRNA genes (*rrnS* and *rrnL*), and 22 tRNA genes (Fig. 1; Table 1); like the reported mitochondrial genomes of other arthropods. The base composition of this chain is adenine 35.6%, thymine 34.8%, cytosine 18.2%, guanine 11.4%. The overall AT-content of the mt genome of *P. fimetorum* is 70.4% was near to the average AT-content of Acari mitochondrial genomes (75.34±4.78%) [24]. Of those, 22 genes, comprising 9 PCG (*cox1-3*, *atp6*, *atp8*, *nad2*, *nad3*, *nad6*, and *cob*) and 13 of the tRNA genes (*trnA*, *trnD*, *trnE*, *trnG*, *trnI*, *trnK*, *trnM*, *trnN*, *trnR*, *trnS*₁, *trnS*₂, *trnT* and *trnW*) were coded on the majority stand (J-stand). The other 15 genes were on the minority strand (N-stand). The mitochondrial genome of *P. fimetorum* is the second sequenced species of Parasitidae, and it shares the same genome structure and content as *P. wangdunqingi*; no gene rearrangement occurs. Gene orders of the mitochondrial genome for two species of mites (*P. fimetorum* and *P. wangdunqingi*) are identical to those of arthropods hypothetical ancestor, but diverge from those of other Parasitiformes species. While the degree of mitochondrial genome rearrangement in Parasitiformes mites is already quite high in comparison to other metazoans, the degree of mitochondrial gene rearrangement in Acariformes mites is even greater: all 27 Acariformes species mitochondrial genomes measured in previous studies exhibit a high degree of rearrangement. Generally speaking, gene rearrangement occurs seldom in the mitochondrial genomes of the majority of arthropod groupings, and gene content and arrangement are conservative at the lower classification levels (families and genera), even though there are significant changes at the higher classification levels (phylum) [27]. So far, only 23 species of Parasitiformes think that their mitochondrial gene sequence is the same as the arthropods hypothetical ancestor arrangement pattern, of 50 species of mitochondrial genomes that have been measured. These species belong to 6 families: 11 species are located in the Argasidae [25–26], 1 species is seen in the Allothyridae [25], 1 species is placed in the Nuttalliellidae [27], 3 species are known in the Diplogyniidae [28], 1 species are found in the Parasitidae [28], and the remaining 6 species are contained in the Ixodidae [27]. In addition, 27 species from 7 families show varying degrees of rearrangement. There seem to be 16 species of Ixodidae [29], 1 species of Varroidae [30], 3 species of Phytoseiidae [28], with the rate of rearrangement of the mitochondrial genome being the highest studied, 2 species of Laelapidae [28], 3 species of Macrochelidae [28], 1 species of Blattisociidae [28], and 1 species of Ologamasidae [28].

Table 1
List of annotated mitochondrial genome of *Parasitus fimetorum*

Genes	Strand	Size	Intergenic length	Start codon	Stop codon	Anticodon
<i>cox1</i>	J	1500	3	ATT	TAA	
<i>cox2</i>	J	679	0	ATG	T	
<i>trnK</i>	J	68	2			CUU
<i>trnD</i>	J	62	0			GUC
<i>atp8</i>	J	162	-4	ATT	TAA	
<i>atp6</i>	J	666	3	ATA	TAA	
<i>cox3</i>	J	778	0	ATG	T	
<i>trnG</i>	J	62	0			UCC
<i>nad3</i>	J	339	-2	ATT	TAG	
<i>trnA</i>	J	63	0			UGC
<i>trnR</i>	J	61	3			UCG
<i>trnN</i>	J	67	-1			GUU
<i>trnS₁</i>	J	59	2			GCU
<i>trnE</i>	J	65	-2			UUC
<i>trnF</i>	N	63	0			GAA
<i>nad5</i>	N	1681	0	GTG	T	
<i>trnH</i>	N	62	0			GUG
<i>nad4</i>	N	1302	37	ATA	TAA	
<i>nad4L</i>	N	276	17	ATG	TAA	
<i>trnT</i>	J	62	-1			UGU
<i>trnP</i>	N	64	1			UGG
<i>nad6</i>	J	435	-1	ATT	TAA	
<i>cob</i>	J	1114	0	ATG	T	
<i>trnS₂</i>	J	62	22			UGA
<i>nad1</i>	N	909	0	GTG	TAA	
<i>trnL₂</i>	N	62	1			UAA
<i>trnL₁</i>	N	64	0			UAG
<i>rrnL</i>	N	1194	0			
<i>trnV</i>	N	63	0			UAC
<i>rrnS</i>	N	707	0			
<i>NCR#2</i>		406	0			
<i>trnI</i>	J	64	-3			GAU
<i>trnQ</i>	N	65	20			UUG

Genes	Strand	Size	Intergenic length	Start codon	Stop codon	Anticodon
<i>trnM</i>	J	64	0			CAU
<i>nad2</i>	J	963	-2	ATT	TAA	
<i>trnW</i>	J	63	-8			UCA
<i>trnC</i>	N	62	0			GCA
<i>trnY</i>	N	63	31			GUA

Table 2
The range of intergenic and overlapping regions of 51 species

Order	Family	Species	Accession number	Interval range	Overlap range	Control region
Holothyrida	Allothyridae	<i>Allothyrus sp.</i>	KC769586	1-20	1-8	348
Ixodida	Argasidae	<i>Antricola mexicanus</i>	KC769591	1-15	1-8	335
		<i>Argas africanus</i>	JQ665720	1-8	1-64	343
		<i>Argas lagenoplastis</i>	KC769587	1-20	1-29	343
		<i>Argas miniatus</i>	KC769590	1-18	1-9	346
		<i>Argas sp.</i>	KC769588	1-18	1-8	344
		<i>Carios capensis</i>	AB075953	1-7	1-57	342
		<i>Ornithodoros brasiliensis</i>	KC769593	1-22	1-9	343
		<i>Ornithodoros moubata</i>	AB073679	1-8	1-47	342
		<i>Ornithodoros porcinus</i>	AB105451	1-8	1-100	338
		<i>Ornithodoros rostratus</i>	KC769592	1-15	1-8	340
		<i>Otobius megnini</i>	KC769589	1-15	1-8	340
	Ixodidae	<i>Amblyomma cajennense</i>	JX573118	1-16	1-24	304 304
		<i>Robertsia elaphensi</i>	JN863729	1-14	1-15	308 286
		<i>Aponomma fimbriatum</i>	JN863730	1-11	1-66	307 269
		<i>Archaeocroton sphenodonti</i>	JN863731	1-13	1-48	317 311
		<i>Amblyomma triguttatum</i>	AB113317	1-14	1-67	307 307
		<i>Bothriocroton concolor</i>	JN863727	1-16	1-23	303 298
		<i>Bothriocroton undatum</i>	JN863728	1-9	1-31	302 302
		<i>Dermacentor nitens</i>	KC503258	1-324	1-11	311 306
		<i>Haemaphysalis flava</i>	AB075954	1-12	1-14	310 310
		<i>Haemaphysalis formosensis</i>	JX573135	1-11	1-20	316 310
		<i>Haemaphysalis inermis</i>	JX573136	1-144	1-8	320 318
		<i>Ixodes hexagonus</i>	AF081828	1-16	1-59	359
		<i>Ixodes holocyclus</i>	AB075955	1-15	1-10	450
		<i>Ixodes pavlovskyi</i>	KJ000060	1-14	1-57	354
		<i>Ixodes persulcatus</i>	AB073725	1-12	1-59	352
		<i>Ixodes ricinus</i>	JN248424	1-354	1-19	354
		<i>Ixodes uriae</i>	AB087746	1-12	1-7	476 388
		<i>Rhipicephalus australis</i>	KC503255	1-27	1-11	310 305
		<i>Rhipicephalus geigy</i>	KC503263	1-117	1-13	306 302
		<i>Rhipicephalus microplus</i>	KC503260	1-11	1-13	310 307

Order	Family	Species	Accession number	Interval range	Overlap range	Control region
		<i>Rhipicephalus sanguineus</i>	AF081829	1-24	1-13	305 303
		<i>Rhipicephalus simus</i>	KJ739594	1-60	1-13	309
	Nuttalliellidae	<i>Nuttalliella namaqua</i>	NC019663	1-5	1-11	339
Mesostigmata	Varroidae	<i>Varroa destructor</i>	AJ493124	1-360	1-62	2174
	Ologamasidae	<i>Stylochyrus ravior</i>	GQ927176	1-16	1-13	471 399
	Parasitidae	<i>Parasitus wandunqingi</i>	MK270528	1-31	1-8	413
		<i>Parasitus fimetorum</i>	OK572962	1-37	1-8	406
	Blattisociidae	<i>Blattisocius tarsalis</i>	MK270529	1-11	1-4	425 513
	Laelapidae	<i>Coleolaelaps c.f. liui</i>	MK270524	1-56	1-32	312 326
		<i>Hypoaspis linteyini</i>	MK270530	1-30	1-11	398 319
	Phytoseiidae	<i>Phytoseiulus persimilis</i>	GQ222414	1-39	1-11	555 578 112 568
		<i>Metaseiulus occidentalis</i>	EF221760	1-129	1-57	311 310 311 311
		<i>Euseius nicholsi</i>	KM999989	1-335	1-11	386 279
	Diplogyniidae	<i>Quadristeroseta c.f. intermedia</i>	MK270521	1-31	1-9	437
		<i>Quadristeroseta c.f. longigynium</i>	MK270522	1-31	1-8	479
		<i>Microdiplogynium sp.</i>	MK270523	1-31	1-8	432
	Machochelidae	<i>Macrocheles glaber</i>	MK270525	1-40	1-7	400
		<i>Macrocheles muscaedomesticae</i>	MK270526	1-32	1-2	368
		<i>Macrocheles nataliae</i>	MK270527	1-38	1-131	496 425

Mitochondrial gene order changes not only between families (Varroidae, Phytoseiidae, Laelapidae, Macrochelidae, Blattisociidae, and Ologamasidae), but also between different genera within the same family (*Coleolaelaps c.f. liui* (Laelapidae: Coleolaelaps) and *Hypoaspis linteyini* (Laelapidae: Hypoaspis); *Metaseiulus occidentalis* (Phytoseiidae: Typhlodrominae) and *Phytoseiulus persimilis* (Phytoseiidae: Amblyseinae)). Furthermore, the mite mitochondrial gene rearrangements of 27 species in 7 families display the following characteristics: the rearrangement rate is high, the degree of rearrangement varies among families, and the rearrangement is primarily tRNA rearrangement in nature. Each sequenced mite had at least two tRNA translocations, and the *cox2-trnD*, *nad4L-trnT* and control areas, as well as the *nad2* gene junction, were all frequent translocations of tRNA rearrangements in the mite mitochondrial genomes.

Gene spacer and gene overlap

Gene overlap region is a section of the mitochondrial genome where the same nucleic acid sequence is involved in the coding of multiple genes. Intergenic region is a portion of the mitochondrial genome that contains non-coding sequences between the coding genes, and its size varies substantially. Gene overlaps were revealed at the 9 gene junctions of *P. fimetorum*, involving a total of 24 bp. The overlap between *trnW* and *trnC* is the longest (7 bp). In addition to the large non-coding regions, the mitochondrial genome of *P. fimetorum* encloses several small non-coding intergenic regions, most of which are distributed at 12 gene junctions and range in diameter from 1 to 37 bp. The *nad4* and *nad4L* genes contain the intergenic region with the longest size. The gene interval and gene overlap range of 51 species sequenced from the Parasitiformes were summarized in this paper (Table 2). The longest spacer

(360bp) is found in *Varroa destructor* (Varroidae), and the overlap area is found in *Macrocheles nataliae* (Machochelidae), which also has the highest overlap area (131bp). There is gene spacer and overlap in each mitochondrial genome of the Parasitiformes, and the positions of the spacer and overlap vary substantially between families and between genera. In most circumstances, the development of gene spacers promotes biological evolution because spacer genes allow for the storage of additional genetic information as well as an increase in the likelihood of mutation.

Analysis of protein coding genes and use of codons in mitochondrial genome

The occurrence of the start and stop codons, as well as the sequence similarity with other complete Parasitiformes mtDNA sequences, confirm the existence of a protein coding gene (PCG). The total length of the 13 PCGs is 10,804bp (just the sum of the lengths of 13 protein-coding genes was computed, the gene spacer was not included), accounting for 73.90% of the entire length of *P. fimetorum* mitochondrial genome. PCGs have an overall A + T content of 69.4%, with a range of 64.3% (*cox3*) to 75.9% (*atp8*) of the total. The traditional ATN codons were used as start codons in most protein-coding genes, and the use of these start codons appeared to follow the patterns that are conserved in the mitochondrial genomes like most Acari species and even some arthropods [16]: five (*cox1*, *atp8*, *nad2*, *nad3* and *nad6*) with ATT, four (*cox2*, *cox3*, *cob*, and *nad4L*) with ATG, two (*atp6* and *nad4*) with ATA, whereas has two (*nad1* and *nad5*) appear to start with GTG (Table 1), which is a rare start codon for animal mitochondrial. Eight PCGs terminate with the conventional stop codons TAA (*cox1*, *nad1*, *nad2*, *nad4*, *nad6*, *nad4L*, *atp6* and *atp8*), one PCG terminate with the conventional stop codons TAG (*nad3*), whereas the remaining four genes (*cox2*, *cox3*, *cob* and *nad5*) ended at a single T residue. A typical occurrence in metazoan mitochondrial genomes is the occurrence of an incomplete stop codon, and these incomplete stop codons are thought to be performed through post-transcriptional polyadenylation [31–33].

In genetic information is available biological function, codons are critical as they serve as a link between genes and proteins. Protein coding genes contain a total of 64 triplet codons in their coding sequences. Synonym codons are used to various codons that encode the same amino acid. The amino acids encoded by these 64 triplet codons are divided into 20 separate categories [34–35]. Using MEGA-X and codon W tools, the amino acid usage and codon usage frequency of 13 protein coding genes sequences from two species of Parasitidae were assessed and compared in this study. The mitochondrial PCGs of two parasitic mites was subjected to a relative synonymous codon usage (RSCU) analysis, which suggested that 62 amino-acid encoding codons identified in the mitochondrial PCGs of two parasitic mites are also discovered in other invertebrates [36]. Among the amino acids found in the mitochondrial genomic PCGs of *P. fimetorum* and *P. wangdunqingi*, the amino acids leucine (12.880~14.361%), serine (9.444~9.530%), and isoleucine (8.260~9.083%) are the most frequently encountered, while cysteine (0.840~0.972%) is the least frequently encountered. The *atp8* gene, for illustration, was appearance in *P. fimetorum*, which has the fewest types of amino acids and does not contain any of the essential amino acids glycine, arginine and aspartic acid, whereas the *nad4L* gene was discovered in *P. wangdunqingi*, which also has the fewest types of amino acids, does not contain any of the essential amino acids proline, glutamine, threonine and tryptophan. The amino acid profile of the proteins encoded by *P. fimetorum* and *P. wangdunqingi* are biased by the fact that the two parasite mites have different bases in the two chains. Table 3 depicts the codon use of the 13 protein-coding genes sequences. *P. fimetorum* has a higher relative codon usage than 1 for 32 codons. Codons with higher relative codon usage than 1 are referred to as preferred codons of mtDNA-encoded protein genes. The preference codon of protein genes encoded by mtDNA in *P. fimetorum* is higher than that of *P. wangdunqingi*. Only 29 codons of *P. wangdunqingi* had a relative usage rate higher than 1. Two mites (*P. fimetorum* and *P. wangdunqingi*) prefers to use U-base-terminated codons (*P. fimetorum* exception of AGU, GAU and CGU, *P. wangdunqingi* exception of CGU, the other are preferred codons), which are followed by A-base-terminated codons (especially *P. fimetorum* AGA and *P. wangdunqingi* UUA, whose relative codon usage is 2.06 and 2.45, respectively). To some extent, *P. fimetorum* should avoid using G-base-terminated codons (other than AGG, AUG and UGG, and every other relative codon usage is less than 1), and *P. wangdunqingi* should avoid using C-base-terminated codons as much as possible. From an evolutionary perspective, the codon usage of the closely related species seems to be more similar than that of the unrelated species. It is estimated that the proportion of U3s in the *P. fimetorum* and *P. wangdunqingi* mtDNA coding protein gene is higher than the proportion of A3s, C3s, and G3s (Table 4). Kimura (1981) genuinely think that codon usage preference is related to GC content, and that codon usage preference related to GC content is affected by mutation pressure, whereas codon usage preference unrelated to GC content is affected by natural selection pressure [37–39]. By comparing the GC and third GC codon content (GC3s) with *P. fimetorum* and *P. wangdunqingi* protein coding genes, the GC and

GC3s contents of *P. fimetorum* were observed to be greater than the *P. wangdunqingi*. Mutation pressure may cause the difference in codon usage preference between *P. fimetorum* and *P. wangdunqingi*.

Table 3
RSCU of preferable codons of mtDNA protein coding sequences in *P. fimetorum* and *P. wangdunqingi*

Amino acid Codon		<i>P.fimetorum</i>		<i>P. wangdunqingi</i>		Amino acid Codon		<i>P. fimetorum</i>		<i>P. wangdunqingi</i>	
		Number	RSCU	Number	RSCU			Number	RSCU	Number	RSCU
Phenylalanine	UUU	177	1.48	271	1.63	Alanine	GCU	41	1.69	43	2.05
	UUC	63	0.52	61	0.37		GCC	32	1.32	11	0.52
Leucine	UUA	121	2.01	210	2.45		GCA	21	0.87	26	1.24
	UUG	49	0.81	69	0.80		GCG	3	0.12	4	0.19
	CUU	75	1.25	104	1.21	Histidine	CAU	58	1.26	49	1.18
	CUC	28	0.47	31	0.36		CAC	34	0.74	34	0.82
	CUA	66	1.10	69	0.80	Glutamine	CAA	74	1.68	40	1.21
	CUG	22	0.37	32	0.37		CAG	14	0.32	26	0.79
Isoleucine	AUU	161	1.36	207	1.54	L-Asparagine	AAU	166	1.26	113	1.48
	AUC	44	0.37	48	0.36		AAC	98	0.74	40	0.52
	AUA	150	1.27	149	1.11	lysine	AAA	182	1.58	74	1.24
Methionine	AUG	35	1.00	49	1.00		AAG	49	0.42	45	0.76
Valine	GUU	37	1.45	63	1.45	Aspartic acid	GAU	17	0.74	48	1.39
	GUC	21	0.82	11	0.25		GAC	29	1.26	21	0.61
	GUA	35	1.37	64	1.47	Glutamic acid	GAA	37	1.51	53	1.29
	GUG	9	0.35	36	0.83		GAG	12	0.49	29	0.71
Serine	UCU	100	1.82	82	1.88	Cysteine	UGU	50	1.28	66	1.52
	UCC	46	0.84	34	0.78		UGC	28	0.72	21	0.48
	UCA	65	1.19	47	1.08	Tryptophan	UGG	27	1.00	30	1.00
	UCG	7	0.13	10	0.23		UGA	51	0.55	59	0.57
	AGU	47	0.86	59	1.35	Arginine	CGU	15	0.58	20	0.83
	AGC	64	1.17	30	0.69		CGC	13	0.51	8	0.33
Proline	CCU	52	1.17	43	1.74		CGA	26	1.01	20	0.83
	CCC	65	1.46	28	1.13		CGG	12	0.47	10	0.42
	CCA	51	1.15	23	0.93		AGA	53	2.06	41	1.71
	CCG	10	0.22	5	0.20		AGG	35	1.36	45	1.88
Threonine	ACU	92	1.63	65	1.70	Glycine	GGU	27	1.23	52	1.78
	ACC	54	0.96	28	0.73		GGC	18	0.82	7	0.24
	ACA	59	1.04	50	1.31		GGA	26	1.18	37	1.26
	ACG	21	0.37	10	0.26		GGG	17	0.77	21	0.72
Tyrosine	UAU	202	1.46	209	1.53	Stop codon	UAA	176	1.91	155	1.49
	UAC	75	0.54	65	0.47		UAG	49	0.53	99	0.95

Table 4

Codon A3s, U3s, G3s, C3s, GC3s, GC content of protein gene encoded by two mitochondrial genomes of Parasitidae. A3s, U3s, G3s and C3s show the codon contents ending with four bases A, U, G and C, respectively; GC3s represents GC content of third synonymous codon

Species	Accession number	A3s(%)	U3s(%)	G3s(%)	C3s(%)	GC3s(%)	GC(%)
<i>Parasitus fimetorum</i>	OK572962	42.78	45.62	13.66	24.66	29.90	32.20
<i>Parasitus wangdunqingi</i>	MK270528	40.69	50.64	18.84	16.20	25.50	29.80

Transfer RNA genes

Using tRNAscan-SE [19] and ARWEN [20] programs, we were able to identify 20 tRNA genes in the mt genomes of *P. fimetorum*. Other than that, the other two mt tRNA genes of *P. fimetorum* (*trnP* and *trnS₇*) could only be found manually through sequence alignment and secondary structure comparison with genes previously identified in other species of Parasitiformes and Acariformes mite. It is common for animals to have a clover secondary structure, which comprises of four arms: the AA-arm, the D-arm, the AC arm, and the T-arm [26]. It has been calculated that the total length of 22 tRNA genes in the mitochondrial genome of *P. fimetorum* is 1390 bp (only the sum of 22 tRNA genes is calculated, excluding gene overlap), that the A + T content is 72.6 %. All of the tRNAs in the secondary structure of *P. fimetorum*, with the exception of the serine tRNA (anticodon GCU), can combine to form a typical secondary structure of clover. It is thought that serine tRNA was deficient in its D-arm in practically all animals and was unable to create a stable secondary structure of clover, which is considered to be a characteristic feature of all chelicerate mitochondrial genomes, but this structure is not found in other tRNAs [10]. For the first time, the absence of the T-arm was discovered in the genes of nematode tRNAs, in which 20 out of 22 tRNAs lack the T-arm and the two tRNAs for serine (anticodons GCT and TGA) lack the D-arm [40]. Following this discovery, other tRNAs with similar structure have been found in the chelicerates, including the Acariformes [41], Araneae [42–44], Scorpiones [45–46], Thelyphonida [46], Pseudoscorpiones [47], Acanthocephala [48], Insecta [49], and Protura [50]. The secondary structures of 50 published mitochondrial genomes of Parasitiformes were observed, except for the loss of D-arm of serine tRNA, 44 species had atypical tRNA structure, and the phenomenon of broken arm of tRNA was fairly prevalent. Holothyrda has 1 species, Ixodida contains 30 species, and Mesostigmata includes 13 species with 22 tRNA secondary structures lacking D-arm or T-arm. No tRNAs of these species are deficient in both the D-and T-arms. The tRNA breaking arm phenomena of Parasitiformes is less prevalent than Acariformes. For particular, in Acariformes, 19 out of the 22 mitochondrial tRNAs of the Tetranychidae were found to be lacking either the D- or T-arm; in fact, in some species, the tRNAs for phenylalanine and glutamine were found to be deficient in both the D-and T-arm [24]. Demodicidae has 15 tRNAs that lack either the D-arm or the T-arm, as well as 5 tRNAs that lack both the D-arm and the T-arm [51]. Pyroglyphidae has 20 tRNAs with either D-arm or T-arm lacking [52], Trombiculidae have 18 tRNA without D-arm or T-arm [41], Acaridae and Psoroptidae have 21 tRNA lacking D-arm or T-arm [53], and Unionicolidae have 15 tRNA with missing arm [31–32]. 13 (*trnD*, *trnG*, *trnA*, *trnR*, *trnF*, *trnH*, *trnT*, *trnS₂*, *trnL₂*, *trnV*, *trnW*, *trnC*, *trnY*) of the 22 tRNA genes found in *P. fimetorum* are within the average range (62.0±1.3 bp) of tRNA genes found in Parasitiformes [24]. The length of 8 tRNA genes (*trnK*, *trnN*, *trnE*, *trnP*, *trnL₁*, *trnI*, *trnQ*, *trnM*) is greater than the average size of Parasitiformes tRNA gene, with a range between 64~68bp. Only one tRNA gene (*trnS₇*) was found to be shorter than the average length of the tRNA gene, and the loss of the D-arm can explain for only 59bp of the total length of the gene. In addition to the standard Watson-Crick pairings of A-U and G-C, there are a number of mispairings, including G-U, U-U, and A-G, among others. *P. fimetorum* secondary structure for 22 tRNA genes consisting of 29 base mismatches and irregular pairings, of which 21 mismatches were G-U mismatches (Fig. 2). Only 15 tRNA genes (*trnA*, *trnD*, *trnG*, *trnI*, *trnK*, *trnL₂*, *trnM*, *trnN*, *trnP*, *trnQ*, *trnR*, *trnS₁*, *trnT*, *trnV*, *trnW*) have complete amino acid receptor arms (7bp), while the remaining 7 tRNA genes have base mismatches (1~2bp varies) in their amino acid receptor. Aside from that, 15 tRNA genes (*trnC*, *trnD*, *trnE*, *trnG*, *trnH*, *trnI*, *trnL₁*, *trnN*, *trnP*, *trnR*, *trnS₂*, *trnT*, *trnV*, *trnW*, *trnY*) were able to generate a totally paired anticodon arm of 5bp, but the anticodon arms of the other 7 tRNA genes had a 1-2 base mismatch. Missmatch which is may be critical for preserving the integrity of the tRNAs secondary structure.

Ribosomal RNA genes and Non-coding region

There have been successful identifications of *rns* gene (707bp) and *rnl* gene (1194bp), which are analogous to other mites, in the whole mitochondrial genome sequence of *P. fimetorum*. Genetic information about the position and structure of rRNA genes is relatively conservative, and the pace of evolution is also conservative. As with other arthropods, two rRNA genes are found on the N-strand between *trnL₁* and the biggest non-coding region [54]. It was found that A+T content of *rns* gene and *rnl* gene was

respectively 69.3% and 75.5%. While *rrnS* gene is situated in the region between *trnL*₁ and *trnV*, *rrnL* gene is located in the region between *trnV* and non-coding region. There is a consistent pattern in the mitochondrial genome of arthropods, in which gene clusters such as *rrnS-trnV-rrnL* are organized.

The control region is the longest non-coding section in the mitochondrial genome, and it is responsible for the beginning of mitochondrial DNA replication and transcription. In most cases, this area contains nucleotides with an adenine (A) and thymine (T) content greater than 85 %; therefore, the control region is sometimes referred to as the A+T-rich region [55]. *P. fimetorum* A+T content of control region were 77.6%, and the similarity with the nucleotide sequence of *P. wandunqingi* control region was 68.1%. It can be inferred that the two parasite mites evolved independently rather than co-evolved. According to Staffan and Anna (2000) [56], only independent evolution can result in a variation in the nucleotide sequence of a non-coding region, which ultimately results in the degeneration or deletion of a specific non-coding region. Non-coding region sections of the mitochondrial genome of the Parasitiformes differ in terms of their location, quantity, and length, and this is one of the primary reasons for the modest difference in overall length between mite genomes. With four control regions, *Phytoseiulus persimilis* (Phytoseiidae) is the species with the greatest number of control regions. Although it is the longest control area in the mites, the control area of *Varroa destructor* (Varroidae) is the longest (up to 2174bp), and the control area of *Rhipicephalus simus* (Ixodidae) is the shortest (only 309bp) (Table 2).

Phylogenetic analysis based on *cox1* gene sequences

In recent years, a large number of researchers have used mixed gene segments for phylogenetic analysis, with the *cox1* gene being one of the most frequently used gene fragment. In terms of nucleotide and amino acid evolution, *cox1* is the slowest-evolving protein, making it a valuable marker for examining phylogenetic relationships at a higher level of classification [24]. In this study, the phylogenetic tree of *cox1* genes from 51 species of Parasitiformes was constructed using the ML method (maximum likelihood method) in MEGA-X software, *Carcinoscorpius rotundicauda* and *Limulus polyphemus* were used as outgroups (Fig. 3). The complete order of Parasitiformes phylogenetic relationship is (Ixodida + Holothyrida + Mesostigmata). The cladistic relationship of the phylogenetic tree showed that 51 species of Parasitiformes were divided into four clades, the first clade was composed of Ixodidae and Nuttalliellidae and formed a sister group with a node support rate of 76; the second clade was composed of Argasidae with the first evolutionary clade formed a sister group and a node support rate of 59; the third clade was composed of Allothryridar and the fourth clade was composed of 8 families of Mesostigmata. The overall node support rate of the phylogenetic tree in this study is strong, but the individual node support rate is poor, which may be owing to the limited sample size. Some families only collect samples from a single species, whereas other families have collected no samples at all. At the base node support rate of the constructed phylogenetic tree is really high. In general, the closer a node is to the tree root, the lower the node support rate will be for that node. This is due to the fact that this study only conducts a phylogenetic analysis on the *cox1* gene of a few parasitic mites belonging to the Parasitiformes. Our results suggested that nine families are monophyletic, which is in accordance with the result of Xue (2019) et al. [28].

Conclusion

As the big data era arrives, even more data will be used to explore animal genetic mitochondria. For the first time, the complete sequence of the *P. fimetorum* mitochondrial genome was determined and analyzed, and have retained the arrangement of arthropods hypothesized ancestor mitochondrial genome. Phylogeny of Parasitiformes mites was evaluated based on *cox1* gene. This research provides potentially a valuable source of information for understanding the phylogeny and evolution of Parasitiformes mites. Due to the obvious large geographical spread of mites, the relevant research has encountered myriad challenges. In addition, there are minor differences in the exterior morphological characteristics of some species, and there are still inconsistencies in the taxonomy and evolutionary position of some of the species studied. We should exploit the advantages of high-throughput sequencing technology in future research, increase the number of species and the number of mites used in research, as well as the number of molecular markers used in analysis, in order to analyze the taxonomic status and phylogenetic relationship of Parasitiformes more thoroughly and systematically.

Abbreviations

ul: Microliter; atp6 and atp8: Genes for ATP synthase subunits 6 and 8; bp: Base pair; cob: Gene for cytochrome b; cox1, cox2 and cox3: Genes for cytochrome c oxidase subunits 1, 2 and 3; DNA: Deoxyribonucleic acid; kb: Kilo base pair; min: Minute; ; mt: Mitochondrial; nad1, nad2, nad3, nad4, nad4L, nad5 and nad6: Mitochondrial genes for NADH dehydrogenase subunits 1-6 and 4 L; PCR: Polymerase chain reaction; RNA: Ribonucleic acid; rRNA: Ribosomal RNA; rrnS and rrnL: Genes for small and large subunits of ribosomal RNA; sec: Second; A: Adenine; T: Thymine; tRNA: Transfer RNA; rRNA: Ribosomal RNA; trnA or A: tRNA gene for alanine; trnC or C: tRNA gene for cysteine; trnD or D: tRNA gene for aspartic acid; trnE or E: tRNA gene for glutamic acid; trnF or F: tRNA gene for phenylalanine; trnG or G: tRNA gene for glycine; trnH or H: tRNA gene for histidine; trnI or I: tRNA gene for isoleucine; trnK or K: tRNA gene for lysine; trnL1 or L1: tRNA gene for leucine (anticodon NAG); trnL2 or L2: tRNA gene for leucine (anticodon YAA); trnM or M: tRNA gene for methionine; trnN or N: tRNA gene for asparagine; trnP or P: tRNA gene for proline; trnQ or Q: tRNA gene for glutamine; trnR or R: tRNA gene for arginine; trnS1 or S1: tRNA gene for serine (anticodon NCU); trnS2 or S2: tRNA gene for serine (anticodon NGA); trnT or T: tRNA gene for threonine; trnV or V: tRNA gene for valine; trnW or W: tRNA gene for tryptophan; trnY or Y: tRNA gene for tyrosine; U: Uracil.

Declarations

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Availability of data and materials

The nucleotide sequence of the mitochondrial genome of the *Parasitus fimetorum*, has been deposited in GenBank (accession number OK572962) and will be released publicly once this manuscript is accepted for publication.

Authors' contributions

HJY, WGD and TC designed the research. HJY performed the research. WGD contributed reagents and materials. HJY and WGD analysed the data. HJY and WGD wrote the manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Capture of *Apodemus chevrieri* was approved by health authorities in Yunnan Province, China. Animal capture protocols and procedures were approved by the animal ethics committees at Dali University (approval # DLDX-20100421-001). All collection methods were carried out in accordance with the approved guidelines and regulations.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests to declare.

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Supplementary Information

Supplementary Information is not available with this version.

Figures

Figure 1

The mitochondrial (mt) genomes of *P. fimetorum*. The direction of the arrow represents the direction of transcription. Abbreviations for gene names. NCR refers to non-coding region. 22 tRNA genes are designated with one letter amino acid codes.

Figure 2

Inferred secondary structures of the mitochondrial tRNAs of *P. fimetorum*.

Figure 3

Phylogenetic relationships of 51 species of parasitic mites based on mitochondrial genomes. The phylogenetic tree was constructed based on nucleotide sequences of *cox1* gene using maximum likelihood. The numbers at the nodes are bootstrap values. The GenBank accession numbers of the 51 mitochondrial genome sequences are listed in Table 2.

Supplementary Files

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