Journal of Helminthology

cambridge.org/jhl

The mitochondrial genome of *Dipetalonema gracile* from a squirrel monkey in China

P. Zhang[1,2], R.K. Ran[1], A.Y. Abdullahi[1,3], X.L. Shi[1], Y. Huang[1], Y.X. Sun[1], Y.Q. Liu[1], X.X. Yan[1], J.X. Hang[1], Y.Q. Fu[1], M.W. Wang[1], W. Chen[2] and G.Q. Li[1]

[1]College of Veterinary Medicine, South China Agricultural University, Guangzhou 510642, People's Republic of China; [2]Guangzhou Zoo & Guangzhou Wildlife Research Center, Guangzhou 510070, People's Republic of China and [3]Animal Science Department, Kano University of Science and Technology, Wudil, PMB 3244, Kano 20027, Nigeria

Research Paper

Cite this article: Zhang P *et al* (2020). The mitochondrial genome of *Dipetalonema gracile* from a squirrel monkey in China. *Journal of Helminthology* **94**, e1, 1–8. https://doi.org/10.1017/S0022149X18000871

Received: 9 August 2018
Accepted: 14 September 2018

Key words:
characteristics; *Dipetalonema gracile*; identification; mitochondrial genome; squirrel monkey

Authors for correspondence:
G.Q. Li, Fax.: +86 20 85280241
E-mail: gqli@scau.edu.cn;
W. Chen, E-mail: guangzhouchenwu@sina.com

Abstract

Dipetalonema gracile is a common parasite in squirrel monkeys (*Saimiri sciureus*), which can cause malnutrition and progressive wasting of the host, and lead to death in the case of massive infection. This study aimed to identify a suspected *D. gracile* worm from a dead squirrel monkey by means of molecular biology, and to amplify its complete mitochondrial genome by polymerase chain reaction (PCR) and sequence analysis. The results identified the worm as *D. gracile*, and the full length of its complete mitochondrial genome was 13,584 bp, which contained 22 tRNA genes, 12 protein-coding genes, two rRNA genes, one AT-rich region and one small non-coding region. The nucleotide composition included A (16.89%), G (20.19%), T (56.22%) and C (6.70%), among which A + T = 73.11%. The 12 protein-coding genes used TTG and ATT as start codons, and TAG and TAA as stop codons. Among the 22 tRNA genes, only *trn*S1^{AGN} and *trn*S2^{UCN} exhibited the TΨC-loop structure, while the other 20 tRNAs showed the TV-loop structure. The *rrn*L (986 bp) and *rrn*S (685 bp) genes were single-stranded and conserved in secondary structure. This study has enriched the mitochondrial gene database of *Dipetalonema* and laid a scientific basis for further study on classification, and genetic and evolutionary relationships of *Dipetalonema* nematodes.

Introduction

Dipetalonema gracile is a common filarioid nematode that inhabits the peritoneal cavity of the primate host (Travi *et al*., 1985). In the case of massive parasitism, the host is roughened, malnourished, anorexic and eventually dies, thus the infection poses a serious threat to the health of squirrel monkey (*Saimiri sciureus*) populations (Notarnicola *et al*., 2007). In the present era of highly advanced technology, mitochondrial DNA (mtDNA) has been characterized as a simple structure with small molecular mass, high mutation rate, fast evolution, and unique maternal hereditary and rare genetic recombination (Bandyopadhyay *et al*., 2006; Hu and Gasser, 2006; Cameron *et al*., 2007). Therefore, the use of mtDNA as a genetic marker is more effective in identifying the hidden species and genotypes of parasites (Liu *et al*., 2013). Lefoulon *et al*. (2015) analysed the *cox*1 and 12S rDNA genes of 48 species of nematodes of Onchocercidae, and found that these nematodes were mainly clustered in the genera *Dipetalonema*, *Setaria*, *Onchocerca*, *Serofilaria* and *Dirofilaria*. Sazmand *et al*. (2016) amplified the *cox*1 gene of the microfilariae and adult stages of *Dipetalonema evansi* from *Camelus dromedarius* in the south-east of Iran, and found that the *cox*1 gene could be used for the accurate diagnosis of nematode infection at different stages. With the development of polymerase chain reaction (PCR) and sequencing technologies, many important breakthroughs have been made in studies on the structural characteristic, gene composition and function, and genetic evolution of the mtDNA from parasitic nematodes (Xu *et al*., 2015; Hu *et al*., 2016; Shi *et al*., 2017). However, little is known about the mitochondrial genome of *Dipetalonema* nematodes.

This study aimed to identify a suspected *D. gracile* worm from a dead squirrel monkey in a zoo in Guangzhou, China, and to amplify its complete mitochondrial genome sequence by conventional or long-range PCR and sequence analysis.

Materials and methods

Parasites and DNA extraction

Three worms were collected from the abdominal cavity of a dead squirrel monkey in a zoo in Guangzhou in April 2016, fixed in 70% ethanol and stored at −20°C until use. Individual worms were put in centrifuge tubes and flushed three times with double-distilled water (ddH_2O). Total genomic DNA from individual worms was extracted using the Wizard® SV

CAMBRIDGE UNIVERSITY PRESS

Table 1. Primers used for PCR amplification of the *Dipetalonema gracile* mitochondrial genome.

Name	Sequence (5'–3')	Amplified region	Expected length (bp)
F1	F: TCTTTGTTTCGTGGGTAT R:ACCAGAGCCAAACAATAACA	1–900	900
F2	F:ATGTTTATAGTGGATTTTTGAGT R:ATAATAATTAAAAGACTTATACG	679–1681	1003
F3	F:TGGTTGCCTAAGGTTCAT R :ACACGAGGAAACGCCATC	1559–2584	1026
F4	F:GCCTGAGTTATCTTTGG R:TACTGCCCACTAACATCC	2752–5931	3179
F5	F:ATTCTGCTTTGGGTCCTT R:CCATACTACAACTTACGC	6006–8099	2093
F6	F: ACTTTGTTGGAGCGTCAT R:TCTGTCTCACGACGAACT	8234–11445	3212
F7	F:TCGTCGTGAGACAGAGCG R: AACCCACATAATCCAAACCAG	11385–13453	2068

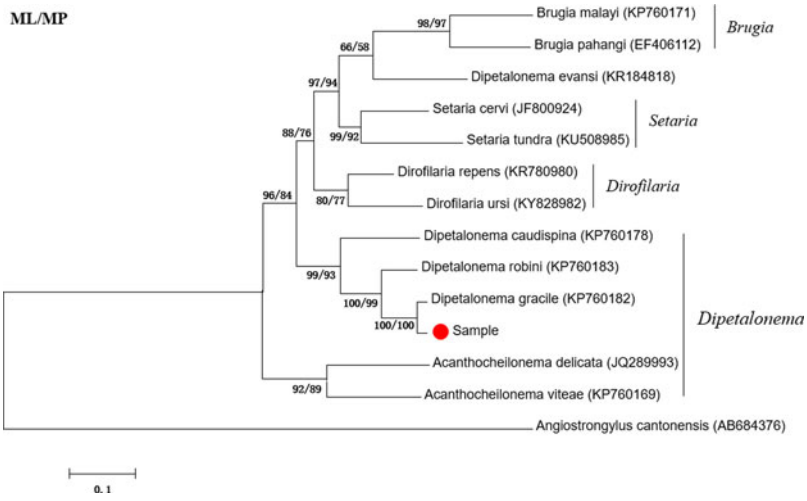

Fig. 1. Phylogenetic tree based on the *cox*1 gene of *Dipetalonema gracile* and other Onchocercidae nematodes by maximum likelihood (ML) and maximum parsimony (MP) methods.

Genomic DNA Purification System (Promega, Madison, Wisconsin, USA) according to the manufacturer's instructions and stored at −20°C.

Molecular identification

The primer CX1 (F): 5'-GACCAGGAAGTAGTTGAA-3' and its complementary primer CX1 (R): 5'-CAGCCTCACTAATAAT ACCA-3' were designed according to published *cox*1 gene sequences of *Dipetalonema* nematodes in GenBank (Lefoulon *et al.*, 2015). PCR reactions were performed in 25 µl, including 12.5 µl of ExTaq polymerase (TaKaRa, Kusatsu, Shiga, Japan), 0.5 µl of each primer (50 pmol/µl), 2 µl of DNA sample and 9.5 µl of ddH_2O. The cycling conditions were initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 52°C for 30 s and extension at 72°C for 1 minute, then a final extension at 72°C for 10 minutes. Amplified fragments were analysed with ethidium bromide stained agarose gel electrophoresis, purified using a DNA gel extraction kit (Omega, Georgia, USA). The purified PCR products were connected with pMD18-T (TaKaRa, Kusatsu, Shiga, Japan) overnight, then transferred into DH5α Competent Cells (TaKaRa, Kusatsu, Shiga, Japan). Positive clones were screened by bacterial PCR and sent to Shanghai Sangon Co., Ltd for sequencing. Homologous comparison was conducted with *cox*1 gene sequences of Onchocercidae nematodes from the GenBank database. Finally, the *cox*1 gene sequences of 12 species of Spirurida nematodes were compared using the MEGA6 software, and the best model was selected by ProtTest 2.4. Using *Angiostrongylus cantonensis* (AB684376) as outgroup, the phylogenetic tree was constructed by maximum likelihood (ML) and maximum parsimony (MP) methods (Zhan *et al.*, 2001).

PCR amplification of complete mitochondrial genome

According to the complete mitochondrial genome sequence of *Dirofilaria immitis* (NC005305) published in GenBank, seven pairs of primers (table 1) were designed in their conserved regions to amplify the entire mitochondrial genome sequence of *D. gracile*. These primers were synthesized by Shanghai Sangon Company in China. PCR reactions for a $\leq$2 kb fragment were performed in 50 µl, including 25 µl of Premix PrimeStar Max

https://doi.org/10.1017/S0022149X18000871 Published online by Cambridge University Press

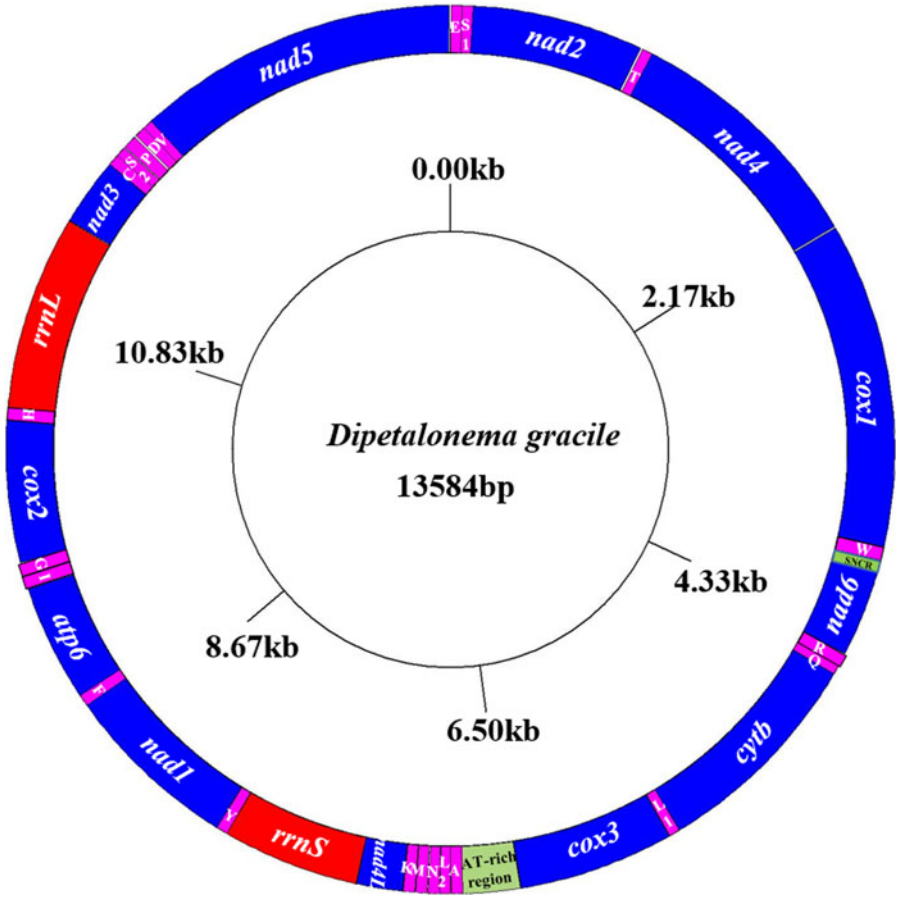

Fig. 2. Arrangement of the mitochondrial genome of *D. gracile*. All genes are predicted to be transcribed in a clockwise direction, and the tRNA genes are designated by single-letter abbreviations for the corresponding amino acids.

(TaKaRa, Kusatsu, Shiga, Japan), 1 µl of each primer (50 pmol/µl), 4 µl of DNA samples and 19 µl of ddH_2O. PCR conditions used were initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 52°C for 30 s and extension at 72°C for 1 minute, followed by a final extension at 72°C for 10 minutes. Long PCR reactions for a > 2 kb fragment were performed in 50 µl, including 25 µl of Premix PrimeStar Max, 1 µl of each primer (50 pmol/µl), 4 µl of DNA samples and 19 µl of ddH_2O. The cycling conditions were initial denaturation at 94°C for 5 minutes; followed by denaturation at 94°C for 30 s, annealing at 42–53°C for 30 s and extension at 68°C for 1.5 minutes for 10 cycles; followed by initial denaturation at 94°C for 5 minutes; denaturation at 94°C for 30 s, annealing at 50–58°C for 30 s and extension at 72°C for 1.5–2.0 minutes for 25 cycles; and then a final extension at 72°C for 7 minutes. Amplified PCR products were analysed with ethidium bromide stained agarose gel electrophoresis, purified using a DNA gel extraction kit (Omega, Georgia, USA). The purified PCR products were connected with pMD18-T (TaKaRa, Kusatsu, Shiga, Japan) overnight, then transferred into DH5α Competent Cells (TaKaRa, Kusatsu, Shiga, Japan). Positive clones were screened by bacterial PCR and sent to Shanghai Sangon Co., Ltd for sequencing.

Sequence analysis

The high-quality sequences obtained using BioEdit version 7.0 were assembled by seqMan software within DNAStar 5.0 (Tamura *et al*., 2011) and adjusted manually. Online software (http://dogma.ccbb.utexas.edu/) was combined with MegAlign software in DNAStar 5.0 (Tamura *et al*., 2011) to identify gene boundaries and composition, as well as translation initiation and termination codons. The AT contents were calculated using Editseq software in DNAStar 5.0 (Tamura *et al*., 2011). The 22 tRNA genes were identified with the aid of the tRNA scan program, available at http://lowelab.ucsc.edu/tRNAscan-SE/, combined with artificial proofreading using *Dirofilaria immitis*. The rRNA genes were identified by aligning sequence with those of *D. immitis* (Hu *et al*., 2003). Their secondary structures were predicted by comparing them with the published structures of *D. immitis* (Hu *et al*., 2003).

Results

Molecular identification of D. gracile

The amplified fragment of the *cox*1 gene was approximately 600 bp in length, which is consistent with the expected size. The sequencing results showed that the *cox*1 gene was 632 bp long. BLAST analysis indicated highest similarity (98.90%) with *D. gracile* (KP760182). Phylogenetic analyses showed that the attained sequence clustered in the same branch as *Dipetalonema gracile* (KP760181) (fig. 1). Thus, the suspected worm was identified as *D. gracile*.

Amplification of complete mitochondrial genome

The amplified fragments from seven pairs of primers (F1–F7) for the complete mitochondrial genome of *D. gracile* were 900 bp,

https://doi.org/10.1017/S0022149X18000871 Published online by Cambridge University Press

Table 2. Organization of the *D. gracile* mitochondrial genome.

Gene/ Region	Position (Fragment size)	Codon (Ini/Ter)	Anticodon	Intergenic nucleotides
trnE	4–56 (53)		UUC	3
trnS1^{AGN}	59–110 (52)		UCU	2
*nad*2	110–956 (847)	TTG/TAG		−1
trnT	962–1018 (57)		UGU	5
*nad*4	1018–2260 (1243)	TTG/TAA		−1
*cox*1	2268–3873 (1606)	ATT/TAG		7
trnW	3876–3931 (56)		UCA	0
SNCR	3932–3977 (46)			0
*nad*6	3978–4429 (452)	TAT/TAG		−13
trnR	4417–4470 (54)		ACG	6
trnQ	4477–4530 (54)		UUG	9
*cyt*b	4540–5620 (1081)	TTG/T		1
trnL1^{CUN}	5622–5676 (55)		UAG	0
*cox*3	5677–6447 (771)	GTT/TAG		−1
AT-rich	6447–6732 (286)			−5
trnA	6728–6785 (58)		UGC	−1
trnL2^{UUR}	6785–6838 (54)		UAA	4
trnN	6843–6899 (57)		GUU	2
trnM	6902–6962 (61)		CAU	5
trnK	6968–7023 (56)		CUU	1
*nad*4L	7025–7253 (229)	GTA/TAA		1
rrnS	7255–7938 (685)			0
trnY	7939–7991 (53)		GUA	−3
*nad*1	7989–8881 (893)	TTG/T		−19
trnF	8863–8918 (56)		GAA	0
*atp*6	8919–9499 (581)	TTG/TAA		8
trnI	9508–9565 (58)		GAU	9
trnG	9575–9630 (56)		UCC	3
*cox*2	9634–10331 (698)	ATT/TTA		0
trnH	10332–10388 (57)		GUG	0
*rrn*L	10389–11356 (968)			2
*nad*3	11359–11695 (337)	CTT/T		0
trnC	11696–11750 (55)		GCA	−1
trnS2UCN	11750–11805 (52)		UGA	4
trnP	11810–11864 (55)		AGG	8
trnD	11873–11926 (54)		GUC	3
trnV	11930–11984 (55)		UAC	2
*nad*5	11987–13584 (1598)	TTG/TAG		

1003 bp, 1026 bp, 3179 bp, 2093 bp, 3212 bp, and 2068 bp in size, respectively, which are consistent with the expected fragments, without non-specific bands.

General features of D. gracile *mitochondrial genome*

The entire mitochondrial genome sequence of *D. gracile* was 13,584 bp in length. There were 36 genes, including 22 tRNA

https://doi.org/10.1017/S0022149X18000871 Published online by Cambridge University Press

Table 3. Nucleotide composition (%) of 12 protein-coding genes of the *D. gracile* mitochondrial genome.

Gene	A	G	T	C	A + T	AT skew	GC skew
*cox*1	17.25	22.54	49.81	10.40	67.06	−0.49	0.37
*cox*2	20.34	23.21	48.42	8.02	68.77	−0.41	0.49
*nad*3	10.68	19.88	66.47	2.97	77.15	−0.72	0.74
*nad*5	13.06	19.33	61.27	6.34	74.33	−0.65	0.51
*nad*6	14.16	18.81	63.50	3.54	77.65	−0.64	0.68
*nad*4L	14.85	21.40	58.95	4.80	73.80	−0.60	0.63
*nad*1	12.88	19.93	59.69	7.50	72.56	−0.65	0.45
*atp*6	13.60	18.76	61.62	6.02	75.22	−0.64	0.51
*nad*2	13.22	20.19	62.34	4.25	75.56	−0.65	0.65
*cyt*b	15.36	18.96	58.19	7.49	73.54	−0.58	0.43
*cox*3	17.12	21.92	54.86	6.10	71.98	−0.52	0.56
*nad*4	14.08	22.04	58.09	5.79	72.16	−0.61	0.58

genes, 12 protein-coding genes, two rRNA genes, one AT-rich region and one small non-coding region (SNCR), which constituted a closed circular structure (fig. 2). There were 16 intergenic regions, ranging from 1 to 9 bp (table 2). The nucleotide composition was A = 16.89%, G = 20.19%, T = 56.22%, and C = 6.70%. Therefore, A + T = 73.11%, with obvious AT preference.

Protein-coding genes

The lengths of 12 protein-coding genes of *D. gracile* were stable. Except *nad*4 and *cox*1, all other protein-coding genes were separated by tRNA genes (table 2). The 12 protein-coding genes were biased towards A and T, where the lowest gene in AT content was *cox*1 (67.06%) and the highest was *nad*6 (77.65%) (table 3). They used TAT, TTG, GTA, CTT, GTT and ATT as the start codons. Among them, TTG was the most common (50.00%), followed by ATT (16.67%) and the others (8.30%). The use of the termination codons was more variable; there were complete TAG (41.67%), TAA (25.00%) and TTA (8.30%) codons, and incomplete T (50.00%) stop codons.

Transfer RNA genes

The 22 tRNA genes in the mitochondrial genome of *D. gracile* formed a local double-helix structure by base pairing. The acceptor arm on the top was composed of 7 base-pairs, and the anticodon area included a stem of 5 base-pairs and a loop of 7 bases. These structures folded to form a stable and atypical cloverleaf pattern 52–61 bp long. The *trn*S1^{AGN} and *trn*S2^{UCN} lacked D-loop, where 4 or 8 bases were connected to amino acid acceptor arm and anticodon loop, and 6 or 5 bases and 3 base-pairs together made up the TΨC loop. The remaining 20 tRNA genes lacked TΨC loops, where 5 to 8 bases were connected to amino acid acceptor arm and anticodon loop, and 4 to 11 bases and 4 base-pairs together made up the TV-loop (fig. 3).

Ribosomal RNA genes

The two rRNA genes in the mitochondrial genome of *D. gracile* encoded a large subunit 16S (*rrn*L) and small subunit 12S (*rrn*S). They were located between *trn*H and *nad*3, and *trn*Y and *nad*4L, respectively. The lengths of *rrn*L and *rrn*S genes were 968 bp and 685 bp, respectively. The AT contents were 72.62% and 75.04%, respectively. Two rRNA genes were single-stranded and relatively conservative in secondary structures. Through A-U and G-C pairings, and even A-A, U-U, G-U and A-G unstable pairings, they formed multiple stem-loop structures (fig. 4).

AT-rich and small non-coding region

The AT-rich region in the mitochondrial genome of *D. gracile* was located between *trn*A and *nad*3, without gene interval. The sequence length was 286 bp, and AT content was up to 80.77%, higher than the 12 protein-coding genes. The small non-coding region (SNCR) was 46 bp in length, and was located between *trn*W and *nad*6, without stem-loop structure.

Discussion

At present, there are only a few morphological and developmental descriptions of *D. gracile* (Travi *et al.*, 1985). In this study, molecular identification of a suspected *D. gracile* worm from a squirrel monkey was conducted, confirming that the worm was *D. gracile*, and its complete mitochondrial genome sequence was obtained for the first time. The mitochondrial genome of *D. gracile* was 13,584 bp in length, and its structure and nucleotide composition are basically similar to other nematodes of Secernentea. This may be because the mitochondrial genes could be under similar evolutionary pressures during the genetic process (Hyman and Azevedo, 1996; Gao *et al.*, 2017). Moreover, mitochondrial dysfunction may result from changes in the structures and lengths of mitochondrial genes. The stop codons of *D. gracile* protein-coding genes were TAG and TAA, which is consistent with most nematodes (Okimoto *et al.*, 1990). The use of incomplete codon T also occurred, possibly as a result of post-transcriptional processing when AA is inserted after T to act as the stop codon for protein translation (Ojala *et al.*, 1981). Among the 12 protein-coding genes, the AT content of *nad*6 gene was the highest (77.65%), and that of *cox*1 gene was the

https://doi.org/10.1017/S0022149X18000871 Published online by Cambridge University Press

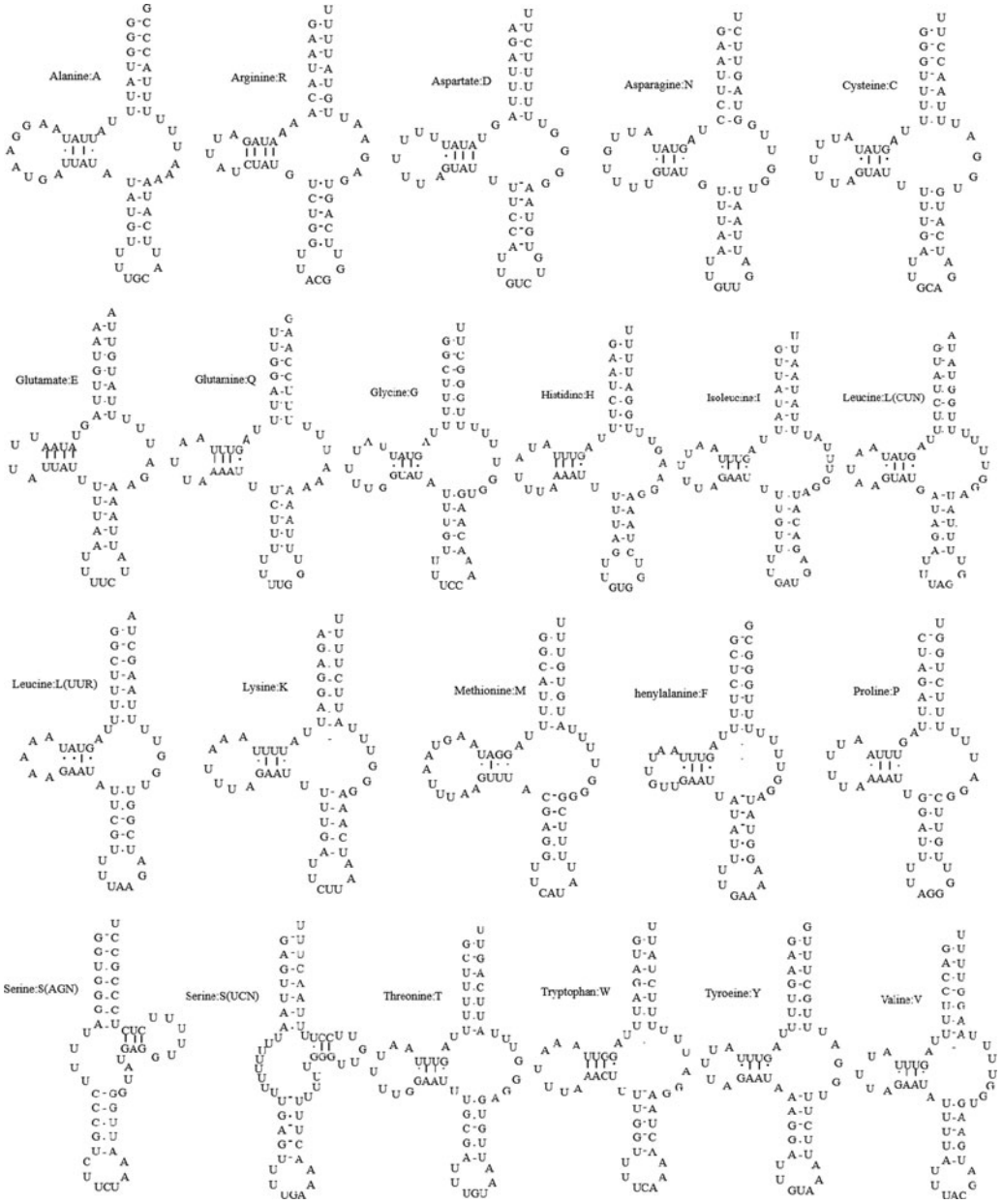

Fig. 3. Secondary structures predicted for the 22 tRNA genes in the mitochondrial genome of *D. gracile*. Canonical base pairs C:G and U:A are indicated by dashes, and G:U pairs by dots.

lowest (67.06%). Therefore, it is speculated that the *cox*1 gene may be under relatively high selection pressure (Dingley *et al.*, 2014), while the *nad*6 gene may be under relatively low selection pressure. As a whole, the 12 protein-coding genes had obvious AT preference; a higher AT preference makes the gene structure more stable and may reduce the probability of gene mutation. This makes multiple protein-coding genes in mitochondria ideal molecular markers for studying molecular classification, phylogenetic evolution, and population genetic variation of the parasite.

In the present study the 22 tRNA genes of *D. gracile* formed a local double-helix structure, as in most nematodes. With the exception of *trn*S1^{AGN} and *trn*S2^{UCN}, the remaining 20 tRNA genes lacked a TΨC loop, showing a TV-loop structure. The polymorphism of the tRNA gene structure may suggest that there are metabolic pathways in this nematode that are different from other organisms (Zhang and Kong, 1997). The rRNA gene of *D. gracile* had multiple unstable pairings, forming many stem and loop structures of different sizes. Such secondary structures were complex but relatively conserved. This means that the differences in rRNA genes among related species can be applied to the classification and phylogenetic studies of nematodes. In addition, there were many common mismatches in the secondary structure of the tRNA and rRNA genes of *D. gracile*. However, no mechanism has been found to correct mitochondrial gene mismatch (Pont-Kingdon *et al.*, 2000). The PCR amplification of the non-coding region of *D. gracile* was difficult, possibly because it does not participate in mitochondrial transcription, with relatively low evolutionary pressure and high mutation rate (Blouin,

https://doi.org/10.1017/S0022149X18000871 Published online by Cambridge University Press

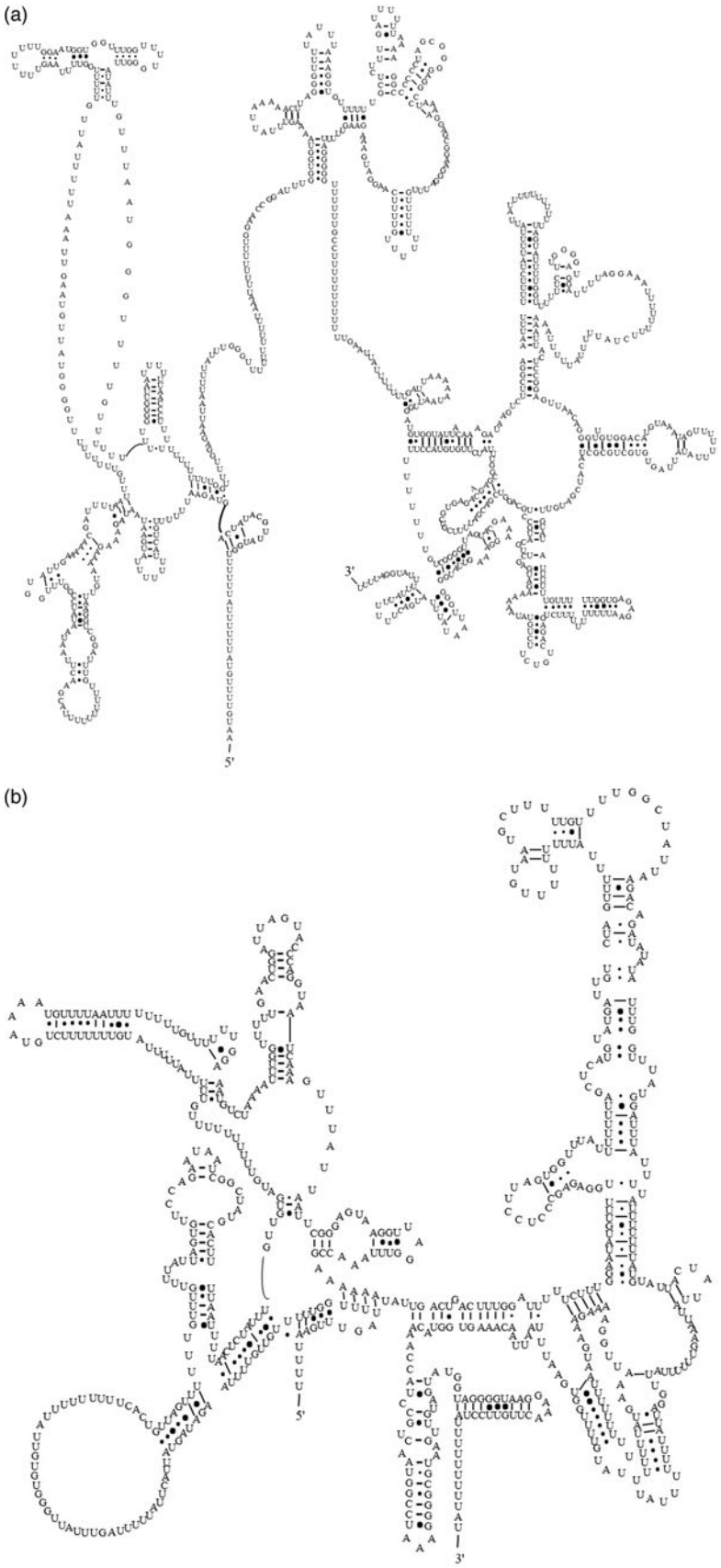

Fig. 4. Predicted secondary structure of the mitochondrial *rrn*L (a) and *rrn*S (b) inferred for *D. gracile*. Canonical base pairs C:G and U:A are indicated by dashes, G:U pairs by large dots, other non-canonical pairings by small dots, and proposed tertiary interactions by lines.

https://doi.org/10.1017/S0022149X18000871 Published online by Cambridge University Press

2002). It is worth noting that the base composition of the *D. gracile* mitochondrial genome had obvious AT bias, which may increase the mutation rate of nucleic acids and the substitution rate of amino acids, making the silent sites more rapidly saturated. This evolutionary trend is conducive to the study of genetic polymorphism and phylogenetics (Zhang *et al.*, 2015).

In conclusion, this study identified *D. gracile* from an infected squirrel monkey in China and obtained its complete mitochondrial genome sequence for the first time, thus enriching the mitochondrial gene database of *Dipetalonema* nematodes. It lays a foundation for studying the classification and genetic evolutionary relationships of *Dipetalonema* nematodes.

Financial support. This work was funded by the National Natural Science Foundation of China (Grant no. 31672541) and the Science and Technology Planning Project of Guangdong Province, China (Grant no. 2014A020214005).

Conflict of interest. None.

References

Bandyopadhyay PK, Stevenson BJ and Cady MT (2006) Complete mitochondrial DNA sequence of a Conoidean gastropod, *Lophiotoma* (*Xenuroturris*) *cerithiformis*: gene order and gastropod phylogeny. *Toxicon* **48**, 29–43.

Blouin MS (2002) Molecular prospecting for cryptic species of nematodes: mitochondrial DNA versus internal transcribed spacer. *International Journal for Parasitology* **32**, 527–531.

Cameron SL, Johnson KP and Whiting MF (2007) The mitochondrial genome of the screamer louse *Bothriometopus* (phthiraptera: ischnocera): effects of extensive gene rearrangements on the evolution of the genome. *Journal of Molecular Evolution* **65**, 589–604.

Dingley SD, Polyak E and Ostrovsky J (2014) Mitochondrial DNA variant in COX1 subunit significantly alters energy metabolism of geographically divergent wild isolates in *Caenorhabditis elegans*. *Journal of Molecular Biology* **426**, 2199–2216.

Gao Y, Qiu JH and Zhang BB (2017) Complete mitochondrial genome of parasitic nematode *Cylicocyclus nassatus* and comparative analyses with *Cylicocyclus insigne*. *Experimental Parasitology* **172**, 18–22.

Hu M and Gasser RB (2006) Mitochondrial genomes of parasitic nematodes - progress and perspectives. *Trends in Parasitology* **22**, 78–84.

Hu M *et al.* (2003) Structure and organization of the mitochondrial genome of the canine heartworm, *Dirofilaria immitis*. *Parasitology* **127**, 37–51.

Hu W *et al.* (2016) Levels of *Ancylostoma* infections and phylogenetic analysis of cox 1 gene of *A. ceylanicum* in stray cat faecal samples from Guangzhou, China. *Journal of Helminthology* **90**, 392–397.

Hyman BC and Azevedo JL (1996) Similar evolutionary patterning among repeated and single copy nematode mitochondrial genes. *Journal of Molecular Evolution* **13**, 221–232.

Lefoulon E, Bain O and Bourret J (2015) Shaking the tree: multi-locus sequence typing usurps current onchocercid (Filarial nematode) phylogeny. *PLoS Neglected Tropical Diseases* **9**, e4233.

Liu GH, Wang Y and Song HQ (2013) Characterization of the complete mitochondrial genome of *Spirocerca lupi*: sequence, gene organization and phylogenetic implications. *Parasites & Vectors* **6**, 1–9.

Notarnicola J, Agustin JF and Gardner SL (2007) A new species of *Dipetalonema* (Filarioidea: Onchocercidae) from *Ateles chamek* from the Beni of Bolivia. *Journal of Parasitology* **93**, 661–667.

Ojala D, Montoya J and Attardi G (1981) tRNA punctuation model of RNA processing in human mitochondria. *Nature* **290**, 470–474.

Okimoto R, Macfarlane JL and Wolstenholme DR (1990) Evidence for the frequent use of TTG as the translation initiation codon of mitochondrial protein genes in the nematodes *Ascaris suum* and *Caenorhabditis elegans*. *Nucleic Acids Research* **18**, 6113–6118.

Pont-Kingdon G, Vassort CG and Warrior R (2000) Mitochondrial DNA of *Hydra attenuata* (Cnidaria): a sequence that includes an end of one linear molecule and the genes for l-rRNA, tRNA(f-Met), tRNA(Trp), COII, and ATPase8. *Journal of Molecular Evolution* **51**, 404–415.

Sazmand A, Eigner B and Mirzaei M (2016) Molecular identification and phylogenetic analysis of *Dipetalonema evansi* (Lewis, 1882) in camels (*Camelus dromedarius*) of Iran. *Parasitology Research* **115**, 1605–1610.

Shi XL *et al.* (2017) The mitochondrial genome of *Ancylostoma tubaeforme* from cats in China. *Journal of Helminthology* **92**, 22–33.

Tamura K, Peterson D and Peterson N (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Journal of Molecular Evolution* **28**, 2731–2739.

Travi BL, Eberhard ML and Lowrie RC (1985) Development of *Dipetalonema gracile* in the squirrel monkey (*Saimiri sciureus*) with notes on its biology. *Journal of Parasitology* **71**, 17–19.

Xu WW, Qiu JH and Liu GH (2015) The complete mitochondrial genome of *Strongylus equinus* (Chromadorea: Strongylidae): comparison with other closely related species and phylogenetic analyses. *Experimental Parasitology* **159**, 94–99.

Zhan B, Li T and Xiao S (2001) Species-specific identification of human hookworms by PCR of the mitochondrial cytochrome oxidase I gene. *Journal of Parasitology* **87**, 1227–1229.

Zhang L and Kong F (1997) Application of mitochondrial DNA to studying the nematological problems. *Acta Parasitologica et Medica Entomologica Sinica* **4**, 178–182.

Zhang Y *et al.* (2015) The complete mitochondrial genome of *Oxyuris equi*: comparison with other closely related species and phylogenetic implications. *Experimental Parasitology* **159**, 215–221.

https://doi.org/10.1017/S0022149X18000871 Published online by Cambridge University Press