Characterization of the complete mitochondrial genome of *Spirocerca lupi*: sequence, gene organization and phylogenetic implications

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Abstract

**Background:** *Spirocerca lupi* is a life-threatening parasitic nematode of dogs that has a cosmopolitan distribution but is most prevalent in tropical and subtropical countries. Despite its veterinary importance in canids, the epidemiology, molecular ecology and population genetics of this parasite still remain unexplored.

**Methods:** The complete mitochondrial (mt) genome of *S. lupi* was amplified in four overlapping long fragments using primers designed based on partial cox1, rrnS, cox2 and nad2 sequences. Phylogenetic re-construction of 13 spirurid species (including *S. lupi*) was carried out using Bayesian inference (BI) based on concatenated amino acid sequence datasets.

**Results:** The complete mt genome sequence of *S. lupi* is 13,780 bp in length, including 12 protein-coding genes, 22 transfer RNA genes and two ribosomal RNA genes, but lacks the *atp8* gene. The gene arrangement is identical to that of *Thelazia callipaeda* (Thelaziidae) and *Setaria digitata* (Onchocercidae), but distinct from that of *Dracunculus medinensis* (Dracunculidae) and *Heliconema longissimum* (Physalopteridae). All genes are transcribed in the same direction and have a nucleotide composition high in A and T. The content of A + T is 73.73% for *S. lupi*, in accordance with mt genomes of other spirurid nematodes sequenced to date. Phylogenetic analyses using concatenated amino acid sequences of the 12 protein-coding genes by BI showed that the *S. lupi* (Thelaziidae) is closely related to the families Setariidae and Onchocercidae.

**Conclusions:** The present study determined the complete mt genome sequence of *S. lupi*. These new mt genome dataset should provide novel mtDNA markers for studying the molecular epidemiology and population genetics of this parasite, and should have implications for the molecular diagnosis, prevention and control of spirocercosis in dogs and other canids.

**Keywords:** *Spirocerca lupi*, Spirocercosis, Mitochondrial genome, Gene organization, Phylogenetic implication

Background

The nematode *Spirocerca lupi* (Rudolphi, 1809) (at the adult stage) parasitizes the oesophagus and aorta of canids, especially in dogs. *S. lupi* is responsible for canine spirocercosis with a worldwide distribution but is usually found in tropical and subtropical countries [1,2]. Canine spirocercosis is usually associated with several clinical signs, such as regurgitation, vomiting and dyspnoea [3,4]. This disease is also fatal when it causes malignant neoplasms or aortic aneurysms [2,4,5]. Fortunately, spirocercosis can be treated efficiently using anthelmintics, such as doramectin [6].

Canine spirocercosis caused by *S. lupi* is often neglected and underestimated by some veterinary scientists and practitioners. However, *S. lupi* is most prevalent in dogs in rural areas, such as in Bangladesh (40%) [7], Greece (10%) [8], Grenada (8.8% in owned dogs and 14.2% in stray dogs) [1], India (23.5%) [9], Iran (19%) [10], South Africa (13%) [11] and Kenya (85% in stray dogs and 38% in owned dogs) [12]. *S. lupi* has been also reported in dogs in China, with a very high prevalence (78.6%) [13]. Although canine spirocercosis is an emerging disease, little is known about
the molecular biology and genetics of *S. lupi* [14]. A previous study has found utility of mitochondrial (mt) cytochrome *c* oxidase subunit 1 (*cox1*) for population genetic and phylogenetic studies of *S. lupi* [14], yet, there is still a paucity of information on *S. lupi* mt genomics.

mt genome sequences provide useful genetic markers not only for genetic and epidemiological investigations and molecular identification of parasites, but also for phylogenetic and population studies [15–18] due to its maternal inheritance, rapid evolutionary rate, and lack of recombination [19,20]. To date, although mt genome sequences have been sequenced for 12 species within the order Spirurida, only one mt genome (for *Thelazia callipaeda*) is available within the family Thelaziidae [21]. Therefore, the objectives of the present study were to determine the complete mt genome sequence of *S. lupi* and to assess the phylogenetic position of this nematode in relation to other spirurid nematodes for which complete mt sequence datasets are available.

**Methods**

**Ethics statement**

This study was approved by the Animal Ethics Committee of Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences (Approval No. LVRIAEC2010-007). The farmed dog from which *S. lupi* adults were collected, was handled in accordance with good animal practices required by the Animal Ethics Procedures and Guidelines of the People’s Republic of China.

**Parasites and DNA extraction**

Adult nematodes representing *S. lupi* were obtained at *post mortem* from the oesophagus of an infected farmed dog in Zhanjiang, Guangdong province, China. These specimens were washed in physiological saline, identified morphologically to species according to existing descriptions [22], fixed in 70% (v/v) ethanol and stored at −20°C until use.

Total genomic DNA was isolated from one *S. lupi* worm using sodium dodecyl sulphate/proteinase K treatment, followed by spin-column purification (TIANamp Genomic DNA Kit). In order to independently verify the identity of this specimen, the mt *cox1* gene was amplified by the polymerase chain reaction (PCR) and sequenced according to an established method [14]. The *cox1* sequence of this *S. lupi* sample had 96.5% similarity with that of *S. lupi* in dogs in South Africa (GenBank accession no. HQ674759).
Amplification and sequencing of partial cox1, rrnS, cox2 and nad2 genes

Initially, a fragment of cox1 (346 bp) was amplified by conserved primers JB3/JB4.5 [23], and rrnS (213 bp), cox2 (300 bp) and nad2 (1200 bp) were amplified by PCR with primers designed (Table 1) based on sequences well conserved in many related taxa. PCR reactions (25 mL) were performed in 10 mM Tris–HCl (pH 8.4), 50 mM KCl, 4 mM MgCl2, 200 mM each of dNTP, 50 pmol of each primer and 2 U Taq polymerase (Takara) in a thermocycler (Biometra) under the following conditions: after an initial denaturation at 94°C for 5 min, then 94°C

Table 2 Mitochondrial genome organization of Spirocerca lupi

| Gene/region | Positions | Size (bp) | Number of aa | Ini/Ter codons | Anticodons | In |
|-------------|-----------|-----------|--------------|----------------|------------|----|
| cox1        | 1-1650    | 1650      | 549          | ATG/TAA        | TCA        | +7 |
| trRNA-Trp (W) | 1657-1714 | 58        |              |                | TAA        | +6 |
| nad6        | 1751-2209 | 459       | 152          | TTG/TAA        | ACG        | +36|
| tRNA-Arg (R) | 2207-2266 | 60        |              |                | TTG        | -3 |
| tRNA-Gln (Q) | 2263-2316 | 54        |              |                | CAT        | -2 |
| cyt b       | 2315-3397 | 1083      | 360          | ATT/TAA        | TAG        | -2 |
| trRNA-LeuCUN (L1) | 3396-3450 | 55       |              |                | TAG        | -2 |
| cox3        | 3448-4230 | 783       | 260          | ATA/TAA        | TAT        | -3 |
| Non-coding region | 4231-4630 | 400       |              |                | 0          | |
| tRNA-Ala (A) | 4631-4692 | 62        |              |                | TGC        | 0 |
| tRNA-Leu/UUR (L2) | 4689-4742 | 54       |              |                | TAA        | -4 |
| tRNA-Asn (N) | 4747-4804 | 58        |              |                | GTT        | +4 |
| tRNA-Met (M) | 4807-4864 | 58        |              |                | CAT        | +2 |
| tRNA-Lys (K) | 4867-4924 | 58        |              |                | TTT        | +2 |
| nad4L       | 4932-5159 | 228       | 75           | ATG/TAG        | 0          | +7 |
| rrnS        | 5170-5855 | 686       |              |                | +10        | |
| tRNA-Tyr (Y) | 5855-5910 | 56        |              |                | GAT        | -1 |
| nad1        | 5908-6816 | 909       | 302          | TTG/TAA        | 0          | -3 |
| tRNA-Phe (F) | 6785-6843 | 59        |              |                | TTG        | -32|
| atp6        | 6847-7431 | 585       | 194          | ATT/TAG        | 0          | +3 |
| tRNA-Ile (I) | 7435-7491 | 57        |              |                | GAT        | +3 |
| tRNA-Gly (G) | 7492-7546 | 55        |              |                | TCC        | 0 |
| cox2        | 7549-8253 | 705       | 234          | ATG/TAG        | 0          | +2 |
| tRNA-His (H) | 8244-8302 | 59        |              |                | GTG        | -10|
| rrnL        | 8301-9288 | 988       |              |                | 0          | -2 |
| nad3        | 9281-9616 | 336       | 111          | TTG/TAA        | 0          | -8 |
| tRNA-Cys (C) | 9616-9670 | 55        |              |                | GCA        | -1 |
| tRNA-SerUCN (S2) | 9673-9726 | 54       |              |                | TGA        | +2 |
| tRNA-Pro (P) | 9730-9787 | 58        |              |                | AGG        | +3 |
| tRNA-Asp (D) | 9847-9900 | 54        |              |                | GTC        | +59|
| tRNA-Val (V) | 9902-9955 | 54        |              |                | TAC        | +1 |
| nad5        | 9959-11551 | 1593    | 530          | TTG/TAG        | 0          | +3 |
| tRNA-Glu (E) | 11550-11606 | 57 | | TCC | -2 |
| tRNA-SerAGN (S1) | 11607-11656 | 50 | | TCT | 0 |
| nad2        | 11637-12485 | 849  | 282          | ATG/TAG        | 0          | -20|
| tRNA-Thr (T) | 12487-12543 | 57 | | TGT | -1 |
| nad4        | 12544-13773 | 1230 | 409 | TTG/TAG | 0 |

*The inferred length of amino acid sequence of 12 protein-coding genes; Ini/Ter codons: initiation and termination codons; In: Intergenic nucleotides.
for 30 s (denaturation), 55°C (for cox1) or 48°C (for cox2) or 50°C (for nadS and rrnS) for 30 s (annealing), 72°C for 1 min (extension) for 36 cycles, followed by 72°C for 10 min (final extension). Two microliters (5–10 ng) of genomic DNA was added to each PCR reaction. Each amplicon (5 μL) was examined by agarose gel electrophoresis to validate amplification efficiency. Then, these amplicons were sent to Sangon Company (Shanghai, China) for sequencing from both directions by using primers used in PCR amplifications.

Long-PCR amplification and sequencing

After we had obtained partial cox1, rrnS, cox2 and nad2 sequences for the S. lupi, we then designed four primers (Table 1) in the conserved regions to amplify the entire mt genome of S. lupi from this representative sample in four overlapping long fragments between cox1 and rrnS (approximately 4.5 kb), between rrnS and cox2 (approximately 2.5 kb), between cox2 and nad2 (approximately 4 kb), and between nad2 and cox1 (approximately 3 kb). Long-PCR reactions (25 μl) were performed in 2 mM MgCl2, 0.2 mM each of dNTPs, 2.5 μl of each primer, 1.25 U LA Taq polymerase (Takara), and 2 μl of DNA sample in a thermocycler (Biometra) under the following conditions: 92°C for 2 min (initial denaturation), then 92°C for 10 s (denaturation), 60°C (for 4.5 kb) or 44°C (for 2.5 kb) or 52°C (for 4 kb) or 48°C (for 3 kb fragment) for 30 s (annealing), and 60°C for 10 min (extension) for 10 cycles, followed by 92°C for 10 s, 60°C (for 4.5 kb) or 44°C (for 2.5 kb) or 52°C (for 4 kb) or 48°C (for 3 kb fragment) for 30 s (annealing), and 60°C for 10 min for 20 cycles, with a cycle elongation of 10 s for each cycle and a final extension at 60°C for 10 min. Each PCR reaction yielded a single band detected in a 0.8% (w/v) agarose gel (not shown). PCR products were sent to Sangon Company (Shanghai, China) for sequencing using a primer-walking strategy.

Sequence analyses

Sequences were assembled manually using the commercial software ContigExpress program of the Vector NTI software package version 6.0 (Invitrogen, Carlsbad, CA), and aligned against the complete mt genome sequences of other spirurid nematodes available using the computer program Clustal X 1.83 [24] and MegAlign procedure within the DNAStar 5.0 [25] to infer gene boundaries. The open-reading frames were analysed with Open Reading Frame Finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html) using the invertebrate mitochondrial code, and subsequently compared with that of T. callipaeda [21]. Protein-coding gene sequences were translated into amino acid sequences using the invertebrate mitochondrial genetic code; amino acid sequences were aligned using default settings with MEGA 5.0 [26]. Translation initiation and termination codons were identified by comparison with those of the spirurid nematodes reported previously [21,27]. For

Table 3 Comparison of A + T content (%) of gene and region of the mt genomes of spirurid nematodes sequenced to date (alphabetical order), including Spirocerca lupi (in bold)

| Gene/region | AV | BM | CQ | DI | DM | HL | LL | OF | OV | SD | SL | TC | WB |
|-------------|----|----|----|----|----|----|----|----|----|----|----|----|----|
| atp6        | 75.21 | 75.09 | 80.14 | 71.88 | 72.40 | 77.89 | 76.46 | 73.71 | 72.99 | 74.23 | 74.87 | 74.23 | 76.63 |
| cox1        | 67.36 | 68.98 | 70.28 | 67.88 | 68.21 | 71.69 | 69.48 | 69.70 | 69.03 | 69.10 | 66.97 | 67.88 | 67.70 |
| cox2        | 66.81 | 68.96 | 73.25 | 69.15 | 68.25 | 74.71 | 71.53 | 68.10 | 69.24 | 69.38 | 68.51 | 67.38 | 70.57 |
| cox3        | 71.54 | 72.69 | 76.92 | 71.79 | 71.54 | 75.93 | 76.20 | 72.18 | 71.79 | 72.56 | 71.39 | 72.41 | 74.33 |
| cytB        | 72.32 | 73.97 | 76.13 | 72.25 | 72.14 | 79.30 | 75.35 | 73.75 | 72.61 | 72.34 | 72.85 | 73.68 | 72.70 |
| nad1        | 73.43 | 73.55 | 75.85 | 72.94 | 72.29 | 75.69 | 72.85 | 71.60 | 69.78 | 72.28 | 72.50 | 73.22 | 72.52 |
| nad2        | 74.68 | 77.61 | 82.39 | 74.39 | 76.93 | 82.92 | 77.26 | 75.56 | 74.30 | 76.49 | 70.91 | 77.35 | 75.71 |
| nad3        | 79.82 | 79.35 | 81.71 | 77.15 | 75.89 | 83.18 | 79.82 | 76.56 | 76.11 | 77.06 | 80.65 | 80.24 | 84.27 |
| nad4        | 73.98 | 76.31 | 78.05 | 74.55 | 72.32 | 80.36 | 75.75 | 74.05 | 73.15 | 76.91 | 74.47 | 75.59 | 73.88 |
| nad4L       | 76.89 | 82.08 | 83.33 | 77.37 | 74.39 | 82.05 | 81.09 | 77.73 | 78.60 | 76.76 | 76.75 | 80.17 | 80.66 |
| nad5        | 71.93 | 74.81 | 78.17 | 73.75 | 73.64 | 78.93 | 74.03 | 73.62 | 72.87 | 74.81 | 72.88 | 73.82 | 74.69 |
| nad6        | 77.19 | 81.46 | 82.89 | 80.57 | 76.26 | 81.74 | 81.98 | 81.11 | 79.11 | 82.44 | 77.56 | 80.17 | 80.04 |
| rrnS        | 75.48 | 76.04 | 76.85 | 75.84 | 73.59 | 80.50 | 76.56 | 75.84 | 74.71 | 74.55 | 76.09 | 75.68 | 75.30 |
| rrnL        | 77.78 | 80.78 | 80.25 | 79.55 | 76.70 | 81.81 | 78.65 | 77.71 | 76.95 | 79.40 | 79.05 | 77.43 | 79.01 |
| AT-loop     | 83.37 | 85.11 | 86.49 | 85.91 | 74.75 | 96.75 | 83.68 | 79.93 | 85.32 | 86.36 | 88.50 | 79.57 | 83.71 |
| Entire      | 73.54 | 75.46 | 77.67 | 74.16 | 72.72 | 79.11 | 75.54 | 74.17 | 73.30 | 75.14 | 73.73 | 74.57 | 74.59 |

*Nematodes: AV: Acanthocheilonema viteae, BM: Brugia malayi, CQ: Chandlerella quiscali, DI: Dirofilaria immitis, DM: Dracunculus medinensis, HL: Helicometra longissimum, LL: Loa loa, OF: Onchocerca flexuosa, OV: Onchocerca volvulus, SD: Setaria digitata, SL: Spirocerca lupi, TC: Thelazia callipaeda, WB: Wuchereria bancrofti, Entire: entire mt genome.*
analyzing ribosomal RNA genes, putative secondary structures of 22 tRNA genes were identified using tRNAscan-SE [28], of the 22 tRNA genes, 14 were identified using tRNAscan-SE, the other 8 tRNA genes were found by eye inspection, and rRNA genes were identified by comparison with that of spirurid nematodes [21,27].

Phylogenetic analysis

The amino acid sequences conceptually translated from individual genes of the mt genome of *S. lupi* were concatenated. Selected for comparison were concatenated amino acid sequences predicted from published mt genomes of key nematodes representing the order Spirurida, including the superfamilies Thelazoidea (*T. callipaeda* [21]), Filarioidea (*Acanthocheilonema vitae* [29], *Brugia malayi* [30], *Chandlerella quisca* [29], *Dirofilaria immitis* [31], *Loa loa* [29], *Onchocerca flexuosa* [29], *O. volvulus* [32], *S. digitata* [27] and *Wuchereria bancrofti* [18]), Dracunculoidea (*Dracunculus medinensis* [33]) and Physalopteroidea (*Heliconema longissimum* [33]) (GenBank accession numbers JX069968, NC_016197, NC_004298, NC_014486, NC_005305, NC_016199, NC_0

| Amino acid | Codon | Number | Frequency (%) | Amino acid | Codon | Number | Frequency (%) |
|------------|-------|--------|---------------|------------|-------|--------|---------------|
| Phe        | TTT   | 591    | 17.03         | Met        | ATA   | 52     | 1.49          |
| Phe        | TTC   | 16     | 0.46          | Met        | ATG   | 103    | 2.96          |
| Leu        | TTA   | 195    | 5.61          | Thr        | ACT   | 81     | 2.33          |
| Leu        | TTG   | 235    | 6.77          | Thr        | ACC   | 3      | 0.08          |
| Ser        | TCT   | 139    | 4.00          | Thr        | ACA   | 2      | 0.05          |
| Ser        | TCC   | 7      | 0.20          | Thr        | ACG   | 3      | 0.08          |
| Ser        | TCA   | 8      | 0.23          | Asn        | AAT   | 87     | 2.50          |
| Ser        | TCG   | 6      | 0.17          | Asn        | AAC   | 6      | 0.17          |
| Tyr        | TAT   | 214    | 6.16          | Lys        | AAA   | 42     | 1.21          |
| Tyr        | TAC   | 6      | 0.17          | Lys        | AAG   | 56     | 1.61          |
| Stop       | TAA   | 7      | 0.20          | Ser        | AGT   | 99     | 2.85          |
| Stop       | TAG   | 5      | 0.14          | Ser        | AGC   | 5      | 0.14          |
| Cys        | TGT   | 75     | 2.16          | Ser        | AGA   | 22     | 0.63          |
| Cys        | TGC   | 3      | 0.08          | Ser        | AGG   | 30     | 0.86          |
| Trp        | TGA   | 36     | 1.03          | Val        | GTT   | 239    | 6.88          |
| Trp        | TGG   | 56     | 1.61          | Val        | GTC   | 5      | 0.14          |
| Leu        | CTT   | 19     | 0.54          | Val        | GTA   | 35     | 1.00          |
| Leu        | CTG   | 2      | 0.05          | Ala        | GCC   | 4      | 0.11          |
| Pro        | CCT   | 55     | 1.58          | Ala        | GCA   | 1      | 0.02          |
| Pro        | CCC   | 7      | 0.20          | Ala        | GCG   | 10     | 0.28          |
| Pro        | CCA   | 6      | 0.17          | Asp        | GAT   | 66     | 1.90          |
| Pro        | CCG   | 9      | 0.25          | Asp        | GAC   | 2      | 0.05          |
| His        | CAT   | 52     | 1.49          | Glu        | GAA   | 31     | 0.89          |
| His        | CAC   | 1      | 0.02          | Glu        | GAG   | 42     | 1.21          |
| Gln        | CAA   | 20     | 0.57          | Gly        | GGT   | 143    | 4.12          |
| Gln        | CAG   | 31     | 0.89          | Gly        | GGC   | 12     | 0.34          |
| Arg        | CGT   | 46     | 1.32          | Gly        | GGA   | 33     | 0.95          |
| Arg        | CGC   | 1      | 0.02          | Gly        | GGG   | 72     | 2.07          |
| Arg        | CGA   | 3      | 0.08          | Ile        | ATT   | 212    | 6.10          |
| Arg        | CGG   | 6      | 0.17          | Ile        | ATC   | 4      | 0.11          |

Table 4 Codon usage of *Spirocerca lupi* mitochondrial protein-coding genes

Total number of codons is 3,470.

Stop = Stop codon.
Results and discussion

General features of the *S. lupi* mt genome

The complete mtDNA sequence of *S. lupi* was 13,780 bp in size (Figure 1), and has been deposited in the GenBank under the accession number KC305876. The mt genome of *S. lupi* contains 12 protein-coding genes (cox1-3, nad1-6, nad4L, atp6 and cyt b), 22 transfer RNA genes, two ribosomal RNA genes (rrnL and rrnS) and a non-coding (control or AT-rich) region, but lacks an atp8 gene (Table 2). All genes are transcribed in the same direction. The gene order is identical to those of *T. callipaeda* and *S. digitata* [21,27], but distinct from those of *H. longissimum* (rearrangement markedly) and *Dracunculus medinensis* (tRNA-Met and tRNA-Val change) [33]. The nucleotide compositions of *S. lupi* mt genome are biased toward A and T, with T being the most favored nucleotide and C being the least favored, in accordance with mt genomes of other spirurid nematodes [27,31]. The content of A + T is 73.73% for *S. lupi*, similar to that of mt genomes of other spirurid nematodes sequenced to date, such as that of *T. callipaeda* (74.57%) [21] and *W. bancrofti* (74.59%) [18] (Table 3). Furthermore, the *S. lupi* mt genes overlap a total of 98 bp in 16 locations ranging from 1 to 32 bp (Table 2). The longest is a 32 bp overlap between *nad1* and tRNA-Phe. The mt genome of *S. lupi* has 150 bp of intergenic regions at 16 locations ranging in size from 1 bp to 59 bp, the longest intergenic region is a 59 bp between tRNA-Pro and tRNA-Asp (Table 2). The mt genome of *T. callipaeda* has 14 intergenic regions, which range from 1 to 62 bp in length. The longest region is 62 bp between tRNA-Pro and tRNA-Asp [21].

Protein-coding genes

The *S. lupi* mt genome encodes 12 protein-coding genes, which are identical to those of *T. callipaeda* and *S. digitata* [21,27]. For *S. lupi*, the sizes of the protein-coding genes were in the order: *cox1* > *nad5* > *nad4* > *cyt b* > *nad1* > *nad2* > *cox3* > *cox2* > *atp6* > *nad6* > *nad3* > *nad4L* (Table 2). The predicted translation initiation and termination codons for the 12 protein-coding genes of *S. lupi* mt genome were compared with that of *T. callipaeda* and *S. digitata* [21,27]. The most common initiation codon for *S. lupi* is TTG (5 of 12 protein genes), followed by ATG (4 of 12 protein genes), ATT (2 of 12 protein genes) and ATA (1 of 12 protein genes) (Table 2). In this mt genome, all protein genes were predicted to have a TAA or TAG as termination codon (Table 2). Although incomplete termination codons (T or TA) are present in some other nematodes, including *Anisakis simplex* (s. l) [38], *A. suum* [39], *Caenorhabditis elegans* [39], *S. digitata* [27], *Toxocara* spp. [40] and *Trichinella spiralis* [41], they were not identified in the *S. lupi* mt genome.

Excluding the termination codons, a total of 3,458 amino acids of protein-coding genes are encoded by the

![Figure 2](http://www.parasitesandvectors.com/content/6/1/45)

**Figure 2** Relationship of *Spirocerca lupi* with other selected spirurid nematodes based on mitochondrial sequence data. The concatenated amino acid sequences of 12 protein-coding genes were subjected to analysis by Bayesian inference (BI) using *Ascaris suum* as the outgroup. Posterior probability (pp) values are indicated.
S. lupi mt genome. Table 4 shows the codon usage. Codons composed of A and T are predominantly used, which seems to reflect the high A + T content of the mt genome of S. lupi. A strong preference for A + T rich codons usage is found in mtDNA of S. lupi. For example, the most frequently used amino acid was Phe (TTT: 17.03%), followed by Leu (TTG: 6.77%), Tyr (ATA: 6.16%) and Ile (ATT: 6.10%). This result is consistent with a recent study [21].

Transfer RNA genes and ribosomal RNA genes
The sizes of 22 tRNA genes identified in the S. lupi mt genome ranged from 50 to 62 bp in size. Secondary structures predicted for the 22 tRNA genes of S. lupi (not shown) are similar to that of S. digitata [27]. The rrnL and rrnS genes of S. lupi were identified by comparison with the mt genomes of T. callipaeda and S. digitata. The rrnL is located between tRNA-His and nad3, and rrnS is located between nad4L and tRNA-Tyr. The lengths of the rrnL and rrnS genes were 988 bp and 686 bp for S. lupi, respectively (Table 2). The A + T contents of the rrnL and rrnS genes for S. lupi are 79.05% and 76.09%, respectively.

Non-coding regions
The majority of nematode mtDNA sequences contain usually two non-coding regions of significant size difference, the long non-coding region and the short non-coding region, including A. lumbricoides and A. suum [34], Contracaecum rudolphi and Oesophagostomum spp. [43], Toxocara spp. [40] and Trichuris spp. [44,45]. However, there is only one non-coding region (AT-rich region) in the mt genome of S. lupi, which is located between cox3 and tRNA-Ala (Figure 1 and Table 2), with 88.50% A + T content (Table 3). This region of the mt genome of S. lupi was considered as a non-coding region (or AT-rich region) due to its location and AT rich feature based on comparison with those of spirurid nematodes reported previously [21,27]. Moreover, in the AT-rich region of S. lupi consecutive sequences [A]13 and [T]12 were found, but there are no AT dinucleotide repeat sequences similar to that of A. simplex s.l. and S. digitata in the this region [27,38].

Phylogenetic analyses
The phylogenetic relationships of 12 spirurid species based on concatenated amino acid sequence datasets, plus the mtDNA sequence of S. lupi obtained in the present study, using BI is shown in Figure 2. The results revealed that S. lupi (Thelaziidae) was a sister taxon to a clade containing S. digitata (Setariidae) and other members of the Onchocercidae, including B. malayi and D. immitis (posterior probability = 1.00), consistent with results of previous studies [14,21,46].

Many studies have demonstrated that mtDNA sequences are valuable genetic markers for phylogenetic studies of members within the Nematoda. A recent study analyzed mt sequence variations in human- and pig-derived Trichuris and demonstrated that they represent separate species [44]. In addition, a previous study sequenced and compared the mt genomes of A. lumbricoides and A. suum from humans and pigs and indicated that A. lumbricoides and A. suum may represent the same species [34]. In the present study, the characterization of the mt genome of S. lupi can promote to re-assess the systematic relationships within the order Spirurida using mt genomic datasets. For many years, there have been considerable debates about the phylogenetic position of members of spirurid nematodes [47,48]. Given this utility of mt genomic datasets, thus, further work should sequence more mt genomes of spirurid nematodes and re-construct the phylogenetic relationships of spirurid nematodes using expanded mt datasets.

Conclusions
The present study determined the complete mt genome sequence of S. lupi, and ascertained its phylogenetic position within the Spirurida. These new mtDNA data will provide useful novel markers for studying the molecular epidemiology and population genetics of S. lupi, and have implications for the diagnosis, prevention and control of spirocercosis in canid animals.

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

Authors’ contributions
XQZ and XLY conceived and designed the study, and critically revised the manuscript. GHL, YW and HQS performed the experiments, analyzed the data and drafted the manuscript. MWL and LA helped in study design, study implementation and manuscript revision. All authors read and approved the final manuscript.

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References
1. Chikweto A, Bahayat MI, Tiwari KP, de Allie C, Sharma RN: Sporoceiosis in owned and stray dogs in Grenada. Vet Parasitol 2012, 190:613–616.
2. van der Meer J, Keibergen RM, Cifor S, Williams M, Keller N, Naidoo V: Sporoceiosis lupi infection in the dog: a review. Vet J 2008, 176:294–309.
3. Mazaki-Tovi M, Baneth G, Aroch I, Harris S, Kess PH, Bondi T, Zir G, Azenberg I, Bark H, Levy T: Canine sporoceiosis: clinical, diagnostic, pathological and epidemiologic characteristics. Vet Parasitol 2002, 107:235–250.
4. Ranen E, Levy A, Azenberg I, Perl S, Harris S: Sporoceiosis associated esophageal sarcomas in dogs. A retrospective study of 17 cases (1997–2003). Vet Parasitol 2004, 119:209–221.
5. Rinas WA, Nesnek R, Kirismitz DM, Defatte: Fatal aortic aneurysm and rupture in a neutropicus dog (Sporoceiosis venenatus) caused by Sporoceiosis lupi. Vet Parasitol 2009, 164:347–349.
6. Levy A, Aroch I, Bark H, Markovics A, Aizenberg I, Bark H: Levy A, Markovics A, Aizenberg I: Sporoceiosis is a widespread zoonotic disease in Egypt. Vet Parasitol 2002, 109:65–73.
7. Shubhagata D, Abdul A, Mohammad MH, Suchandan S, Muraduzzaman M: Investigation of parasites in dogs in Sichuan province. Int J Parasitol 2003, 33:153–158.
8. Ranen E, Levy A, Aroch I, Bark H, Markovics A, Aizenberg I: Sporoceiosis is a widespread zoonotic disease in Egypt. Vet Parasitol 2002, 109:65–73.
9. Chikweto A, Bahayat MI, Tiwari KP, de Allie C, Sharma RN: Sporoceiosis in owned and stray dogs in Grenada. Vet Parasitol 2012, 190:613–616.
10. van der Meer J, Keibergen RM, Cifor S, Williams M, Keller N, Naidoo V: Sporoceiosis lupi infection in the dog: a review. Vet J 2008, 176:294–309.
11. Mazaki-Tovi M, Baneth G, Aroch I, Harris S, Kess PH, Bondi T, Zir G, Azenberg I, Bark H, Levy T: Canine sporoceiosis: clinical, diagnostic, pathological and epidemiologic characteristics. Vet Parasitol 2002, 107:235–250.
12. Ranen E, Levy A, Azenberg I, Perl S, Harris S: Sporoceiosis associated esophageal sarcomas in dogs. A retrospective study of 17 cases (1997–2003). Vet Parasitol 2004, 119:209–221.
13. Rinas WA, Nesnek R, Kirismitz DM, Defatte: Fatal aortic aneurysm and rupture in a neutropicus dog (Sporoceiosis venenatus) caused by Sporoceiosis lupi. Vet Parasitol 2009, 164:347–349.
14. Levy A, Aroch I, Bark H, Markovics A, Aizenberg I, Bark H: Levy A, Markovics A, Aizenberg I: Sporoceiosis is a widespread zoonotic disease in Egypt. Vet Parasitol 2002, 109:65–73.
15. Shubhagata D, Abdul A, Mohammad MH, Suchandan S, Muraduzzaman M: Investigation of parasites in dogs in Sichuan province. Int J Parasitol 2003, 33:153–158.
16. Ranen E, Levy A, Aroch I, Bark H, Markovics A, Aizenberg I: Sporoceiosis is a widespread zoonotic disease in Egypt. Vet Parasitol 2002, 109:65–73.
17. Chikweto A, Bahayat MI, Tiwari KP, de Allie C, Sharma RN: Sporoceiosis in owned and stray dogs in Grenada. Vet Parasitol 2012, 190:613–616.
18. van der Meer J, Keibergen RM, Cifor S, Williams M, Keller N, Naidoo V: Sporoceiosis lupi infection in the dog: a review. Vet J 2008, 176:294–309.
19. Mazaki-Tovi M, Baneth G, Aroch I, Harris S, Kess PH, Bondi T, Zir G, Azenberg I, Bark H, Levy T: Canine sporoceiosis: clinical, diagnostic, pathological and epidemiologic characteristics. Vet Parasitol 2002, 107:235–250.
20. Ranen E, Levy A, Azenberg I, Perl S, Harris S: Sporoceiosis associated esophageal sarcomas in dogs. A retrospective study of 17 cases (1997–2003). Vet Parasitol 2004, 119:209–221.
21. Rinas WA, Nesnek R, Kirismitz DM, Defatte: Fatal aortic aneurysm and rupture in a neutropicus dog (Sporoceiosis venenatus) caused by Sporoceiosis lupi. Vet Parasitol 2009, 164:347–349.
22. Levy A, Aroch I, Bark H, Markovics A, Aizenberg I, Bark H: Levy A, Markovics A, Aizenberg I: Sporoceiosis is a widespread zoonotic disease in Egypt. Vet Parasitol 2002, 109:65–73.
23. Shubhagata D, Abdul A, Mohammad MH, Suchandan S, Muraduzzaman M: Investigation of parasites in dogs in Sichuan province. Int J Parasitol 2003, 33:153–158.
24. Ranen E, Levy A, Aroch I, Bark H, Markovics A, Aizenberg I: Sporoceiosis is a widespread zoonotic disease in Egypt. Vet Parasitol 2002, 109:65–73.
25. Chikweto A, Bahayat MI, Tiwari KP, de Allie C, Sharma RN: Sporoceiosis in owned and stray dogs in Grenada. Vet Parasitol 2012, 190:613–616.
26. van der Meer J, Keibergen RM, Cifor S, Williams M, Keller N, Naidoo V: Sporoceiosis lupi infection in the dog: a review. Vet J 2008, 176:294–309.
27. Mazaki-Tovi M, Baneth G, Aroch I, Harris S, Kess PH, Bondi T, Zir G, Azenberg I, Bark H, Levy T: Canine sporoceiosis: clinical, diagnostic, pathological and epidemiologic characteristics. Vet Parasitol 2002, 107:235–250.
28. Ranen E, Levy A, Azenberg I, Perl S, Harris S: Sporoceiosis associated esophageal sarcomas in dogs. A retrospective study of 17 cases (1997–2003). Vet Parasitol 2004, 119:209–221.
29. Rinas WA, Nesnek R, Kirismitz DM, Defatte: Fatal aortic aneurysm and rupture in a neutropicus dog (Sporoceiosis venenatus) caused by Sporoceiosis lupi. Vet Parasitol 2009, 164:347–349.
30. Levy A, Aroch I, Bark H, Markovics A, Aizenberg I, Bark H: Levy A, Markovics A, Aizenberg I: Sporoceiosis is a widespread zoonotic disease in Egypt. Vet Parasitol 2002, 109:65–73.
using mitochondrial cytochrome c oxidase subunit 1 (cox1) gene
analysis. Parasitol Res 2009, 104:979–984.
47. De Ley P, Blaxter M: Systematic position and phylogeny. In The Biology of
Nematodes. Edited by Lee DL. London and New York: Taylor & Francis;
2002:1–30.
48. Nadler SA, Carreno RA, Mejía-Madrid H, Ullberg J, Pagan C, Houston R,
Hugot JP. Molecular phylogeny of clade III nematodes reveals multiple
origins of tissue parasitism. Parasitology 2007, 134:1421–1442.

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