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The complete mitochondrial genome of *Strongylus equinus* (Chromadorea: Strongylidae): Comparison with other closely related species and phylogenetic analyses

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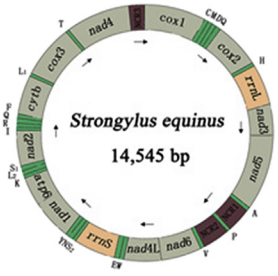
HIGHLIGHTS

- The complete mitochondrial sequence of *Strongylus equinus* was determined.
- The complete mtDNA sequence of *S. equinus* was 14,545 bp in length.
- *S. equinus* was genetically closer to *Strongylus vulgaris* than to *Cylicocyclus insignis*.

GRAPHICAL ABSTRACT



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ABSTRACT

The roundworms of genus *Strongylus* are the common parasitic nematodes in the large intestine of equine, causing significant economic losses to the livestock industries. In spite of its importance, the genetic data and epidemiology of this parasite are not entirely understood. In the present study, the complete *S. equinus* mitochondrial (mt) genome was determined. The length of *S. equinus* mt genome DNA sequence is 14,545 bp, containing 36 genes, of which 12 code for protein, 22 for transfer RNA, and two for ribosomal RNA, but lacks *atp*8 gene. All 36 genes are encoded in the same direction which is consistent with all other Chromadorea nematode mtDNAs published to date. Phylogenetic analysis based on concatenated amino acid sequence data of all 12 protein-coding genes showed that there were two large branches in the Strongyloidea nematodes, and *S. equinus* is genetically closer to *S. vulgaris* than to

Abbreviations: *S. vulgaris*, *Strongylus vulgaris*; *S. equinus*, *Strongylus equinus*; mt, mitochondrial; mtDNA, mitochondrial DNA; PCR, polymerase chain reaction; BI, Bayesian inference; MP, maximum parsimony; ML, maximum likelihood; *cox*1, cytochrome *c* oxidase subunit 1 gene; *nad*1, nicotinamide adenine dinucleotide dehydrogenase subunit 1 gene; *cyt*b, cytochrome b; *atp*6, adenosine triphosphatase subunit 6; tRNA, transfer RNA; rRNA, ribosomal RNA; AA, amino acid.

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Cylicocyclus insignis in Strongylidae. This new mt genome provides a source of genetic markers for the molecular phylogeny and population genetics of equine strongyles.

1. Introduction

Roundworms of the genus *Strongylus* are the common parasitic nematodes in the large intestine of horse, donkey, mule and other equine animals and can cause significant economic losses to the livestock industries worldwide (Tang and Tang, 2009). There are three important species in genus *Strongylus*, namely *S. equinus*, *S. edentatus* and *S. vulgaris*. The larvae of these worms migrate and cause aneurysms and emboli in the small arterioles of circulatory system, peritoneum inflammation, liver and pancreas damage (Tang and Tang, 2009; Gundłach and Sadzikowski, 2004; Studzińska et al., 2012). The mature nematodes live in the colon and cecum, fixating with large mouth capsules to intestinal mucous, and take up the blood, which may lead to anemia, diarrhea and edema in the case of a great number of worms (Tang and Tang, 2009; Studzińska et al., 2012). *S. equinus* is the common large intestine parasitic nematode affecting equines and has a worldwide distribution (Lichtenfels et al., 1998; Hinney et al., 2011; Kuzmina et al., 2012). China is also a major epidemic area (Tang and Tang, 2009; Shen and Huang, 2006).

In recent years, numerous complete mitochondrial DNA (mtDNA) genomes of metazoan species, including helminth, protozoa and arthropod have been deposited in GenBank and extensively used to investigate population genetic structures, systematics and phylogenetics (Hu et al., 2003; Hu and Gasser, 2006; Ogedengbe et al., 2013; Duan et al., 2015). Although Nematoda is the second largest animal phylum (Li et al., 2008), only ~90 complete mt genomes of animal parasitic nematodes have been sequenced to date. In horse parasitic nematodes, only *S. vulgaris*, *Parascaris univalens* and *Cylicocyclus insignis* mt genomes have been determined (Jex et al., 2010; Jabbar et al., 2014). The paucity of information on mt genome dataset of parasitic nematodes is a limitation for population genetics and phylogenetics studies of horse's parasitic nematodes. The objectives of the present study were to determine the complete *S. equinus* mt genome sequence, another important parasitic nematode of genus *Strongylus*, and to make comparative analysis with those of other closely related Strongyloidea worms.

2. Materials and methods

2.1. Parasites and extraction of genomic DNA

This study was approved by the Animal Ethics Committee of Heilongjiang Bayi Agricultural University (Approval No. HBAU 2010-007). Adult specimens of *S. equinus* were collected from colon and cecum of naturally infected horse in Daqing, Heilongjiang Province, in accordance with the Animal Ethics Procedures and Guidelines of the People's Republic of China. Nematodes were washed in physiological saline, identified to species according to their primarily morphologically characters using existing keys and descriptions (Lichtenfels et al., 2008; Tang and Tang, 2009), fixed in 70% (v/v) ethanol, and stored at −20 °C until DNA extraction. Total genomic DNA was extracted from single adult worm using sodium dodecyl-sulphate/proteinase K treatment (Gao et al., 2014), followed by spin-column purification (Wizard Clean-Up, Promega; Madison, WI, USA).

2.2. The complete mtDNA enzymatic amplification, sequencing and full mt cox1 sequence variation analysis of 16 individual S. Equines worms

The whole mt genome of *S. equinus* was amplified by PCR using ten primers (Table 1) which designed directly from conserved regions of *S. vulgaris* (GQ888717.2) and other Strongylidae nematodes. One microlitre of DNA template was used in a PCR reaction of 25 μL containing 18.3 μL of H_2O, 2.5 μL of Ex *Taq* buffer (pH 8.5) (Takara; Dalian, China), 2 μL of dNTP mixture (2.5 mM) (Takara; Dalian, China), 0.5 μL of each primer (10 pmol/mL) and 0.2 μL of Ex *Taq* DNA polymerase (5 U/mL) (Takara; Dalian, China) in a thermocycler (TaKaRa TP600; Dalian, China) under the following conditions: 94 °C for 5 min (initial denaturation), followed by 35 cycles of 94 °C for 1 min (denaturation); 44.9–51.5 °C for 1 min (annealing); 72 °C for 1.5 min (extension), and then followed by a final extension at 72 °C for 7 min. Each amplicon was examined by agarose gel (Sangon Biotech; Shanghai, China) (1%) electrophoresis and ethidium bromide staining. The positive PCR products were sent to Life Technology Company (Beijing, China) for sequencing using the primers used in primary amplifications. The full mt *cox*1 sequence was PCR-amplified from 16 individual DNA samples using primers designed as above and then sequence variation in full *cox*1 gene were assessed among 16 individuals *S. equinus* and *S. vulgaris* worms.

2.3. The features of complete mitochondrial genome sequence and comparative analysis with other closely related Strongyloidea worms

The gene annotation for the 12 protein-coding genes and two ribosomal RNA genes was made by identifying each gene boundary based on sequence comparison with other nematode species. Putative secondary structures of 22 tRNA genes were identified using the tRNAscan-SE program (Lowe and Eddy, 1997) or by manually

Table 1
PCR primer used in this study.

Primer	Sequence (5′–3′)
*cox*1-F	TATAAAAAGTATCAAGGCGGTTTGT
*cox*1-R	ATTACTAAACGATAACCCCCTAATG
*cox*1-*rrn*L-F	TATGGATTATCCCGATGTGTATTCT
*cox*1-*rrn*L-R	AATTTTTAATTTTTCTACCTTTTAA
*rrn*L-*nad*5-F	TTTTAAGAAAATTTTATGGTTTATA
*rrn*L-*nad*5-R	TAACCCCTAAAAACAGCTCCACTAA
*nad*5-*nad*6-F	ACGGCTTTAACTAATCGAATTGGTG
*nad*5-*nad*6-R	TTATAATTCCATTAAAACTACATAC
*nad*6-*rrn*S-F	GGGTTATTAAGAAGAATTTTACTGA
*nad*6-*rrn*S-R	ATAAAAGTAGTTAATGAGGGTTCTC
*rrn*S-*nad*1-F	TTCTAAATTATCTTTGGAGGTTGAG
*rrn*S-*nad*1-R	CAATGAAATAGGCAAAAACTTAAAC
*nad*1-*nad*2-F	TTTATTCGAAGGTCATATCCACGTT
*nad*1-*nad*2-R	AATACCAATATAGTTTCTCAACCCA
*nad*2-*cyt*b-F	ATTTTTAATATTGTTAATTATTCCT
*nad*2-*cyt*b-R	TAAATAATAAAGCCACCACACCTAA
*cyt*b-*nad*4-F	ATTGAGGCTGATCCTATAATAAGTC
*cyt*b-*nad*4-R	AGGAAATGAACAAATGGCCGCATAA
*nad*4- *cox*1-F	TTAGTTATAATTTTGGGTTATGGAG
*nad*4- *cox*1-R	ATGTCCCAATATCTTTATGATTAGA