

## The complete mitogenome of *Toxascaris leonina* from the Siberian tiger (*Panthera tigris altaica*)

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### ABSTRACT

*Toxascaris leonina* is a polyxenical parasite and commonly found in canids and felids. In this study, we used the Illumina high throughput sequencing and assembly to determine the complete mitogenome of a representative of this parasite from the Siberian tiger (*Panthera tigris altaica*). The genome was 14,248 bp in size and encoded 12 protein-coding genes, 22 transfer RNAs, and two ribosomal RNAs. Phylogeny showed that two canid (dog)-originated *T. leonina* were phylogenetically distinct from two felid-originated *T. leonina* (tiger isolate and cheetah isolate), suggesting at least two distinct subclades of *T. leonina* infecting these hosts and supporting once again that *T. leonina* represents a species complex. Furthermore, four isolates of *T. leonina* grouped together and were more closely related to other species from the family Ascarididae than species of families Toxocaridae, Anisakidae and Ascaridiidae, demonstrating phylogenetic stability of these paraphyletic groups characterized in this study. These cumulative mitochondrial DNA data provide a better understanding of phylogenetic relationships of this polyxenical and zoonotic roundworm species.

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The roundworm *Toxascaris leonina* is a polyxenical parasite and its host range includes the domestic dogs and cats as well as wild canids and felids, such as wolves, foxes, tigers, and lions (Okulewicz et al. 2012; Fogt-Wyrwas et al. 2019; Jin et al. 2019). Unlike other roundworms, *T. leonina* lifecycle is simple and infections often follow by oral ingestion of the infective eggs (Sprent 1959). Then, the egg-hatched larvae penetrate the mucosal lining of the small intestine and settle down there for development. After several molts, these larvae return to the intestinal lumen where they mature and mate, and the females release eggs into the feces. The clinical symptoms contain diarrhea, emesis, abdominal discomfort, and even life-threatening blockage of intestinal obstruction (Lee et al. 2010). Humans can also be infected through interaction with dogs or cats, or ingestion of eggs in contaminated food. Until now there have been several clinic cases of human infections with *T. leonina* characterized and often involved to aberrant larva migrans in the eye (ocular larva migrans, OLM) and the viscera (visceral larva migrans, VLM) (Robertson and Thompson 2002; Okulewicz et al. 2012). Moreover, increased epidemiological evidences suggest that *T. leonina* is emerging as an underestimated zoonotic agent because of close relationships between humans and their pets (e.g. dogs and cats) and increased interactions between

people and wildlife hosts in zoos (Robertson and Thompson 2002; Li et al. 2007; Li et al. 2008; Okulewicz et al. 2012; Xie et al. 2020). Such situations highlight the significance and necessity for diagnosis and identification of *T. leonina*. Unfortunately, current diagnosis and identification of this parasite still rely on morphological characteristics, host sources and geographical distributions although these criteria are not always sufficient to distinguish the morphologically similar species (Gasser 2006; Chen et al. 2012; Fogt-Wyrwas et al. 2019; Xie et al. 2020). Under such context, obtaining a more efficient approach to identify *T. leonina* infections has become urgent for clinical diagnosis and epidemiological investigation, and achieving this goal is foreseeable only through utilization of molecular methodologies. Numerous studies have demonstrated that the mitogenomic DNA is an informative marker and has been widely used for species-specific identification in many zoonotic nematodes (Hu and Gasser 2006; Hu et al. 2004). Herein, we reported the complete mitogenome of a representative *T. leonina* isolated from its wildlife host, the Siberian tiger.

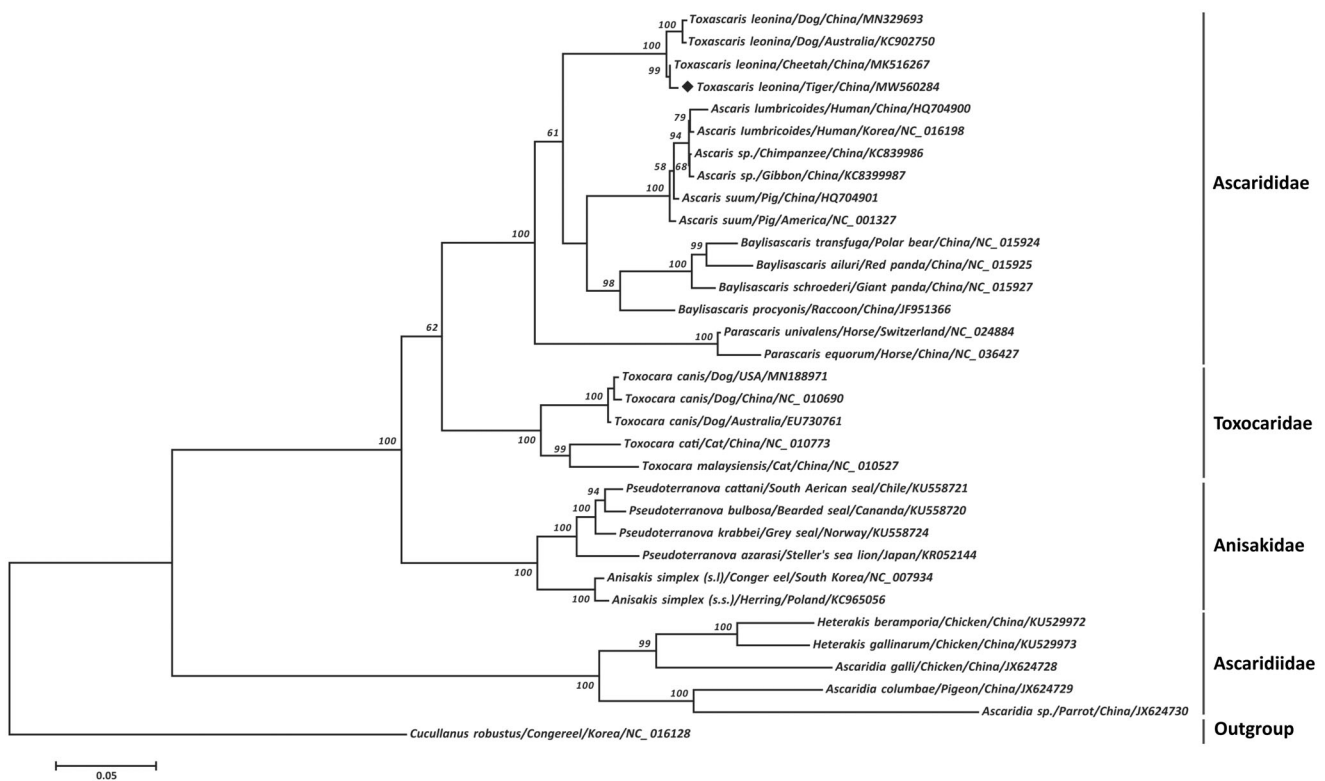
In November 2018, four roundworm samples were collected from a naturally infected Siberian tiger (*Panthera tigris altaica*) provided by the Veterinary Hospital, Chengdu Zoo (30°42'N, 104°06'E), Sichuan of China, after treatment with

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**Figure 1.** Phylogenetic relationships among roundworm nematodes. The maximum likelihood phylogenetic tree was inferred from concatenated amino-acid sequences of 12 mitochondrial protein-coding genes of *T. leonina* and other related nematodes, utilizing MtArt + I model selected by TREEFINDER under the corrected Akaike's Information Criterion and 1,000,000 bootstrap replications (<50% support not shown). The solid black diamond represents the species in this study.

pyrantel pamoate. Following morphological identification (Sprent 1959) and molecular sequencing (Zhu et al. 1999), these parasite specimens were determined as *T. leonina* adults with one male and three females. One female was subsequently used for extraction of genomic DNA and the remaining were formalin-fixed and archived in the Parasitological Museum of Sichuan Agricultural University (<https://dop.sicau.edu.cn/>; xyue1985@gmail.com (Yue Xie)) under collection numbers XY2018\_23-25. Total genomic DNA was extracted using the Universal Genomic DNA Extraction Kit (TaKaRa, Dalian, China). After quality and quantity assessment, ~5 µg genomic DNA was sheared into c. 350 bp fragments to construct a paired-end (PE) library, followed by sequencing on the Illumina HiSeq X Ten platform (BerryGenomics, Beijing, China). The clean reads (~3.5 Gb) were assembled with MITObim (Hahn et al. 2013) using the mitogenome of cheetah *T. leonina* as the reference. The completeness and accuracy of this assembly was validated by multiple whole-genome alignment at the nucleotide level using species of *Ascaris* and *Baylisascaris* for which complete mitogenome sequences are available. Annotation was performed using MITOS (Bernt et al. 2013) with manual adjustment on the basis of whole genome-guided gene alignments and online BLAST against Nt and Nr databases. The complete genome sequence was deposited in GenBank under accession number: MW560284.

The mitogenome of the tiger *T. leonina* was 14,248 bp in size and encoded 12 protein-coding genes (PCGs), 22 tRNAs, and two rRNAs. Similar to congeneric species including those from the dog and cheetah (Liu et al. 2014; Jin et al. 2019; Xie

et al. 2019), all of these genes were located on the same strand and transcribed in one direction. Twelve PCGs, except *nad2* and *nad5* genes deduced to use an incomplete stop codon 'T', were predicted to use the typical TAG as the stop codons. Twenty-two tRNAs ranged from 52 bp (tRNA<sup>(AGN)</sup>-Ser) to 62 bp (tRNA-Lys) in size, the same as those of the dog *T. leonina* (Xie et al. 2019). Two rRNAs, the small rRNA (rrnS; 700 bp) and large (rrnL; 959 bp) subunits, presented between tRNA-Glu and tRNA<sup>(UCN)</sup>-Ser and between tRNA-His and *nad3*, respectively. In addition, two non-coding regions, namely NC1 (also known as AT-rich region; 1000 bp) and NC2 (100 bp), were located between tRNA<sup>(UCN)</sup>-Ser and tRNA-Asn and between *nad4* and *cox1*, respectively, in accordance with other roundworm species, suggesting their conservation and function in regulation of transcription and DNA replication (Clayton 1991).

On the basis of the available complete mitogenomes of Ascaridida species retrieved from GenBank, an alignment of 3,445 amino acid loci was yielded from the concatenated 12 PCGs of 33 nematode parasites using MAFFT v7.271 (Katoh 2002) and GBLOCKS v0.91b (Castresana 2000) for phylogeny. A maximum-likelihood (ML) tree was subsequently constructed with PHYML 3.0 (Guindon and Gascuel 2003) using *Cucullanus robustus* as the outgroup and edited for display using FigTree v1.4.3 (<http://tree.bio.ed.ac.uk/software/figtree/>). As shown in Figure 1, this phylogenetic tree clearly showed that two dog-originated *T. leonina* (Chinese isolate and Australian isolate) were phylogenetically distinct from two felid-originated *T. leonina* (tiger isolate and cheetah isolate), consistent with recent molecular studies (Jin et al. 2019;

Fogt-Wyrwas et al. 2019; Xie et al. 2020), suggesting at least two distinct subclades of *T. leonina* infecting these hosts and supporting that *T. leonina* represents a species complex. Furthermore, four *T. leonina* isolates of grouped together, regardless of isolate origins and hosts, and were more closely related to other species from the family Ascarididae than species of families Toxocaridae, Anisakidae, and Ascaridiidae, demonstrating phylogenetic stability of these paraphyletic groups characterized in this study. Taken together, these cumulative mitochondrial DNA data provide a better understanding of classification and phylogenetic relationships of this polyxenical and zoonotic roundworm species.

## Disclosure statement

No potential conflict of interest was reported by the authors.

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## Data availability statement

The data that support the findings of this study are openly available in GenBank of NCBI at <https://www.ncbi.nlm.nih.gov>, under the accession number MW560284. Associated BioProject, SRA, and BioSample numbers are PRJNA673292, SRR12950074, and SAMN16598390, respectively.

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