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# Characterization of the complete mitochondrial genome sequence of the dog roundworm *Toxascaris leonina* (Nematoda, Ascarididae) from China

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**ABSTRACT**

*Toxascaris leonina* (Nematoda, Ascarididae) is a cosmopolitan nematode of canids and felids and poses potential threats to public health due to aberrant larva migrans. Herein, the complete mitochondrial genome sequence of a representative of this nematode from the dog in China was determined using next-generation sequencing technology. The assembled genome was 14,357 bp in length and encoded 36 genes, including 12 protein-coding genes, 22 transfer RNAs and 2 ribosomal RNAs. The phylogeny revealed that the canid-originated *T. leonina* were phylogenetic distinctiveness from the felid-originated *T. leonina* within the genus Toxascaris of Ascarididae, supporting that *T. leonina* may represent a species complex.

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*Toxascaris leonina* (Nematoda, Ascarididae) is a common intestinal nematode parasite of canids and felids (Okulewicz et al. 2012; Fogt-Wyrwas et al. 2019). Unlike other roundworms, the life cycle of *T. leonina* is simple and infections generally follow by oral ingestion of the infective eggs, and then the egg-hatched larvae mature in the small intestine of the definitive hosts (Sprent 1959). Humans as accidental hosts become infected by direct contact with dogs or cats or ingestion of eggs-contaminated food (Robertson and Thompson 2002). Although rare cases of human infections with *T. leonina* were reported so far, the aberrant larva migrans may substantially affect the eye (ocular larva migrans, OLM) and the viscera (visceral larva migrans, VLM) (Robertson and Thompson 2002; Okulewicz et al. 2012). For instance, one OLM case was found in a child in East Africa by Beaver and Bowman (1984). Epidemiological studies suggest that after *T. leonina* may be emerging as another underestimated zoonotic agent because of close relationships between humans and their pets (such as dogs and cats) and increased interactions between people and wildlife hosts (such as wolves and foxes) in conservation centres and zoos (Robertson and Thompson 2002; Li et al. 2007, 2008; Okulewicz et al. 2012). Such situations highlight the significance of diagnosis and identification of *T. leonina*. However, current diagnosis and identification of this worm is largely based on morphology and often misdiagnosed even by experienced microscopists (Gasser 2006; Chen et al. 2012; Fogt-Wyrwas et al. 2019). In such context, obtaining a more efficient approach to identify *T. leonina* infection has become crucial for clinical diagnosis and epidemiological investigation, and achieving this goal is foreseeable only through utilization of molecular methodologies. Mitochondrial DNA (mtDNA) is regarded as an important molecular marker and has been widely used for species-specific identification and differentiation in many zoonotic nematodes (Hu et al. 2004; Hu and Gasser 2006). Here, we reported the complete mitochondrial genome sequence of a representative *T. leonina* from the dog in China.

The parasite samples were obtained from a stray dog housed in an animal shelter at Wenjiang (30°44′N, 103°55′E), Sichuan of China, after treatment with pyrantel pamoate. After morphological identification (Sprent 1959) and molecular sequencing (Zhu et al. 1999), two worm specimens were identified as adult females of *T. leonina*. Then, one worm was used for mtDNA extraction and another was fixed in 5% formalin solution and archived in the Parasitological Museum of Sichuan Agricultural University (Sichuan, China) under collection numbers XY2018_11. Total mtDNA was sequenced using the Illumina HiSeq platform (Novogene, Tianjin, China), and the assembly and annotation of the mitochondrial genome were carried out as previously described (Xie et al. 2019).

The complete mitochondrial genome sequence of *T. leonina* was 14,357 bp in length (GenBank accession no. MN329693) and encoded 12 protein-coding genes, 22 tRNAs,

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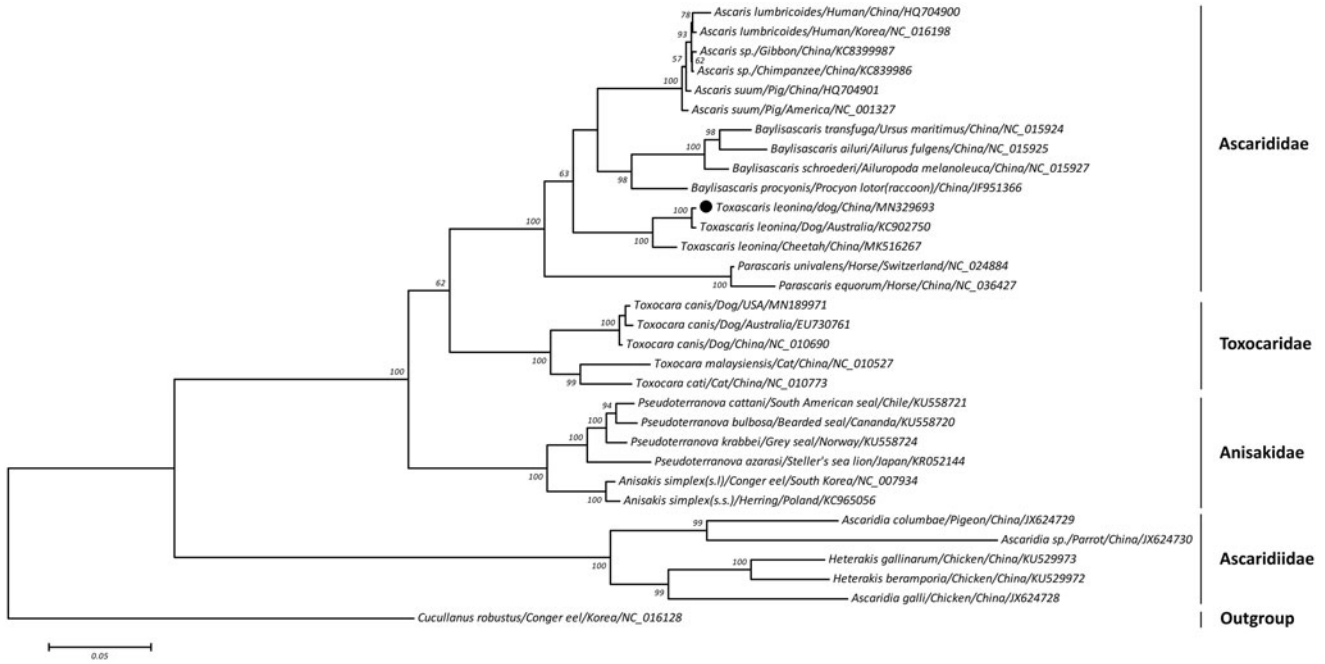


**Figure 1.** Maximum-likelihood tree inferred from concatenated amino-acid sequences of 12 mt protein-coding genes of *T. leonina* and other related nematodes, utilising MtArt + I + G model and after 10,000 bootstrap replications (<50% support not shown). The solid black circle represents the species in this study.

and 2 rRNAs. All genes were unidirectionally transcribed on the same strand, typical for other roundworms reported to date. Among the 12 protein-coding genes, except *nad2* and *nad5* deduced to use an incomplete stop codon 'T', the rest were predicted to use the typical TAG as the stop codons. Twenty-two tRNA genes ranged from 52 bp ($tRNA^{(AGN)}$-*Ser*) to 62 bp (*tRNA-Lys*) in length. Both *12S* and *16S rRNAs* were 700 and 959 bp in length, respectively, and located between *tRNA-Glu* and $tRNA^{(UCN)}$-*Ser* and between *tRNA-His* and *nad3*, respectively. Two non-coding regions, namely NC1 (also known as AT-rich region; 1000 bp) and NC2 (115 bp), were present between $tRNA^{(UCN)}$-*Ser* and *tRNA-Asn* and between *nad4* and *cox1*, respectively.

The maximum-likelihood (ML) phylogeny inferred from a concatenated amino acid dataset of 12 protein-coding genes from 32 nematode parasites clearly placed *T. leonina* together with other species from the family Ascarididae and separated from species of the families Toxocaridae, Anisakidae, and Ascaridiidae, with high bootstrap values (Figure 1). Within the genus *Toxascaris* of Ascarididae, two canid-originated *T. leonina* (one was from China and another was from Australia) clustered together, and were phylogenetically distinctive from felid-originated *T. leonina* (Chinese isolate), consistent with recent molecular studies (Fogt-Wyrwas et al. 2019; Jin et al. 2019), supporting that *T. leonina* may represent a species complex. Overall, the sequenced mitochondrial genome of *T. leonina* in this study adds novel molecular evidence for phylogenetic and taxonomic position of this roundworm species.

## Disclosure statement

No potential conflict of interest was reported by the authors.

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