

## Sequencing and analysis of the complete mitochondrial genome of dog roundworm *Toxocara canis* (Nematoda: Toxocaridae) from USA

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### ABSTRACT

The dog roundworm *Toxocara canis* (Nematoda: Toxocaridae) is an important zoonotic parasitic nematode and cause toxocarosis in human with a worldwide distribution. Herein, the complete mitochondrial genome of a representative of this nematode from USA was determined through next generation sequencing platform. The whole genome was 14,309 bp in size and encoded 12 protein-coding genes, 22 transfer RNAs, and 2 ribosomal RNAs. Phylogeny showed that although *T. canis* from USA and Australia were more closely related to each other than to that from Chinese, three *T. canis* isolates clustered together and formed paraphyletic relationships with *T. cati* and *T. malayensis*, supporting them as sister species among the family Toxocaridae. These cumulative mitochondrial DNA data should contribute to a better understanding of the phylogenetic relationship of this species.

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*Toxocara canis*; toxocarosis; mitochondrial genome; phylogeny

The dog roundworm *Toxocara canis* (Nematoda: Toxocaridae) serves canine species as definitive hosts and can be also transmitted to other animals including humans, in which the migrating larvae of *T. canis* cause visceral larva migrans, ocular larva migrans, neurological larva migrans and covert or common toxocarosis (Despommier 2003; Ma et al. 2018). The life cycle of *T. canis* is complex and high prevalence is frequently found in young canid populations. Adult roundworms inhabit in the small intestine of pups and shed huge eggs to the environment through feces. Humans as accidental hosts become infected by direct contact with dogs, ingestion of eggs-contaminated food or soil, or eating infected meat of paratenic host rodents (Ma et al. 2018; Smith et al. 2009). Human toxocarosis has more than 90% seroprevalence in tropical settings, and even for industrialized countries, the prevalence can range up to 42% in rural areas and to 5% in urban areas (Fan et al. 2015). For example, within the USA, a large-scale national survey showed a 13.9% age-adjusted seroprevalence of toxocarosis (Won et al. 2008; Hotez and Wilkins 2009). Although until now there has been significant knowledge progress about the biology of *T. canis*, there are still major gaps in the understanding of this parasite, especially in genetics and molecular epidemiology because of lacking suitable genetic markers (Gasser et al. 2005; Holland 2017). Mitochondrial DNA (mtDNA) is proven to be a valuable source of molecular markers and has been widely used for genetics and molecular identification of many zoonotic

parasites (Hu and Gasser 2006; Hu et al. 2004). Here, we reported the complete mitochondrial genome sequence of a representative *T. canis* from USA and added novel mtDNA data to this zoonotic parasite.

The parasite samples were obtained from an infected household dog raised by a 72-year-old female nursing home resident at Silver Spring (39°0'N, 77°1'E), Maryland, USA, after treatment with pyrantel pamoate. After morphological identification, the worm specimens ( $n=3$ ) were identified as *T. canis* females according to the taxonomic key of Skrjabin et al. (1991) and molecular confirmation by amplification and sequencing of the first and second internal transcribed spacers (ITS1/2) of nuclear ribosomal DNA (Zhu et al. 1998; Zhou et al. 2010). One worm was used for mtDNA extraction, and the remaining two were fixed in 5% formalin solution and archived in the Parasitological Museum of Sichuan Agricultural University (Sichuan, China) under voucher numbers XY2018\_5-6. The mtDNA was sequenced using the Illumina HiSeq platform (Novogene, Tianjin, China). The genome assembly was achieved using MITObim (Hahn et al. 2013), and gene annotation was developed by MITOS (Bernt et al. 2013).

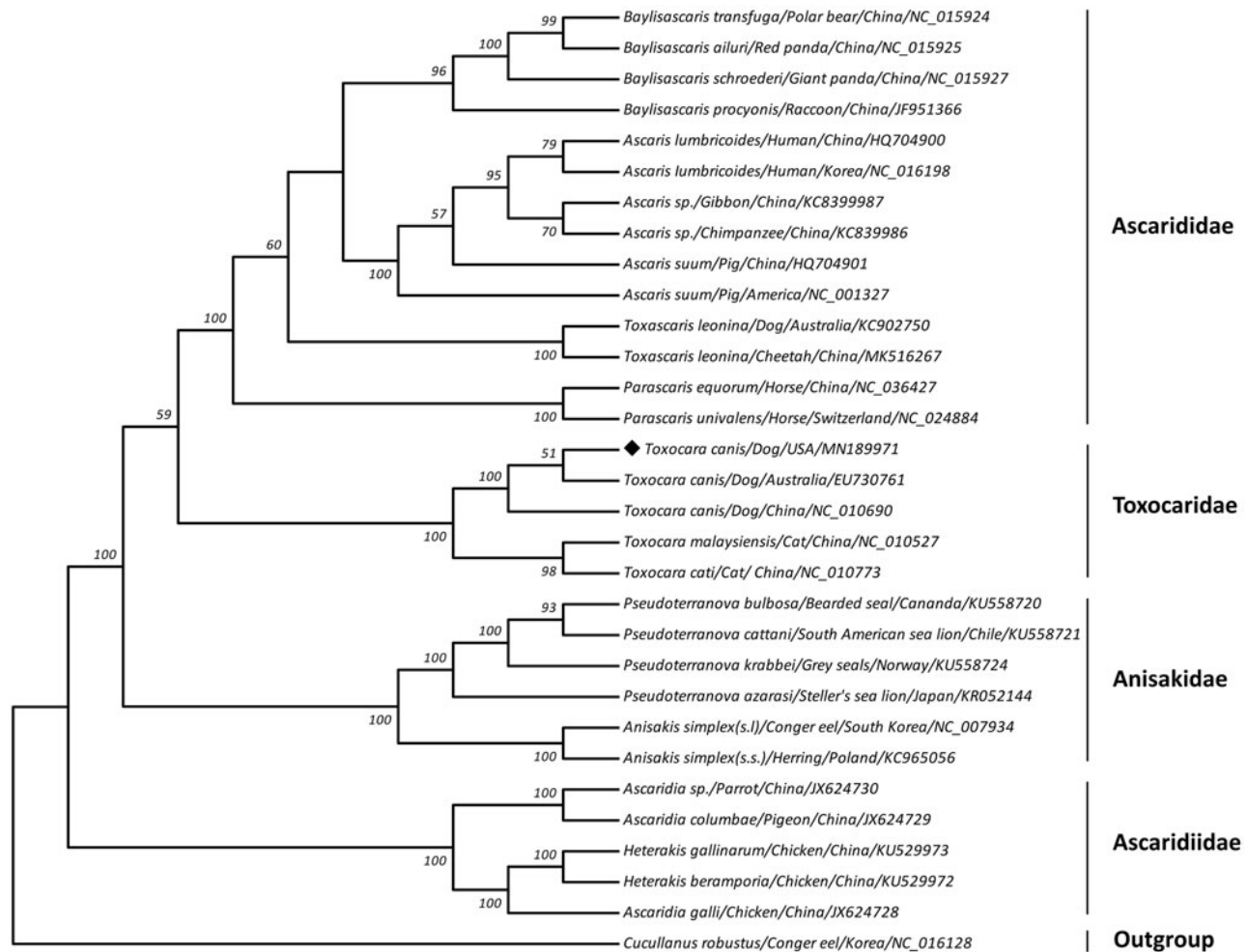
The complete mtDNA of *T. canis* was 14,309 bp in size (GenBank accession no. MN189971) and encoded 12 protein-coding genes, 22 tRNA genes, and 2 rRNA genes. All genes were transcribed in the same direction on the same strand. Among the 12 protein-coding genes, except *atp6*, *nad2*,

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

*nad5*, and *nad4L* deduced to use an incomplete stop codon 'T', the rest were predicted to use the typical TAG or TAA as the stop codons. Twenty-two tRNA genes ranged from 52 bp (tRNA<sup>(AGY)</sup>-Ser) to 64 bp (tRNA-Glu) in size. Both rRNAs were 695 bp (12S) and 963 bp (16S) in size, respectively, and located between tRNA-Glu and tRNA<sup>(UCN)</sup>-Ser and between tRNA-His and *nad3*, respectively. Two non-coding regions, namely NCR1 (also known as AT-rich region; 203 bp) and NCR2 (174 bp), were placed between tRNA<sup>(UCN)</sup>-Ser and tRNA-Asn and between *nad4* and *cox1*, respectively.

A maximum-likelihood (ML) phylogeny was reconstructed on a concatenated amino acid dataset of 12 protein-coding genes from 31 nematode parasites, using *Cucullanus robustus* as outgroup. This phylogenetic tree showed that *T. canis* from USA and Australia were more closely related to each other than to that from Chinese, nevertheless, three *T. canis* isolates clustered together and formed a branch that was paraphyletic with the congeneric *Toxocara cati* and *Toxocara malaysiensis* with 100% bootstrap confidence, supporting their sister-species relationships within the family Toxocaridae (Figure 1). In addition, within this topology, each family Toxocaridae, Ascarididae, Anisakidae or Ascaridiidae was treated as a monophyletic group in the order Ascaridida, consistent with results of morphological and molecular studies

(Hartwich 1974; Li et al. 2018). In conclusion, the complete mtDNA of *T. canis* sequenced here provides a novel marker resource for genetic and evolutionary biological studies of this zoonotic nematode.

## Disclosure statement

No potential conflict of interest was reported by the authors.

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