


The mitochondrial genome of the sheep roundworm *Ascaris ovis* (Ascaridida: Nematoda) from Southwest China

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ABSTRACT

The *Ascaris* roundworms (Ascaridida: Nematoda), one of the commonest soil-transmitted helminths (STHs), can cause ascariasis with significant socioeconomic and public health impact. In this study, the mitochondrial genome of *Ascaris ovis*, a representative of this genus from the sheep in Southwest China was determined using Illumina sequencing technology. The assembled genome was 14,205 bp in size and encoded 36 genes, including 12 protein-coding genes, 22 transfer RNA genes, and two ribosomal RNA genes. Phylogenetic analysis showed that *A. ovis* grouped with the congeneric *Ascaris lumbricoides* of humans, *Ascaris* spp. of non-human primates and *Ascaris suum* of pigs and together formed a monophyletic group relationship with either species of *Baylisascaris/Toxascaris/Parascaris*, species of *Toxocara*, species of *Anisakis/Pseudoterranova* or species of *Ascaridia/Heterakis* in the order Ascaridida, supporting its genetic similarity with *A. lumbricoides*, *A. suum*, and other congeneric species. The cumulative mitochondrial DNA data of this genus should contribute to a better understanding of the phylogenetic relationships among these roundworms.

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The *Ascaris* roundworms (Ascaridida: Nematoda), one of the commonest soil-transmitted helminths (STHs), can cause the socioeconomically important disease ascariasis in various mammals (Dold and Holland 2011; Xie et al. 2013). However, owing to the sampling and survey biases, the current targeted species in this genus are mostly toward the roundworms that are of medical and veterinary importance, such as the human *Ascaris lumbricoides* and pig *Ascaris suum* (Dold and Holland 2011; Betson and Stothard 2016; Sadaow et al. 2018). Little or no attention is paid to roundworms infecting other hosts including domestic animals. *Ascaris ovis* Rudolphi, 1819, one common parasitic nematode of sheep and goats, represents a significant threat to both populations, especially the lambs and kids (Neveu-Lemaire 1923; Goodey 1926; Mozgovoi and Nosik 1951). The damage to the host body includes inflammation and scarring of the intestinal wall and parenchyma of the liver and lung (caused by larvae), as well as intestinal inflammation, obstruction, and even death (caused by adults) (Shcherbinin 1959; Niu et al. 2018). Although there has been substantial progress in studies on the morphology and biology of *A. ovis* so far, the knowledge gaps to understand this parasite at the molecular level, especially in genetics and molecular epidemiology, are still not sufficiently explored due to lacking suitable genetic markers (Niu et al. 2018). Moreover, given remarkable similarities in

morphological characteristics and chromosome karyotypes between *A. ovis* and *A. suum* (Goodey 1926; Vassilev and Mutafova 1974), it is still unclear whether *Ascaris* species from sheep and goats represent *A. suum* or a different species. Under such context, we sequenced the mitochondrial genome of a representative *A. ovis* from the sheep using Illumina sequencing technology, because the mitochondrial DNA (mtDNA) has been not only proven to be an important source for molecular markers but its complete data can also provide novel insights into the genome-wide comparative, phylogenetic-based analyses of *Ascaris* (Hu and Gasser 2006; Jex et al. 2010; Xie et al. 2013).

The parasite specimens ($n=2$) were obtained from a naturally infected stray sheep housed in a mixed pastoral-farming area at Yajiang (30°03'N, 101°01'E), Sichuan Province of Southwest China, after treatment with pyrantel pamoate. After morphological identification, both worms were identified as the adult females of *A. ovis* according to the taxonomic key of Goodey (1926). One specimen was used for DNA extraction and another was fixed in 5% formalin solution and archived in the Parasitological Museum of Sichuan Agricultural University (Ya'an, China) under collection numbers XY2018_19. Whole genomic DNA was extracted from a small portion (1.5 cm) of the worm specimen using the Universal Genomic DNA Extraction Kit Ver. 3.0 (TaKaRa,

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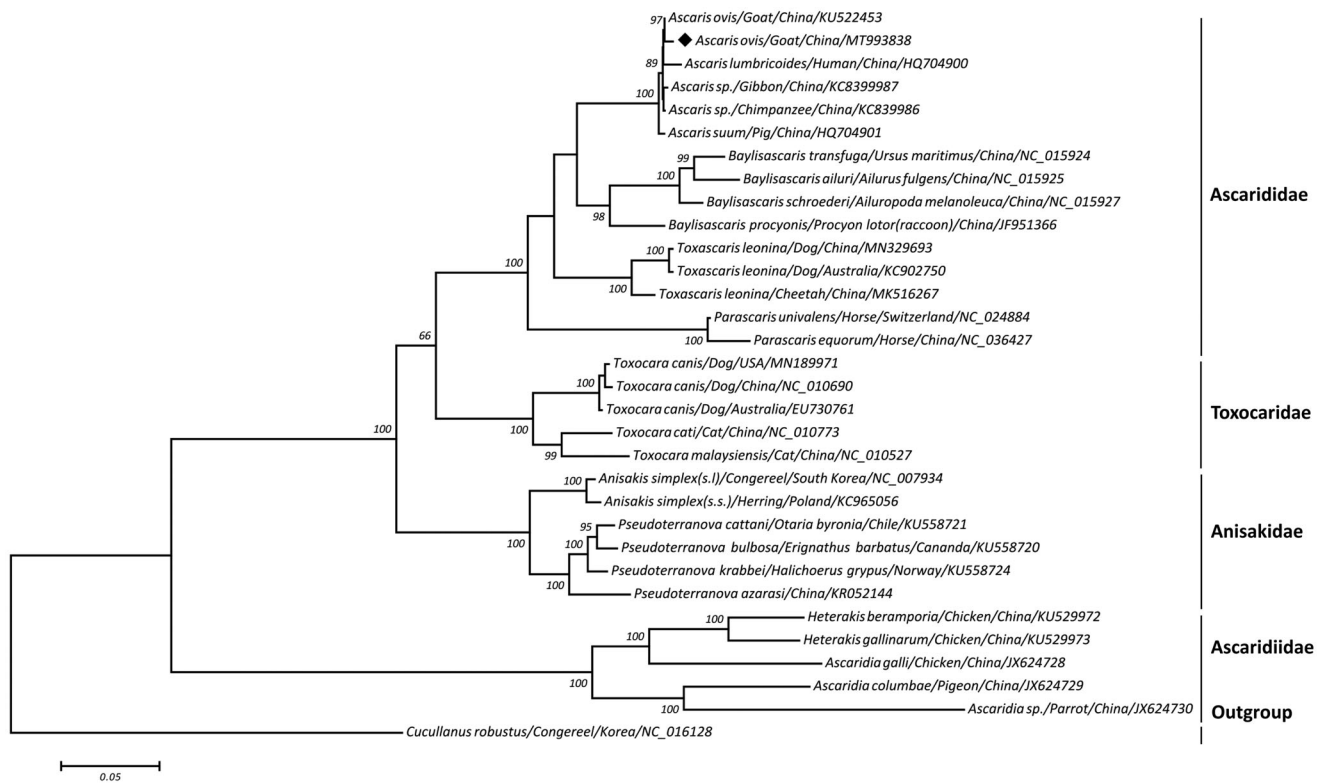


Figure 1. Maximum-likelihood tree inferred from concatenated amino-acid sequences of 12 mt protein-coding genes of *T. leonine* and other related nematodes, utilizing MtArt + I model and after 100,000 bootstrap replications (<50% support not shown). The solid black diamond represents the species in this study.

Dalian, China). After DNA yield and integrity assessment, a 300-bp paired-end (PE) genomic library was constructed according to manufacturer's instructions (Illumina, San Diego, CA). The sequencing was carried out on HiSeq platform (BGI, Shenzhen, China) and reads were exported to the FASTQ format. The complete mtDNA was assembled using MITObim (Hahn et al. 2013) and annotated by MITOS (Bernt et al. 2013). The complete genome sequence was deposited in GenBank under accession number: MT993838.

The complete mitochondrial genome of *A. ovis* was 14,205 bp in size with 72.0% AT and encoded 12 protein-coding genes, 22 tRNA genes, and two rRNA genes. All genes were present on the same strand and unidirectionally transcribed, typical for other roundworms characterized so far (Park et al. 2011; Xie et al. 2011, 2013, 2019; Liu et al. 2012). Nine of the 12 protein-coding genes were predicted to use the TAA or TAG (*atp6*, *cytb*, *cox2-3*, *nad1*, *nad4*, and *nad6*) as the stop codons, while the remaining genes (*cox1*, *nad2*, and *nad5*) were deduced to end with an incomplete codon 'T' or 'TA'. Twenty-two tRNA genes ranged in size from 51 bp (tRNA^(AGN)-Ser and tRNA-Thr) to 61 bp (tRNA-Lys, tRNA-Ile, and tRNA-Met) and had distinctly different stem-loop structures when compared to those of other metazoan mtDNAs (Park et al. 2011; Liu et al. 2012; Xie et al. 2013; Xie et al. 2019). Two rRNAs, the small rRNA (*rrnS*; 692 bp) and large (*rrnL*; 960 bp) subunits, were 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; 868 bp) and NC2 (93 bp), were present between tRNA^(UCN)-Ser and tRNA-Asn and between *nad4* and *cox1*, respectively.

Based on a concatenated amino acid dataset of 12 protein-coding genes from 32 nematode parasites, a maximum-likelihood (ML) tree was reconstructed using *Cucullanus robustus* as the outgroup. As shown in Figure 1, this tree topology clearly placed *A. ovis* together with the congeneric *A. lumbricoides* of humans, *Ascaris* spp. of non-human primates and *A. suum* of pigs and together formed a monophyletic group relationship with either species of *Baylisascaris*/*Toxascaris*/*Parascaris* in the family Ascarididae, species of *Toxocara* in the family Toxocaridae, species of *Anisakis*/*Pseudoterranova* in the family Anisakidae or species of *Ascaridia*/*Heterakis* in the family Ascaridiidae under the order Ascaridida, with high bootstrap values, supporting its genetic similarity with *A. lumbricoides*, *A. suum*, and other congeneric species (Niu et al. 2018). Further, a closer relationship was observed between *A. ovis* and *A. lumbricoides* than between *A. ovis* and *A. suum*, suggesting that *A. ovis* may share more genetic similarity to *A. lumbricoides*. Taken together, the sequenced mitochondrial genome of *A. ovis* in this study provides novel molecular evidence for its phylogenetic and taxonomic position in the genus *Ascaris*.

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 at <https://www.ncbi.nlm.nih.gov/nucleotide/MT993838>. Associated BioProject, SRA, and BioSample accession numbers are PRJNA673292 (<https://www.ncbi.nlm.nih.gov/bioproject/PRJNA673292>), SRR12950074 (<https://www.ncbi.nlm.nih.gov/sra/SRR12950074/>), and SAMN16598390 (<https://www.ncbi.nlm.nih.gov/biosample/SAMN16598390/>), respectively.

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