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Characterization of the complete mitochondrial genomes of two whipworms *Trichuris ovis* and *Trichuris discolor* (Nematoda: Trichuridae)

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ABSTRACT

For many years, whipworms (*Trichuris* spp.) have been described with a relatively narrow range of both morphological and biometrical features. Moreover, there has been insufficient discrimination between congeners (or closely related species). In the present study, we determined the complete mitochondrial (mt) genomes of two whipworms *Trichuris ovis* and *Trichuris discolor*, compared them and then tested the hypothesis that *T. ovis* and *T. discolor* are distinct species by phylogenetic analyses using Bayesian inference, maximum likelihood and maximum parsimony) based on the deduced amino acid sequences of the mt protein-coding genes. The complete mt genomes of *T. ovis* and *T. discolor* were 13,946 bp and 13,904 bp in size, respectively. Both mt genomes are circular, and consist of 37 genes, including 13 genes coding for proteins, 2 genes for rRNA, and 22 genes for tRNA. The gene content and arrangement are identical to that of human and pig whipworms *Trichuris trichiura* and *Trichuris suis*. Taken together, these analyses showed genetic distinctiveness and strongly supported the recent proposal that *T. ovis* and *T. discolor* are distinct species using nuclear ribosomal DNA and a portion of the mtDNA sequence dataset. The availability of the complete mtDNA sequences of *T. ovis* and *T. discolor* provides novel genetic markers for studying the population genetics, diagnostics and molecular epidemiology of *T. ovis* and *T. discolor*.

1. Introduction

Whipworms are widespread soil-transmitted helminthes (=geohelminths) that can be found in a broad range of hosts, including humans (*Trichuris trichiura*), pigs (*Trichuris suis*), sheep, goats and bovines (*Trichuris ovis* and *Trichuris discolor*), dogs (*Trichuris vulpis*) and non-human primates (*Trichuris* spp.) (Beer and Lean, 1973; Cutillas et al., 2007; Khalafalla et al., 2011; Hotez et al., 2012). These parasites are transmitted via a direct, fecal-oral route. The thick-shelled, infective larvated eggs are ingested and then hatch, following gastric passage, in the small intestine. First-stage larvae are released and then burrow into the wall of the caecum and proximal colon, where they develop to adult worms (~30–50 mm in length) (Beer, 1973). Whipworms are among the most common intestinal parasites of humans and animals, causing significant diseases and economic losses globally (Jex et al., 2011; Roepstorff et al., 2011).

Based on current knowledge, *Trichuris* species are usually identified based on species of hosts and a relatively narrow set of morphological features of the adult worms (e.g. spicule and pericloacal papillae) (Cutillas et al., 1995; Robles, 2011). However, it is not always possible to unequivocally identify and sufficiently differentiate between congeners based on these criterion of adult worms alone (Chandler, 1930; Cutillas et al., 2009). *T. ovis* and *T. discolor* are not considered to be host specific because they are often found in the caecum and colon of bovines, sheep and goats. A morphological study revealed differences between *T. ovis* and *T. discolor* isolated from bovines (Callejón et al., 2012). A molecular study, using the first and second internal transcribed spacers (ITS-1 and ITS-2) of nuclear ribosomal DNA (rDNA) and a portion of large subunit ribosomal RNA (16S) gene of the mitochondrial (mt) genome as genetic markers, supported that *T. ovis* and *T. discolor* represent two distinct species (Callejón et al., 2012). However, ITS-1 and ITS-2 rDNA sequences can be difficult or unsuitable to

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provide reliable genetic marker for systematic studies of enoplid nematodes because significant level of size and sequence polymorphism (heterogeneity) exists in these rDNA regions within individual nematodes (Gasser, 1997; Gasser et al., 2001).

mtDNA sequences have been widely used as genetic markers not only for population genetics, but also for the study of systematics and evolutionary relationship among a range of animals due to their maternal inheritance, fast rate of evolutionary change, lack of recombination and relatively conserved genome structures (Wolstenholme, 1992; Boore, 1999; Zhao et al., 2012). To date, 63 complete or near-complete mt genome sequences of nematode species have been deposited in GenBank. In the family Trichuridae, however, the mt genomes of only two species are available (Liu et al., 2012a). Particularly, the complete mt genome sequences, critical information for the study of taxonomic and phylogenetic relationship, remain unavailable for *T. ovis* and *T. discolor*. Therefore, in the present study, we (i) characterized the mt genomes of *T. ovis* and *T. discolor*, (ii) compared these genomes and (iii) tested the hypothesis that *T. ovis* and *T. discolor* are distinct species via phylogenetic analyses of protein-coding sequence data sets representing both genomes and those derived from all currently known mt genomes of enoplid nematodes.

2. Materials and methods

2.1. Parasites and DNA extraction

Adult samples representing *T. ovis* were obtained from the caecum of an infected antelope in Guangzhou zoo, Guangdong Province, China. Adult samples representing *T. discolor* were obtained from the caecum of wild yak during surgery in Noel Cover Grassland in Sichuan Province, China. The whipworms were washed in physiological saline, identified preliminarily to species based on host preference, morphological characters and predilection sites (Cutillas et al., 1995; Callejón et al., 2012), fixed in 70% (v/v) ethanol and stored at −20 °C until use.

Total genomic DNA was isolated separately from two individual whipworms using sodium dodecyl sulfate/proteinase K treatment, followed by spin-column purification (Wizard® SV Genomic DNA Purification System, Promega). The region spanning ITS-1, the 5.8S rDNA and ITS-2 was amplified from each of these individuals by PCR using previously reported primers (Zhu et al., 1999; Kralova et al., 2001) and sequenced directly. The ITS-1 and ITS-2 sequences of the *T. ovis* sample had 98% similarity with that of *T. ovis* in Spain (GenBank accession nos. AJ310662 and AJ238220, respectively). The ITS-1 and ITS-2 sequences of the *T. discolor* sample had 99.0% and 100.0% similarity with that of *T. discolor* from bovine in Iran, respectively (GenBank accession nos. HE608852 and HE608855, respectively).

2.2. Amplification and sequencing of partial cox1, nad5 and cytb

Initially, fragments of *cox*1, *nad*5 and *cyt*b genes were amplified individually from *T. ovis* and *T. discolor* by PCR with primers (Table 1) designed based on mtDNA sequences of *T. trichiura* and *T. suis* (Liu et al., 2012a) using Primer Primer 5 Design Program (PREMIER Biosoft International). One microliter (5–10 ng) of genomic DNA was added to each PCR reaction (25 μL). PCR was performed in 10 mM Tris–HCl (pH 8.4), 50 mM KCl, 4 mM MgCl2, 200 mM each of dNTP, 50 pmol of each primer and 2 U *Taq* polymerase (Takara) in a thermocycler (Biometra) under the following conditions: after an initial denaturation at 94 °C for 5 min, then 36 cycles at 94 °C for 30 s (denaturation), 55 °C (for p*ncox*1 and p*nad*5) or 50 °C (for p*cyt*b) for 30 s (annealing), 72 °C for 30 s (extension), followed by 72 °C for 5 min (final extension). Each amplicon (5 μL) was examined by (1%) agarose gel electrophoresis. The purified amplicons were sequenced directly from both directions using the same primers used in primary PCR amplifications (BigDye terminator v3.1, ABI PRISM 3730).

Table 1
Sequences of primers used to amplify PCR fragments from *Trichuris ovis* and *Trichuris discolor*.

Primer	Sequence (5′ to 3′)
Short-PCR	
For p*cox*1	
COX1F	TATACAGGAAATCATAAAAAAAT
COX1R	GCAGGCAATACTAAAATATATACTT
For p*nad*5	
NAD5F	CAAGGATTTTTTTGAGATCTTTTTC
NAD5R	TAAACCGAATTGGAGATTTTTGTTT
For p*cytb*	
CytbF	GAGTAATTTTTATAATACGAGAAGT
CytbR	AATTTTCAGGGTCTCTGCTTCAATA
Long-PCR	
For *T. ovis*	
TOCO1F	ATTTTTCTTCACATTTCATTATTTAGCACCTT
TOND5R	TGTCTTGGGCACCTACTATGATTGGGAG
TOND5F	CACCTATTCAGGGGTATTTGTGCTCTT
TOCOBR	GAGAGTTCGTCCCCTAATCCTGTA
TOCOBF	CTCCCAATCATAGTAGGTGCCCAAGAC
TOCO1R	GATTACCTATTGCTATGGCTGCTCCTA
For *T. discolor*	
TBCO1F	CACCTATTCAGGGGTATTTGTGCTCTT
TBND5R	GAGAGTTCGTCCCCTAATCCTGTA
TDND5F	GTTTTCTCTCTGGACCGATTACCTATGTT
TDCOBR	CTACTCCAGTCAGTTCTCTTGTTCATTCT
TDCOBF	CTTATTTGTGAAACTTGGGTAGATT
TDCO1R	ACATAGGTAATCGGTCCAGAGAGA

2.3. Long-PCR amplification and sequencing

After we had obtained partial *cox*1, *nad*5 and *cyt*b sequences for *T. ovis* and *T. discolor* primers (Table 1) were designed in the conserved regions to amplify the complete mt genome of *T. ovis* and *T. discolor*. The complete mt genome of *T. ovis* and *T. discolor* were amplified in 3 overlapping long fragments between *cox*1 and *nad*5 (approximately 5 kb), between *nad*5 and *cyt*b (approximately 4 kb), and between *cyt*b and *cox*1 (approximately 6 kb). Long-PCR reactions (25 μL) were performed in 2 mM $MgCl_2$, 0.2 mM each of dNTPs, 2.5 μL 10× r*Taq* buffer, 2.5 μM of each primer, 1.25 U r*Taq* polymerase (Takara), and 1 μL of DNA in a thermocycler (Biometra) under the following conditions: 92 °C for 2 min (initial denaturation), then 92 °C for 10 s (denaturation), 48–57 °C for 30 s (annealing), and 60 °C for 10 min (extension) for 10 cycles, followed by 92 °C for 10 s, 48–57 °C for 30 s, and 60 °C for 10 min for 20 cycles, with a cycle elongation of 10 s for each cycle and a final extension at 60 °C for 10 min. A no-DNA control was included in each amplification run, and in neither case were amplicons detected in the no-DNA controls (data not shown). Each amplicon (5 μL) was examined by agarose (1%) gel electrophoresis, stained with ethidium bromide and photographed using a gel documentation system (UVItec). PCR products were sent to Sangon Company (Shanghai, China) for sequencing from both directions using a primer walking strategy.

2.4. Sequence analyses

Sequences were assembled manually and aligned against the complete mt genome sequences of *T. trichiura* and *T. suis* (Liu et al., 2012a) using Clustal X 1.83 (Thompson et al., 1997) to identify gene boundaries. The open-reading frames were analyzed with Open Reading Frame Finder (<http://www.ncbi.nlm.nih.gov/