Gene 585 (2016) 71–75

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Research paper

Comparative analyses of the complete mitochondrial genomes of the two murine pinworms *Aspiculuris tetraptera* and *Syphacia obvelata*

Chun-Ren Wang [a,b,*], Yan Lou [b], Jun-Feng Gao [b,c], Jian-Hua Qiu [b], Yan Zhang [b], Yuan Gao [b], Qiao-Cheng Chang [b,**]

[a] College of Life Science and Technology, Heilongjiang Bayi Agricultural University, Daqing, Heilongjiang Province 163319, PR China
[b] College of Animal Science and Veterinary Medicine, Heilongjiang Bayi Agricultural University, Daqing, Heilongjiang Province 163319, PR China
[c] Department of Parasitology, Heilongjiang Institute of Veterinary Science, Qiqihar, Heilongjiang Province 161006, PR China

ARTICLE INFO

Article history:
Received 5 February 2016
Received in revised form 13 March 2016
Accepted 20 March 2016
Available online 23 March 2016

Keywords:
Aspiculuris tetraptera
Syphacia obvelata
Complete mitochondrial genome
Comparative analysis
Phylogenetic analysis

ABSTRACT

Pinworms *Aspiculuris tetraptera* and *Syphacia obvelata* are important parasitic nematodes of laboratory mice, rat and other rodents. However, the mitochondrial (mt) genome of these parasites have not been known yet. In the present study, the complete mt genomes of *A. tetraptera* and *S. obvelata* were sequenced, which were 13,669 bp and 14,235 bp in size, respectively. Both genomes included 12 protein-coding genes, two rRNA genes, 22 tRNA genes and one non-coding region. The mt genomes of *A. tetraptera* and *S. obvelata* preferred bases A and T, with the highest for T and the lowest for C. The mt gene arrangements of the two pinworms were the same as that of the GA8 type. Phylogenetic analysis using mtDNA data revealed that the Bayesian inference (BI) trees contained two big branches: species from Oxyuridomorpha, Rhabditomorpha and Ascaridomorpha formed one branch, and those from Spiruromorpha formed another branch with high statistical support. The two murine pinworms *A. tetraptera* and *S. obvelata* have closer relationship than to other pinworms. This study provides a foundation for studying the population genetics, systematics and molecular phylogeny of pinworms.

1. Introduction

Aspiculuris tetraptera and *Syphacia obvelata* are pinworms which inhabit in the caecum and colon of laboratory mice, rats and other rodents, and can cause enterobiasis (Baker 1998). They are common parasites infecting conventional colonies or specific pathogen free (SPF) laboratory mice or rats with high prevalence even in well-managed colonies (Baker 1998; Bazzano, 2002). Customarily, pinworm infection imposes no obvious clinical symptoms in mice, but in the case of serious infections, it can cause rectal prolapse, intussusception, enteritis, fecal impaction and weight loss (Sato et al. 1995). More importantly, the enterobiasis would influence the accuracy of animal experiments by generating host humoral and cellular immune response (Michels et al. 2006). Therefore, it is imperative to prevent and control enterobiasis of laboratory mice to ensure the accuracy of scientific experiments.

Previous studies on *A. tetraptera* and *S. obvelata* have mainly focused on their morphology, life cycle and epidemiological investigation (Sato et al. 1995; Baker 1998; Chen et al. 2011). There were only a few studies at molecular level, including genetic variability in partial mitochondrial (mt) DNA sequences, internal transcribed spacer (ITS) rDNA sequences, and 28S rDNA sequences among pinworms from different geographical origins (Okamoto et al. 2007, 2009; Parel et al. 2008; Lou et al. 2015; Wang et al. 2015; Qiu et al. 2016). Recently, the complete mitochondrial sequences have received increased attention, and clearly indicated that complete mtDNA sequences are useful genetic marker for the identification and phylogenetic analysis of different parasite groups, including helminthes, arthropods and protozoa (Liu et al. 2013a; Ogedengbe et al. 2014; Duan et al. 2015).

Although Nematoda is the second largest animal phylum, and many species of nematodes can infect plants, animals and humans, causing significant impacts on agriculture, animal husbandry, and human health. However, so far, only about 110 complete mt genomes of animal parasitic nematodes have been sequenced. In the Oxyuridomorpha, mt genome sequences of only four species (*Oxyuris equi*, *Passalurus ambiguus*, *Enterobius vermicularis* and *Wellcomia siamensis*) have been deposited in GenBank. The paucity of information on mt genomes of parasitic nematodes belonging to Oxyuridomorpha is a limitation

Abbreviations: Mt, mitochondrial; mtDNA, mitochondrial DNA; PCR, polymerase chain reaction; *cox*1–3, *nad*1–6 and *nad*4L, *atp*6, *rrn*S and *rrn*L, and *cyt*b, genes encoding cytochrome coxidase subunits I and III, NADH dehydrogenase subunits 1–6 and 4L, ATPase subunits 6, small and large subunits ribosomal RNA, and cytochrome oxidase *b*; ITS rDNA, the internal transcribed spacers of nuclear ribosomal DNA; BI, Bayesian inference.

* Correspondence to: C.-R. Wang, College of Life Science and Technology, Heilongjiang Bayi Agricultural University, Daqing, Heilongjiang Province 163319, PR China.
** Corresponding author.
E-mail addresses: chunrenwang@sohu.com (C.-R. Wang), changqiaocheng2001@163.com (Q.-C. Chang).

http://dx.doi.org/10.1016/j.gene.2016.03.037
0378-1119/

for population genetic and phylogenetic studies of these parasites. Therefore, the purposes of this study were to determine and comparatively analyze the complete mt genome sequences of *A. tetraptera* and *S. obvelata* and use these sequences to study the phylogenetic relationships of pinworms with other nematodes.

2. Materials and methods

2.1. Parasites and extraction of genomic DNA

Adult pinworms *A. tetraptera* and *S. obvelata* were collected from the large intestine of the infected laboratory mice in Heilongjiang Province and Beijing, China. The pinworms were washed in physiological saline and individual pinworms were identified to species according to their morphological characteristics and molecular-biological method (Taffs 1976; Parel et al. 2008). The ribosomal DNA ITSs were amplified by the polymerase chain reaction (PCR) using universal primers NC5 (forward; 5′-GTA GGT GAA CCT GCG GAA GGA TCA TT-3′) and NC2 (reverse; 5′ - TTA GTT TCT TTT CCT CCG CT - 3′) described previously (Zhu et al. 2002). The ITS sequences obtained were identical to that of *A. tetraptera* (EU263107) and *S. obvelata* (EU263105) available in GenBank. Then the worms fixed in 70% ethanol until the extraction of genomic DNA. Total genomic DNA was extracted from each individual pinworms using a commercial Genomic DNA kit (TIANGEN Biotech, Beijing/China) according to the manufacturer's protocol, followed by purification over a mini-column and eluted into 35 μL double-distilled water. DNA samples were stored at −20 °C until use.

2.2. PCR amplification and sequencing

Primers for amplifying the mtDNA of *A. tetraptera* and *S. obvelata* were designed based on mtDNA sequences of *E. vermicularis* (NC_011300) and *W. siamensis* (NC_016129) available in GenBank. The complete mtDNA sequences of *A. tetraptera* and *S. obvelata* were amplified in 13 and 7 overlapping amplicons, respectively (See Supplementary, Table S1). Notably, the *nad*1-*rrn*L fragment of *S. obvelata* was amplified using the previous designed primers 5F and 40R (Hu et al. 2002a). PCR reactions (50 μL) were performed in 20 mM Tris–HCl (pH 8.4), 100 mM KCl, 8 mM $MgCl_2$, 400 mM each of dNTPs, 100 pmol of each primer, 5 U Ex*Taq* polymerase and 2 μL of DNA sample in a thermocycler (BioRad, USA). The amplification was executed under the following conditions: 95 °C for 2 min (initial denaturation); then 95 °C for 30 min (denaturation), 50 °C for 30 s (annealing), 72 °C (~1 kb region) for 1 min (extension) for 35 cycles, and a final extension at 72 °C for 10 min. Each amplicon yielded a single band on agarose gel (0.8%) upon ethidium-bromide staining. PCR products were sent to Sangon Company (Shanghai, China) for sequencing from both directions using a primer walking strategy, and the primers used for amplification and sequencing of the complete genomes of the two pinworms are listed in Table S1 (see Supplementary).

2.3. Sequence assembly and phylogenetic analyses

Sequences were assembled using the program DNAStar 5.0 (Burland 2000). The boundaries of the 12 protein-coding genes (PCGs) and two ribosomal RNA genes were identified by comparing with the mt genome sequences of *E. vermicularis* and *W. siamensis* available in the GenBank. The putative secondary structures of the 22 transfer RNA genes were identified using the program tRNAscan-SE or manually by finding the anticodon and comparing with tRNA secondary structures of *E. vermicularis* and *W. siamensis* nematodes. Translation initiation and translation termination codons were identified using genetic codon table for mitochondrion in MEGA 5 or based on comparison with the mt genomes of *E. vermicularis* and *W. siamensis* reported previously (Tamura et al. 2011).

The phylogenetic relationships of *A. tetraptera* and *S. obvelata* with 15 other nematode species were reconstructed based on the combined amino acid sequences of 12 mt PCGs utilizing Bayesian inference (BI) with *Trichuris ovis* (NC_018597) as the outgroup. These species included four species in Oxyuridomorpha: *E. vermicularis* (NC_011300), *W. siamensis* (NC_016129), *P. ambiguus* (KT879302), *O. equi* (KP404095); three in Ascaridomorpha: *Ascaris suum* (NC_001327), *Toxocara cati* (NC_010773), *Ascaridia galli* (JX624728); six in Rhabditomorpha: *Ancylostoma caninum* (NC_012309), *Bunostomum trigonocephalum* (KF255998), *Mecistocirrus digitatus* (NC_013848), *Metalastronglyus salmi* (NC_013815), *Dictyocaulus eckerti* (NC 019809.1), *Trichostrongylus axei* (NC 013824); and two in Spiruromorpha: *Dirofilaria immitis* (NC_005305), *Setaria digitata* (NC_014282). Phylogram was drawn using the Tree View program version 1.65 (Page 1996).

3. Results and discussion

3.1. General features of A. tetraptera and S. obvelata mt genomes

The complete mtDNA sequences of *A. tetraptera* (GenBank accession no. KT764937) and *S. obvelata* (KT900946) obtained in this study were 13,669 bp and 14,235 bp in length, respectively (Fig. 1, Table 1), which was similar to the mt genomes of other nematodes previouly reported (Hu et al. 2002b; Kang et al. 2009), but far less than those of the Enoplea, Mermithida nematodes, such as *Hexamermis agrotis* (24,606 bp) and *Romanomermis culicivorax* (26,194 bp). Each of the circular mt genome contains 36 genes, including 12 PCGs (*cox*1–3, *cyt*b, *atp*6, *nad*1–6 and *nad*4L), 22 tRNA genes and two rRNA genes, which was consistent with mt genomes of other Chromadorea nematodes (such as *Triodontophorus brevicauda*) (Duan et al. 2015), but different from those of Enoplea nematodes (such as *Trichinella spiralis*) and ticks (*Rhipicephalus sanguineus*), which have an *atp*8 gene (Lavrov and Brown 2001; Liu et al. 2013a). All mt genes of the two pinworms were encoded on the same strand and transcribed in the same direction (5′ to 3′), as reported for those of Chromadorea nematodes (Hu et al.

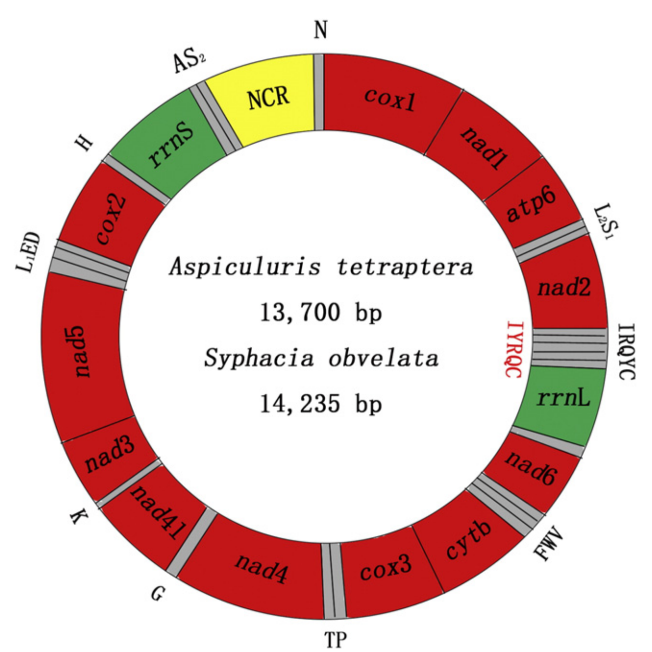

Fig. 1. Arrangement of the mitochondrial genomes for *Aspiculuris tetraptera* and *Syphacia obvelata*. Gene scaling is only approximate. All genes have standard nomenclature including the 22 tRNA genes, which are designated by the one-letter code for the corresponding amino acid, with numerals differentiating each of the two leucine- and serine specifying tRNAs (L1 and L2 for codon families CUN and UUR, respectively; S1 and S2 for codon families AGN and UCN, respectively). IRQYC indicates the order of five tRNAs of *A. tetraptera* and IYRQC indicates the order of five tRNAs of *S. bovelata*.