

Complete mitochondrial genome of *Cylicocyclus auriculatus*: molecular structure and phylogenetic analysis

Xuankai Li^{a*}, Lidan Wang^{a*}, Yijun Chen^a, Xianxiang Wang^b, Xuan Zhou^a and Yue Xie^a 

^aDepartment of Parasitology, College of Veterinary Medicine, Sichuan Agricultural University, Chengdu, China; ^bCollege of Science, Sichuan Agricultural University, Ya'an, China

ABSTRACT

Cylicocyclus spp. (Nematoda: Strongylida: Cyathostominae) are the common and important parasitic nematodes found in horses and donkeys worldwide. In this study, the complete mitochondrial genome of *Cylicocyclus auriculatus* Looss 1900, a representative member of this genus from the donkey in Southwest China was determined using the next-generation DNA sequencing technology. The genome was 13,851 bp in size and consisted of 36 genes including 12 protein-coding genes (*atp6*, *cox1-3*, *cytb*, *nad1-6* and *nad4L*), 22 transfer RNA genes and two ribosomal RNA genes (*rrnL* and *rrnS*), as well as two non-coding regions. Phylogenetic analysis showed that *C. auriculatus* and *Cylicocyclus insigne* Boulenger 1917 were closely related, and then both grouped with other congeneric species and formed a monophyletic relationship with either species of *Cyathostomum*, *Coronocyclus*, *Cyathostomum*, *Cylicostephanus* or *Cylicodontophorus*, demonstrating their phylogenetic stability within Cyathostominae. These cumulative mitochondrial DNA data provide novel and useful genetic markers for molecular diagnostic, systematic and evolutionary biological studies of Cyathostominae nematodes.

ARTICLE HISTORY

Received 25 August 2021
Accepted 14 February 2022

KEYWORDS






Bursate nematode;
Cylicocyclus; mtDNA;
phylogenetic relationships

Cylicocyclus parasites (Nematoda: Strongylida: Cyathostominae), commonly known as the bursate nematodes of equines, represent a common and severe threat to the livestock breeding industry worldwide (Lichtenfels et al. 2008; Zhang 2017; Hu et al. 2020). There are 13 valid species in the genus *Cylicocyclus* responsible for such morbidity and socio-economic burdens. *Cylicocyclus auriculatus* Looss 1900 is a significant member of the *Cylicocyclus* nematodes and frequently found in donkeys (Lichtenfels et al. 2008; Kuzmina and Kuzmin 2008; Bu et al. 2009, 2013). This parasite inhabits in the large intestine of donkeys and can cause abdominalgia, diarrhea, weight loss and even death. Although there have been substantial advances in morphology and biology of *C. auriculatus* so far, knowledge gaps to understand this nematode at the molecular level, especially in its genetics and molecular epidemiology, are still not sufficiently explored because of lacking suitable genetic markers (Lichtenfels et al. 2008; Bu et al. 2013). More importantly, until now the morphology-based classification of *Cylicocyclus* has been controversial (Lichtenfels et al. 2008; Zhang and Kong 2002; Gao et al. 2021). Under this context, we decoded the mitochondrial genome of *C. auriculatus*, a representative member of this genus from the Dezhou donkey in Southwest China using the Illumina sequencing technology, as the mitochondrial DNA is not only a rich resource for molecular markers but its

complete data can also provide novel insights into phylogenetic relationships of *Cylicocyclus* (Jex et al. 2010; Gao et al. 2017; Hu et al. 2020; Gao et al. 2021).

A total of six parasite specimens were obtained from a Dezhou donkey housed in an original breeding farm at Dazhou (31°92'N, 103°29'E), Sichuan Province of Southwest China, after treatment with pyrantel pamoate. These specimens were identified as adults of *C. auriculatus* with two males and four females, according to morphological keys of Lichtenfels et al. (2008). One male specimen was used for DNA isolation and the remaining were fixed in 5% formalin solution and archived in the Parasitological Museum of Sichuan Agricultural University (<https://dop.sicau.edu.cn/>; xyue1985@gmail.com (Yue Xie)) under collection numbers XY2018_28-32. After quality and quantity assessment, ~2 µg genomic DNA was fragmented to construct a 350-bp paired-end (PE) library, followed by sequencing on an Illumina HiSeq X-TEN platform. The clean reads (~1.5 Gb) were used to assemble a mitochondrial genome with MITObim (Hahn et al. 2013). Gene annotation was achieved using MITOS (Bernt et al. 2013). The complete genome sequence was deposited in GenBank under accession number: MZ888509.

The mitochondrial genome of *C. auriculatus* was 13,851 bp in size and encoded 12 protein-coding genes (*atp6*, *cox1-3*, *cytb*, *nad1-6* and *nad4L*), 22 tRNA genes and two rRNA genes

CONTACT Xianxiang Wang  xianxiangwang@hotmail.com  College of Science, Sichuan Agricultural University, Ya'an 625014, China; Xuan Zhou  zhouxuan198866@163.com; Yue Xie  xyue1985@gmail.com  Department of Parasitology, College of Veterinary Medicine, Sichuan Agricultural University, Chengdu 611130, China

*Authors have contributed equally to this work

© 2022 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

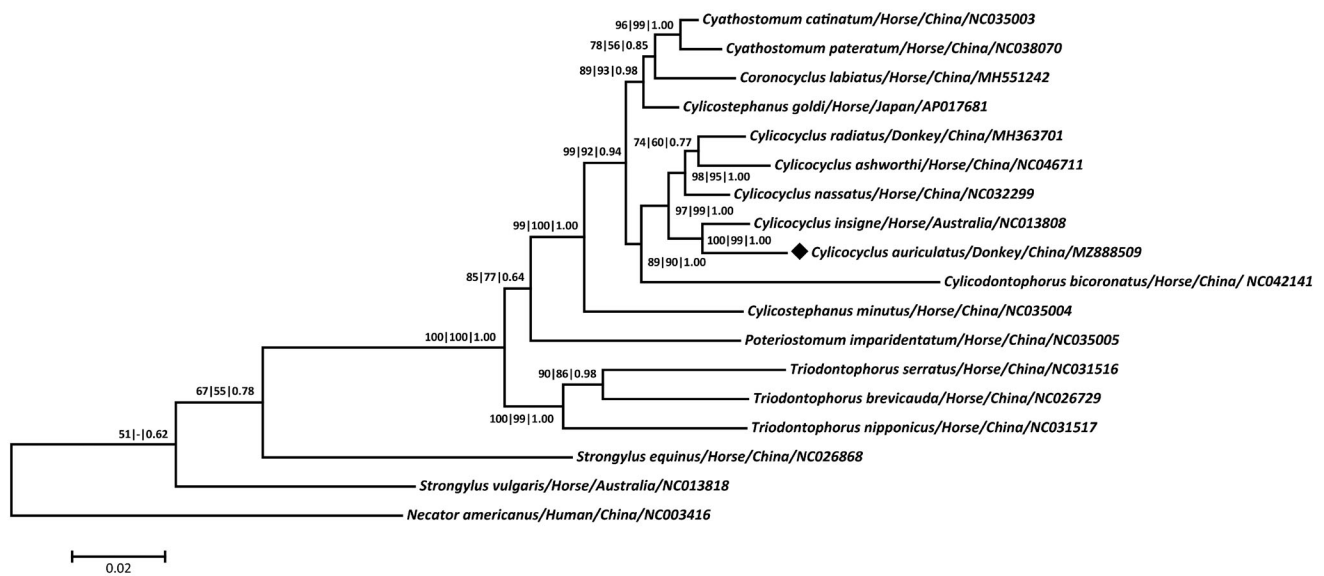


Figure 1. Phylogeny was inferred from maximum parsimony (MP), maximum-likelihood (ML) and Bayesian inference (BI) analyses based on concatenated amino-acid sequences of 12 mt protein-coding genes of *C. auriculatus* and other related nematodes. Numbers along the branches represent bootstrap values calculated from different analyses in the order: MP/ML/BI; values < 50% are not shown. The scale indicates an estimate of substitutions per site, using the optimized model setting. The solid black diamond represents the species in this study.

(*rrnL* and *rrnS*). All genes were unidirectionally transcribed on the same strand. Among these twelve protein-coding genes, *atp6*, *cytb*, *cox1-3*, *nad3*, *nad5*, *nad6* and *nad4L* started with ATT, while *nad1*, *nad2* and *nad4* used the TTG as the initiation codon. Correspondingly, except for *cox3* and *nad2* deduced to end with 'T' or 'TAG', the remaining genes were predicted to use the TAA as the stop codons. Twenty-two tRNA genes ranged from 54 bp (tRNA^(AGN)-Ser) to 63 bp (tRNA-Lys) in size. In addition to tRNA-Ser genes, all had a DHU arm and a TV-replacement loop instead of the T ψ C arm (Xu et al. 2015; Gao et al. 2017, 2021; Li et al. 2019; Qiu et al. 2019; Hu et al. 2020). Two rRNAs, the small rRNA (*rrnS*; 700 bp) and large (*rrnL*; 968 bp) subunits, were located between tRNA-Glu and tRNA^(UCN)-Ser and between tRNA-His and *nad3*, respectively. Two non-coding regions, namely the long non-coding region (LNCR; 273 bp) and short non-coding region (SNCR; 88 bp), were located between tRNA-Ala and tRNA-Pro and between *nad4* and *cox1*, respectively.

Building on a concatenated amino acid dataset of 12 protein-coding genes from 17 strongyloid parasites, the maximum parsimony (MP), maximum-likelihood (ML) and Bayesian inference (BI) methods were used to construct phylogenetic trees using *Necator americanus* Stiles 1902 as the outgroup because of its relationship with equine Strongyloidea nematodes (Jex et al., 2009; Liu et al., 2013; Gao et al., 2021). The three identical trees clearly grouped *C. auriculatus* with *Cylicocyclus ashworthi* Le Roux 1924, *Cylicocyclus insigne* Boulenger 1917, *Cylicocyclus nassatus* Looss 1900 and *Cylicocyclus radiates* Krecek 2011 and together formed a monophyletic relationship with either species of *Cyathostomum*, *Coronocyclus*, *Cyathostomum*, *Cylicostephanus* or *Cylicodontophorus* within Cyathostominae (Figure 1). Further, *C. auriculatus* was determined to be more closely related to *C. insigne* than to other species in

Cylicocyclus, with high statistical supports (all bootstrap values ≥ 99 or =1.00), consistent with recent molecular studies (Hu et al. 2020; Gao et al. 2021), demonstrating the phylogenetic stability of these bursate nematodes found in equines. Taken together, the sequenced mitochondrial genome of *C. auriculatus* not only provides novel molecular evidence for its phylogenetic position in *Cylicocyclus* but also enriches the marker resource for molecular diagnostic, systematic and evolutionary biological studies of strongyloid nematodes.

Disclosure statement

No potential conflict of interest was reported by the authors. The study was reviewed and approved by the Animal Ethics Committee of Sichuan Agricultural University (Sichuan, China; approval no. SYXK 2014-187).

Funding

This study was supported by the Sichuan International Science and Technology Innovation Cooperation/Hong Kong, Macao and Taiwan Science and Technology Innovation Cooperation Project [No. 2019YFH0065] and Fund Program for the Scientific Activities of Selected Returned Overseas Professionals in Sichuan Province [No. 2022999016].

ORCID

Yue Xie  <http://orcid.org/0000-0001-9062-6928>

Data availability statement

The genome sequence data that support the findings of this study are openly available in GenBank of NCBI at <https://www.ncbi.nlm.nih.gov>, under the accession number MZ888509. The associated BioProject, SRA and Bio-Sample numbers are PRJNA794955, SRR17475793 and SAMN24665689, respectively.

Author contributions

Conceived and designed the research: YX, XZ, XW. Performed the experiments: XL, LW, YC. Analyzed the data and wrote the paper: XL, LW, YX. Revised the paper: YX, XZ, XW. All authors read and approved the final version of this manuscript.

References

- Bernt M, Donath A, Jühling F, Externbrink F, Florentz C, Fritzsche G, Pütz J, Middendorf M, Stadler PF. 2013. MITOS: improved de novo metazoan mitochondrial genome annotation. *Mol Phylogenet Evol.* 69(2): 313–319.
- Bu Y, Niu H, Gasser RB, Beveridge I, Zhang L. 2009. Strongyloid nematodes in the caeca of donkeys in Henan Province. *China Acta Parasitol.* 54:263–268.
- Bu Y, Niu H, Zhang L. 2013. Phylogenetic analysis of the genus *Cylicocyclus* (Nematoda: Strongylidae) based on nuclear ribosomal sequence data. *Acta Parasitol.* 58(2):167–173.
- Gao Y, Qiu JH, Zhang BB, Su X, Fu X, Yue DM, Wang CR. 2017. Complete mitochondrial genome of parasitic nematode *Cylicocyclus nassatus* and comparative analyses with *Cylicocyclus insigne*. *Exp Parasitol.* 172:18–22.
- Gao Y, Wang XX, Ma XX, Zhang ZH, Lan Z, Qiu YY, Wang S, Song MX, Wang CR. 2021. Characterization of the complete mitochondrial genomes of *Coronocyclus labiatus* and *Cylicodontophorus bicoronatus*: comparison with Strongylidae species and phylogenetic implication. *Vet Parasitol.* 290:109359.
- Hahn C, Bachmann L, Chevreux B. 2013. Reconstructing mitochondrial genomes directly from genomic next-generation sequencing reads—a baiting and iterative mapping approach. *Nucleic Acids Res.* 41(13): e129–e129.
- Hu L, Zhang M, Sun Y, Bu Y. 2020. Characterization and phylogenetic analysis of the first complete mitochondrial genome of *Cylicocyclus radiatus*. *Vet Parasitol.* 281:109097.
- Jex AR, Littlewood DT, Gasser RB. 2010. Toward next-generation sequencing of mitochondrial genomes—focus on parasitic worms of animals and biotechnological implications. *Biotechnol Adv.* 28(1):151–159.
- Jex AR, Waeschenbach A, Hu M, Van Wyk JA, Beveridge I, Littlewood DTJ, Gasser RB. 2009. The mitochondrial genomes of *Ancylostoma caninum* and *Bunostomum phlebotomum*—two hookworms of animal health and zoonotic importance. *BMC Genomics.* 10:79.
- Kuzmina TA, Kuzmin YI. 2008. The community of strongylids (Nematoda, Strongylida) of working donkeys (*Equus asinus*) in Ukraine. *Vest Zool.* 2008(42):e18–e23.
- Li Q, Gao Y, Wang XX, Li Y, Gao JF, Wang CR. 2019. The complete mitochondrial genome of *Cylicocyclus ashworthi* (Rhabditida: Cyathostominae). *Mitochondrial DNA B Resour.* 4(1):1225–1226.
- Lichtenfels JR, Kharchenko VA, Dvojnos GM. 2008. Illustrated identification keys to strongylid parasites (Strongylidae: Nematoda) of horses, zebras and asses (Equidae). *Vet Parasitol.* 156(1-2):4–161.
- Liu GH, Shao R, Li JY, Zhou DH, Li H, Zhu XQ. 2013. The complete mitochondrial genomes of three parasitic nematodes of birds: a unique gene order and insights into nematode phylogeny. *BMC Genomics.* 14:414.
- Qiu YY, Zeng MH, Diao PW, Wang XX, Li Q, Li Y, Gao Y, Wang CR. 2019. Comparative analyses of the complete mitochondrial genomes of *Cyathostomum pateratum* and *Cyathostomum catinatum* provide new molecular data for the evolution of Cyathostominae nematodes. *J Helminthol.* 93(5):643–647.
- Xu WW, Qiu JH, Liu GH, Zhang Y, Liu ZX, Duan H, Yue DM, Chang QC, Wang CR, Zhao XC. 2015. The complete mitochondrial genome of *Strongylus equinus* (Chromadorea: Strongylidae): comparison with other closely related species and phylogenetic analyses. *Exp Parasitol.* 159:94–99.
- Zhang SY. 2017. Sequence and phylogenetic analysis of COI and ND1 genes for Strongylidae isolated from Equus. Henan Normal University. 1–71.
- Zhang LP, Kong FY. 2002. Review of the systematics of Cyathostominae (Nematoda: strongylidae). *Acta Zootax Sin.* 27:435–446.