Characterization of the complete mitochondrial genome of the cloacal tapeworm *Cloacotaenia megalops* (Cestoda: Hymenolepididae)

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**Abstract**

**Background:** The cloacal tapeworm *Cloacotaenia megalops* (Hymenolepididae) is one of the most common cestode parasites of domestic and wild ducks worldwide. However, limited information is available regarding its epidemiology, biology, genetics and systematics. This study provides characterisation of the complete mitochondrial (mt) genome of *C. megalops*.

**Methods:** The complete mt genome of *C. megalops* was obtained by long PCR, sequenced and annotated.

**Results:** The length of the entire mt genome of *C. megalops* is 13,887 bp; it contains 12 protein-coding, 2 ribosomal RNA and 22 transfer RNA genes, but lacks an *atp8* gene. The mt gene arrangement of *C. megalops* is identical to that observed in *Anoplocephala magna* and *A. perfoliata* (Anoplocephalidae), *Dipylidium caninum* (Dipylidiidae) and *Hymenolepis diminuta* (Hymenolepididae), but differs from that reported in taeniids owing to the position shift between the tRNA (L1) and tRNA (S2) genes. The phylogenetic position of *C. megalops* was inferred using Maximum likelihood and Bayesian inference methods based on the concatenated amino acid data for 12 protein-coding genes. Phylogenetic trees showed that *C. megalops* is sister to *Anoplocephala* spp. (Anoplocephalidae) + *Pseudanoplocephala crawfordi* + *Hymenolepis* spp. (Hymenolepididae) indicating that the family Hymenolepididae is paraphyletic.

**Conclusions:** The complete mt genome of *C. megalops* is sequenced. Phylogenetic analyses provided an insight into the phylogenetic relationships among the families Anoplocephalidae, Hymenolepididae, Dipylidiidae and Taeniidae. This novel genomic information also provides the opportunity to develop useful genetic markers for studying the molecular epidemiology, biology, genetics and systematics of *C. megalops*.

**Keywords:** Tapeworm, Cestoda, Hymenolepididae, *Cloacotaenia megalops*, Mitochondrial genome, Phylogenetic analyses

**Abbreviations:** BI, Bayesian inference; Bpp, Bayesian posterior probabilities; ML, Maximum likelihood; Mt, Mitochondrial

**Background**

The cloacal tapeworm, *Cloacotaenia megalops* Nitzsch in Creplin, 1829 (Cestoda: Hymenolepididae), is one of the most common hymenolepidid tapeworms parasitising waterfowl, with a global distribution. The life-cycle of this tapeworm is complex. Seed shrimpos (Ostracoda) act as intermediate hosts and many waterfowl species (including ducks, geese and swans) serve as definitive hosts [1]. In China, *C. megalops* is considered as a predominant cestode species in ducks and geese [2, 3].

Comparison of entire mitochondrial (mt) genomes has been used for reconstructing phylogenetic relationships among parasitic Platyhelminthes [4, 5], including cestodes [6–11]. Cestode mt genomes usually encode 36 genes, including 12 protein-coding genes, 2 ribosomal RNA (rRNA) genes and 22 transfer RNA (tRNA) genes [6–11]. Cestoda is a large class of parasitic flatworms...
with many species representing a health danger for animals and humans worldwide. Despite the availability of advanced DNA technologies and bioinformatic methods, there is still a paucity of knowledge of mt genomes for many tapeworms of socioeconomic importance, such as the members of the family Hymenolepididae. Although complete mt genomes are available for Hymenolepis diminuta [8], H. nana (=Rodentolepis nana or Vampirolepis nana) [9], and Pseudanoplocephala crawfordi [10], no mt genomes are available from the genus Cloacotaenia. Furthermore, little is known about the epidemiology, genetics and biology of the type-and only species of this genus, C. megalops.

The taxonomic status of C. megalops has been controversial for many years, and is still debated. Czaplinski & Vaucher [12] considered Cloacotaenia a synonym of Hymenolepis but Makarikov et al. [13] have recently restored the independent status of the genus Cloacotaenia based on remarkable morphological differences between C. megalops and Hymenolepis (sensu stricto). To tackle these issues, in the present study, the complete mt genome of C. megalops was determined and its phylogenetic relationships with selected cestode species were inferred based on analysis of the concatenated mt amino acid sequences.

Methods
Parasites and DNA extraction
Cloacotaenia megalops were collected from the cloaca of ducks from a small abattoir in Xinjiang Uygur Autonomous Region, China. The adult tapeworm was isolated from cloaca of a duck. Cestode identification was conducted by morphological criteria including the features of the scolex and mature and gravid proglottids [14]; the scolex was observed in stereoscan photographs and mature and gravid proglottids were examined after hematoxylin staining. The remaining fragment was fixed in 70 % alcohol and stored at -20 °C until use. Total genomic DNA was extracted from one of these specimens using Tissue DNA Kit (OMEGA, Doraville, USA) according to the manufacturer’s instructions.

PCR amplification and sequencing
Three pairs of PCR primers (Additional file 1: Table S1) were designed based on well-conserved regions within the mt genomes of tapeworms [6, 7]. These primers were used to amplify three overlapping segments of the complete mt genome of C. megalops by long PCR technology. Long PCR reactions (50 μl) were conducted in 5.0 μl 10× LA Mixture (Takara), 10 pmol of each primer (1 μl), 1.5 μl of DNA sample and 41.5 μl of H2O in a thermocycler (Eppendorf, Hamburg, Germany) under the following conditions: 94 °C for 5 min (initial denaturation), followed by 35 cycles of 98 °C for 10 s (denaturation), 50 °C for 20 s (annealing), and 68 °C for 8 min (extension), and with a final extension step at 68 °C for 10 min. Amplicons were examined on 0.8 % agarose gels stained with ethidium bromide. PCR products were subsequently sent to Sangon Biotech Co. Ltd. (Shanghai, China) for sequencing using a primer-walking strategy.

Sequence analyses
Sequences were assembled using CAP3 Server online. The complete mt genome of C. megalops was aligned against the complete mt genome sequences of H. diminuta and A. perfoliata using the computer program MAFFT 7.122 [15] to identify gene boundaries. Each gene was translated into its amino acid sequence using the flatworm mt genetic code (Translation table 9) in MEGA 5 [16]. The translation start and stop codons were identified based on the similarity of the gene lengths and usual codons between H. diminuta and A. perfoliata mt genomes. Twenty-two tRNA genes were predicted using the program tRNAscan-SE [17] and then confirmed by recognizing anticodon sequences and potential secondary structures by visual inspection, and two rRNA genes were identified by comparison with that of H. diminuta and A. perfoliata [7, 8].

Phylogenetic analyses
A total of 20 tapeworm species were selected for phylogenetic analyses using one trematode Schistosoma japonicum (GenBank accession number NC_002544) as the outgroup [18]. The 12 amino acid sequences of protein-coding genes were aligned independently using MAFFT 7.122. Ambiguously aligned sites and regions were excluded using GBLOCKS (http://molevol.cmima.csic.es/castresana/Gblocks_server) [19]. Phylogenetic analyses were performed using Bayesian inference (BI) and Maximum likelihood (ML) methods. The Akaike information criterion as implemented in ProtTest 2.4 [20] was used to choose the most suitable model of evolution. BI was performed in MrBayes using the MtArt + I + G + F model of evolution. BI was set up to perform two runs, each of four simultaneous chains for the Monte Carlo Markov Chain. In each run, the number of generations was set to 1,000,000 and a tree was sampled every 100 generations in MrBayes 3.1.1 [21]; the average standard deviation of split frequencies of less than 0.01 and the potential scale reduction factor approaching 1 were used to ensure the convergence of the two runs. The first 25 % of the trees were discarded as ‘burn-in’. A 50 % majority rule consensus tree was used to calculate Bayesian posterior probabilities (Bpp). ML analysis was conducted using PhyML 3.0 [22]. A BioNJ tree was used as a starting tree to search for the ML tree with the MtArt + I + G model of evolution. The subtree pruning
and regrafting method was chosen. The middle of each discretized substitution rate class was determined using the median. ML analyses were checked on the basis of 100 bootstrap replicates (Bf). Phylogenograms were drawn using the program FigTree v.1.4 (http://tree.bio.ed.ac.uk/software/figtree).

Results and discussion

Features of the C. megalops mt genome

The complete mt genome of C. megalops is a 13,887 bp (KU641017) long circular DNA molecule (Fig. 1). All 36 genes expected for tapeworm mt genomes have been identified. This AT rich (71.6 %) mt genome includes 12 protein-coding genes (atp6, cox1–3, cytb, nad1–6 and nad4L), 22 tRNA genes and two rRNA genes, but lacks an atp8 gene (Table 1). Thirty-six mt genes are transcribed from the same direction (Fig. 1). The size of C. megalops mt genome is similar to other tapeworm mt genomes, such as H. diminuta (13,900 bp) [8], H. nana (13,764 bp) [9] and P. crawfordi (14,192 bp) [10]. The mt gene arrangement of C. megalops is identical to that in Anoplocephala magna and A. perfoliata (Anoplocephalidae), Dipylidium caninum (Dipylidiidae) and Hymenolepis diminuta (Hymenolepididae), but differs from that in taeniids owing to the position shift between the tRNA-Gly and cox3 genes. The two leucine tRNA genes are designated by L1 (CUN) and L2 (UUR), respectively, and two serine tRNA genes by S1 (AGN) and S2 (UCN), respectively. Gene scaling is only approximate

Annotation

A total of 3352 amino acids are encoded in the C. megalops mt genome. The aggregate length of all of the 12 protein-coding genes is 10,092 bp. In terms of the length of individual protein-coding genes, cox1 gene is the largest (1593 bp) and nad4L gene is the shortest (261 bp). In this mt genome, all protein-coding genes use ATG as start codon (Table 1). All protein-coding genes have complete termination codons (TAA and TAG) (Table 1). However, some studies have indicated that the incomplete termination codons T or TA are present in the protein-coding genes of some tapeworm mt genomes [7, 23]. A total of 22 tRNA (ranging from 56 to 72 nucleotides in length) genes were identified. Their predicted secondary structures (not shown) are similar to those in H. diminuta and A. perfoliata [8]. The tRNA-Cys gene separates rrnL from rrnS. The size of the rrnL gene is 959 bp and the size of the rrnS gene is 722 bp (Table 1). One larger non-coding region (NC2; 446 bp) is located between the tRNA-Gly and cox3 genes, and one shorter non-coding region (NC1; 221 bp) is located between the tRNA-Tyr and tRNA-Ser genes (Table 1; Fig. 1). In the NC1 region, there were two sets of short inverted repeats and one set of long inverted repeats (33 bp), each of them could be folded into a stem-loop hairpin structure (Additional file 2: Figure S1A). The NC2 region consists of six identical tandem repeats with 31 bp sequences (Additional file 2: Figure S1B). Similar stable hairpin structures and tandem repeats in C. megalops may play the same role as those in vertebrates, which have been shown to initiate replication and transcription [24].

Sequence comparisons

Pairwise comparisons of C. megalops mt protein-coding genes with those of three other hymenolepidid tapeworms revealed 12.9–35.3 % differences in the nucleotide sequences, and 10.6–43.6 % differences in amino acid sequences (Table 2). Among twelve protein-coding genes, cox1 and cytb genes were relatively conserved whilst nad5 and nad6 genes were the most different in all four species (Table 2). These results are useful to design primers to capture high sequence variability within and between mt genes of these species as genetic markers for population genetics and diagnostics.

Phylogenetic analyses

Phylogenetic analyses showed three distinct groups with high statistical support (BPP = 1.0; Bf = 98 %) with C. megalops as a sister taxon to Anoplocephala spp. (Anoplocephalidae) + P. crawfordi + Hymenolepis spp. (Hymenolepididae) (Fig. 2), indicating that the family Hymenolepididae is paraphyletic. The result is consistent with the maximum likelihood analysis in the study by
von Nickisch-Rosenegk et al. [25] in which 12S rDNA data of a wider set of taxa representing a larger number of families. The families Taeniidae, Anoplocephalidae and Diphyllobothriidae were monophyletic with maximum support in all analyses (Bpp = 1.0; Bf = 100 %) (Fig. 2), in agreement with previous studies [7, 9, 26]. In addition, our results show that *H. diminuta* is more closely related to *P. crawfordi* than to *H. nana* (Fig. 2).

In this study, the phylogenetic tree revealed that *C. megalops* is distantly related to the genus *Hymenolepis*, supporting the view of Makarikov et al. [13] who preferred to retain the independent status of *Cloacotaenia*

| Gene/region    | Position | Size (bp) | Start codon | Stop codon |
|---------------|----------|-----------|-------------|------------|
| cox1          | 1–1593   | 1593      | ATG         | TAA        |
| tRNA-Thr (T)  | 1574–1637| 64        | –           | –          |
| rrnL          | 1638–2596| 959       | –           | –          |
| tRNA-Cys (C)  | 2597–2660| 64        | –           | –          |
| rrnS          | 2661–3382| 722       | –           | –          |
| cox2          | 3383–3961| 579       | ATG         | TAG        |
| tRNA-Glu (E)  | 3963–4029| 67        | –           | –          |
| nad6          | 4033–4482| 450       | ATG         | TAA        |
| tRNA-Tyr (Y)  | 4487–4548| 62        | –           | –          |
| Non-coding region (NC1) | 4549–4759 | 211 | – | – |
| tRNA-SerUCN (S2) | 4760–4824 | 65 | – | – |
| tRNA-LeuUCN (L1) | 4841–4904 | 64 | – | – |
| tRNA-LeuUUR (L2) | 4907–4971 | 65 | – | – |
| tRNA-Arg (R)  | 4972–5027| 56        | –           | –          |
| nad4          | 5031–6602| 1572      | ATG         | TAA        |
| tRNA-Gly (G)  | 6609–6680| 72        | –           | –          |
| Non-coding region (NC2) | 6681–7126 | 446 | – | – |
| cox3          | 7127–7780| 654       | ATG         | TAG        |
| tRNA-His (H)  | 7771–7837| 67        | –           | –          |
| cyt b         | 7841–8932| 1092      | ATG         | TAG        |
| nad4L         | 8936–9196| 261       | ATG         | TAA        |
| nad5          | 9157–10,410| 1254     | ATG         | TAG        |
| tRNA-Gln (Q)  | 10,411–10,473| 63| – | – |
| tRNA-Phe (F)  | 10,475–10,533| 59| – | – |
| tRNA-Met (M)  | 10,532–10,596| 65| – | – |
| atp6          | 10,600–11,115| 513| ATG | TAG |
| nad2          | 11,121–11,999| 879| ATG | TAG |
| tRNA-Val (V)  | 12,009–12,072| 64| – | – |
| tRNA-Ala (A)  | 12,073–12,135| 63| – | – |
| tRNA-Asp (D)  | 12,149–12,211| 63| – | – |
| nad1          | 12,215–13,105| 891| ATG | TAG |
| tRNA-Asn (N)  | 13,105–13,169| 65| – | – |
| tRNA-Pro (P)  | 13,184–13,247| 64| – | – |
| tRNA-Ile (I)  | 13,248–13,308| 61| – | – |
| tRNA-Lys (K)  | 13,320–13,384| 65| – | – |
| nad3          | 13,388–13,741| 354| ATG | TAG |
| tRNA-SerAGN (S1) | 13,749–13,809 | 61 | – | – |
| tRNA-Trp (W)  | 13,820–13,884| 65| – | – |
based on the morphological characteristics. The present results also suggest that *Pseudanoplocephala crawfordi* is a member of the genus *Hymenolepis* as shown in previous studies [10, 27]. *Hymenolepis nana* is one of the most common tapeworms infecting humans and rodents. However, there is confusion regarding the nomenclature of this species [28]. A taxonomic revision of hymenolepidids with armed rostellae was suggested by Vaucher [29] in which *Hymenolepis nana* was regarded as a member of the genus *Rodentolepis*. This classification is currently accepted by some cestode taxonomists [12]. Despite the nomenclature being revised,
the name *H. nana* persists in textbooks and medical papers [30]. Additionally, whether the Anoplocephalidae should also be rigorously evaluated in further studies based on more extensive taxon sampling of hymenolepidids. This will also help a better understanding of the evolution of hymenolepidid cestodes and a re-evaluation of the morphological traits employed in their systematics.

**Conclusions**

The complete mt genome of *C. megalops* is characterised. Phylogenetic analyses of the concatenated amino acid sequence dataset for 12 protein-coding mt genes of *C. megalops* and selected cestode representatives indicated that the family Hymenolepididae is paraphyletic. This mt genome provides a unique genetic marker for studying the molecular biology, genetics and systematics of *C. megalops*.

**Additional files**

**Additional file 1: Table S1.** Primers used to amplify PCR fragments for *Cloacotaenia megalops*. (DOC 37 kb)

**Additional file 2: Figure S1.** Putative secondary structures for the two non-coding regions in *Cloacotaenia megalops* mtDNA. The NC1 (A) consists of two identical repeats of 34 nt shown in the box. The NC2 region (B) consists of six identical tandem repeats of a 31 nt sequence and part of the seventh repeat (10 nt). Arrows represent inverted repeats. (DOC 330 kb)

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**Availability of data and material**

The datasets supporting the conclusions of this article are included within the article and its additional files. The complete mt genome sequence is submitted to the GenBank database under accession no. KU641017.

**Competing interests**

The author declares that she has no competing interests.

**Consent for publication**

Not applicable.

**Ethics approval and consent to participate**

Not applicable.

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