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The mitochondrial genome of *Ancylostoma tubaeforme* from cats in China

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Abstract

Ancylostoma tubaeforme may infect canids, felids and humans, and pose a potential risk to public health. Polymerase chain reaction (PCR) techniques were used to amplify the complete mitochondrial (mt) genome sequence of *A. tubaeforme* from cats and to analyse its sequence characteristics after molecular identification based on the internal transcribed spacer ITS1+ sequence. The results show that the complete mt genome sequence (GenBank accession number KY070315) of *A. tubaeforme* from cats was 13,730 bp in length, including 12 protein-coding genes, 22 transfer RNA (tRNA) genes, two ribosomal RNA (rRNA) genes, two non-coding regions and an AT-rich region. The nucleotide content of A and T was 77.93%, biased toward A and T. Twelve protein-coding genes used ATT, TTG and GTG as initiation codons, and TAA, TAG, TA and T as termination codons. The length of the 22 tRNA genes ranged from 52 to 62 bp, their predicted secondary structures were D loops and V loops. The lengths of the two rRNAs were 958 and 697 bp. Phylogenetic analyses showed that *A. tubaeforme* from cats was in the lineage of *Ancylostoma*, having a close phylogenetic relationship with *A. caninum*. This study reports for the first time the mt genome of *A. tubaeforme* from cats in China, which could enhance the mt genome database of Ancylostomatidae nematodes, and it offers the scientific basis for further studies in the genetic diversity of hookworms among different hosts.

Introduction

Hookworms are soil-transmitted parasites that inhabit the small intestines of mammals, including humans, canids and felids. They may cause dyspepsia, abdominal pain and diarrhoea, as well as iron deficiency anaemia and even death under heavy infection (Chilton & Gasser, 1999; Landmann & Prociv, 2003; Jex *et al*., 2009). The common hookworms of dogs and cats are *Ancylostoma caninum, A. tubaeforme, A. ceylanicum, A. braziliense* and *Uncinaria stenocephala* (Prociv, 1998). They can also infect humans, which can lead to cutaneous larva migrans (CLM) and eosinophilic enteritis (EE) (Landmann & Prociv, 2003; Bowman *et al*., 2010).

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Ancylostoma tubaeforme, a common species of felids, is prevalent throughout warmer regions of the world (Gasser *et al*., 1996). It has been reported that cats were infected with *A. tubaeforme* in many countries, such as Australia (Kelly & Ng, 1975), America (Gates & Nolan, 2009), Spain (Millan & Blasco-Costa, 2012), Brazil (Labarthe *et al*., 2004) and Italy (Riggio *et al*., 2013). Several studies have shown that rDNA internal transcribed spacers (ITS1 and ITS2) provide genetic markers to study the epidemiology of hookworm infections (Gasser *et al*., 1996). However, the ITS1 and ITS2 regions are not suitable for assessing levels of sequence variation within individual species (intraspecific variation) to study the genetic structure within and among hookworm populations (Gasser *et al*., 1998). In contrast, mitochondrial (mt) genes have been used extensively as genetic markers to study the intraspecific variation of hookworm species, due to their rapid evolutionary rate and high mutation

rate (Boore, 1999; Jex *et al*., 2009; Gao *et al*., 2014). In addition, the mt genome belongs to extranuclear DNA, its inheritance has a maternal genetic mode and less gene recombination. Accordingly, mtDNA has been used to study molecular epidemiology and population genetics, as well as phylogenetic and evolutionary relationships (G. Liu *et al*., 2015; Zhang *et al*., 2015).

At present, the complete mt genome sequences of *A. duodenale*, *Necator americanus* and *A. caninum* have been reported (Hu *et al*., 2002; Jex *et al*., 2009), but knowledge of the mt genome of *A. tubaeforme* is scanty. The objectives of the present study were to amplify the complete mt genome sequence of *A. tubaeforme* isolated from cats and to analyse its sequence characteristics after molecular identification based on the ITS1+ sequence. This study can augment mt genome databases of Ancylostomatidae nematodes and could provide the scientific basis for further studies of the genetic diversity of hookworms among different hosts.

Materials and methods

Parasites and DNA extraction

The hookworms used in this study were from Guangzhou, China. Adult *A. tubaeforme* were obtained from stray cats in a humane shelter following treatment with Drontal Plus. Samples were identified morphologically, fixed in 75% ethanol and stored at −20°C until use. Individual worms were put in centrifuge tubes and flushed five times with double-distilled water (ddH_2O). Total genomic DNA from individual worms was extracted using the Wizard® SV Genomic DNA Purification System (Promega, Guangzhou, China) according to the manufacturer's instructions, and stored at −20°C for use.

Molecular identification

The primer AF (5′-GACTGCGGACTGCTGTAT-3′) and its complementary primer AR (5′-AAGTTCAGCGGG TAGTCA-3′) were designed according to Y.J. Liu *et al.* (2015) to amplify the ITS1+ sequence of *A. tubaeforme* and compare it with the same sequence of relevant hookworms in GenBank.

PCR amplification of the complete mt genome

Based on the complete mt genome sequences of *A. duodenale*, *N. americanus* and *A. caninum* downloaded from GenBank, eight pairs of primers (table 1) were designed in their conserved regions to amplify the entire mt genome sequence of *A. tubaeforme*. Polymerase chain reactions (PCR) for a <2-kb fragment were performed in 25 µl, including 2.5 µl of 10× PCR buffer (Mg^{2+} free), 3.0 µl of $MgCl_2$ (25 mmol/l), 2.0 µl of deoxyribonucleoside triphosphates (dNTPs) (2.5 mmol/l), 1.0 µl of each primer (25 µmol/ml), 1 µl of DNA samples, 0.25 µl of ExTaq polymerase (TaKaRa, Dalian, China) and 13.25 µl of ddH_2O. PCR conditions were: initial denaturation at 94°C for 5 min; followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 47–55°C for 30 s and extension at 72°C for 1 min; then a final extension at 72°C for 5 min. Long PCR reactions for a >2-kb fragment were performed in 12.5 µl of Premix PrimeStar Max (TaKaRa), 1.0 µl for each of two primers (25 µmol/ml), 2 µl of genomic DNA and ddH_2O was added to 25 µl. The cycling conditions were: initial denaturation at 94°C for 5 min; then denaturation at 94°C for 30 s, annealing at 45–50°C for 50 s and extension at 68°C for 1.5 min for 10 cycles; followed by initial denaturation at 94°C for 2 min; denaturation at 94°C for 30 s, annealing at 48–53°C for 1.5 min and extension at 68°C for 1.5–2.0 min for 25 cycles; and then a final extension at 68°C for 7 min. Amplified PCR products were detected on 1% agarose gels. The PCR products were cloned in *Escherichia coli* and connected with pMD18-T (TaKaRa, Dalian, China), then transferred into DH5 Competent Cells (TaKaRa). Positive clones were screened by bacterial PCR, the plasmids were extracted and sent to Shanghai Sangon Co., Ltd for sequencing.

Sequence analysis

The high-quality sequences obtained using BioEdit version 7.0 were assembled by seqMan software within DNAStar 5.0

Table 1. Primers used for PCR amplification of the mitochondrial genome of *Ancylostoma tubaeforme* from cats.

Name	Sequence (5′–3′)	Amplified region	Expected length
F1	F: TTGTTATTGGGTAATGGTC R: AACCCACAATAACAAAGG	*cox1*	~1 kb
F2	F: TAGTTGACTTTGTGCTGGT R: TGGAACTCAATAAAAGCA	*cox1–rrnL*	~2.7 kb
F3	F: TTTAGAAATGGCAGTCTTAG R: TAAAGTCCCATAAAAAAATA	*rrnL–nad5*	~2.6 kb
F4	F: AGCGTTGGTTGAGGAAGA R: TTACGCAAAGCCCCTGAA	*nad5–nad6*	~1.8 kb
F5	F: GTTTCGTTAGTTGGAGGTG R: GACGCTCATACAACGTAATAAA	*nad6–nad1*	~1.8 kb
F6	F: GGAGGTTGAGTAATAATTGAG R: GAGCAGGATAACCTAAAAC	*rrnS–nad2*	~1.8 kb
F7	F: CGTAGTTCTTATCCTCGTT R: CAACTTTCACCCAACTCA	*nad1–cox3*	~3.5 kb
F8	F: GTGCATATTGTGCCTGAG R: CAGAGTTCTTAAAGGAGGA	*cytb–cox1*	~3.0 kb

F, forward; R, reverse.

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(Tamura *et al.*, 2011) and adjusted manually. Utilizing online software (http://dogma.ccbb.utexas.edu/) combined with Megalign software in DNAStar 5.0 (Tamura *et al.*, 2011) to identify gene boundaries and composition, as well as translation initiation and termination codons. 22 transfer RNA genes of *A. tubaeforme* were identified with the aid of the tRNA scan program available at http://lowelab.ucsc.edu/tRNAscan-SE/ combined with artificial proofreading using Ancylostomatidae nematodes. The rRNA genes were identified by aligning sequences with those of *A. duodenale* and *N. americanus* (Hu *et al.*, 2002). Their secondary structures were predicted by comparing them with the published structures of *A. duodenale* and *N. americanus* (Hu *et al.*, 2002).

Phylogenetic analyses

Phylogenetic analyses were performed using 25 Strongylata nematodes (see fig. 6) as ingroups, and *Toxocara* nematodes (*T. canis* and *T. cati*) as the outgroup, based on amino acid sequences of 12 protein-coding genes. The amino acid sequence for each gene was individually aligned using Clustal X under the default setting, and then concatenated into a single alignment for phylogenetic analyses. The (LG+G+F) model of amino acid evolution was selected as the most suitable model of evolution by ProtTest 2.4 (Abascal *et al.*, 2005) based on Akaike information criterion (AIC). Maximum parsimony (MP) was performed in PAUP* 4.0 Beta 10 (Swofford, 2002), maximum likelihood (ML) was implemented by PhyML3.0 (Guindon & Gascuel, 2003). Branch supports were estimated by bootstrap analysis of 1000 replicates for MP trees, and 100 replicates for the ML tree. Baynesian inference (BI) was conducted with four independent Markov chains, run for 2,000,000 metropolis-coupled MCMC generations, sampling a tree for every 100 generations in MrBayes 3.1.1 (Ronquist & Huelsenbeck, 2003). Phylograms were drawn using FigTreev1.4 software (Page, 1996).

Results

Species identification

There were a pair of prominent chitinous plates bearing three teeth in the ventral oral capsule. Three lateral costae of the copulatory bursa in the male had a single origin, the mediolateral and posterolateral were close together. There was an abrupt narrowing posterior to the anus in the female, and the tail was often curved ventrally (fig. 1). The amplified ITS1+ fragment was 404 bp in length and the generated sequence data were submitted to GenBank (KY474056). BLAST analysis indicated highest similarity (99%) with *A. tubaeforme* from America (GenBank accession number: JQ812691). Thus, the hookworm was identified as *A. tubaeforme*.

Amplification of A. tubaeforme *mt genome*

The amplified fragments from eight pairs of primers for the complete mt genome of *A. tubaeforme* from cats were 1020, 2700, 2638, 1837, 1818, 1830, 3595 and 3068 bp in size, respectively, which are consistent with predicted fragments (fig. 2).

General features of A. tubaeforme *mt genome*

The complete mt genome sequence (GenBank accession number KY070315) of *A. tubaeforme* from cats was 13,730 bp in length. There were 36 genes, including 12 protein-coding genes, 22 transfer RNA genes and two ribosomal RNA genes, two non-coding regions (SNCR, LNCR) and one AT-rich region (fig. 3). All mt genes were transcribed in the same direction and located on the heavy strand. The arrangement of the mt genome was compact, with only two overlap regions of 2 bp and 1 bp between *trn*E and *rrn*S, *trn*S2^{UCN} and *trn*N. There were 13 intergenic regions ranging from 1 to 19 bp in size, the longest one was located between *trn*K and *trn*L2^{UUR} and the shortest were located between genes *cox*2 and *trn*H, *trn*R and *trn*Q, *cox*3 and *trn*T (table 2). The base composition of the mt genome was biased toward A and T. The nucleotide composition of the entire mt genome was A = 29.24%, G = 15.57%, T = 48.54% and C = 6.65%; while the content of A+T was 77.78%.

Protein genes and codon usage patterns

The most conserved genes in the 12 protein-coding genes of *A. tubaeforme* were *nad*4L, *cox*1 and *cox*3, while the *nad2*, *nad1* and *nad5* were the least conserved ones.

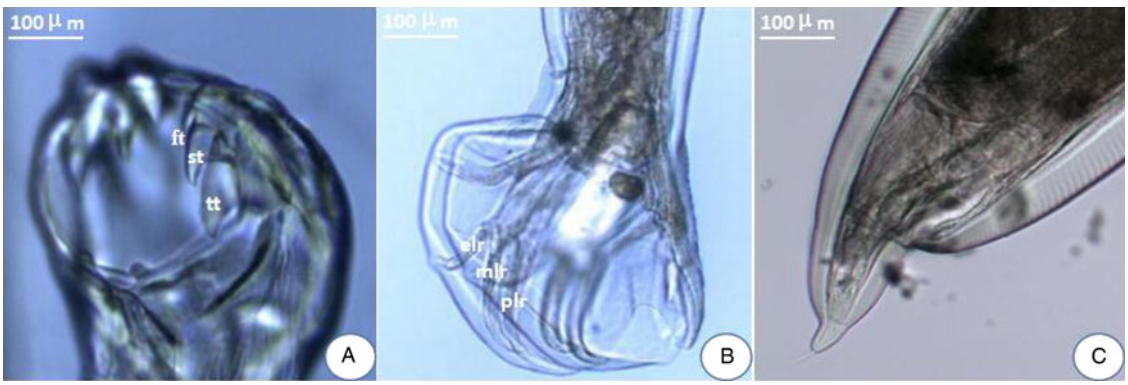

Fig. 1. Morphological characters of adult *Ancylostoma tubaeforme*. (A) A pair of plates bearing three teeth – first tooth (ft), second tooth (st) and third tooth (tt) – in the ventral oral capsule. (B) In the male, three lateral costae: mediolateral ray (mlr), posterolateral ray (plr) and externolateral ray (elr) of copulatory bursa. (C) An abrupt narrowing posterior to the anus in the female.

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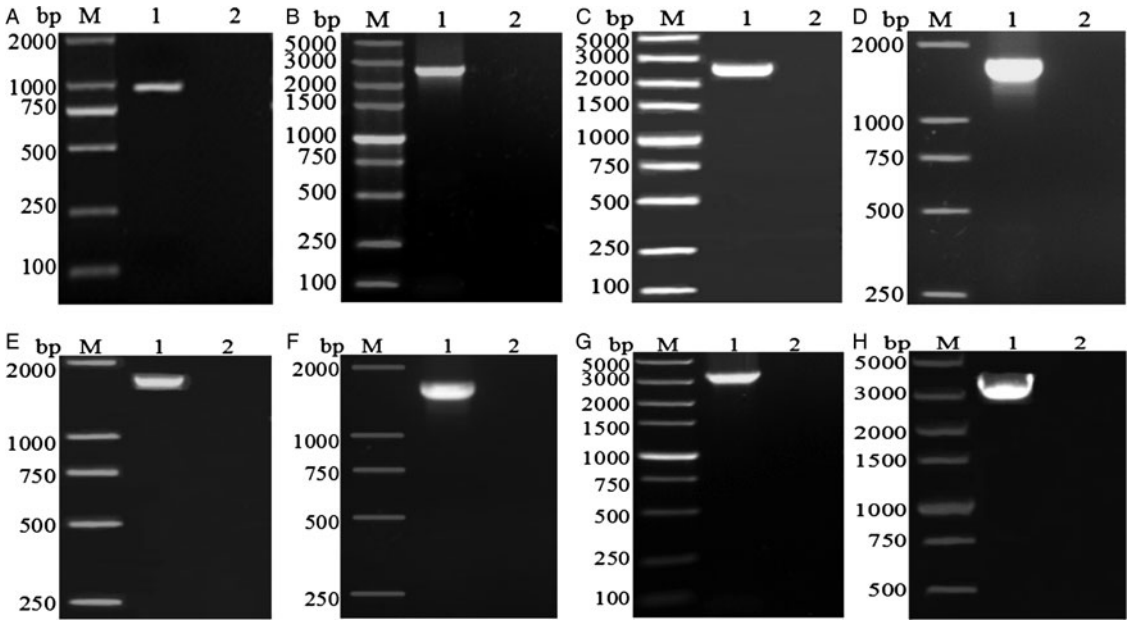

Fig. 2. PCR amplification of the mitochondrial genome of *A. tubaeforme* from cats. (A–H) PCR products from primers F1–F8, respectively; Lanes: M, DL2000 marker or DL5000 marker; 1, target fragment; 2, negative control.

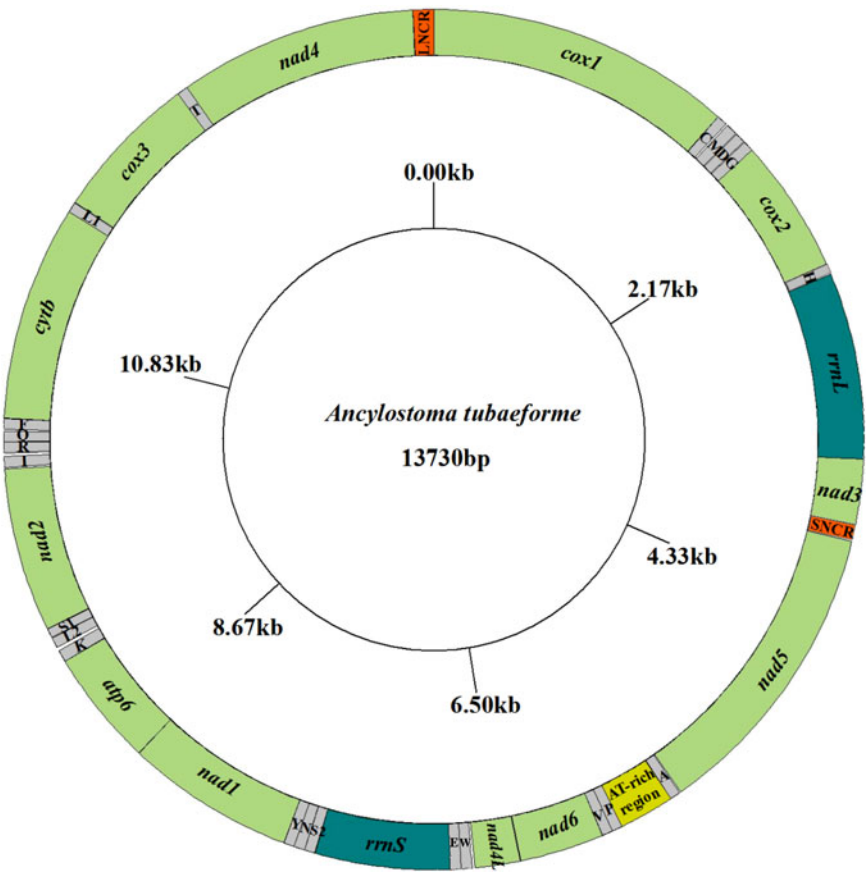

Fig. 3. Arrangement of the mitochondrial genome of *A. tubaeforme* from cats.

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Table 2. Organization of the mitochondrial genome of *Ancylostoma tubaeforme* from cats.

Gene/Region	Position (fragment size)	Codon (Ini/Ter)	Anticodon	Intergenic nucleotides
cox1	1–1578 (1578)	ATT/TAA		0
*trn*C	1579–1632 (54)		GCA	10
*trn*M	1643–1701 (59)		CAT	6
*trn*D	1708–1761 (54)		GTC	2
*trn*G	1764–1819 (56)		TCC	0
cox2	1820–2515 (696)	ATT/TAA		1
*trn*H	2517–2570 (54)		GTG	0
rrnL	2571–3528 (958)			0
nad3	3529–3864 (336)	ATT/TAG		0
SNCR	3865–3952 (88)			0
nad5	3953–5528 (1576)	ATT/T		6
*trn*A	5535–5589 (55)		TGC	0
AT-rich region	5590–5875 (284)			0
*trn*P	5876–5929 (54)		TGG	0
*trn*V	5930–5984 (55)		TAC	0
nad6	5985–6419 (435)	GTG/TAG		2
nad4L	6422–6655 (234)	ATT/TAG		11
*trn*W	6667–6724 (58)		GTA	0
*trn*E	6725–6781 (57)		TTC	−2
rrnS	6780–7476 (697)			0
*trn*S2^{UCN}	7477–7531 (55)		TGA	−1
*trn*N	7531–7587 (57)		GTT	0
*trn*Y	7588–7643 (56)		GTA	0
nad1	7644–8516 (873)	ATT/TAA		0
atp6	8517–9115 (599)	ATT/TA		0
*trn*K	9116–9177 (62)		TTT	19
*trn*L2^{UUR}	9197–9251 (55)		TAA	0
*trn*S1^{AGN}	9252–9303 (52)		TCT	0
nad2	9304–10149 (846)	TTG/TAA		7
*trn*I	10157–10215 (59)		GAT	4
*trn*R	10220–10283 (64)		ACG	1
*trn*Q	10285–10339 (55)		TTG	7
*trn*F	10347–10403 (57)		GAA	0
cytb	10404–11515 (1112)	ATT/TA		0
*trn*L1^{CUN}	11516–11571 (56)		TAG	0
cox3	11572–12337 (766)	ATT/T		1
*trn*T	12339–12393 (56)		TGT	0
nad4	12394–13623 (1230)	TTG/TAA		0
LNCR	13624–13730 (107)			0

Ini, initiation codon; Ter, termination codon.

The content of base A, G, T and C was 24.59–28.49%, 12.93–19.54%, 44.83–54.02% and 2.68–10.01%, respectively; while the AT content ranged from 70.60 to 81.68% (table 3). The 12 protein-coding genes in the mt genome used ATT, TTG and GTG as initiation codons. Among them, ATT was the most common (75%, for 9 of 12 protein genes), followed by TTG (17%) and GTG (8% only). The use of the termination codons was more variable, there were complete TAA and TAG codons, and incomplete TA and T stop codons, which is slightly different from the mt genome of other Ancylostomatidae nematodes (table 4).

Transfer RNA genes

The 22 tRNA genes had compact structure and varied from 52 to 62 bp in length. The predicted secondary structures were almost all TV loops except for *trn*S1^{AGN} and *trn*S2^{UCN}, which were D-loop structures. The acceptor arm was composed of 7 bp and the anticodon area included an anticodon stem of 5 bp and a loop of 7 bp. Because the D-loop structure lacked a DHU arm, there were six bases of loop connecting the anticodon area

Table 3. Nucleotide composition (%) of the 12 protein-coding genes of the *A. tubaeforme* mitochondrial genome.

Gene	A	G	T	C	A+T
cox1	24.59	19.39	46.01	10.01	70.60
cox2	28.02	19.54	44.83	7.61	72.84
nad3	27.08	16.67	53.57	2.68	80.65
nad5	28.17	15.86	49.87	6.09	78.05
nad6	27.36	15.17	54.02	3.45	81.38
nad4L	25.21	17.52	53.42	3.85	78.63
nad1	27.64	15.14	49.20	8.03	76.83
atp6	28.05	15.19	50.75	6.01	78.80
nad2	28.49	13.71	53.19	4.61	81.68
cytb	26.71	17.45	48.20	7.64	74.91
cox3	26.71	17.45	48.20	7.64	74.91
nad4	28.05	12.93	52.03	6.99	80.08

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Table 4. Nucleotide codon usage for 12 protein-coding genes of the mitochondrial genome of *A. tubaeforme* and other Ancylostomatidae nematodes.

Gene	At (Ini/Ter)	Ad (Ini/Ter)	Aca (Ini/Ter)	Ace (Ini/Ter)	Na (Ini/Ter)
cox1	ATT/TAA	ATT/TA	ATT/TAA	ATA/TAA	ATT/TAA
cox2	ATT/TAA	ATT/TAA	ATA/TAA	ATT/TAG	ATA/TAA
nad3	ATT/TAG	ATT/TAG	ATT/TAA	ATT/TAG	ATT/TAA
nad5	ATT/T	ATT/T	ATT/T	TTG/T	ATT/T
nad6	GTG/TAG	ATG/TAG	GTG/TAA	ATT/TAA	GTG/TAA
nad4L	ATT/TAG	ATT/TAA	ATT/TAG	ATT/TAA	ATT/TAG
nad1	ATT/TAA	ATT/TAA	ATT/T	ATT/TAA	ATT/TAG
atp6	ATT/TA	ATT/TA	ATT/TAG	ATT/TAA	ATT/TAG
nad2	TTG/TAA	TTG/TAA	TTG/TAT	TTG/TAA	TTG/TAA
cytb	ATT/TA	ATT/TA	ATT/TAA	ATT/TAG	ATT/TTA
cox3	ATT/T	ATT/T	ATT/T	ATT/T	ATT/T
nad4	TTG/TAA	TTG/TAA	TTG/TAA	TTG/TAA	TTG/TAA

At, *A. tubaeforme*; Ad, *A. duodenale* (AJ417718); Aca, *A. caninum* (FJ483518); Ace, *A. ceylanicum* (AP017674); Na, *N. americanus* (AJ556134); Ini, initiation codon; Ter, termination codon.

and acceptor arm. The TV-loop structure evolved from the TΨC area and variable area, and varied between 6 and 12 bp in size (fig. 4).

Ribosomal RNA genes

The two rRNA genes in the mt genome of *A. tubaeforme* from cats encoded a large subunit 16S (*rrnL*) and small subunit 12S (*rrnS*). They were located between *trn*H and *nad3*, and *trn*E and *trn*S2^{UCN}, respectively. The lengths of *rrnL* and *rrnS* genes were 958 bp and 697 bp, respectively. There was only one copy of each rRNA gene and an overlap region of 2 bp between *rrnS* and *trn*E (fig. 5).

AT-rich and non-coding regions

An AT-rich region and two non-coding regions (SNCR, LNCR) in the mt genome of *A. tubaeforme* from cats, were located between *trn*A and *trn*P, *nad*3 and *nad*5, and *nad*4 and *cox*1, respectively, while their sequence lengths were 284 bp, 88 bp and 107 bp, respectively. The AT contents were 92.25, 78.05 and 82.5%, respectively. There were a large number of A and T multi-copy fragments in the AT-rich region, and a repeat sequence of TATATTTAGT in SNCR.

Phylogenetic tree based on the mt genome

A phylogenetic tree of *A. tubaeforme* from cats was constructed by ML, MP and BI methods based on concatenation of the 12 protein-coding genes of the mt genome (fig. 6). The result showed that *A. tubaeforme* from cats was in the branch of *Ancylostoma* and its closest phylogenetic relationship was with *A. caninum*, followed by *A. duodenale* and *A. ceylanicum*.

Discussion

There are many unique characteristics of gene composition, arrangement, heredity and variation, as well as codon usage, of the mt genome. It has been shown that mt gene sequence has the same or similar characteristics between two species of similar taxonomic status (Hu & Gasser, 2006). In the present study, the complete mt genome of *A. tubaeforme* from cats was 13,730 bp in length. Compared with other nematodes reported to date, it is similar to those of Strongylata nematodes in size (13,300–13,869 bp) (Hu *et al*., 2002; Jex *et al*., 2009; Gasser *et al*., 2012; Lin *et al*., 2012; Zhao *et al*., 2013; Gao *et al*., 2014), whereas it is slightly smaller than the mtDNA sequence of Ascaridida (13,916–14,898 bp) (Jex *et al*., 2008; Li *et al*., 2008; Xie *et al*., 2011; Kim *et al*., 2012; Liu *et al*., 2013). This is mainly due to differences in length of the AT-rich region and non-coding regions (SNCR, LNCR) on the mt genome.

There are three non-coding regions in the *A. tubaeforme* mt genome. The longest one is located between *trn*A and *trn*P. The content of bases A and T in this region is as high as 92.25%, and it is therefore called an 'AT-rich region'. The size of the AT-rich region is consistent with that of *A. duodenale* and has slight differences from that of other Ancylostomatidae nematodes, but apparent differences from those of other nematodes, such as *Ascaris suum*, *Toxocara canis* and *T. malaysiensis* (Okimoto *et al*., 1992; Li *et al*., 2008). The other non-coding regions are located between *cox1* and *nad4*, and *nad3* and *nad5*, like those of *A. duodenale* and *N. americanus*. These non-coding regions can form complex secondary structures with stem and loop arrangements (D loops), and this is also the main reason for the great differences in the length of the mt genome (Le *et al*., 2002).

The predicted secondary structures of the 22 tRNA genes in *A. tubaeforme* from cats are almost all TV loops, except for *trn*S1^{AGN} and *trn*S2^{UCN} genes, which appear to be D loops. Notably, there is an overlap region of 1 bp between *trn*S2^{UCN} and *trn*N, as found in *A. duodenale* and *N. americanus*. It has been demonstrated that these two genes share same base in the process of mitochondrial transcription and translation (Ojala *et al*., 1981; Yokobori & Paabo, 1997). The lengths of the two rRNA genes (*rrnL*: 958 bp; *rrnS*: 697 bp) are similar to those of Secernentea nematodes (944–978 bp, 672–703 bp), whereas there is a greater difference with Adenophorea nematodes (729–947 bp, 569–688 bp) (Jia *et al*., 2011). *Ancylostoma tubaeforme* from cats can be classified as type GA7 according to the arrangement

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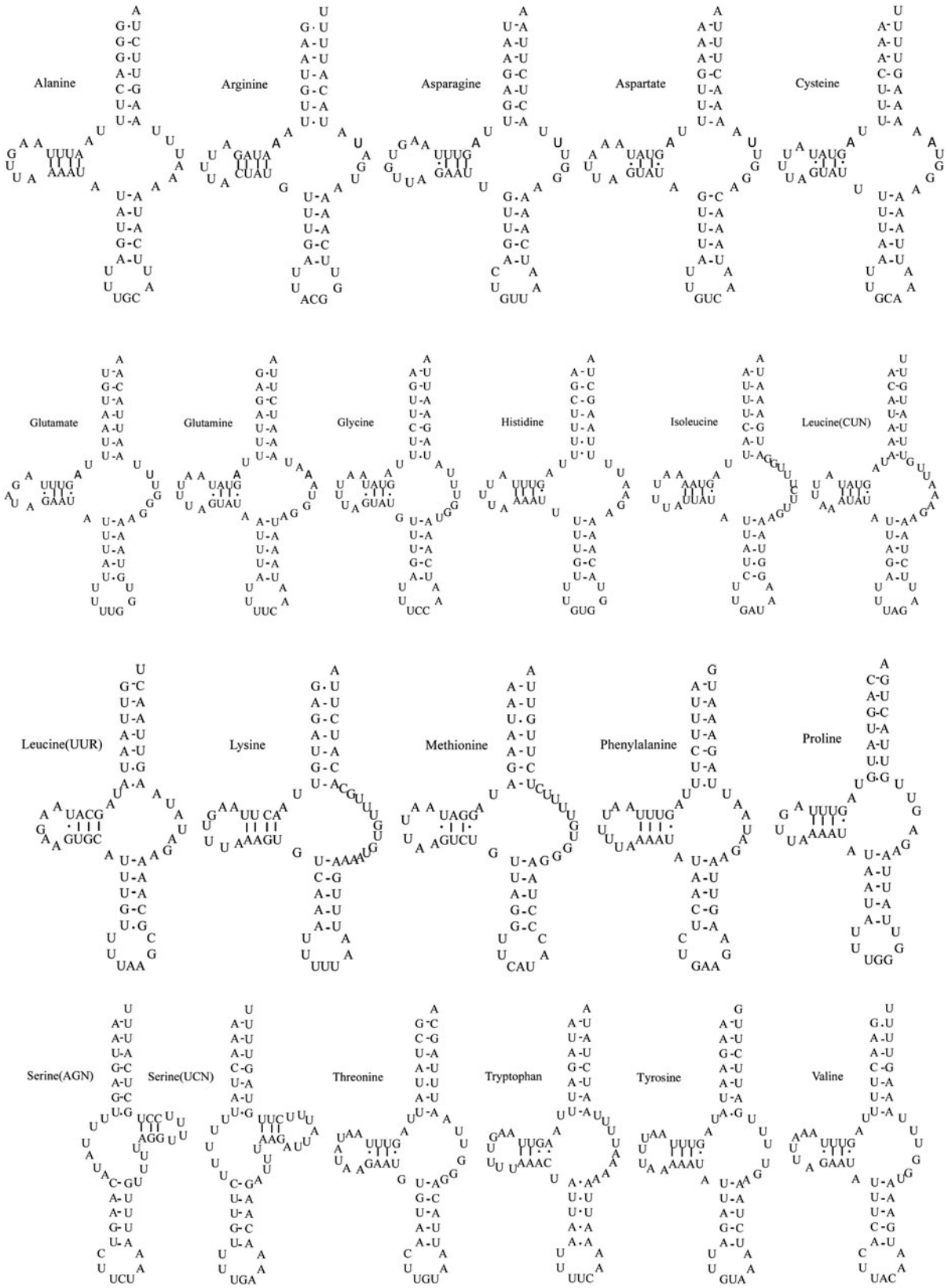

Fig. 4. Secondary structures predicted for the 22 *trn* genes in the mitochondrial genome of *A. tubaeforme*.

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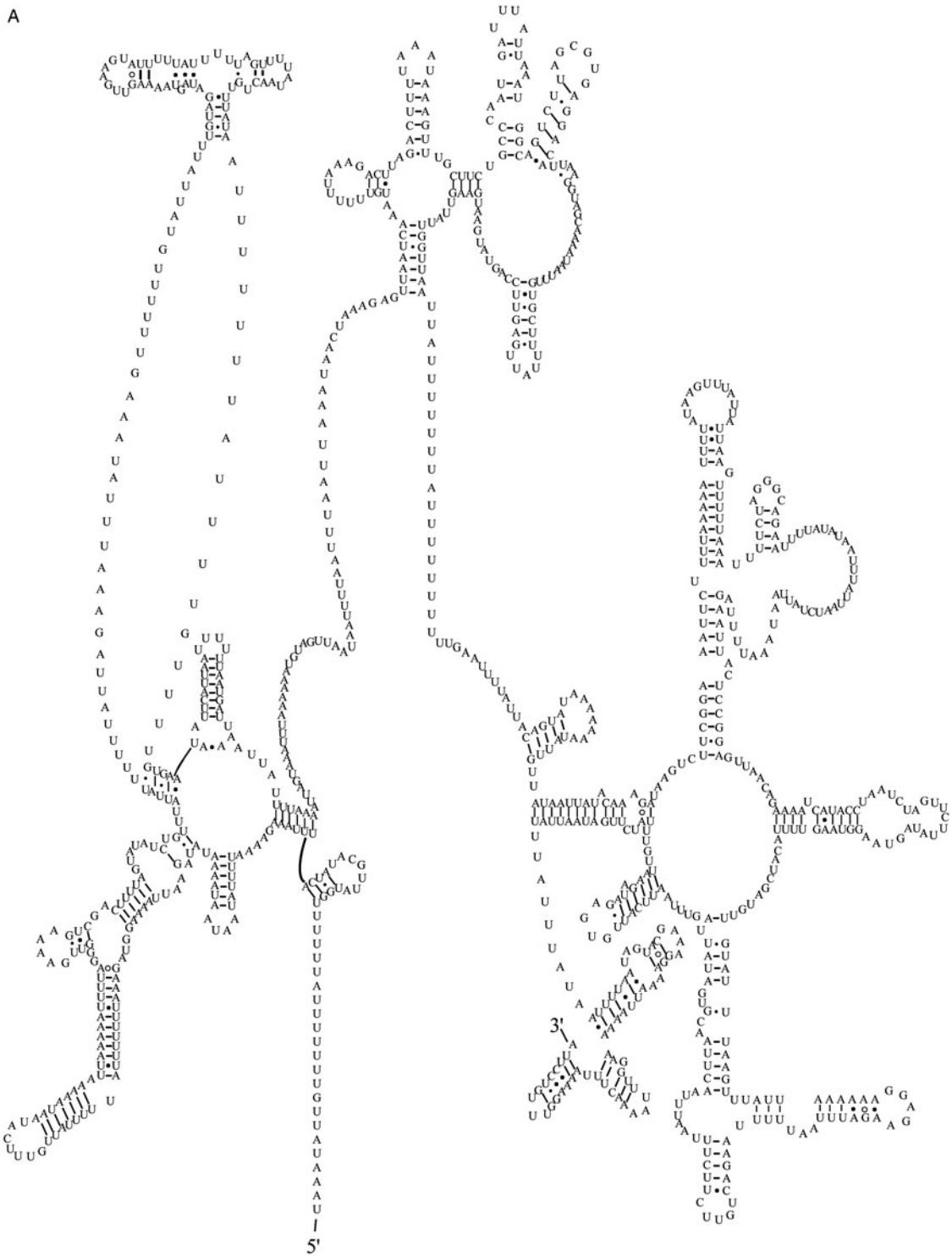

Fig. 5. Predicted secondary structure of the mitochondrial rRNA genes inferred for *A. tubaeforme*: (A) *rrnL*.

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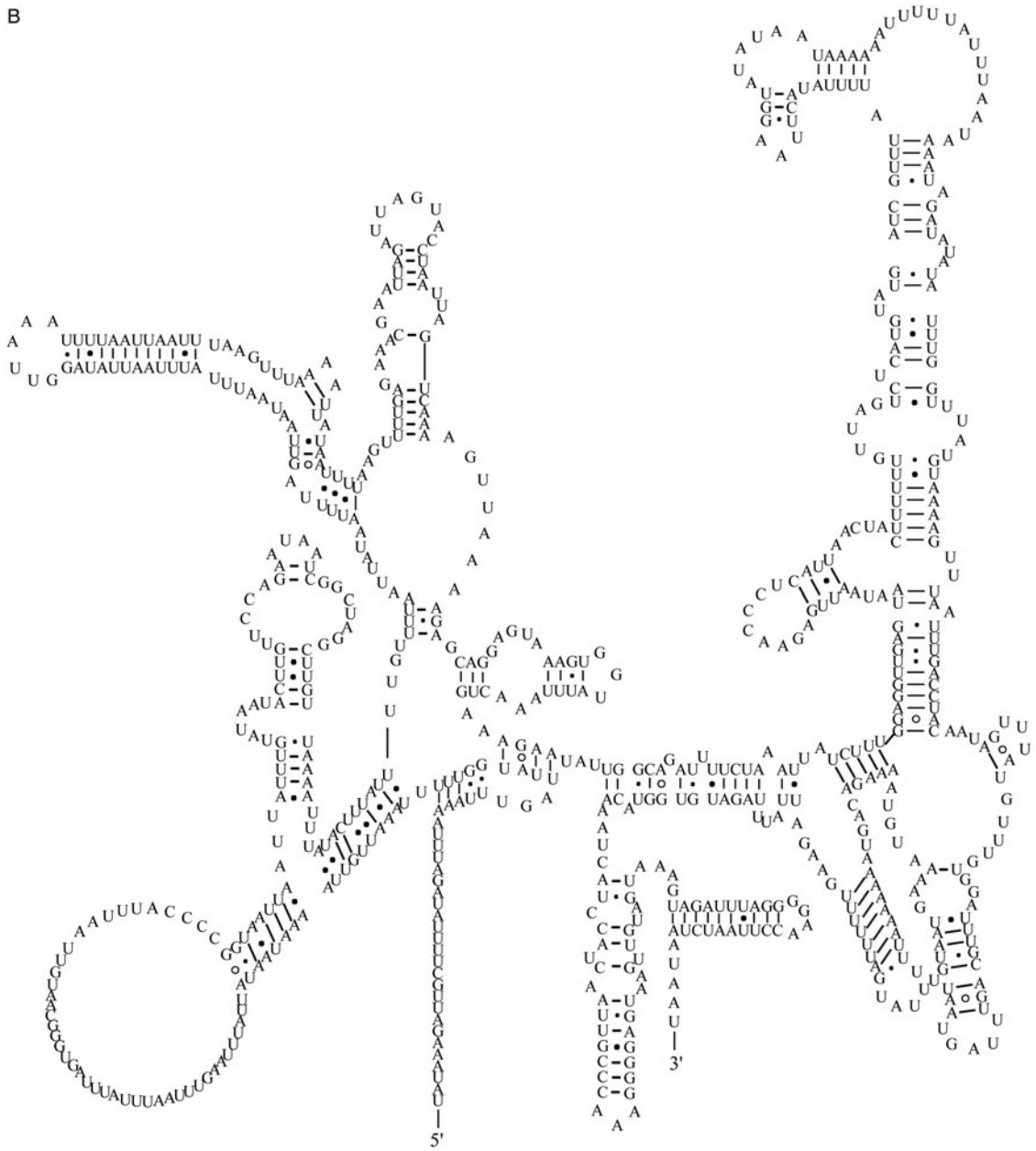

Fig. 5. (*Contd*) Predicted secondary structure of the mitochondrial rRNA genes inferred for *A. tubaeforme*: (B) *rrnS*.

of *rrnL* and *rrnS* genes in whole mt genomes (Yatawara *et al.*, 2010). Most nematodes belong to GA7, including Ancylostomatidae nematodes (*A. caninum*, *A. duodenale*, *N. americanus*, *Bunostomum phlebotomum*, *B. trigonocephalum*) and Ascaridata nematodes (*Ascaris suum*, *Toxocara canis*, *T. cati*), as well as *Caenorhabditis elegans* (Okimoto *et al.*, 1992; Hu *et al.*, 2002; Li *et al.*, 2008; Jex *et al.*, 2009). In addition, the consensus secondary structures inferred for the two rRNA genes of these nematodes are composed of lots of stem and loop structures with G–C, A–U and unstable G–U.

Mitochondrial (mt) genes are known to provide useful genetic markers for species classification and phylogenetic analysis (Le *et al.*, 2002; Piganeau *et al.*, 2009). In this study, the phylogenetic tree showed that, in the Strongylata lineage, the classification of Ancylostomatidae, Strongylidae and Trichostrongylidae is confirmed based on the mt genome of these nematodes (Hu *et al.*, 2002; Gao *et al.*, 2014), and, in the lineage of Ancylostomatidae, the classification of *Ancylostoma*, *Necator* and *Bunostomum* is the same as in the phylogenetic tree constructed by Hu *et al.* (2016). In addition, the classification of *Ancylostoma* nematodes (*A.*

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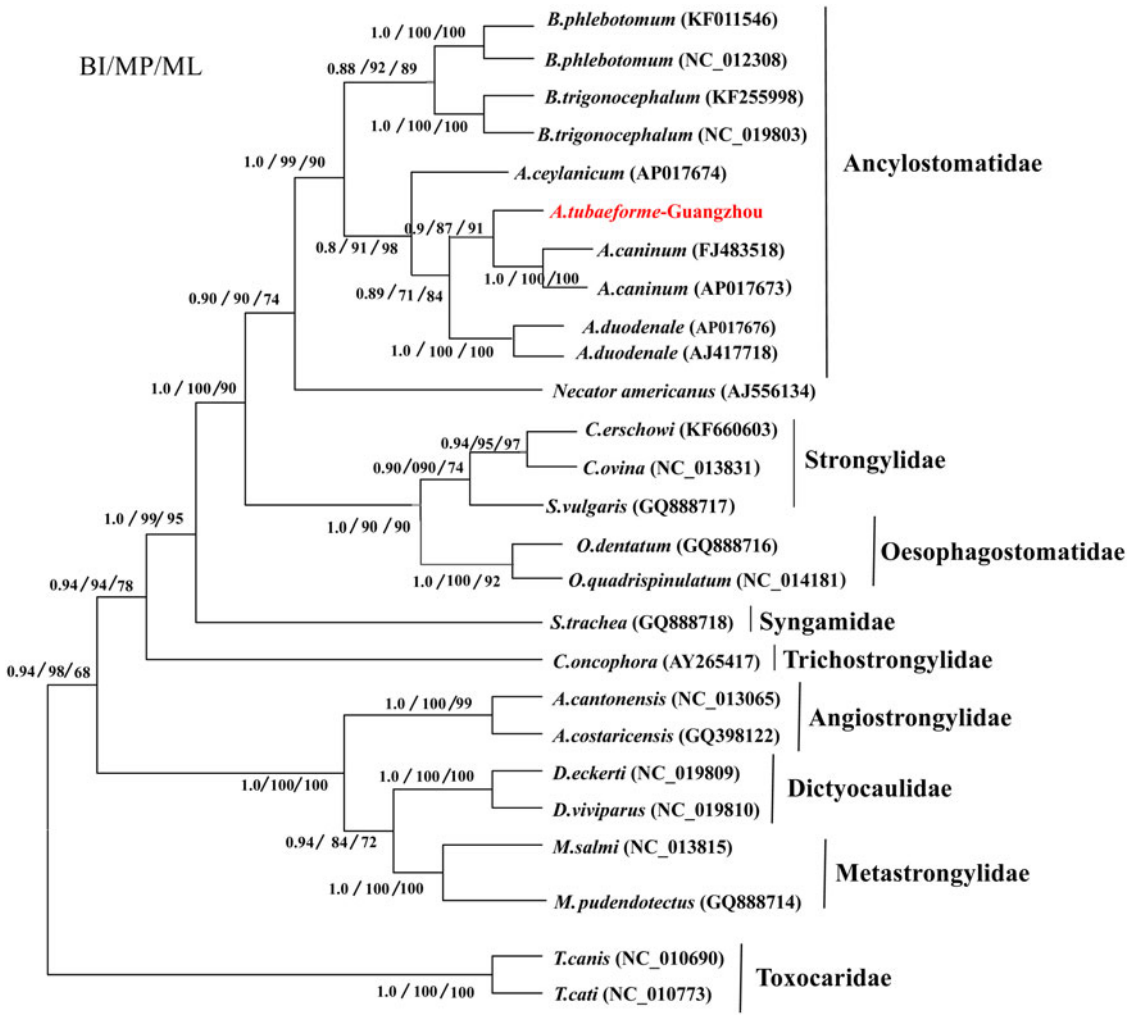

Fig. 6. Phylogenetic tree based on mtDNA protein-coding genes of *A. tubaeforme* and other Strongylata nematodes by BI, MP and ML methods.

tubaeforme, *A. caninum*, *A. duodenale* and *A. ceylanicum*) confirms that of a phylogenetic tree based on the rDNA ITS sequence (Chilton & Gasser, 1999).

This study found *A. tubaeforme* infecting cats from Guangzhou in China, and determined its complete mitochondrial genome sequence, which could enrich the mt genome database of Ancylostomatidae nematodes and provide a scientific basis for further studies on the genetic diversity of hookworms among different hosts.

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Conflict of interest

None.

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