

# The complete mitochondrial genome of the dwarf tapeworm *Hymenolepis nana*—a neglected zoonotic helminth

Tian Cheng<sup>1,2,3</sup> · Guo-Hua Liu<sup>1</sup> · Hui-Qun Song<sup>1</sup> · Rui-Qing Lin<sup>2,3</sup> · Xing-Quan Zhu<sup>1,4</sup>

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**Abstract** *Hymenolepis nana*, commonly known as the dwarf tapeworm, is one of the most common tapeworms of humans and rodents and can cause hymenolepiasis. Although this zoonotic tapeworm is of socio-economic significance in many countries of the world, its genetics, systematics, epidemiology, and biology are poorly understood. In the present study, we sequenced and characterized the complete mitochondrial (mt) genome of *H. nana*. The mt genome is 13,764 bp in size and encodes 36 genes, including 12 protein-coding genes, 2 ribosomal RNA, and 22 transfer RNA genes. All genes are transcribed in the same direction. The gene order and genome content are completely identical with their congener *Hymenolepis diminuta*. Phylogenetic analyses based on concatenated amino acid sequences of 12 protein-coding

genes by Bayesian inference, Maximum likelihood, and Maximum parsimony showed the division of class Cestoda into two orders, supported the monophylies of both the orders Cyclophyllidea and Pseudophyllidea. Analyses of mt genome sequences also support the monophylies of the three families Taeniidae, Hymenolepididae, and Diphyllobothriidae. This novel mt genome provides a useful genetic marker for studying the molecular epidemiology, systematics, and population genetics of the dwarf tapeworm and should have implications for the diagnosis, prevention, and control of hymenolepiasis in humans.

**Keywords** Tapeworm · *Hymenolepis nana* · Mitochondrial genome · Phylogenetic analyses

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- ✉ Rui-Qing Lin  
rqlin@scau.edu.cn
- ✉ Xing-Quan Zhu  
xingquanzhu1@hotmail.com

- <sup>1</sup> State Key Laboratory of Veterinary Etiological Biology, Key Laboratory of Veterinary Parasitology of Gansu Province, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou, Gansu Province 730046, People's Republic of China
- <sup>2</sup> College of Veterinary Medicine, South China Agricultural University, Guangzhou, Guangdong Province 510642, People's Republic of China
- <sup>3</sup> Key Laboratory of Zoonosis Prevention and Control of Guangdong Province, Guangzhou, Guangdong Province 510642, People's Republic of China
- <sup>4</sup> Jiangsu Co-innovation Center for the Prevention and Control of Important Animal Infectious Diseases and Zoonoses, Yangzhou University College of Veterinary Medicine, Yangzhou, Jiangsu Province 225009, People's Republic of China

## Introduction

*Hymenolepis nana* is the most common tapeworm of humans, particularly in young children in developing countries (Willcocks et al. 2015). It is often referred to as the “dwarf tapeworm” due to its small size (about 2–4 cm long and only 1 mm wide). Human hymenolepiasis, caused by *H. nana* and *H. diminuta*, is a globally widespread zoonosis, and it is endemic in Asia, Southern and Eastern Europe, Central and South America, and Africa (Thompson 2015). *H. nana* that causes many clinical symptoms, such as headaches, weakness, anorexia, abdominal pain, and diarrhea, has been seriously neglected (Sirivichayakul et al. 2000). The life cycle of *H. nana* may be either direct or indirect. Direct human to human transmission is the most common route of infection with *H. nana*, particularly in poor hygiene and inadequate sanitation (Willcocks et al. 2015). Fortunately, hymenolepiasis can be treated effectively using drugs such as praziquantel and albendazole.

The metazoan mitochondrial (mt) genomes, ranging in size from 14 to 18 kb, are typically circular and usually encode 36–37 genes, including 12–13 protein-coding genes (PCGs), 2 ribosomal RNA (rRNA) genes and 22 transfer RNA (tRNA) genes (Wolstenholme 1992). There are no introns within genes and only limited spacer regions between genes. Mt genomes have been extensively used as genetic markers in molecular phylogenetic studies due to its several useful properties (i.e., haploidy, compactness, maternal inheritance, relatively high mutation rates, and the lack of recombination) (Boore 1999; Tao et al. 2014). To date, there are over 4000 complete mt genome sequences of metazoans available in GenBank, including some from parasites, including tapeworm (Wolstenholme 1992; Boore 1999; Tao et al. 2014; Liu et al. 2013, 2014a, b; Knapp et al. 2011). Currently, The class Cestoda comprises more than 5000 described species, and many tapeworm species threaten the health of animals and humans on a global scale (Macnish et al. 2002; Mbaya et al. 2014). In spite of the availability of advanced DNA technologies, however, there is still a major gap in our knowledge of mt genomes of the hymenolepidid tapeworm. The hymenolepidid tapeworms have been assigned to many genera based on their morphological traits (Widmer et al. 2013; Makarikov et al. 2015). *Pseudanoplocephala crawfordi* is first described in the small intestine of a wild pig and recorded as one member of the genus *Pseudanoplocephala* based on its morphological features (sizes, acetabulum numbers, testicular quantities, and locations) (Jiang et al. 1990). However, recent studies using ribosomal DNA (rDNA) and mitochondrial DNA (mtDNA) sequences proposed that *P. crawfordi* may represent a member of the genus *Hymenolepis* (Jia et al. 2014; Zhao et al. 2015). However, molecular phylogenetic studies of hymenolepidid tapeworms are still scarce (Okamoto et al. 1997). In addition, limited information is available about mt genomes of hymenolepidid tapeworms (Von Nickisch-Roseneck et al. 2001; Zhao et al. 2015).

In the present study, we sequenced the complete mt genome of the dwarf tapeworm *H. nana* (Cyclophyllidea: Hymenolepididae), which is a neglected zoonotic helminth. Also, we inferred the phylogenetic relationships with the concatenated mt amino acid sequences of the *H. nana* and 40 other tapeworm species that have been sequenced to date.

## Materials and methods

### Parasites and DNA extraction

*H. nana* samples were collected from a naturally infected Kunming mouse in Lanzhou, Gansu Province, China. The adult tapeworms were isolated from the small intestine of mice. The presence of tapeworms was detected by light

microscopic examination, and identification of *H. nana* was conducted by morphological criteria and site of predilection (Jia et al. 2010), then fixed in 70 % alcohol and stored at  $-20^{\circ}\text{C}$  until use. Total genomic DNA was isolated from these specimens using sodium dodecyl sulfate/proteinase K treatment, followed by spin-column purification (WizardSV Genomic DNA Purification System, Promega), and the DNA was extracted by shaking with 250  $\mu\text{L}$  Wizard SV Lysis Buffer. After centrifugation, the DNA was washed, eluted, and stored.

### PCR amplification and sequencing

Five pairs of PCR primers were used to amplify overlapping segments of the complete mt genome of *H. nana* as shown in Table 1. Briefly, the segment of *nad1* was amplified using the primers JB11/JB12 (Bowles and McManus 1993). The other segments were amplified using designed primers (Table 1) based on sequences well conserved in the *H. diminuta* (Von Nickisch-Roseneck et al. 2001). All PCR reactions (25  $\mu\text{L}$ ) were performed in 4.0 mM dNTP mixture, 3.0  $\mu\text{L}$  10 $\times$ LA Taq buffer (Mg<sup>2+</sup> Plus), 10 pmol of each primer, 1 U LA Taq polymerase (Takara), 1  $\mu\text{L}$  of DNA sample in a thermocycler (Eppendorf, Germany) under the following conditions: 94  $^{\circ}\text{C}$  for 2 min (initial denaturation); then followed by 5 cycles of 92  $^{\circ}\text{C}$  for 30 s (denaturation), 48–55  $^{\circ}\text{C}$  for 30 s (annealing), 68  $^{\circ}\text{C}$  for 1~6.5 min (extension), and 92  $^{\circ}\text{C}$  for 2 min (initial denaturation); then followed by 30 cycles of 92  $^{\circ}\text{C}$  for 30 s (denaturation), 48–55  $^{\circ}\text{C}$  for 30 s (annealing), 68  $^{\circ}\text{C}$  for 1~6.5 min (extension) according to the product length; and with a final extension step at 68  $^{\circ}\text{C}$  for 10 min. Each PCR reaction yielded a single band as detected in a 1 % (W/V) agarose gel upon ethidium bromide staining (not shown). PCR products were subsequently sent to Invitrogen Biotechnology Company (Shanghai, China) for sequencing using a primer-walking strategy.

**Table 1** Sequences of primers used to amplify PCR fragments from *Hymenolepis nana*

Primer	Sequence (5' to 3')
ND4-COX1 F	TGGTCATCTGCTTATACGAAGGGAA
ND4-COX1 R	ACCATATCACCGGATCACTCTTT
COX1-ND4 F	AGAGTGATCCGGTGATATGGTGA
COX1-ND4 R	ACCATTCCCTTCGTATAAGCAGA
N54 F	GTCGTTGGGATAAATTTTCTT
N54 R	AGCCAACAATTAATTCAACAA
JB3	TTTTTTGGGCATCCTGAGGTTTAT
JB4.5	TAAAGAAAGAACATAATGAAAATG
JB11	AGATTTCGTAAGGGGCCTAATA
JB12	ACCACTAATAATTCACCTTC

## Sequence analyses

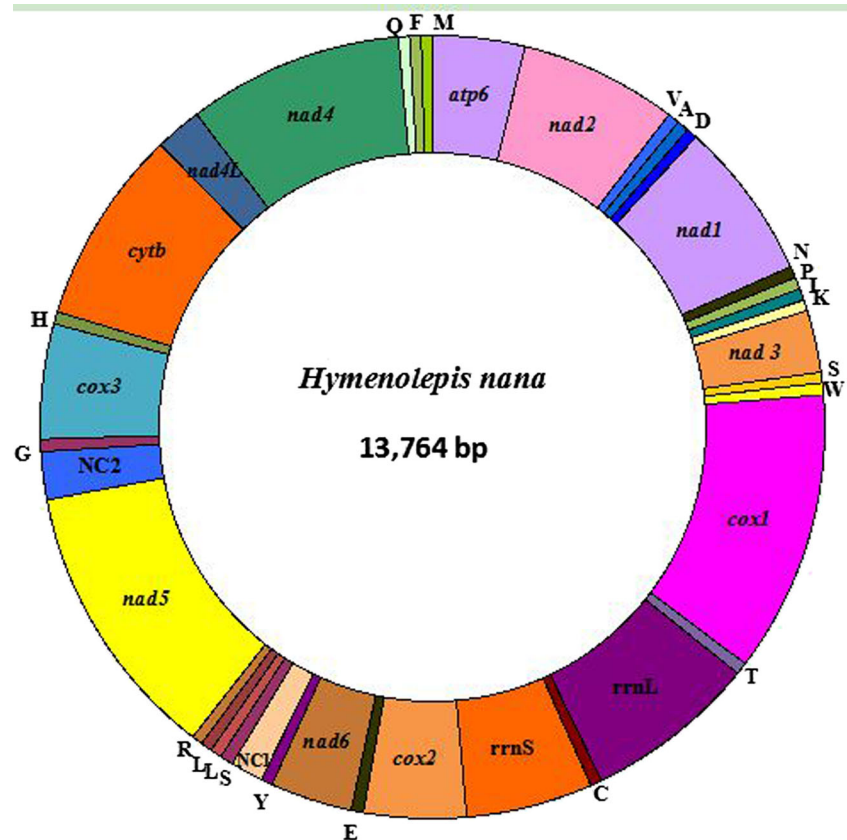
Sequences were assembled manually and aligned against the complete mt genome sequences of *H. diminuta* available using the computer program MAFFT 7.122 (Katoh and Standley 2013) to identify gene boundaries. Each gene was translated into an amino acid sequence using the invertebrate mitochondrial genetic code in MEGA 5 (Tamura et al. 2011) and aligned based on its amino acid sequence using default settings. The translation initiation and termination codons were identified to avoid gene overlap and to optimize the similarity between the gene lengths of *H. diminuta* mt genomes. For analyzing tRNA genes, the program tRNAscan-SE (Lowe and Eddy 1997) were identified by recognizing potential secondary structures and anticodon sequences by eye, and two rRNA genes were predicted by comparison with that of *H. diminuta* (Von Nickisch-Roseneck et al. 2001).

## Phylogenetic analyses

For comparative purposes, amino acid sequences predicted from published mt genomes of 40 species were also included in the present analysis, using the one trematode *Dicrocoelium dendriticum* (GenBank accession number JQ966973) as an outgroup (Liu et al. 2014b). The 12 amino acid sequences

were single aligned using MAFFT 7.122 and then concatenated, and ambiguously aligned regions were excluded using Gblocks online server ([http://molevol.cmima.csic.es/castresana/Gblocks\\_server.html](http://molevol.cmima.csic.es/castresana/Gblocks_server.html)) with the default parameters (Talavera and Castresana 2007) using the options for a less stringent selection. Phylogenetic analyses were conducted using three methods: Bayesian inference (BI), Maximum likelihood (ML), and Maximum parsimony (MP). The JTT+I+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 the Akaike information criterion (AIC). As the JTT model is not implemented in the current version of MrBayes, an alternative model, MtREV, was used in BI, and four chains were run simultaneously for the Monte Carlo Markov Chain. Two independent runs were made for 1,000,000 metropolis-coupled MCMC generations, sampling a tree every 100 generation in MrBayes 3.1.1 (Ronquist and Huelsenbeck 2003); the first 2500 trees represented burn-in and the remaining trees were used to calculate Bayesian posterior probabilities (Bpp). The analysis was performed until the potential scale reduction factor approached 1, and the average standard deviation of split frequencies was less than 0.01. ML analysis was performed with PhyML 3.0 (Guindon and Gascuel 2003) using the subtree pruning and regrafting (SPR) method with a BioNJ starting tree, and the MtArt model of amino acid substitution with proportion of invariant sites (I)

**Fig. 1** Arrangement of the mitochondrial genome of *Hymenolepis nana*. 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 (L<sub>1</sub> and L<sub>2</sub> for codon families CUN and UUR, respectively; S<sub>1</sub> and S<sub>2</sub> for codon families AGN and UCN, respectively)



**Table 2** Organization of *Hymenolepis nana* mitochondrial genome

Genes/regions	Position	Size (bp)	Start codons	Stop codons
<i>atp6</i>	1/516	516	ATG	TAG
<i>nad2</i>	525/1409	885	ATG	TAA
<i>tRNA-Val</i> (V)	1436/1501	66		
<i>tRNA-Ala</i> (A)	1500/1566	67		
<i>tRNA-Asp</i> (D)	1572/1633	62		
<i>nad1</i>	1637/2530	894	ATG	TAG
<i>tRNA-Asn</i> (N)	2537/2602	66		
<i>tRNA-Pro</i> (P)	2622/2687	66		
<i>tRNA-Ile</i> (I)	2684/2749	66		
<i>tRNA-Lys</i> (K)	2749/2811	63		
<i>nad3</i>	2813/3160	348	ATG	TAG
<i>tRNA-Ser</i> (S)	3165/3224	60		
<i>tRNA-Trp</i> (W)	3233/3298	66		
<i>cox1</i>	3302/4885	1584	ATG	TAG
<i>tRNA-Thr</i> (T)	4875/4942	68		
<i>rrnL</i>	4940/5906	967		
<i>tRNA-Cys</i> (C)	5905/5969	65		
<i>rrnS</i>	5971/6680	710		
<i>cox2</i>	6699/7271	573	ATG	TAG
<i>tRNA-Glu</i> (E)	7273/7341	69		
<i>nad6</i>	7348/7806	459	ATG	TAG
<i>tRNA-Tyr</i> (Y)	7805/7870	66		
<i>NC1</i>	7871/8052	182		
<i>tRNA-Ser</i> (S)	8053/8127	75		
<i>tRNA-Leu</i> (L)	8139/8203	65		
<i>tRNA-Leu</i> (L)	8212/8275	64		
<i>tRNA-Arg</i> (R)	8276/8336	61		
<i>nad5</i>	8339/9913	1575	ATG	TAA
<i>NC2</i>	9914/10,181	268		
<i>tRNA-Gly</i> (G)	10,182/10,250	69		
<i>cox3</i>	10,253/10,897	645	ATG	TAA
<i>tRNA-His</i> (H)	10,898/10,966	69		
<i>cytb</i>	10,970/12,067	1098	ATG	TAA
<i>nad4L</i>	12,068/12,328	261	GTG	TAA
<i>nad4</i>	12,340/13,548	1209	ATG	TAA
<i>tRNA-Gln</i> (Q)	13,550/13,611	62		
<i>tRNA-Phe</i> (F)	13,610/13,673	64		
<i>tRNA-Met</i> (M)	13,671/13,733	63		

and gamma distribution (G) parameters estimated from the data with four discretized substitution rate classes, the middle of which was estimated using the median. Bootstrap frequency (Bf) was calculated using 100 bootstrap replicates. MP analysis was conducted using PAUP 4.0 Beta 10 program (Swofford 2002), with indels treated as missing character states; 1000 random additional searches were performed using TBR. Bf was calculated using 1000 bootstrap replicates and 100 random taxon additions in PAUP. Phylograms were

drawn using the program FigTree v.1.4 (<http://tree.bio.ed.ac.uk/software/figtree>).

## Results and discussion

### General features of the mt genome of *H. nana*

The complete mt genome of *H. nana* was 13,764 bp in length (Fig. 1). This size is within the range of other mite mt genomes (Table 2). The sequences have been deposited in GenBank under the accession number KT951722. This circular mt genome contains 12 PCGs (*cox1-3*, *nad1-6*, *nad4L*, *cytb*, and *atp6*), 22 tRNA genes, two rRNA genes, and two noncoding regions (Table 2). The genes are transcribed in same directions (Fig. 1). The nucleotide composition of the complete mt genome of *H. nana* is A=26.96 %, T=46.01 %, G=18.19 % and C=8.83 %, with a typically high A+T content of 72.97 % within the range of values found in *H. diminuta* (71.04 %) and *P. crawfordi* (69.70 %) (Table 2).

### Annotation

The length of PCGs of *H. nana* was in the following order: *cox1* > *nad5* > *nad4* > *cytb* > *nad1* > *nad2* > *cox3* > *cox2* > *atp6* > *nad6* > *nad3* > *nad4L* (Table 2). A total of 3337 amino acids are encoded in the mt genome of *H. nana*. In this mt genome, all genes (*cox1*, *nad3*, *nad6*, *cox2*, *cox3*, *atp6*, *nad4*, *nad1*, *nad5*, *nad2*, and *cytb*) use ATG as start codon, except *nad4L* uses GTG, respectively (Table 2). All genes have complete termination codon and half (*cox3*, *nad2*, *nad4*, *nad5*, *nad4L*, and *cytb*) use TAA, and the other genes (*atp6*, *cox1*, *cox2*, *nad1*, *nad3*, and *nad6*) use TAG as termination codon, respectively (Table 2). A total of 22 tRNA sequences (ranging from 60 to 75 nucleotides in length) were identified in the *H. nana* mt genome. Their predicted secondary structures (not shown) are similar to that of *H. diminuta* (Liu et al. 2011). The *rrnS* of *H. nana* is located between tRNA-Cys and *cox2*, and *rrnL* is located between tRNA-Thr and tRNA-Cys. The length of the *rrnS* gene is 710 bp and *rrnL* gene is 967 bp (Table 2). The A+T contents of the *rrnS* and *rrnL* are 71.41 and 71.66 %, respectively. The longer non-coding region (NC2) is located between the *nad5* and tRNA-Gly, and the shorter one (NC1) is located between genes tRNA-Tyr and tRNA-Ser. Their sizes are 268 bp (NC2) and 182 bp (NC1) (Table 2). The A+T contents of the NC2 and NC1 are 79.10 and 83.52 %, respectively (Table 2).

### Comparative analyses among *H. nana*, *H. diminuta*, and *Pseudanoplocephala crawfordi*

The mt genome sequence of *H. nana* was 13,764 bp in length, 136 bp shorter than that of *H. diminuta*, and 428 bp shorter

**Table 3** Nucleotide and/or predicted amino acid (aa) sequence differences for mt protein-coding and ribosomal RNA genes among *Hymenolepis nana* (HN), *Hymenolepis diminuta* (HD), and *Pseudanoplocephala crawfordi* (PC)

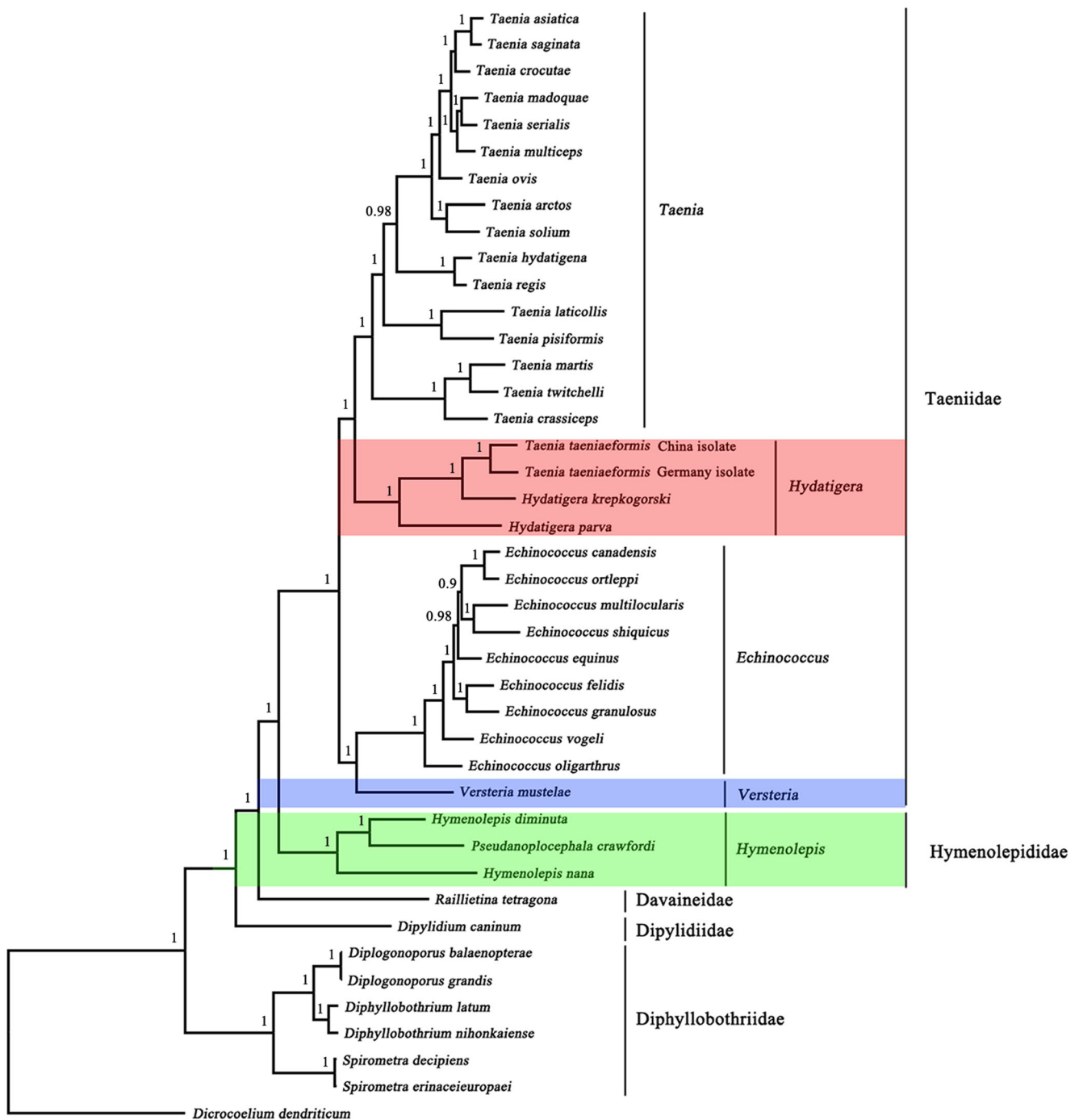
Gene/region	Nucleotide length (bp)			Nucleotide difference (%)			Number of aa			aa difference (%)		
	HN	HD	PC	HN/HD	HN/PC	HD/PC	HN	HD	PC	HN/HD	HN/PC	HD/PC
<i>atp6</i>	516	516	516	26.9	28.2	21.9	171	171	171	33.6	33.1	27.3
<i>nad1</i>	894	891	891	21.7	19.8	16.4	297	296	296	21.9	20.9	15.8
<i>nad2</i>	885	882	897	26.7	26.4	24.1	294	293	298	37.1	37.2	25.9
<i>nad3</i>	348	348	348	25.2	27.2	21.8	115	115	115	27.6	33.6	25
<i>nad4</i>	1209	1230	1230	29.2	32.6	25.5	402	409	409	36.3	37.3	27.3
<i>nad4L</i>	261	261	261	20.6	20.2	16	86	86	86	25.3	23	23
<i>nad5</i>	1575	1575	1575	31.2	31.9	25.9	524	524	524	38	38.3	28.5
<i>nad6</i>	459	459	459	32.2	30.2	23.8	152	152	152	39.7	39.6	28.1
<i>cox1</i>	1584	1552	1582	20.6	19.4	17.3	527	517	527	21.5	18.8	16.2
<i>cox2</i>	573	579	579	26	28.1	18.3	190	192	192	33.5	31.6	18.1
<i>cox3</i>	645	651	651	30.2	30	21.2	214	216	216	36.2	34	25.8
<i>cytb</i>	1098	1098	1095	22.9	23.4	20.3	365	365	364	21.9	24.7	17.5
<i>rrnS</i>	710	709	724	16.1	16.7	12.9	–	–	–	–	–	–
<i>rrnL</i>	967	967	963	21.5	22	19.2	–	–	–	–	–	–

than that of *P. crawfordi*. The arrangement of the mt genes (i.e., 13 protein genes, 2 rrn genes, and 22 tRNA genes) and NCRs were the same. A pairwise comparison of the nucleotide sequences of each mt gene and the amino acid sequences conceptually translated from individual protein genes was made among the three taxa of tapeworms (Table 3). The sequence lengths of individual genes varied among these taxa, except for the *atp6*, *nad3*, *nad4L*, *nad5*, and *nad6* genes, which were the same (Table 3). The magnitude of sequence variation in each gene among the three taxa of tapeworms ranged from 12.9–32.6 % for nucleotide sequences and 16.2–39.7 % for amino acid sequences (Table 3). The sequence difference across the entire mt genome between *H. nana* and *H. diminuta* was 42.9 %. This difference across the entire mt genome between *H. nana* and *P. crawfordi* was 43.4 %. The sequence difference across the entire mt genome between *H. diminuta* and *P. crawfordi* was 21.0 %. The greatest variation among the three taxa of tapeworms was in the *nad5* (25.9–31.9 %), whereas least differences (12.9–16.7 %) were detected in the *rrnS* gene (Table 3).

Amino acid sequences inferred from individual mt protein genes of *H. nana* were compared with those of *H. diminuta* and *P. crawfordi*. The difference across amino acid sequences of the 12 protein genes between the *H. nana* and *H. diminuta* was 30.7 and 33.9 % between the *H. nana* and *P. crawfordi*, respectively. The difference across amino acid sequences of the 12 protein genes between the *H. diminuta* and *P. crawfordi* was 26.6 %. The amino acid sequence differences among three taxa of tapeworms ranged from 16.2 to 39.7 %, with *cox1* being the most conserved and *nad6* the least conserved protein.

### Phylogenetic analyses

Of the 40 tapeworm species included in the phylogenetic analyses in this study, 34 species belonged to the order Cyclophyllidea while six belonged to the order Pseudophyllidea. Both orders Cyclophyllidea and Pseudophyllidea were monophyletic in all of the trees inferred by the BI, ML, and MP methods. The monophyly of the order Cyclophyllidea was strongly supported with a Bpp of 1 in BI analyses (Figs. 2, 3 and 4). The 34 species of Cyclophyllidea tapeworm included in this study were from four families: Taeniidae (29 species), Hymenolepididae (three species), Davaineidae (one species), and Dipylidiidae (one species). The monophyly of the families Taeniidae and Hymenolepididae were strongly supported in all of the three phylogenetic analyses in the present study (Bpp=1, Fig. 2; Bf=100 %, Fig. 3; Bf=100 %, Fig. 4). The family Dipyllobothriidae was monophyletic with strong support in all of the three phylogenetic analyses (Bpp=1, Fig. 2; Bf=100 %, Fig. 3; Bf=100 %, Fig. 4). The family Taeniidae consists of four valid genera. Of the 16 species of tapeworm in the genus *Taenia*, four species were from the genus *Hydatigera*, nine species were from the genus *Echinococcus* and one species from the order *Versteria*. The genus *Taenia* was monophyletic with strong support in BI analysis (Bpp=1, Fig. 2), and with weak support in ML analysis (Bf=59 %, Fig. 3), and was moderately supported in MP analysis (Bf=75 %, Fig. 3). Both the genera *Hydatigera* and *Echinococcus* were monophyletic with strong support in all of the three phylogenetic analyses (Bpp=1, Fig. 2; Bf=100 %, Fig. 3; Bf=100 %, Fig. 4).



0.1

**Fig. 2** Phylogenetic relationships among 40 species of tapeworms inferred by Bayesian analysis of deduced amino acid sequences of 12 mitochondrial proteins. Bayesian posterior probability (Bpp) values were indicated at nodes

### Significance and implications

The majority of helminth parasites are considered by WHO to be the cause of neglected diseases, and most are zoonotic with animal reservoirs playing a role in their epidemiology (Palmeirim et al. 2014; Thompson 2015). In the present study,

we determined the complete mt genome of the dwarf tapeworm *H. nana*, which is a neglected zoonotic helminth. The characterization of the *H. nana* mt genome provides a foundation for the improved diagnosis of human hymenolepiasis using molecular methods. Molecular tools, using genetic markers in mt genes, would be developed to support clinical diagnosis and to

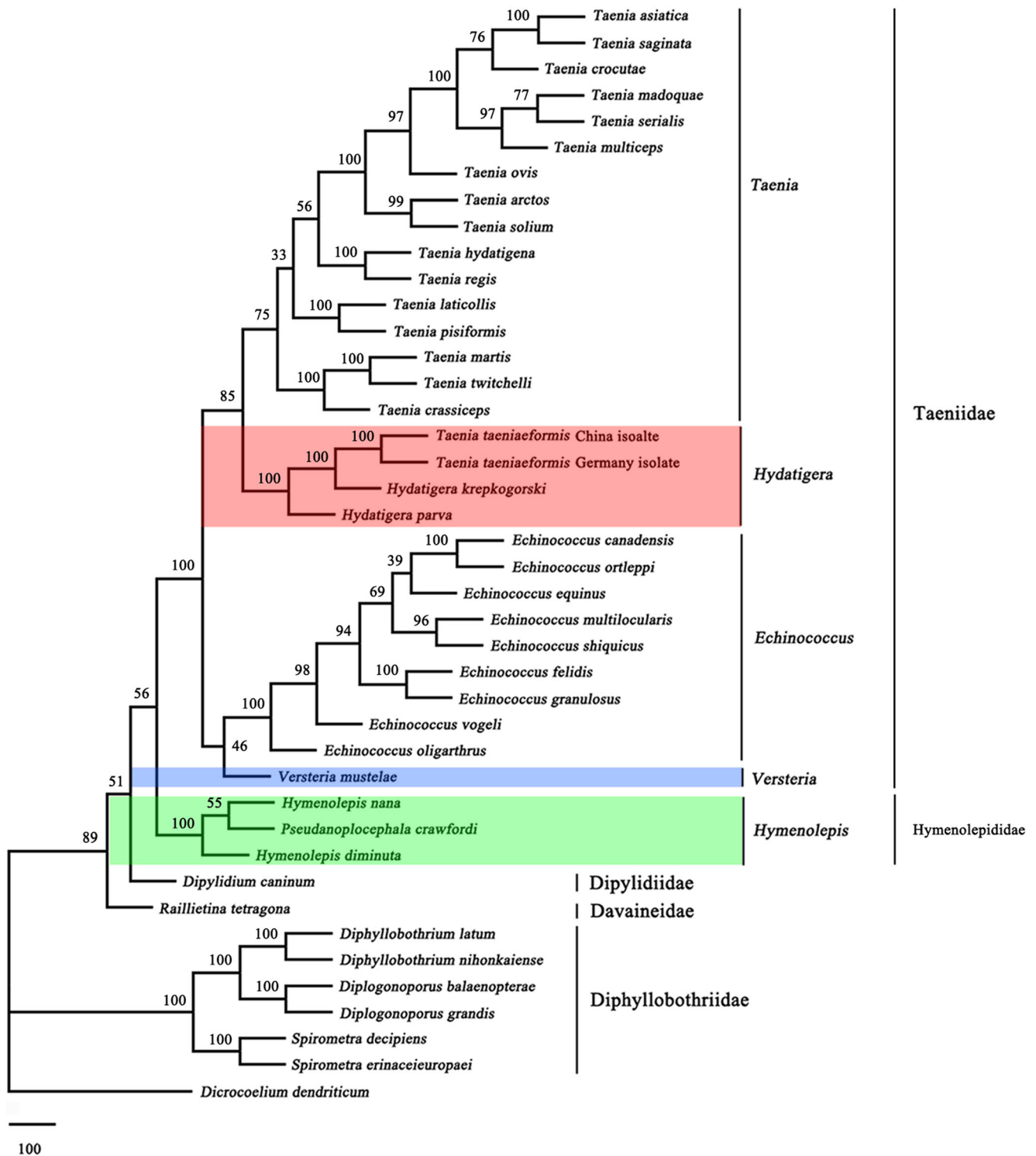


**Fig. 3** Phylogenetic relationships among 40 species of tapeworms inferred by maximum likelihood (ML) of deduced amino acid sequences of 12 mitochondrial proteins. Bootstrapping frequency (Bf) values were indicated at nodes

assist in undertaking molecular epidemiological investigations of *H. nana*. In addition, there is considerable significance employing mtDNA markers to investigate the genetic variation of *H. nana*, particularly at the larval stages or cryptic/sibling species because morphological features of *H. nana* is still scarce. Macnish *et al.* (2002) suggested that isolates of *H. nana* in

Australia actually exist as two cryptic or sibling species because they are morphologically identical, but genetically different. The availability of the complete *H. nana* mt genome herein now allows to identify and differentiate these cryptic/sibling species.

The characterization of the mt genome of *H. nana* can generate genetic markers for future systematic studies. Mt



**Fig. 4** Phylogenetic relationships among 40 species of tapeworms inferred by maximum parsimony (MP) of deduced amino acid sequences of 12 mitochondrial proteins. Bootstrapping frequency (Bf) values were indicated at nodes

genome sequences provide useful genetic markers in examining taxonomic status of helminths, particularly when protein-coding gene sequences are used as markers in comparative analyses (Liu et al. 2011, 2012, 2016; Yamasaki et al. 2012; Jeon et al. 2007). In the present study, both the orders

Cyclophyllidea and Pseudophyllidea were monophyletic in all of the trees inferred by the BI, ML, and MP methods. These results were consistent with those of previous studies (Liu et al. 2011, 2012; Yamasaki et al. 2012). The results of the present study support that the genus *Taenia* was



monophyletic with strong support in BI analysis (Bpp=1, Fig. 2), and with weak support in ML analysis (Bf=59 %, Fig. 3), and was moderately supported in MP analysis (Bf=75 %, Fig. 3). Previous studies from both nuclear and mt genes indicated that *Taenia mustelae* is a sister to *Echinococcus* (Olson and Tkach 2005). Recently, Nakao *et al.* (2013) proposed the erection of a new genus, *Versteria*, for *T. mustelae* (*Versteria mustelae*) because this species is more closely related to *Echinococcus* than to *Taenia*. Our present results support the erection of the new genus *Versteria*. Based on molecular phylogenies, Nakao *et al.* (2013) also proposed the resurrection of *Hydatigera*, and the members of *Hydatigera* included *Hydatigera taeniaeformis*, *Hydatigera krepkogorski*, and *Hydatigera parva*. In the present study, the genus *Hydatigera* (four 4 species) were monophyletic with strong support in all of the three phylogenetic analyses (Bpp=1, Fig. 2; Bf=100 %, Fig. 3; Bf=100 %, Fig. 4), and is a sister to *Taenia*. The present results suggested that *P. crawfordi* would be one member of the genus *Hymenolepis* but not a member of the genus *Pseudanoplocephala*. Although several distinct morphological features for the two genera *Pseudanoplocephala* and *Hymenolepis* in the family Hymenolepididae have been reported, many similar morphological characters confused accurate identification of species in these two genera (Wang 2002). Based on genetic analyses using ribosomal and mtDNA sequences, Jia *et al.* (2014) and Zhao *et al.* (2015) suggested that *P. crawfordi* should be a member of the genus *Hymenolepis*. To date, mt genomes of many lineages of hymenolepidid tapeworms are still underrepresented or not represented. Therefore, expanding taxon sampling is necessary for future phylogenetic studies of hymenolepidid cestodes using mt genomic datasets.

## Conclusion

The present study determined the mt genome of the dwarf tapeworm *H. nana* of human health significance. Phylogenetic analyses of the mt genome sequences of *H. nana* with that of 40 other tapeworm species support the monophylies of the orders Cyclophyllidea and Pseudophyllidea, the families Taeniidae, Hymenolepididae, and Diphyllbothriidae. Analyses of mt genome sequences suggest that *Taenia taeniaeformis* is a member of the genus *Hydatigera*, and *P. crawfordi* is a member of the genus *Hymenolepis*. Our results indicate the further need to study the phylogeny of the class Cestoda with more taxa and different molecular markers.

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