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Characterization of the complete mitochondrial genome of *Nippotaenia mogurndae* Yamaguti and Miyata, 1940 (Cestoda: Nippotaeniidae)

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Abstract

In this study, we report the first complete mitochondrial genome of the tapeworm *Nippotaenia mogurndae* in the order Nippotaeniidea Yamaguti, 1939. This mitogenome, which is 14,307 base pairs (bp) long with an A + T content of 72.2%, consists of 12 protein-coding genes, 22 transfer RNA (tRNA) genes, two rRNA genes, and two non-coding regions. Most tRNAs have a conventional cloverleaf structure, but *trnS1* and *trnR* lack dihydrouridine arms of tRNA. The two largest non-coding regions, NCR1 (220 bp) and NCR2 (817 bp), are located between *trnY* and *trnS2* and between *nad5* and *trnG*, respectively. Phylogenetic analyses of mitogenomic data indicate that *N. mogurndae* is closely related to tapeworms in the order Cyclophyllidea.

Introduction

Nippotaenia mogurndae Yamaguti and Miyata, 1940, is a common nippotaeniid tapeworm found in the gastrointestinal tracts of the fish *Odontobutis obscura* and *Perccottus glenii* in Japan, China and Russia. Nippotaeniid tapeworms comprise a small group of eucestodes, and most species have a single powerful terminal sucker, anapolytic stobila, close-knit bilobed vitellarium and many interconnecting longitudinal excretory canals (Hine, 1977; Sokolov *et al.*, 2018). To date, only six species have been placed in the family Nippotaeniidae and the monotypic order Nippotaeniidea (Hine, 1977). These cestodes are mostly found from freshwater fish in China, Japan, New Zealand, Russia, Slovakia, Poland, and Ukraine (Hine, 1977; Bray, 1994; Košuthová *et al.*, 2008; Mierzejewska *et al.*, 2010; Kvach *et al.*, 2013). However, it is difficult to identify and taxonomically classify these species using their limited and simple morphological characters. The six nippotaeniid cestodes were assigned into two genera, *Nippotaenia* (Yamaguti, 1939) and *Amurotaenia* (Achmerow, 1941); however, the validity of genus *Amurotaenia* is in doubt (Bray, 1994; Hoberg *et al.*, 1997; Waeschenbach *et al.*, 2007, 2012; Caira *et al.*, 2014). Based on phylogenetic analysis with 18S rRNA sequences, Sokolov *et al.* (2018) confirmed that the division of nippotaeniid cestodes into these two genera was improper.

Since *N. mogurndae* was first reported in Asia, it has spread through European waters with its fish hosts and has shown great adaptivity in colonized areas (Mierzejewska *et al.*, 2010). With increasing international fish trade, the transmission of invasive parasites along with fish hosts has drawn considerable attention (e.g. Košuthová *et al.*, 2008; Mierzejewska *et al.*, 2010). Mitochondrial DNA is a powerful marker that is used for species identification and phylogenetic analysis because it is maternally inherited and evolves rapidly (Xi *et al.*, 2018). In this study, the complete mitogenome of *N. mogurndae* was sequenced and annotated. The results presented herein will facilitate a better understanding of the evolution and taxonomy of nippotaeniideans.

Materials and methods

Specimen collection and DNA extraction

In September 2019, six Chinese sleepers (*Perccottus glenii*, Dybowski, 1877) caught from the Amur River (Heilongjiang River, Tongjiang, China) were purchased from the local fish market and immediately examined for parasites. The tapeworm *N. mogurndae* was found in the intestines of Chinese sleepers with high infective prevalence (100%; 6/6 fish) and intensity of 2–11. The tapeworms were rinsed with saline, fixed in 100% ethanol, and then identified using the method described by Sokolov *et al.* (2018). A Blood and Tissue DNA Mini Kit (Aidlab, China)

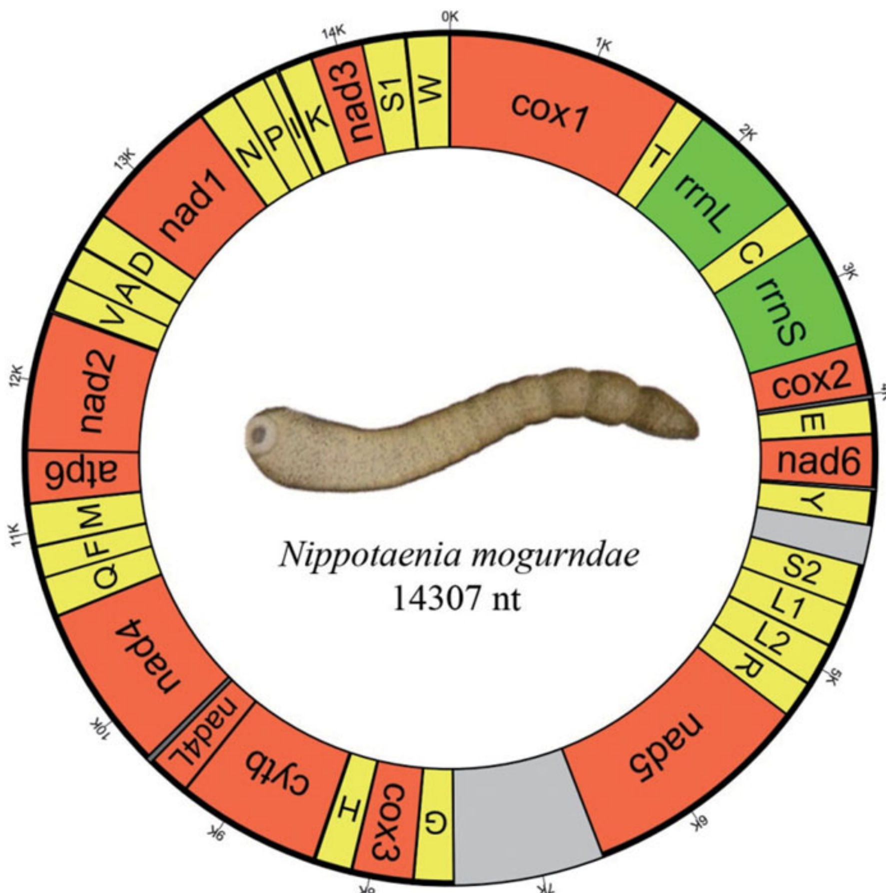

Fig. 1. Organization of the complete mitochondrial genome of *Nippotaenia mogurndae*.

was used to extract total genomic DNA and samples were immediately stored at −20°C until further analysis.

Polymerase chain reaction (PCR) and DNA sequencing

We amplified the whole mitogenome using primers (supplementary table S1) synthesized by Bio-Transduction Laboratory (Wuhan, China). PCR reactions were performed in a 50 μl reaction mixture, containing 33.5 μl double-distilled water, 5 μl 10 × LA Taq Buffer II (Mg^{2+} plus), 1 μl of each primer (10 μM), 0.5 μl LA Taq (Takara, China), 8 μl dNTP Mixture (2.5 mM each) and 60 ng DNA template. The procedure for amplification was as follows: initial denaturation at 95°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 44°C–62°C (T_m of the primers used were listed in supplementary table S1) for 30 s, elongation at 72°C for 1 min/kb, and then final extension at 72°C for 10 min. PCR products were sequenced bidirectionally at Bio-Transduction Laboratory (Wuhan, China).

Sequence annotation and analyses

The amplified fragments were confirmed by Nucleotide Basic Local Alignment Search Tool (Altschul *et al.*, 1990), and mitochondrial genome sequences were assembled using DNAstar software (Burland, 2000). The mitogenomic data of *Mesocestoides vogae* (LC102498) was used as a reference, and gene boundaries were determined with Multiple Alignment using Fast Fourier Transform (MAFFT) (Katoh & Standley, 2013) and Geneious (Kearse *et al.*, 2012). Mitogenome annotations and characterizations were conducted as previously described (Li *et al.*, 2017; Zhang *et al.*, 2017a, b; Zou *et al.*, 2017). Protein-coding genes (PCGs) and non-coding regions (NCRs) were determined with Geneious by searching for open reading frames (using genetic code 9) and comparing nucleotide alignments with reference genomes. All transfer RNAs (tRNAs) were identified and confirmed using ARWEN software (Laslett & Canback, 2008) and the MITOS web server (Bernt *et al.*, 2013). Similarly, we used MITOS to search *rrnL* and *rrnS* with the reference genomes to determine their boundaries. The statistics tables and National Center for Biotechnology Information submission file was generated by PhyloSuite v1.2.2 (Zhang *et al.*, 2020). The tandem repeats (TRs) in non-coding regions were identified using the Tandem Repeats Finder (Benson, 1999). The broken line graph of A + T content and scatter diagram of nucleotide skews were produced in ggplot2 (Wickham, 2009), and PhyloSuite was employed to make a nucleotide composition table and relative synonymous codon usage (RSCU) figures for PCGs.

Phylogeny and gene order

In addition to the newly sequenced mitogenome of *N. mogurndae*, 32 selected cestode mitochondrial DNA sequences retrieved from GenBank were used for phylogenetic analyses, including 30 complete mitogenome sequences (supplementary table S2). Two trematode species served as outgroups; namely, *Dicrocoelium dendriticum* (Rudolphi, 1819) (NC_025280) and *Dicrocoelium chinensis* (Tang & Tang, 1978) (NC_025279). PhyloSuite was used to extract PCG,

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Table 1. Organization and length of genes in the mitochondrial genome of *Nippotaenia mogurndae* (Cestoda: Nippotaeniidae).

Gene/region	Position	Length	Intergenic nucleotides	Genetic codes		Transfer RNA anti-codes
				Ini.	Ter.	
cox1	1–1602	1602		ATG	TAG	
trnT	1602–1663	62	−1			TGT
rrnL	1664–2629	966				
trnC	2630–2695	66				GCA
rrnS	2696–3422	727				
cox2	3423–4002	580		ATG	T	
trnE	4003–4067	65				TTC
nad6	4072–4527	456	4	ATG	TAA	
trnY	4545–4608	64	17			GTA
trnS2	4829–4894	66	220			TGA
trnL1	4896–4961	66	1			TAG
trnL2	4966–5028	63	4			TAA
trnR	5032–5090	59	3			ACG
nad5	5091–6662	1572		ATG	TAA	
trnG	7480–7545	66	817			TCC
cox3	7549–8193	645	3	GTG	TAA	
trnH	8199–8265	67	5			GTG
cytb	8276–9373	1098	10	ATG	TAA	
nad4L	9378–9638	261	4	ATG	TAG	
nad4	9605–10852	1248	−34	ATG	TAG	
trnQ	10857–10917	61	4			TTG
trnF	10917–10980	64	−1			GAA
trnM	10977–11042	66	−4			CAT
atp6	11046–11561	516	3	ATG	TAA	
nad2	11561–12436	876	−1	ATG	TAG	
trnV	12451–12511	61	14			TAC
trnA	12514–12578	65	2			TGC
trnD	12586–12649	64	7			GTC
nad1	12653–13537	885	3	ATG	TAG	
trnN	13543–13607	65	5			GTT
trnP	13611–13673	63	3			TGG
trnI	13673–13737	65	−1			GAT
trnK	13752–13813	62	14			CTT
nad3	13817–14164	348	3	ATG	TAG	
trnS1	14166–14226	61	1			GCT
trnW	14236–14302	67	9			TCA

rRNA and tRNA nucleotide sequences from GenBank files. PCGs were translated into amino acid sequences (with genetic code 9), aligned in batches with MAFFT using codon-alignment mode, and refined with MACES v2.03 (Ranwez *et al.*, 2018). We aligned the RNA sequences using MAFFT's normal alignment mode. Ambiguously aligned fragments of all of the alignments were removed using Gblocks v0.91b (Talavera & Castresana, 2007) with default settings. The aligned and trimmed PCGs and RNA sequences were concatenated using PhyloSuite. We conducted phylogenetic analyses simultaneously using maximum likelihood (ML) and Bayesian inference (BI). The optimal nucleotide substitution models were selected based on the lowest Bayesian information criterion scores in ModelFinder (Kalyaanamoorthy *et al.*, 2017) (supplementary table S3). ML analysis was performed in IQ-TREE Web Server

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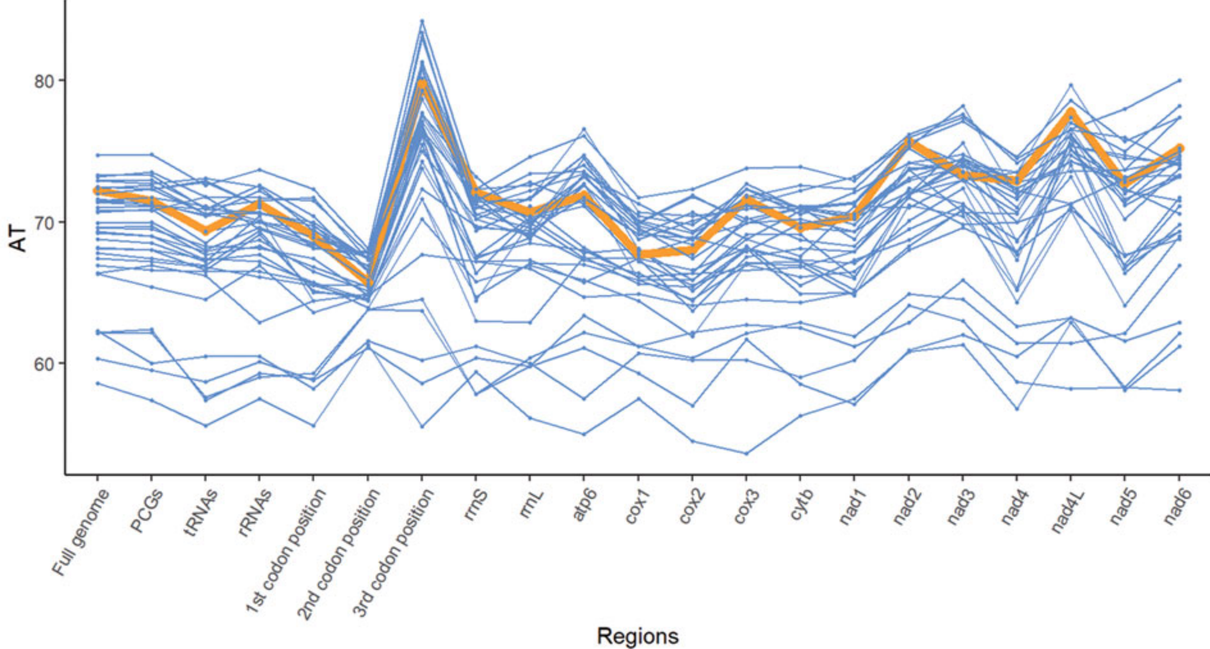

Fig. 2. A+T content of complete genomes and different regions for the mitogenome of *Nippotaenia mogurndae* (red) and other cestodes.

(Trifinopoulos *et al.*, 2016) using IQ-TREE v1.6.12. Branch support was estimated with 5000 bootstrap replicates. BI analysis was performed in MrBayes v3.2.7a (Ronquist *et al.*, 2012) on CIPRES Science Gateway (Miller *et al.*, 2010) with the default settings, and 5×10^6 metropolis-coupled Markov chain Monte Carlo generations. MAFFT, MACES, Gblocks, and ModelFinder were used as plugins in PhyloSuite. The tree was annotated using iTOL web-based tool (Letunic & Bork, 2021).

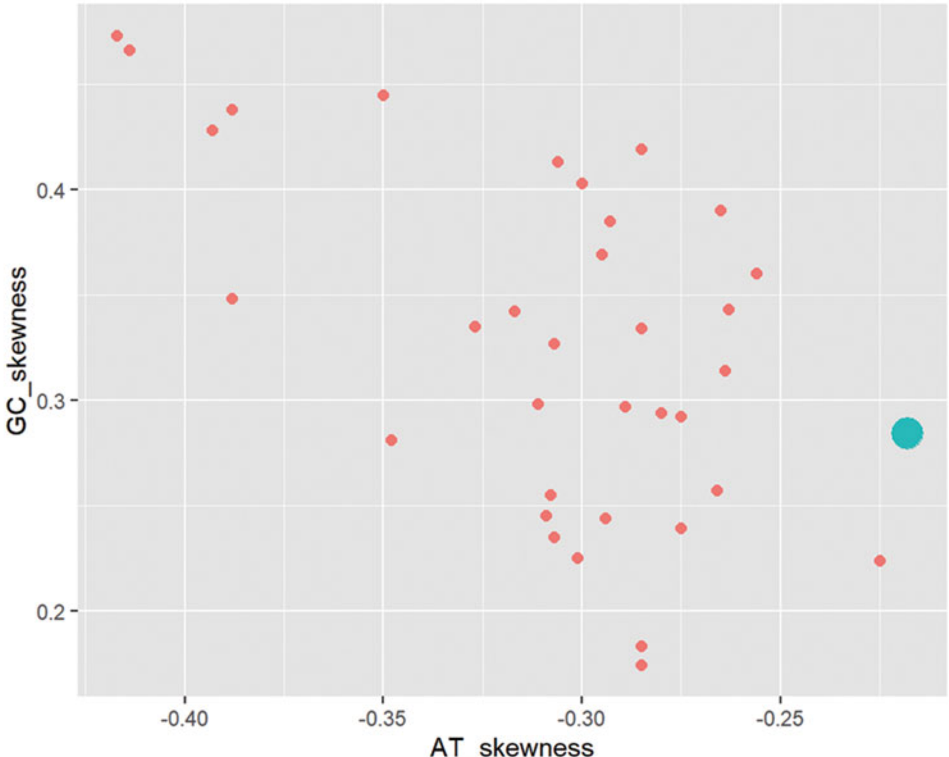

Fig. 3. Comparison of nucleotide skewness of the full genomes for the mitogenome of *Nippotaenia mogurndae* (green) and other cestodes.

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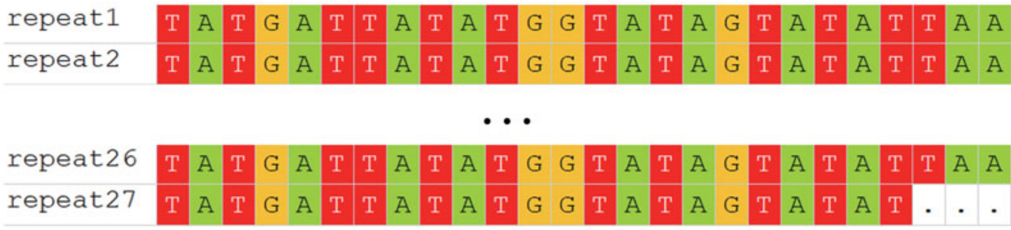

Fig. 4. Tandem repeats in the second main non-coding region of *Nippotaenia mogurndae*.

Results and discussion

Genome organization and base composition

The mitochondrial genome of *N. mogurndae* is 14,307 base pairs (bp) in length (GenBank accession number: ON640728), and consists of 12 PCGs, 22 tRNA genes, two rRNA genes and two NCRs. The mitogenome also lacks the *atp8* gene, similar to other platyhelminths (fig. 1), and all of the genes are transcribed along the same strand. Six overlapping regions and 23 intergenic regions were identified (table 1). The mitogenome of *N. mogurndae* shows a higher A + T content (72.2%) among the cestodes studied in this report (fig. 2), meanwhile it contains G-skew and T-skew, like those of other cestodes (fig. 3).

PCGs and codon usage

Coalesced PCGs are 10,113 bp in size, with 71.5% A + T content (supplementary table S2). A + T content of individual PCGs ranges from 66.9% (*cox2*) to 77.8% (*nad4L*) (supplementary table S4). GTG is identified as the initial codon for *cox3,* and ATG for the rest of the 12 PCGs. Among the terminal codons, six (*cox1*, *nad4L*, *nad4*, *nad2*, *nad1* and *nad3*) are identified as TAG, and that of the remaining terminal codons five (*nad6*, *nad5*, *cox3*, *cytb* and *atp6*) as TAA, while that of *cox2* is T (table 1).

The RSCU and codon family proportion of *N. mogurndae* is shown in supplementary fig. S1. The four most abundant codon families (Leu2, Phe, Ile and Val) are found to account for 41.99%. In all of the codon families, A + T-rich codons are more commonly seen as synonymous codons in contrast to the lower A + T content in *N. mogurndae* (supplementary fig. S1). Accordingly, the relatively high A + T content fits well with this tendency (supplementary table S2).

Transfer and ribosomal RNA genes

The two rRNAs, *rrnL* and *rrnS*, are 966 and 727 bp in size, with 70.7% and 72.1% A + T content, respectively (supplementary table S2). The mitogenome of *N. mogurndae* contains all 22 tRNAs, which range in size from 59 bp (*trnR*) to 67 bp (*trnH* and *trnW*), and have a combined total size of 1408 bp (table 1

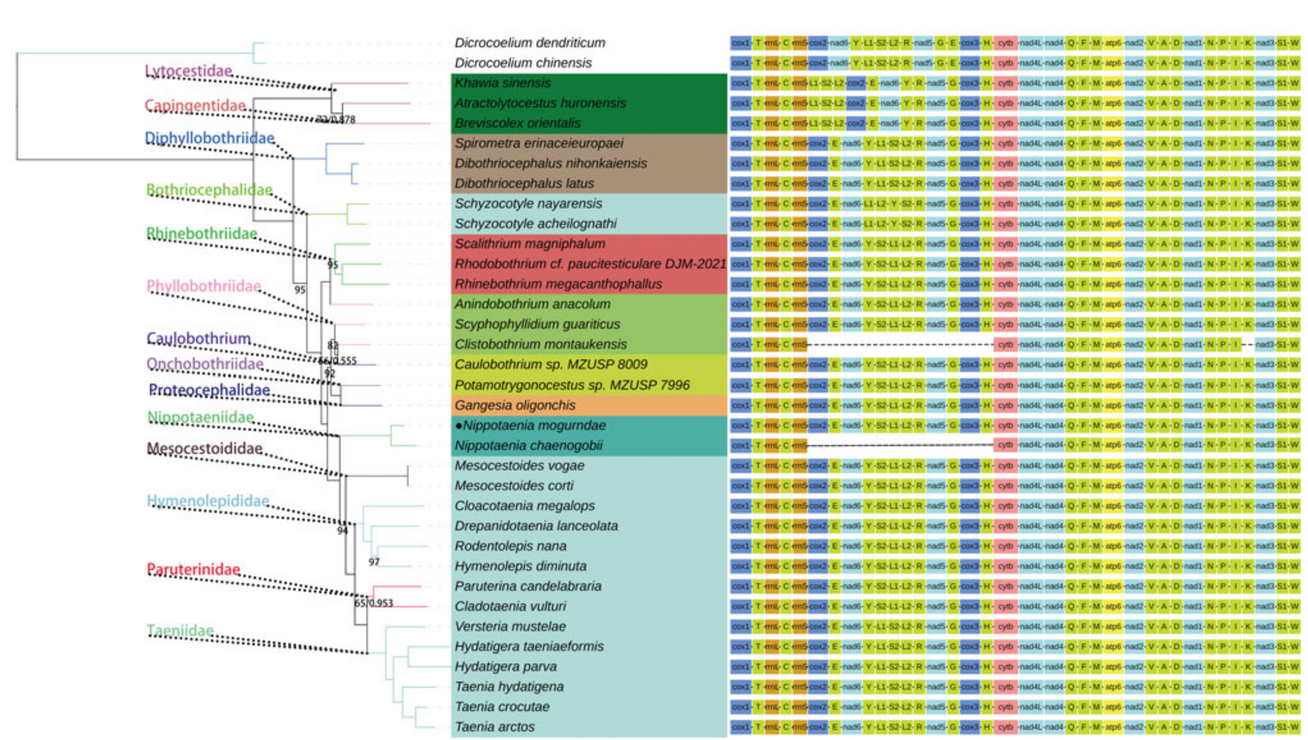

Fig. 5. Phylogenetic tree with gene order of cestodes species in nine orders inferred with 34 genes using maximum likelihood analyses. Bootstrap (BS)/Bayesian posterior probability (BPP) support values are shown above the nodes. Displayed sequences are BS <99 or BPP <1.

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and supplementary table S2). Most tRNAs have a conventional cloverleaf structure, but *trnS1* and *trnR* lack dihydrouridine arms of tRNA which also were determined in caryophyllidean and anoplocephalidaen tapeworms (Guo, 2017; Li *et al.*, 2017; Xi *et al.*, 2018).

Non-coding regions

The NCR1 (220 bp) and NCR2 (817 bp), the two largest non-coding regions, are located between *trnY* and *trnS2*, *nad5* and *trnG*, respectively. At similar positions, NCRs also have been found in other segmented tapeworms (e.g. von Nickisch-Rosenegk *et al.*, 2001). The A + T content in the two largest NCRs (81.8% and 83.1%) are higher than other regions in mitogenome (supplementary table S4). NCR2 contains 27 TRs. The first 26 repeat units are identical in nucleotide composition and size (26 bp), while the last one is truncated with 23 bp (fig. 4).

Phylogeny and gene order

The phylogenetic topologies constructed with ML and BI show concordant branches, and high statistical support that are above 99 (bootstrap support values) or near 1.00 (Bayesian posterior probabilities) for most nodes. The complete mtDNA data set contributes significantly informative characters to the study of cestode evolution (Waeschenbach *et al.*, 2012). In this study, *N. mogurndae* clusters with the congener tapeworm *Nippotaenia chaenogobii* (JQ268550.1) and forms a monophyletic clade at the basal branch consisting of tetrafossate tapeworms from families Nippotaeniidae, Mesocestoididae, Hymenolepididae, Paruterinidae and Taeniidae (fig. 5). The *Nippotaenia* clade shows a close relationship with the Mesocestoididae, and this finding is also supported by the cytogenetic data reported by Bombarová *et al.* (2005).

Gene order and arrangement in tapeworm mitogenome is conserved (Li *et al.*, 2017), and only four types of gene order are found among the 31 mtDNA data used in this study (fig. 5). The mtDNA of *N. mogurndae* showed consistent gene order with other segmented tapeworms.

Conclusion

In this study, we sequenced, annotated, and characterized the complete mitogenome of tapeworm *N. mogurndae* collected from Chinese sleeper *P. glenii*. Phylogenetic analysis based on mitogenomic data further confirmed that *Nippotaenia* is closely related to tetrafossate tapeworms, especially in the family Mesocestoididae.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/S0022149X22000530.

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Conflicts of interest. The authors have no conflicts of interest to declare.

Ethical standards. The animal study was reviewed and approved by the protocols used on the experimental fish followed the guidelines of the Institutional Animal Care and Ethics Committee of Nanjing Agricultural University, Nanjing, China. [Permit number: SYXK (Su) 2011-0036].

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