Comparative mitochondrial genome analysis of Spirometra tapeworms from different hosts

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Research Article

Keywords: Spirometra erinaceieuropaei, mitochondrial genome, phylogenetic relationship, molecular genetic marker

Posted Date: July 29th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1889916/v1

Abstract

Background: *Spirometra erinaceieuropaei*, the causative agent of food/water-borne sparganosis, has been widely reported worldwide. However, the taxonomy of the genus *Spirometra* has always been complicated. The main objectives of this study were to assemble 7 complete mitochondrial genomes of *Spirometra erinaceieuropaei,* collected from different hosts in the Hunan province of China, and to analyze the phylogenetic relationship and genetic diversity of cestode species.

Methods: In this study, seven *Spirometra erinaceieuropaei* (three spargana and 4 adults) were collected from different hosts in Hunan province, China. The long-PCR was performed to amplify the four large fragments of the *Spirometra* mitochondria (mt) genome by using specific primers reported in a previous study. Then, the mt genome of each *S. erinaceieuropaei* was assembled and annotated after overlapping four large fragments. Sliding window analysis was carried out to explore the nucleotide variation of the mt genome between 7 isolates obtained in this study and 8 reported *Spirometra*. The genetic diversity of cestode species was also investigated by Bayesian analysis based on 12 protein-coding genes.

Results: Seven mt genomes of *S. erinaceieuropaei* obtained in this study were successfully assembled and annotated. The genome features of *S. erinaceieuropaei* are similar to other reported cestode species, containing 12 protein-coding genes (PCGs), 22 transfer RNA genes (tRNAs), and two ribosomal RNA genes (rRNA), large non-coding regions (NC), and small non-coding regions (NR). Sequencing analysis revealed 97.40%-99.90% sequence similarity among seven mt genomes identified in this study. Sliding window analysis indicated that the Korea isolate (KJ599680) might be the differential specie of genus *Spirometra*, and *nad*4L, *cox*3, and *nad*6 were the top three genes with the lowest variation rates in mt DNA. Phylogenetic analysis based on 12 PCGs further demonstrated that *S. mansoni* (AB374543) might be the misnamed *S. erinaceieuropaei*.

Conclusion: The results of the current study supports the previously reported conclusion that multiple genotypes exist within *S. erinaceieuropaei*, and the Korean isolate (KJ599680) be a novel genotype or even a novel species of the genus *Spirometra*, and we strongly suggests that *S. decipines* may be a misnamed of *S. erinaceieuropaei* like *S. mansoni* is. *nad*4L, *cox*3 and *nad*6 are more suitable molecular genetic markers than *cox*1 for *S. erinaceieuropaei* identification. However, the authentic relationships among *S. erinaceieuropaei* isolates from different hosts and geographical sites are still unknown. More samples must be collected in different host and geographical positions to help us understand the genus *Spirometra*.

Background

Spirometra erinaceieuropaei, the first identified tapeworms of the genus *Spirometra*, has been widely reported worldwide as the causative agent of food/water-borne sparganosis [1, 2]. In 1882, Patrick Manson reported the first human sparganosis in Xiamen, Fujian Province, China [3]. To date, more than 2,000 patients with sparganosis have been diagnosed, most of which most of them reported in Asia [3].

Hosts can acquire *S. erinaceieuropaei* by ingesting raw or undercooked meat of tadpoles, frogs, snakes, and other animals or water containing parasite eggs or larvae [4]. In humans, sparganosis occurs when drinking water containing infected copepods or ingesting raw or undercooked meat of second intermediate hosts containing plerocercoids [1]. After infection, *S. erinaceieuropaei* can causes cerebral sparganosis, endermic sparganosis, ocular sparganosis and splanchnic sparganosis, even death in some individuals [1, 3]. After parasitizing the human intestine, *S. erinaceieuropaei* can develop into an adult tapeworm, causing vomit, stomachache, and diarrhea and misdiagnosing as diphyllobothriasis [2].

Mitochondrial (mt) genomes were widely used to identify the metazoan, phylogenetic relationship, and evolutional analysis, due to their maternal inheritance, rapid evolutionary rate, lack of recombination, and relatively conserved genome structures [5–9]. Mitochondrial *c* oxidase subunit I (*cox*1) was considered the most conserved gene in *Spirometra* and was widely used to identify accurate *Spirometra* species [2]. Jeon et al. identified the *S. erinaceieuropaei*, *S. decipiens*, and *S. ranarum* by amplifying the mitochondrial *cox*1 and *cyt*b or *nad*1 genes of *Spirometra* in Korea, Japan, China, and Myanmar [10–12]. In Tanzania, *S. theileri* was firstly identified from leopard and spotted hyena-derived isolates based on *cox*1 and *nad*1 genes of *Spirometra* [13]. Otherwise, plerocercoids were found in snakes from Poland, identified as *S. erinaceieuropaei*, by amplifying the evolutionary conserved nuclear 18S rRNA gene [14]. In China, Zhang et al. revealed two clusters of *S. erinaceieuropaei* by amplifying the *cyt*b, *cox*1, *rrn*S, and 28S rRNA of frog-derived plerocercoid, and they started to divergence in the middle Pliocene [15]. However, the latest document in 2021 indicated the existence of two species of *Spirometra* by *cox*1 gene amplification in Asia and is highly divergent from known *S. erinaceieuropaei* [10]. Collectively, the taxonomy of *Spirometra* has always been complicated. 4 out of 64 identified species of *Spirometra* are considered valid, which are *S. erinaceieuropaei*, *S. mansonoides*, *S. pretoriensis*, and *S. theileri* [16, 17]. The possible reason for the difficulty in the taxonomy of the genus *Spirometra* is varied, including high intraspecific variability, lack of species-specific morphological markers, and non-specific imaging examinations [18].

Comparison with single or multi genes of mt, complete mt genomes could be a better accurate tool to analyze the phylogenetic or genetic evolutionary relationship [19, 20]. In this study, the DNA genomic of seven *S. erinaceieuropaei* Chinese isolates were extracted, and the complete mt genome was assembled based on long-PCR amplification. In the present study, we identified seven *Spirometra* samples collected from different hosts by assembling and annotating their mt genomes. Furthermore, the mt genome features and structure, nucleotide homology, and nucleotide diversity Pi (π) were calculated by sequence analysis. Besides, the phylogenetic relationships were reconstructed based on mt DNAs of cestode species, including 7 mt genomes obtained in this study. The objectives of this study were to (i) expand the hosts and enrich mt genome data of gene *Spirometra*; (ii) clarify the population structure and genetic differentiation of *S. erinaceieuropaei*; and (iii) to clarify the origin and development of genus *Spirometra*.

Materials And Methods

Collection of samples

This study collected three spargana and four adult tapeworms from Hunan province, China. As shown in Table 1, three spargana were collected from three wild snakes (*Elaphe taeniura*, *Elaphe carinata*, and *Ptyas dhumnades*), each in different cities of Hunan province. Four adult tapeworms were obtained from four different Felidae (*Panthera tigris* ssp. *altaica*, *Panthera tigris* ssp. *tigris*, *Prionailurus bengalensis*, *Felis catus*) in Changsha city, Hunan province. Before genomic DNA extraction, 7 isolates were washed twice with physiological saline and stored at -20°C with 70% ethanol.

Genomic DNA extraction and long-PCR amplification

After washing the tapeworm with phosphate-buffered solution (PBS), approximately 300 mg of tissue from each tapeworm was used to extract the genomic DNA using TIANamp Genomic DNA Kit (TianGen, Beijing city, China) following the manufacturer's instructions and stored at -20℃ until used for subsequent PCR amplification. The primers used for long-PCR amplification in this study were described in a previous study by Liu et al. [5] and summarized in Table 2. Briefly, the PCR reactions (50 μL) contain 25 μL of *Premix* Taq polymerase (Takara, Dalian city, China), 1 μL each of forward and reverse primer, 21 μL of ddH_2O, and 2 μL of DNA template. The PCR systems are as follows: initial denaturation at 92℃ for 2 min, then 10 cycles of denaturation at 92℃ for 10 s, annealing at 50℃ (for *cox*2-*nad*4 and *cox*1-*rrn*S amplification) or 55℃ (for *nad*4-*cox*1 and *rrn*S-*cox*3 amplification) for 30 s, extension at 60℃ for 10 min, followed by denaturation at 92℃ for 10 s, annealing at 55℃ (for *cox*2-*nad*4 and *cox*1-*rrn*S amplification) or 57℃ (for *nad*4-*cox*1 and *rrn*S-*cox*3 amplification) for 30 s, extension at 60℃ for 10 min and 10 s, and a final extension at 60℃ for 10 min. Then, the PCR products were checked using 1.5% agarose gel with ethidium bromide and visualized under UV light.

Sequencing, assemblage, and annotation of the mitochondrial genome

All PCR products were sent to BGI Inc. (Shenzhen, China) for two-directional sequencing. The obtained sequences were overlapped and assembled using ChromasPro V2.1.3 software, and the gene boundaries were clarified after alignment with other *S. erinaceieuropaei* mitochondrial genome sequences deposited in GenBank. The open reading frame and codon usage of protein-coding genes (PCGs) were analyzed using MEGA7.0 software, and the initiation codons and termination codons of PCGs and two rRNAs were compared and verified with other *S. erinaceieuropaei*. The TBI-forna online website was performed to identify and predict the secondary structures of 22 tRNAs obtained in our work. EditSeq software was used to analyze the nucleotide content of each gene in the mitochondrial genome of *S. erinaceieuropaei*. Then, 7 complete mitochondrial genomes of *S. erinaceieuropaei* obtained in this study were annotated using SeqBuilder software and then deposited to GenBank under the accession number: OM935775-OM935781.

Sliding window analysis of nucleotide variation

To explore the nucleotide variation of *S. erinaceieuropaei* mitochondrial PCGs, software DnaSP V6.12 were performed to analyze the mitochondrial genomes and mitochondrial PCGs of 7 *S. erinaceieuropaei* isolates and 8 representatives of *S. erinaceieuropaei* mt sequences reserved in GenBank database. A

sliding window of 300 bp and steps of 10 bp were used to estimate nucleotide diversity Pi (π) for the complete alignment. Nucleotide diversity for the complete alignment was plotted against mid-point positions of each window, and gene boundaries were indicated. Notably, a Korean isolate of *S. erinaceieuropaei* (KJ599680) identified by Eom et al. in 2014 showed a significant difference with other reported mt sequences of *S. erinaceieuropaei*. Thus, sliding window analysis was performed for 12 PCGs based on 15 (contains KJ599680) and 14 (without KJ599680) mt genomes, respectively. Meanwhile, all mt DNA sequences of genus *Spirometra* recorded in GenBank were also used to analyze the nucleotide difference by sliding window analysis with 300 bp sliding window and steps of 10 bp.

Phylogenetic analyses

In the present study, 75 representative mtDNA sequences of cestode species, including 7 mt sequences of *S. erinaceieuropaei* identified in this study and 68 known mtDNA sequences recorded in GenBank, were recorded and used to construct the phylogenetic relationships and to explore the genetic diversity of cestode. Details of the 75 sequences used in this study are summarized in **Additional file 1: Table S1**. Briefly, the sequences of the 12 PCGs in each mtDNAs were separately aligned in MEGA v7 using translated amino acid sequences. Then, Bayesian inference (BI) was performed in PhyloSuite V1.2.2 for phylogenetic construction based on 12 concatenated PCGs and 12 amino acid sequences of PCGs, respectively [21]. BI analyses were conducted with four independent Markov chains run for 2,000,000 metropolis-coupled MCMC generations and sampling trees every 100 generations. The initial 25% tree was discarded as burin-in, and the remained tree was calculated as Bayesian posterior probabilities (PP) [22]. In this study, GTR + G + I and CPREV + I + G were chosen as the best model for nucleotide and amino acid sequences in Bayesian analysis, respectively. Finally, obtained Bayesian tree was visualized by using the software Figtree v1.4.4.

Results

Mitochondria genome structure of S. erinaceieuropae

In the present study, four long fragments of 7 *S. erinaceieuropaei* isolates were successfully amplified and assembled into 7 complete mt genomes, ranging from 13,640 to 13,680 bp in length A + T contents ranging from 66.14–66.48%. Sequencing analysis revealed 97.40–99.90% nucleotide similarity of seven isolates mt DNAs. Remarkably, the mt DNA sequences similarity within sample 1 (OM9335775) and sample 5 (OM9335779) was 99.7%. However, 97.6–98.2% similarity of mt DNAs were observed in comparison with the other five mt DNAs identified in this study, although the other five mt DNAs have 99.1% ~ 99.9% sequence similarity.

However, compared with a reported mt DNA of *S. erinaceieuropaei*, Korea isolate (KJ599680), the mt DNAs of seven isolates have 87.20%-87.60% sequences similarity, and 97.60%-99.90% sequences similarity was investigated with other reported *S. erinaceieuropaei* mt DNAs (without KJ599680). Additionally, seven isolates mt DNAs obtained in this study have 85.10–85.60% nucleotide similarity with *S. theileri*, a valid *Spirometra* species.

The boundary of each gene was investigated by comparison with known mt genome sequences, while each gene was simultaneously annotated. The mt genomes of 7 isolates also contain 12 PCGs (*cox*1-3, *nad*1-6, *nad*4L, *atp*6, and *cyt*b), 22 transfer RNA genes, two ribosomal RNA genes, and two non-coding regions. Besides 22 tRNA genes, 2 ribosomal RNA genes (*rrn*L and *rrn*S) and 2 non-coding regions. The size of each gene and its arrangement are shown in Additional file 2: Table S2. Among the 12 PCGs of the mt genome, the longest gene was *nad*5 with 1,569 bp, followed by the *cox*1 gene (1566 bp); the minimum length gene was *nad*4L was 261 bp. As the most frequently used initiation codon, ATG was detected among 11 out of 12 PCGs, except for *cox*3, which uses GTG as the initiation codon. The termination codon of TAA and TAG were used among 6 (*cox*2, *nad*1, *nad*6, *nad*5, *cyt*b, and *atp*6) and 4 (*cox*1, *nad*2, *nad*4L, *nad*4) PCGs of the mt genome, respectively, and an incomplete termination codon (T) were used in the gene *cox*3 and *nad*3. Also, 12 PCGs of the seven mt genomes have a similarity of 97.70–100.00% and 99.00-99.90% nucleotides homology and amino acid sequences.

In 12 transfer RNAs (tRNAs), the tRNA-Thr and tRNA-Arg were the largest and smallest tRNAs with lengths of 70 bp and 57 or 56 bp, respectively. The tRNA secondary structure was predicted by the online website (TBI-forna, http://nibiru.tbi.univie.ac.at/forna/), differences in stem and loop sizes of dihydrouridine (D) and TCC arms (data not shown). The two rRNA genes were *rrn*L and *rrn*S, with the sizes ranging from 972 ~ 973 bp and 729 ~ 730 bp, respectively.

Nucleotide variation in mt genome among S. erinaceieuropaei

Overall, 15 *Spirometra* mtDNA, including 7 mtDNA assembled in this study and 8 mtDNA recorded in the GenBank database, were performed to investigate the nucleotide diversity (π) within and between mt genes. As shown in Fig. 1, the gray and blue curves represent the nucleotide variation within and between 15 mt genomes and 14 mt genomes (without KJ599680). The results demonstrated that the nucleotide variation within and between mt genes among the aligned 15 or 14 *Spirometra* mt genomes could be visualized within a window of 300 bp and a step of 10 bp, with the π ranging from 0.00832 to 0.04625 (15 mt genomes). In combination with the calculation of the number of variable positions per unit length of the gene, the sliding window showed that the PCGs with low sequence variability included *cox*2 (0.01793), *nad4*L (0.01835), and *nad*1 (0.01999), while the genes with high sequence variability included *nad*5 (0.0331), *nad*6 (0.03027), and *cox*1 (0.02706). The isolate of KJ599680 was the most relatively diverged among all isolates. After excluding the data of KJ599680 isolate, the π ranging from 0.00088 to 0.02007, and show the π of PCGs are the following: *nad*4L (0.00307) < *cox*3 (0.00579) < *nad*6 (0.00674) < *nad*2 (0.00677) < *cox*2 (0.00684) < *atp*6 (0.00669) < *nad*1 (0.00747) < *nad*3 (0.00845) < *cyt*b (0.00879) < *nad*4 (0.00972) < *nad*5 (0.01021) < *cox*1 (0.01644). As shown in Fig. 2, the nucleotide diversity of each gene in the 19 mt DNAs of genus *Spirometra* ranged from 0.01355 to 0.08025, with the lowest variation level of gene *cox*1 (0.03551), *nad*4L (0.03733) and *cox*2 (0.03881), whereas *nad*5 (0.0662), *nad*6 (0.0557) and *nad*3 (0.05392) were the top three genes with the highest variation level.

Phylogeny analysis

In the present study, the phylogenetic tree was constructed using BI based on nucleotide and amino acid sequences of 12 PCGs of cestode mt genomes, respectively. As shown in Fig. 3., seven isolates obtained in this study were clustered into the genus *Spirometra*, and the same species were assembled in one branch. However, *S. mansoni* (AB374543) and *S. decipines* (MN121695 and NC_026852) are separate branches located in the middle of the *S. erinaceieuropaei*. Additionally, based on amino acid sequences of 12 PCGs, the Bayesian tree showed that all members of the genus *Spirometra* were also clustered into one root.

Discussion

In the present study, seven isolates obtained from different hosts in Hunan province were identified as *S. erinaceieuropaei* by overlapping four large fragments of the mitochondrial genome. The length of mt genome sequences of three sparganum and one adult *S. erinaceieuropaei* isolates (13,640 ~ 13,643 bp) were close to the lengths of the mt genome of adult *S. erinaceieuropaei* isolated from a dog in Hunan province (JQ267473, 13,641 bp) [5]. The length of the mt genome of three *S. erinaceieuropaei* isolates (13,676 ~ 13,680 bp) approaches the mt genome of *S. erinaceieuropaei*, identified in the frog in China [6]. The difference in nucleotide size might be responsible for the difference in their hosts, developmental stage, and geographical locations. The mt genome of *S. erinaceieuropaei* obtained in this study was also observed with 12 PCGs, 22 tRNAs, two rRNAs, and two non-coding genes transcribed in the same direction as other cestode species [5, 6, 23–25]. Among 12 PCGs, 11 PCGs use ATG as an initiation codon, and 10 PCGs use TAA or TAG as a termination codon, following the mt genome reported in the previous studies [5, 6]. Otherwise, GTG was usually used as the initiation codon of *cox*3, and an incomplete termination codon (T) was used as the termination codon of *cox*3 and *nad*3, which is consistent with other cestode species [24, 26]. The size of 22 tRNA genes obtained in this study ranged from 56 to 70 bp, which was different from the size of the tRNA gene (57 bp to 69 bp) reported by Liu et al. (2011), but these tRNA genes showed similar putative secondary structures[5, 6]. Also, a similar length of rrnL (972 bp to 973 bp) and rrnS (729 bp to 730 bp) in the mt genome among 7 isolates with the 63.96–63.37% of A + T content, representing the same function in genus *Spirometra*. A large non-coding region (NC) was inserted between rRNA-Tyr and tRNA-LeuCUN with 204 bp length among seven isolates. A small non-coding region (NR) was inserted between gene nad5 and tRNA-Gly with a different length of 173 to 211 bp among seven isolates. These results are consistent with the previous study (2017), Which analyzed the mt genome of *S. erinaceieuropaei* isolated from wild frogs in China [6]. The mitochondrial genomes of the seven cestodes obtained in this study were consistent with the gene sequences and transcriptional directions of other cestodes and also lacked the atp8 gene, suggesting that the mitochondrial genes of cestodes are relatively conserved [5, 6, 23, 24].

Currently, the accurate species identification of cestodes mainly depends on the complete cox1 gene amplification and sequencing [2]. Sliding window analysis as a population genetic tool was performed in this study to explore the nucleotide diversity Pi (π) among 15 *S. erinaceieuropaei* mt genomes. The gene of *cox*2 (0.01793), *nad*1 (0.01999), and *nad*4L (0.01835) has a lower sequence variability than *cox*1 (0.02706), which was widely used to identify the Pseudophyllidea cestodes. The previous study

suggested that the Korean isolate (KJ599680) may be a novel genotype or species[6]. Thus, the blue curve (Fig. 1; without KJ599680) represents lower sequence variability among 14 mt genomes. Among them, *nad*4L (0.00307), *cox*3 (0.00579), and *nad*6 (0.00674) can be considered the more suitable genetic marker than frequently-used *cox*1 (0.01644) to investigate the population genetics of Pseudophyllidea cestodes. These results are inconsistent with the previously reported studies, suggesting *cox*2 and *nad*6 or *cox*1, *cyt*b, and *nad*4 as the optimal genetic marker [5, 6]. The sliding window analysis of 19 mt DNAs showed that *cox*1 had the lowest nucleotide diversity and was the most accurate genetic marker of the genus *Spirometra*, which was consistent with the previous study [2]. Nevertheless, gene *nad*4L may be the ideal genetic marker for detecting *S. erinaceieuropaei*. Our results indicated that sliding window analyses could define genetic markers for population genetics and systematics studies of cestodes.

As shown in Fig. 3 and Fig. 4, phylogenetic analysis of cestodes using the BI method revealed similar tree topologies based on concatenated nucleotide sequences and concatenated amino acid sequences of 12 PCGs, all revealed distinct groups with high statistical support and demonstrated that *S. mansoni* and *S. decipines* might be the synonym of *S. erinaceieuropaei*. Also, the position of the Korea isolate (KJ599680) on the phylogenetic tree demonstrated that it can be a novel genotype of the genus *Spirometra*, consistent with that of a previous study [6]. However, the reasons *S. mansoni* and *S. decipines* inserted into the middle of *S. erinaceieuropaei* isolates need more future samples to understand the exact relationship among isolates further. The present study also supports the previously reported conclusion that multiple genotypes exist within *S. erinaceieuropaei* [15, 27, 28], and the phylogenetic tree indicates that there may be two genotypes among *S. erinaceieuropaei*, one of which is OM9335775 and OM9335779.

Additionally, two phylogenetic trees also showed that no correlation was not clustered into the same branch among *S. erinaceieuropaei* isolates, which were collected from the same host or same developmental stage or geographic region, consistently with the previous studies that genetic evolution based on complete mitochondrial *cox*1 of cestode by Okamoto [27]. We speculated that this variation is caused by human migration or economic activity, resulting in the migration of many hosts to different areas, such as pets and rare animals. For better adaptation to hosts, different cestode species can infect the same host, and the same cestode species can infect different hosts. However, the authentic relationships among *S. erinaceieuropaei* isolates from different hosts and geographical sites are still unknown, and more samples need to be collected from different hosts and geographical positions to help us understand the genus *Spirometra*.

Conclusion

In the present study, seven *S. erinaceieuropaei* isolates from different hosts in Hunan province were successfully assembled and annotated. The genome features contain 12 PCGs, 22 tRNA genes, two rRNA genes, and two non-coding regions. The nucleotide sequences of the mtDNA of these isolates were remarkably similar. Sliding window analysis revealed that *nad*4L, *cox*3, and *nad*6 are more suitable as a molecular genetic marker than *cox*1 for *S. erinaceieuropaei* identification. Phylogenetic analysis indicated

that the mt DNA of cestode species was not correlated with sampling site, hosts, and developmental stage, and *S. decipines* may be the misnamed of *S. erinaceieuropaei* like *S. mansoni* is. It is important to understand the taxonomic status of the genus *Spirometra*, which is essential for detecting and controlling sparganosis.

Declarations

Acknowledgments

Not applicable.

Funding

Project support was provided by the Natural Science Foundation of Hunan Province, China (2021JJ30335), Scientific Research Fund of Hunan Provincial Education Department, China (21A0141).

Availability of data and materials

The data sets supporting the results of this article have been submitted to GenBank, and the accession number is shown in the article. Further inquiries can be directed to the corresponding authors.

Author contributions

WL and GHL conceived and designed the experiments. JLH, TFG, SYC XRX, and WCL performed the experiments. JLH, SCX, and GHL analyzed the data. JLH and GHL drafted the paper. All authors critically revised the paper. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Tables

Tables 1-2 are not available with this version.

Figures

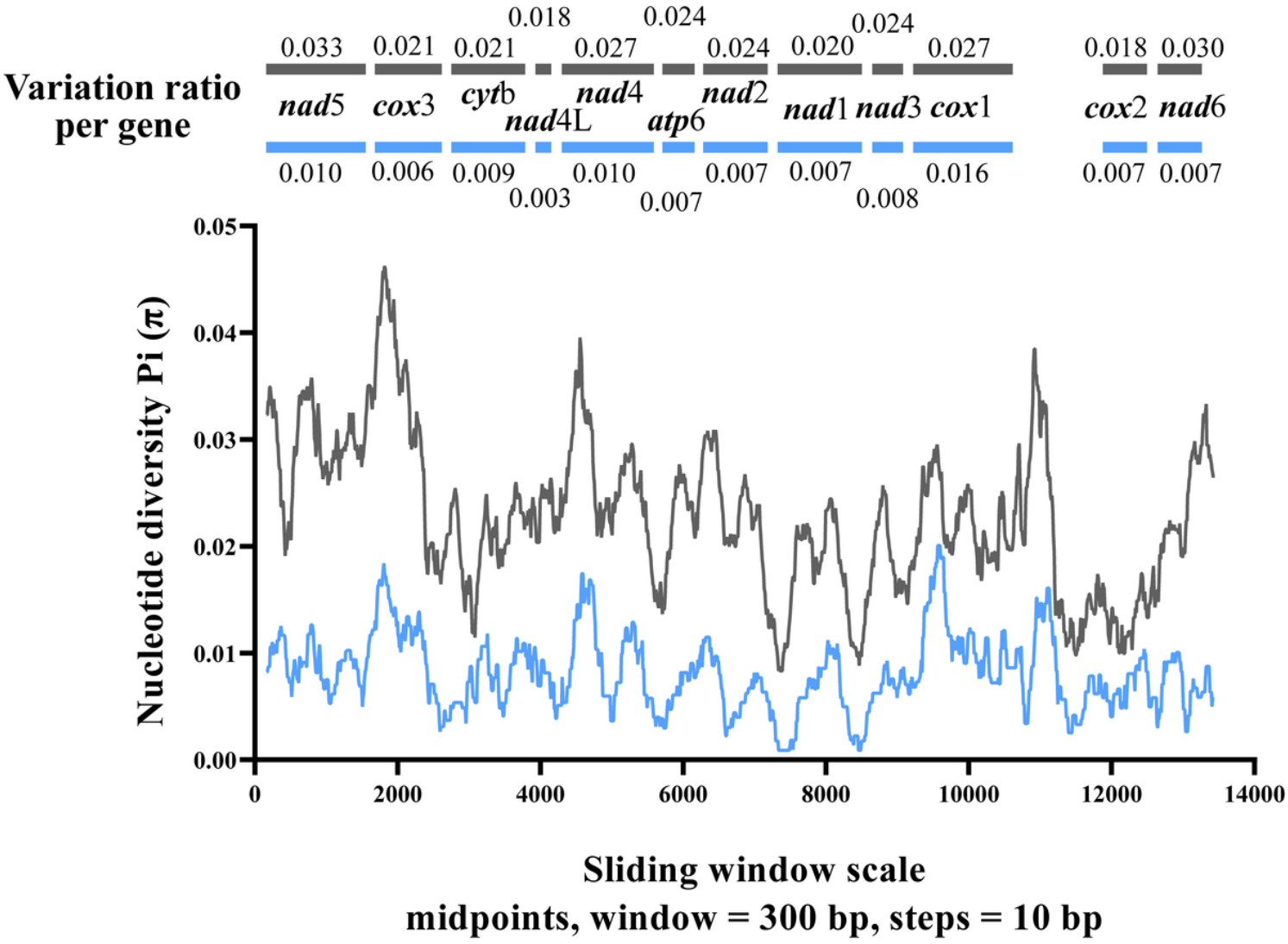

Figure 1

Sliding window analysis of the alignment of complete mtDNAs of *Spirometra erinaceieuropaei*. The black and blue lines showed the value of nucleotide diversity Pi (π) of 15 mt DNAs and 14 mt DNAs (without KJ599680) in a sliding window analysis of window size 300 bp with step size 10, and the value is inserted at its mid-point. Gene boundaries are indicated with a variation ratio per gene.

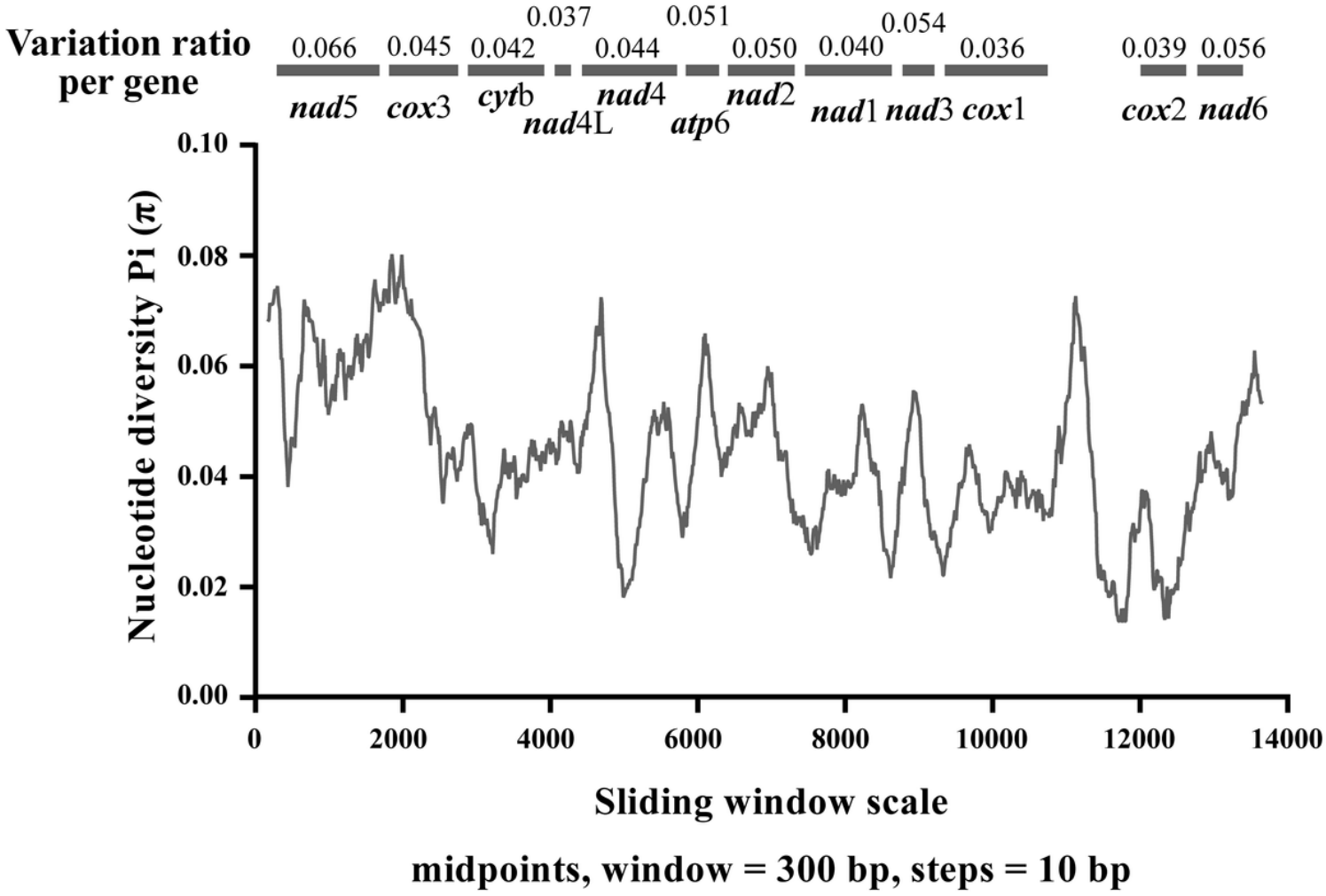

Figure 2

Sliding window analysis of the alignment of complete mt DNAs of genus *Spirometra*. The black line shows the value of nucleotide diversity Pi (π) in a sliding window analysis of window size 300 bp with step size 10, and the value is inserted at its mid-point. Gene boundaries are indicated with a variation ratio per gene.

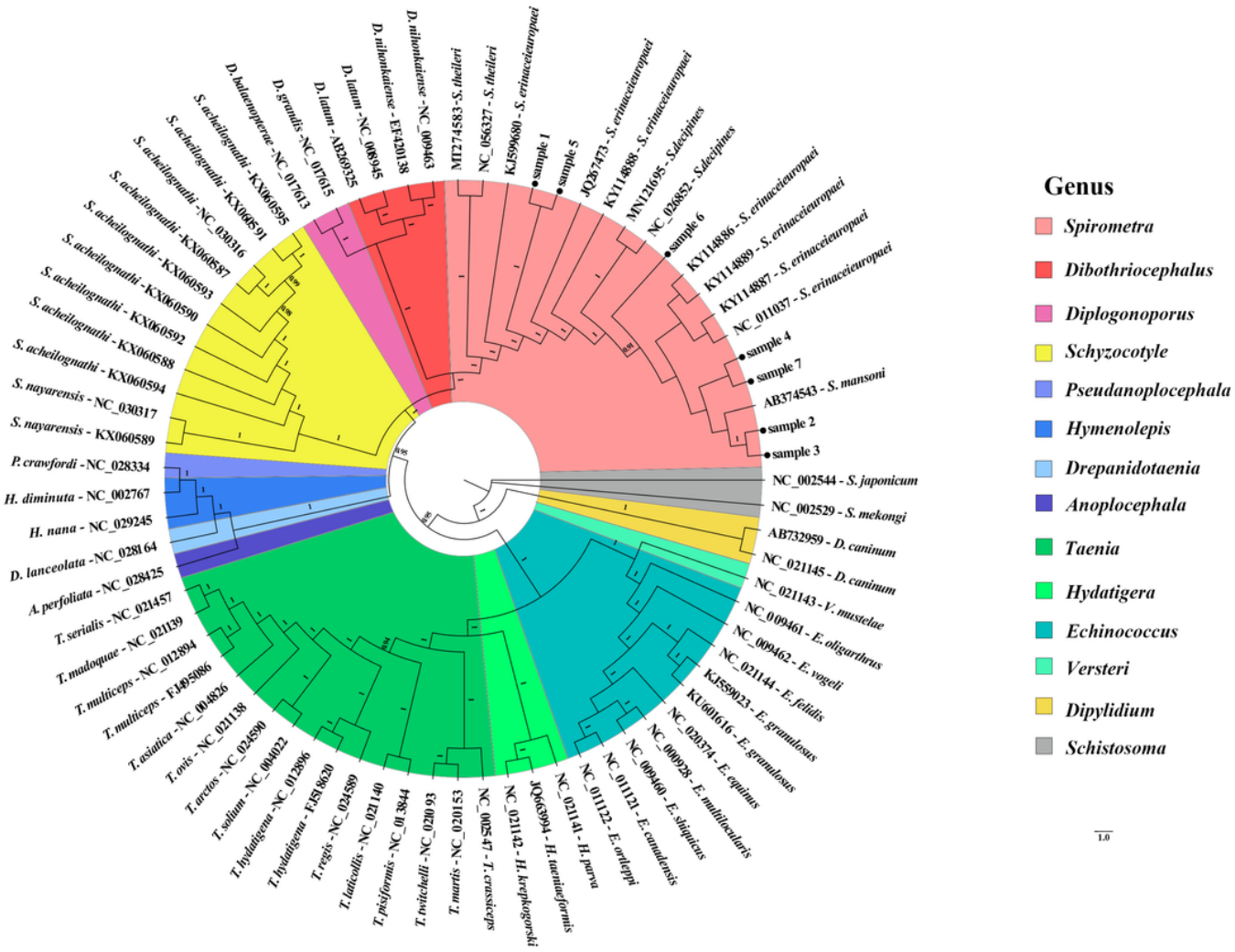

Figure 3

Phylogenetic relationship among 75 cestode species based on concatenated nucleotide sequences of 12 protein-coding gene loci using Bayesian inference (Bayes) analysis. The genus *Schistosoma* (*S. japonicum*: NC_002544; *S. mekongi*: NC_002529) was used as the outgroup. The number above the branches indicates posterior probability (PP) values for Bayesian inference. One for each color represents a genus.

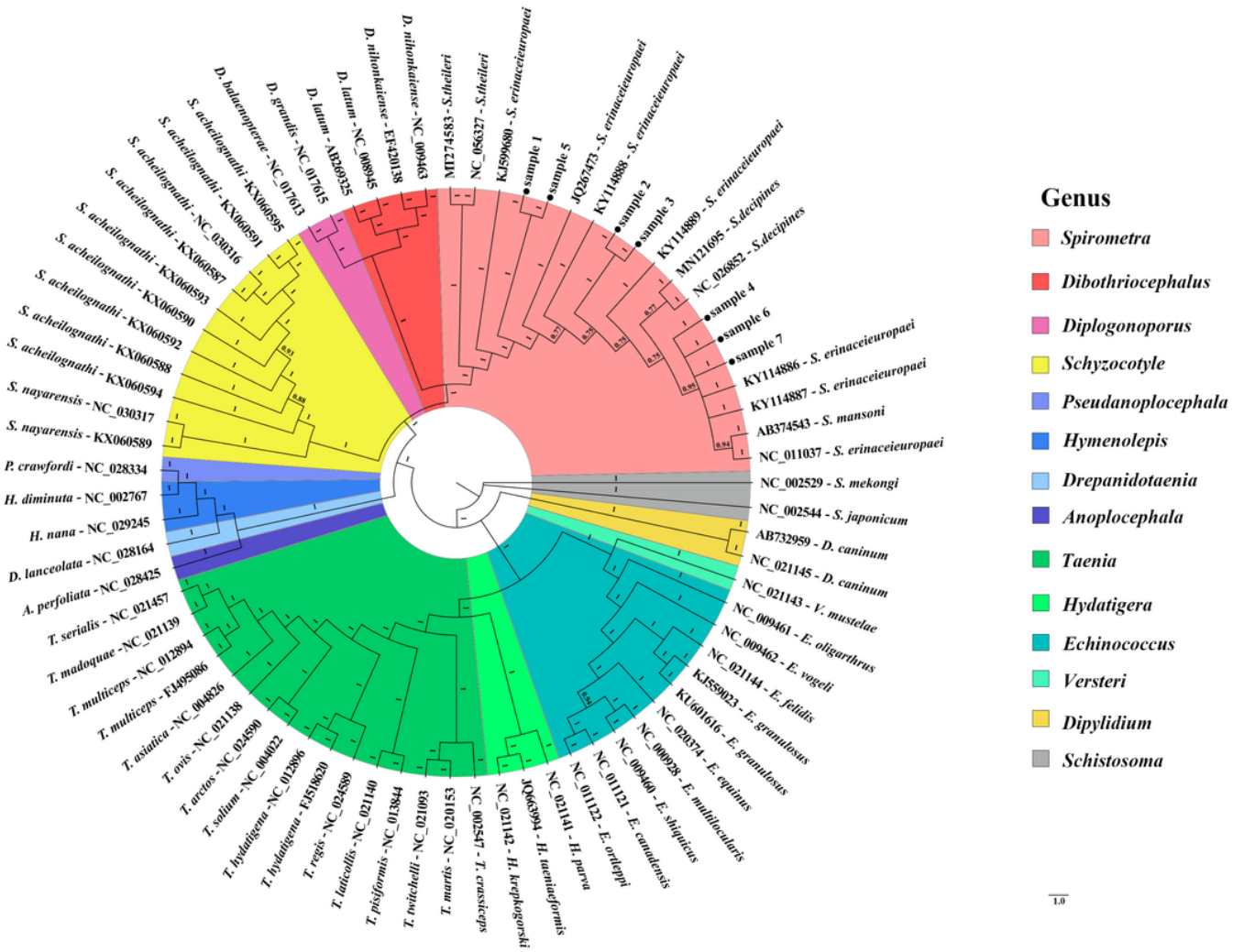

Figure 4

Phylogenetic relationship among 75 cestode species based on concatenated amino acid sequences selected from 12 protein-coding gene loci using Bayesian inference (Bayes) analysis. The genus *Schistosoma* (*S. japonicum*: NC_002544; *S. mekongi*: NC_002529) was used in this study as the outgroup. The number above the branches indicates posterior probability (PP) values for Bayesian inference. One for each color represents a genus.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS1.docx
- TableS2.docx