Analysis of the complete *Fischoederius elongatus* (Paramphistomidae, Trematoda) mitochondrial genome

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Abstract

**Background:** *Fischoederius elongatus* is an important trematode of Paramphistomes in ruminants. Animals infected with *F. elongates* often don't show obvious symptoms, so it is easy to be ignored. However, it can cause severe economic losses to the breeding industry. Knowledge of the mitochondrial genome of *F. elongates* can be used for phylogenetic and epidemiological studies.

**Findings:** The complete mt genome sequence of *F. elongates* is 14,120 bp in length and contains 12 protein-coding genes, 22 tRNA genes, two rRNA genes and two non-coding regions (LNR and SNR). The gene arrangement of *F. elongates* is the same as other trematodes, such as *Fasciola hepatica* and *Paramphistomum cervi*. Phylogenetic analyses using concatenated amino acid sequences of the 12 protein-coding genes by Maximum-likelihood and Neighbor-joining analysis method showed that *F. elongates* was closely related to *P. cervi*.

**Conclusion:** The complete mt genome sequence of *F. elongates* should provide information for phylogenetic and epidemiological studies for *F. elongates* and the family Paramphistomidae.

**Keywords:** *Fischoederius elongatus*, Mitochondrial genome

Findings

**Background**

Paramphistomes are distributed worldwide and have been reported in many countries, such as Bulgaria, France, Poland, Hungary, Italy, India, Russia, Sardinia and Yugoslavia [1]. The paramphistome can infect fishes, reptiles, birds and mammals, some of which can lead to huge economic losses related to seriously gastrointestinal diseases, low productivity or death in ruminants [2]. In Arumeru District, the prevalence rate of paramphistomes is as high as 56.7% in cattle [3].

*Fischoederius elongates* is an important member of paramphistomes, the parasite usually inhabits the rumen of cattle, buffaloes, sheep and goats. Ruminants are usually infected by ingesting snails, such as *Lymnaea acuminata*, *Lymnaea succinea* or *Gyraulus euphraticus* [4]. Ruminants infected with *F. elongates* show weakness, mental fatigue and eventually death. More seriously, *F. elongates* maybe a zoonotic trematode, a Chinese woman from Guangdong Province was reported to be the first human infection case [5], but it is still unknown how she was infected.

Until now, the most common diagnostic method for *F. elongates* is the microscopical examination, but it's time-consuming, and hard to distinguish with other paramphistomes. As a useful marker, mt genome has been widely used for species identification [6–10]. The complete mt genome of *F. elongates* can provide alternative molecular markers for the species identification, epidemiology and genetic diversity of paramphistomes.

In the present study, we got the full sequence and gene arrangement of mt genome of *F. elongates* and compared it with selected trematodes. We found that *F. elongates* had the closest relationship with *P. cervi*.
Methods
Ethical approval
The study was performed under the instructions and approval of Laboratory Animals Research Centre of Hubei province in P. R. China and the ethics committee of Huazhong Agricultural University (Permit number: 4200695757).

Parasite collection and DNA isolation
*F. elongates* adults were collected from the rumen and reticulum of naturally infected cattle in Zhanggang, Tianmen, Hubei province, PR China, according to the Animal Ethics Guidelines of Huazhong Agricultural University. Then, the adult worms were washed extensively in 0.9 % sodium chloride solution, and identified through morphological examinations [2]. Subsequently, one worm was stained for identification [11], and the rest were fixed in 75% alcohol (V/V) and stored at −20 °C until use [12]. Total genomic DNA was isolated from one worm [13]. The ITS-2 region of *F. elongates* was amplified and sequenced as reported previously [14], it was 100 % similar to that of *F. elongates* (GenBank accession no. JQ688410.1).

Amplification and sequencing of *F. elongates* mt genome
Firstly, we designed 12 oligonucleotide primers according to the conserved regions from reported mt genome sequences of *F. hepatica* [15], *Clonorchis sinensis* [16] and *P. cervi* [17] to amplify partial fragments from *cox*3, *cyt* *b*, *nad*4, *cox*1, *rrn*S and *nad*5 (Table 1). PCRs (25 μl) were performed in the following reaction: 10 mM Tris–HCl (pH 8.4), 50 mM KCl, 4 mM MgCl2, 200 mM each of dNTP, 50 pmol of each primer, 2 U *Taq* polymerase (Takara) and 2.5 μl genomic DNA. Reactions were run under the following conditions: 94 °C for 5 min, followed by 35 cycles of 94 °C/30 s, 50 °C/30 s and 72 °C/1 min. Amplicons were sent to Sangon Company (Shanghai, China) for sequencing.

Then, 12 additional primers (Table 1) were designed based on the obtained sequencing results to amplify six regions from genomic DNA (~40-80 ng) by long-PCR.

Table 1 Primers used in the present study

| Primer codes | Sequences (5′-3′) | Target gene | References |
|--------------|-------------------|-------------|------------|
| XCCOX3F      | AGYACDGTDGDDTTRCATTT | cox31 | Present study |
| XCCOX3R      | CANAYATAATCMACARAATGNA | cox31 | Present study |
| nxcobF       | ATGTCTWTGTRGGCKGBACNGT | cyrb1 | Present study |
| nxcobR       | GADVCTNCGGRTGRCAVGCHCC | cyrb1 | Present study |
| nxcND4F      | GAKTCBCCDATTCDGARCG | nad41 | Present study |
| nxcND4R      | ACHCNGCAGHANGNMCRTGMCC | nad41 | Present study |
| TXCOCX1F     | GGHGTAACHRWTAYCCHCC | cox11 | Present study |
| TXCOCX1R     | GTGRTGRGCGYCAWCDAYAMHCC | cox11 | Present study |
| X12SF        | AAWAAYAGAGAGGYACGGGGCG | rrnS1 | Present study |
| X12SR        | TARACTAGGATTAGATACCC | rrnS1 | Present study |
| NxcNDSF      | TGTGGCTBNCNGCAGTGGNGATG | nad51 | Present study |
| NxcNDSR      | TAAACCTTCAHNMCCRTGHGT | nad51 | Present study |
| 3CF1         | TGCATGTAGTATAGGTTTGG | cox3-cyro2 | Present study |
| 3CR1         | AACTAAAGTAAACATTTGTAC | cox3-cyro2 | Present study |
| 3CF2         | TTGGTTTTGTTGCTTC | cyrb-nad42 | Present study |
| 3CR2         | AACGTAATATTCACTCCC | cyrb-nad42 | Present study |
| 3CF3         | TGGCGTTTTGAGTTTTC | nad4-cox12 | Present study |
| 3CR3         | TCAACGAACTAATATCTTG | nad4-cox12 | Present study |
| 3CF4         | TGTTTTGGGCTTGACAG | cox1-rrnS2 | Present study |
| 3CR4         | ACCAAGCAAAAAATCTACC | cox1-rrnS2 | Present study |
| 3CF5         | TGTTAAAAGGCTTGTCTGGT | rrnS-nad52 | Present study |
| 3CR5-1       | ACCAAACAACTACATC | rrnS-nad52 | Present study |
| 3CF6-1       | TTACATGGATTGGCTTGTG | nad5-cox32 | Present study |
| 3CR6         | TTACATTTTTAATTAAACCTTTC | nad5-cox32 | Present study |

1 short regions amplified by PCR from cox3 (139 bp), cyrb (613 bp), nad4 (354 bp), cox1 (497 bp), rrnS (500 bp) and nad5 (458 bp). 2 large fragments that were amplified by long-range PCR from cox3-cyrb (724 bp), cyrb-nad4 (1008 bp), nad4-cox1 (4675 bp), cox1-rrnS (2198 bp), rrnS-nad5 (1981 bp) and nad5-cox3 (1718 bp).
PCRs (50 μl) were performed in reactions containing 0.4 mM each of dNTPs, 5 μl 10× LA Taq buffer II(Mg²⁺ Plus), 2.5 μM of each primer, 2.5 U LA Taq polymerase (Takara) and 2.5 μl genomic DNA. And the reactions were run under the following program: 94 °C for 5 min, followed by 35 cycles of 94 °C/30 s, 50 °C/30 s and 72 °C/1-5 min (depending on the size of *F. hepatica*). Amplicons were cloned into pGEM-T-Easy vector (Promega, USA) and then sequenced using a primer-walking strategy [18].

Sequence analyses

*F. elongates* mt genome sequences were assembled manually and then aligned with the mt genome sequences of *F. hepatica*, *C. sinensis* and *P. cervi* using the program Clustal X 1.83 [19]. Open reading frames were identified by ORF Finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html) using the echinoderm and flatworm mitochondrial code. Initiation and termination codons of the 12 protein-coding genes were identified as reported [15]. The 22 tRNA genes were predicted using tRNAscan-SE or manual adjustments [20,21]. The two rRNA genes were predicted by comparison with those of *F. hepatica* [15], *C. sinensis* [16] and *P. cervi* [17]. Amino acid sequences of 12 protein-coding genes were inferred using ExPASy Translate tool (http://web.expasy.org/translate/) using the echinoderm and flatworm mitochondrial codes, and aligned using MEGA 5.0 with default settings [22].

Nucleotide variation analysis

The nucleotide variation between *F. elongates* and *P. cervi* was analysed by sliding window analysis as reported [17].

Phylogenetic analysis

Amino acid sequences translated from individual genes of the mt genome of *F. elongates* were aligned with those predicted from mt genomes of selected trematodes, including *C. sinensis* (NC_012147) [16], *Dicrocoelium dendriticum* (NC_025280.1) [23], *F. hepatica* (NC_002546) [15], *Haplorchis taichui* (NC_022433.1) [24], *Metagonimus yokogawai* (KC330755.1), *Opisthorchis viverrini* (JF739555.1) [25], *P. cervi* (NC_023095.1) [17], *Schistosoma haematobium* (NC_008074) [26], *Schistosoma japonicum* (AF215860) [15], *Schistosoma mekongi* (NC_002529) [27], *Schistosoma spindale* (NC_008067) [26], and the cestode *Taenia solium* (outgroup) (NC_004022.1) [28]. The amino acid sequences of selected trematodes were aligned using MEGA 5.0 [22], and phylogenetic analysis of the aligned amino acid sequences was conducted in MEGA 5.0 using the Maximum Likelihood (ML) method.
Table 2 The organization of the mitochondrial genome of *Fuscoebaenus elongatus*

| Gene/region | Positions | Size (bp) | Number of aa | Ins/Ter codons | Anticodons | In^2 |
|-------------|-----------|-----------|--------------|----------------|------------|------|
| cox3        | 1-645     | 645       | 215          | ATG/TAG        | 0          |      |
| trnH        | 648-715   | 68        |              | GTG            | +2         |      |
| cyt b       | 717-1829  | 1113      | 371          | ATG/TAA        | +1         |      |
| SNR         | 1830-1892 | 63        |              |                | 0          |      |
| nad4L       | 1893-2156 | 264       | 88           | ATG/TAG        | 0          |      |
| nad4        | 2117-3397 | 1281      | 427          | GTG/TAA        | −38        |      |
| trnQ        | 3409-3471 | 63        |              | TTG            | +11        |      |
| trnF        | 3486-3549 | 65        |              | GAA            | +14        |      |
| trnM        | 3549-3612 | 64        |              | CAT            | −1         |      |
| atp6        | 3613-4128 | 516       | 172          | ATG/TAG        | 0          |      |
| nad2        | 4133-5008 | 876       | 292          | GTG/TAG        | +4         |      |
| trnV        | 5039-5102 | 64        |              | TAC            | +30        |      |
| trnA        | 5109-5179 | 71        |              | TGC            | +6         |      |
| trnD        | 5328-5397 | 70        |              | GTC            | +148       |      |
| nad1        | 5400-6296 | 897       | 299          | ATG/TAG        | +2         |      |
| trnN        | 6314-6379 | 66        |              | GTT            | +17        |      |
| trnP        | 6384-6447 | 64        |              | TGG            | +4         |      |
| trnI        | 6449-6511 | 63        |              | GAT            | +1         |      |
| trnK        | 6518-6852 | 65        |              | CTT            | +6         |      |
| nad3        | 6587-6943 | 357       | 119          | ATG/TAG        | +4         |      |
| trnS1       | 6955-7014 | 60        |              | GCT            | +11        |      |
| trnW        | 7027-7091 | 65        |              | TCA            | +12        |      |
| cox1        | 7095-8636 | 1542      | 514          | GTG/TAA        | +3         |      |
| trnT        | 8646-8709 | 64        |              | TGT            | +9         |      |
| rnl^4       | 8710-9704 | 995       |              |                | 0          |      |
| trnC        | 9707-9767 | 61        |              | GCA            | +2         |      |
| rns^4       | 9768-10518| 751       |              |                | 0          |      |
| cox2        | 10519-11100| 582    | 194         | ATG/TAG        | 0          |      |
| nad6        | 11046-11546| 501  | 167         | ATG/TAG        | −53        |      |
| trnY        | 11568-11632| 65   |              | GTA            | +21        |      |
| trnL1       | 11652-11715| 64   |              | TAG            | +19        |      |
| trnS2       | 11717-11785| 69   |              | TGA            | +1         |      |
| trnL2       | 11792-11856| 65   |              | TAA            | +6         |      |
| trnR        | 11860-11925| 66   |              | TCG            | +3         |      |
| nadS        | 11926-13506| 1581 | 527         | GTG/TAG        | 0          |      |
| trnG        | 13510-13574| 65   |              | TCC            | +3         |      |
| trnE        | 13587-13651| 65   |              | TTC            | +12        |      |
| LNR         | 13652-14120| 469  |              |                | 0          |      |

The inferred length of amino acid sequence of 12 protein-coding genes: 
1 amino acid; 2 initiation and termination codons; 3 intergenic nucleotides; 
4 initiation or termination positions of ribosomal RNAs defined by adjacent gene boundaries.

Results and discussion

Features of the mt genome of *F. elongatus*

The complete mitochondrial genome of *F. elongatus* (GenBank accession no. KM_397348) is 14,120 bp in length. The length of the *F. elongatus* mt genome is larger than the mtDNA genomes of *C. sinensis* (13,875 bp) and *S. japonicum* (14,085 bp), but smaller than *D. dendriticum* (14,884 bp), *F. hepatica* (14,462 bp), *H. taichui* (15,130 bp), *M. yokogawai* (15,258 bp), *S. haematobium* (15,003 bp), *S. mekongi* (14,072 bp) and *S. spindale* (16,901 bp).

The circular mt genome of *F. elongatus* includes 12 protein-coding genes (cox1-3, nad1-6, nad4L, cyt b and atp6), 22 tRNA genes, two rRNA genes (rrnS and rrnL) and two non-coding regions (SNR and LNR). All the 12 protein-coding genes are transcribed in the same direction (Fig. 1), which is the same as in *F. hepatica* [15], *C. sinensis* [16] and *P. cervi* [17]. The gene arrangement order is as follows: cox3-cyt b-nad4L-nad4-atp6-nad2-nad3-cox1-rrnL-rrnS-cov2-nad6, which is consistent with *F. hepatica*, *O. viverrini*, *P. cervi*, *S. japonicum* and *S. mekongi*, except for *S. haematobium* and *S. spindale* [26].

Overlapping nucleotides between mt genes of *F. elongatus* ranged from 1 to 53 bp (Table 2). The *F. elongatus* mt genome has 26 intergenic spacers ranging from 1 bp to 148 bp in length (Table 2). The nucleotide contents of A, C, T and G in the mt genome are 19.78 %, 9.62 %, 44.10 % and 26.50 %, respectively (Table 3), with T being the most favored nucleotide, followed by G, A and C, which is also the same as the mt genomes of *F. hepatica*.

Table 3 Nucleotide contents of genes and the non-coding region within the mitochondrial genome of *Fuscoebaenus elongatus*

| Gene | A(%) | C(%) | G(%) | T(%) | A + T(%) |
|------|------|------|------|------|---------|
| cox3 | 18.29 | 8.53  | 24.50 | 48.68 | 66.97   |
| cyt b| 18.96 | 8.89  | 26.33 | 48.52 | 64.78   |
| SNR  | 20.63 | 4.76  | 31.75 | 42.86 | 63.49   |
| nad4L| 21.97 | 8.33  | 25.38 | 44.32 | 66.29   |
| nad4 | 16.55 | 9.52  | 25.45 | 48.48 | 65.03   |
| atp6 | 17.64 | 10.08 | 24.42 | 47.87 | 65.50   |
| nad2 | 15.64 | 7.99  | 25.11 | 51.26 | 66.89   |
| nad1 | 16.39 | 7.47  | 28.21 | 47.94 | 64.33   |
| nad3 | 15.97 | 7.84  | 28.01 | 48.18 | 64.15   |
| cox1 | 18.87 | 11.02 | 24.51 | 45.59 | 64.46   |
| rnl  | 25.83 | 10.35 | 26.73 | 37.09 | 62.91   |
| rns  | 24.37 | 12.25 | 28.10 | 35.29 | 59.65   |
| cox2 | 19.93 | 11.11 | 27.49 | 41.58 | 61.51   |
| nad6 | 17.44 | 8.61  | 26.71 | 47.24 | 64.68   |
| nad5 | 16.32 | 8.29  | 28.78 | 46.62 | 62.93   |
| LNR  | 26.01 | 9.17  | 26.44 | 38.38 | 64.39   |
C. sinensis and P. cervi. The A + T content of 12 protein coding genes and 22 rRNA genes of F. elongates ranged from 59.65% (rrnS) to 66.97% (cox3), and the overall A + T content of the mt genome is 63.88%.

The present F. elongates mt genome can provide useful information for the studies of epidemiology, species identification and genetic diversity of Fischoederius spp. At the same, it will also make contribution to the taxonomy study of Fischoederius spp. With the full mt genome of F. elongates, we can undertake a study within F. elongates from different regions or among Fischoederius spp, by combining the morphological features with genetic analyses (with molecular markers from mitochondria or ribosome, such as cox1, nad4, 18S, ITS-1 and ITS-2). Meanwhile, the mt genome of F. elongates may also provide information for the prevention and diagnosis of Fischoederius spp and perhaps, this mt genome information may assist in the new drug, since mitochondria is the target of some drugs, such as decoquinate.

### Protein-coding genes

The F. elongates mt genome has 12 protein-coding genes, including cox3, cyt b, nad4L, nad4, atp6, nad2, nad1, nad3, cox1, cox2, nad6 and nad5. For these protein coding genes, ATG (eight of 12 protein genes) is the most common initiation codon, followed by GTG (four of 12 protein

| Amino acid | Codon | Number | Frequency(%) | Amino acid | Codon | Number | Frequency(%) |
|------------|-------|--------|--------------|------------|-------|--------|--------------|
| Phe        | TTT   | 325    | 9.65         | Ile        | ATT   | 127    | 3.77         |
| Phe        | TTC   | 28     | 0.83         | Ile        | ATC   | 6      | 0.18         |
| Leu        | TTA   | 167    | 4.96         | Ile        | ATA   | 71     | 2.11         |
| Leu        | TTG   | 290    | 8.61         | Met        | ATG   | 105    | 3.12         |
| Ser        | TCT   | 118    | 3.50         | Met        | GTG   | 165    | 4.90         |
| Ser        | TCC   | 6      | 0.18         | Thr        | ACT   | 54     | 1.60         |
| Ser        | TCA   | 22     | 0.65         | Thr        | ACC   | 3      | 0.09         |
| Ser        | TCG   | 25     | 0.74         | Thr        | ACA   | 19     | 0.56         |
| Tyr        | TAT   | 169    | 5.02         | Thr        | ACG   | 16     | 0.47         |
| Tyr        | TAC   | 11     | 0.33         | Asn        | AAT   | 54     | 1.60         |
| Stop       | TAA   | 3      | 0.09         | Asn        | AAC   | 2      | 0.06         |
| Stop       | TAG   | 9      | 0.27         | Asn        | AAA   | 23     | 0.68         |
| Cys        | TGT   | 112    | 3.32         | Lys        | AAG   | 50     | 1.48         |
| Cys        | TGC   | 9      | 0.27         | Ser        | AGT   | 92     | 2.73         |
| Trp        | TGA   | 41     | 1.22         | Ser        | AGC   | 9      | 0.27         |
| Trp        | TGG   | 72     | 2.14         | Ser        | AGA   | 31     | 0.92         |
| Leu        | CTT   | 43     | 1.28         | Ser        | AGG   | 35     | 1.04         |
| Leu        | CTC   | 3      | 0.09         | Val        | GTT   | 177    | 5.25         |
| Leu        | CTA   | 17     | 0.50         | Val        | GTC   | 12     | 0.36         |
| Leu        | CTG   | 23     | 0.68         | Val        | GTA   | 58     | 1.72         |
| Pro        | CCT   | 53     | 1.57         | Ala        | GCT   | 95     | 2.82         |
| Pro        | CCC   | 4      | 0.12         | Ala        | GCC   | 4      | 0.12         |
| Pro        | CCA   | 11     | 0.33         | Ala        | GCA   | 13     | 0.39         |
| Pro        | CCG   | 15     | 0.45         | Ala        | GCG   | 33     | 0.98         |
| His        | CAT   | 41     | 1.22         | Asp        | GAT   | 62     | 1.84         |
| His        | CAC   | 7      | 0.21         | Asp        | GAC   | 2      | 0.06         |
| Gln        | CAA   | 13     | 0.39         | Glu        | GAA   | 17     | 0.50         |
| Gln        | CAG   | 14     | 0.42         | Glu        | GAG   | 67     | 1.99         |
| Arg        | CGT   | 45     | 1.34         | Gly        | GGT   | 165    | 4.90         |
| Arg        | CGC   | 0      | 0            | Gly        | GGC   | 16     | 0.47         |
| Arg        | CGA   | 6      | 0.18         | Gly        | GGA   | 22     | 0.65         |
| Arg        | CGG   | 11     | 0.33         | Gly        | GGG   | 51     | 1.51         |

[15], C. sinensis [16] and P. cervi [17]. The A + T content of 12 protein coding genes and 22 rRNA genes of F. elongates ranged from 59.65% (rrnS) to 66.97% (cox3), and the overall A + T content of the mt genome is 63.88%.

The present F. elongates mt genome can provide useful information for the studies of epidemiology, species identification and genetic diversity of Fischoederius spp. At the same, it will also make contribution to the taxonomy study of Fischoederius spp. With the full mt genome of F. elongates, we can undertake a study within F. elongates from different regions or among Fischoederius spp, by combining the morphological features with genetic analyses (with molecular markers from mitochondria or ribosome, such as cox1, nad4, 18S, ITS-1 and ITS-2). Meanwhile, the mt genome of F. elongates may also provide information for the prevention and diagnosis of Fischoederius spp and perhaps, this mt genome information may assist in the new drug, since mitochondria is the target of some drugs, such as decoquinate.
genes) (Table 2), which is the same as other trematodes, such as *F. hepatica* [15], *C. sinensis* [16], *P. cervi* [17], *S. mekongi* [27]. TAG (seven of 12 protein genes) or TAA (five of 12 protein genes) are the termination codons, this is in agreement with other digeneans, except for *P. cervi* (Only TAG was used as termination codons). Excluding the termination codons, 10,107 nucleotides encode 3,369 amino acids of protein-coding genes in the *F. elongates* mt genome. The most frequently used amino acid is TTT (Phe), with the frequency of 9.65 %, followed by TTT (Phe), TTG (Leu: 8.61 %), GTT (Val: 5.25 %) and TAT (Tyr: 5.02 %) (Table 4). The least used codons are AAC (Asn: 0.06 %), GAC (Asp: 0.06 %) and CGC (Arg: 0).

Transfer RNA and ribosomal RNA genes
The *F. elongates* mt genome encodes 22 tRNAs, and the length of 22 tRNA genes ranged from 60 bp to 71 bp (Table 2). There are two non-coding regions in *F. elongates* mt genome, *rrnS* (751 bp) and *rrnL* (995 bp) (Table 2). The location of *rrnS* is between tRNA-Cys and *cox2* and the *rrnL* is between tRNA-Thr and tRNA-Cys, which is the same as other trematodes, such as *F. hepatica* [15], *C. sinensis* [16] and *P. cervi* [17].

Non-coding regions
Many flatworms have non-coding regions, it’s common to find two non-coding regions in trematodes: one long...
non-coding region (LNR) and one short non-coding region (SNR). In *F. elongates*, there is a short non-coding region (SNR: 62 nucleotides), which is located between *cytb* and *nad4L*. In addition, there is also a long non-coding region (LNR: 468 nucleotides) between tRNA-Phe and *cox3* (Table 2), the LNR has two obvious features, one is microsatellite-like sequences, such as (TA)n (n <5); the other is homopolymer sequences, such as (T)n (n <7). People still don’t understand clearly why the non-coding regions exist, and the function of them, people just knew the non-coding regions may participate in the replication of mitochondria [26].

**Nucleotide variability between *F. elongates* and *P. cervi***

A sliding window analysis of *F. elongates* and *P. cervi* using full mt genome sequences reflected the nucleotide diversity (\(\pi\)) for all the protein-coding genes (Fig. 2). The highest and lowest level of nucleotide variability was within *nad6* and *cox3*, respectively. In our study, *nad6* and *cox2* are the most conserved genes, and *cox3* and *atp6* are the least conserved. With sliding window analysis, we could know the conserved regions of mt genome among species.

**Genetic relationships**

Concatenated amino acid sequence data representing 12 protein-coding genes of 11 digenean species (*C. sinensis*, *D. dendriticum*, *F. hepatica*, *F. hydrolapathica*, *H. taichui*, *M. yokogawai*, *O. viverrini*, *P. cervi*, *S. haematobium*, *S. japonicum*, *S. mekongi*, and *S. spiridale*) and one tapeworm (*T. solium*) were used for genetic relationship analysis (Fig. 3). In the tree, we can find two large clades with strong support (100 %): one clade consists of eight members representing five families (Heterophyidae, Opisthorchiidae, Fasciolidae, Paramphistomidae and Dicrocoeliidae); the other clade is Schistosomatidae. In the present analysis, *F. elongates* has the closest genetic relationship with *P. cervi* (100 %), followed by Fasciolidae, this is consistent with their relationship in the classification of biology. At the same time, we also used NJ method analysis (not shown), and there was no difference between these two methods.

**Competing interests**

The authors declare that they have no competing interests.

**Authors’ contributions**

RF conceived and designed the study. XY and YYZ wrote the manuscript with input from other coauthors. XY, YYZ and LXW performed the experiments; HLF, LT, WQJ, and KQZ analyzed the data. MH assisted in study design and editing. All authors read and approved the final manuscript.

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