Research Article

Characterization of the Complete Mitochondrial Genome of *Fischoederius elongatus* Derived from Cows in Shanghai, China

Zhaoqing Han,1 Kun Li,2 Houqiang Luo,3 Muhammad Shahzad,4 and Khalid Mehmood4

1College of Agriculture and Forestry Science, Linyi University, Linyi 276005, China
2College of Veterinary Medicine, Huazhong Agricultural University, Wuhan, China
3College of Animal Science, Wenzhou Vocational College of Science and Technology, Wenzhou 325006, China
4University College of Veterinary & Animal Sciences, The Islamia University of Bahawalpur, Bahawalpur, Pakistan

Correspondence should be addressed to Zhaoqing Han; nlkxxy@lyu.edu.cn and Kun Li; kl@mail.hzau.edu.cn

Received 23 October 2019; Revised 9 December 2019; Accepted 23 December 2019; Published 13 January 2020

A study was conducted to reveal the characterization of the complete mitochondrial genome of *Fischoederius elongatus* derived from cows in Shanghai, China. Results indicated that the complete mt genome of *F. elongatus* was 14,288bp and contained 12 protein-coding genes (cox1-3, nad1-6, nad4L, atp6, and cytb), 22 transfer RNA genes, and two ribosomal RNA genes (l-rRNA and s-rRNA). The overall A+T content of the mt genome was 63.83%, and the nucleotide composition was A (19.83%), C (9.75%), G (26.43%), and T (44.00%). A total of 3284 amino acids were encoded by current *F. elongatus* mt genome, TTT (Phe) (9.84%) and TTG (Leu) (7.73%) codon were the most frequent amino acids, whereas the ACC (Thr) (0.06%), GCC (Ala) (0.09%), CTC (Leu) (0.09%), and AAC (Asn) (0.09%) codon were the least frequent ones. At the third codon position of *F. elongatus* mt protein genes, T (50.82%) was observed most frequently and C (5.85%) was the least one. The current results can contribute to epidemiology diagnosis, molecular identification, taxonomy, genetic, and drug development researches about this parasite species in cattle.

1. Introduction

*Fischoederius elongatus* is a representative of *Paragonimus* genus, which was frequently discovered in ruminants in tropical and subtropical regions [1]. Weight loss and decease of milk production were the common effects of infections caused by *F. elongatus* [2]. Due to the serious economic losses caused by this trematode, a growing trend of attentions was paid towards the *F. elongatus* [3]. According to National Bureau of Statistics of China, population of 89.15 and 297.13 million heads of cattle and sheep, respectively, was estimated in 2018 (http://data.stats.gov.cn/easyquery.htm?cn=C01). In a study, the prevalence of *F. elongatus* infection in cattle and sheep was found to be 50% and 10%, respectively, in Jiangjin, China [4]. However, little knowledge is known about the genetic information of *F. elongatus* in cows in China.

As a maternal inheritance circular genome, mitochondrial genome is popularly utilized in studies for taxonomy and phylogenetic analysis [5, 6]. A higher mutational rate has been observed in mt DNA than that of nuclear DNA [7], and due to the alterations in contents of mt DNA, it has been reported to be highly related to various diseases [8]. Hundreds of parasitic mt genomes are available at NCBI (https://www.ncbi.nlm.nih.gov/nucleotide/); however, there is relatively a lack of data about the mt genomes of trematodes. Until now, to the best of our knowledge, only one report is available about the mt genome of *F. elongatus* isolated from cattle [3]. The present research herein was aimed to reveal the characterization of the complete mt genome of *F. elongatus* derived from cows in Shanghai, China.
2. Materials and Methods

2.1. Ethical Statement. All procedures adopted in the current research were followed according to the laws, regulations, and guidelines of the Laboratory Animals Research Centre of Hubei province, P. R. China, and the Ethics Committee of Huazhong Agricultural University.

2.2. Parasite Collection. Adult trematodes were collected from the cows in Shanghai in 2019. Morphological examination was conducted after extensive washing in 0.9% sodium chloride solution [9]. All the samples were fixed in 75% alcohol (V/V) and kept at –2°C for further utilization as narrated in a previous research [5].

2.3. Mitochondrial DNA Sequencing. The extraction of mt DNA of F. elongatus was performed by employing a commercial Mitochondria Isolation Kit (Sigma-Aldrich, China). The agarose gel electrophoresis method and nanodrop detection were used for the integrity and purity of DNA. All the DNA samples were quantified via a Qubit Fluorometer (3.0). DNA samples were fragmented randomly via the ultrasonic method. End repair, A-tailing, index adapter adding, amplification, and purification were performed for library construction according to the manufacturer’s instructions (Illumina). These libraries were sent to commercial sequencing via an Illumina HiSeq X sequencing system at Personalbio in Shanghai, China.

2.4. Sequencing Analysis and Genome Annotation. To obtain high accurate sequencing clean data, all the obtained raw reads were filtered with quality score (Q < 10) (90%), uncalled bases (“N” characters) (>10%), and duplicated sequences. The mt genome of F. elongatus was assembled via SPAdes v3.11.1 (http://cab.spbu.ru/software/spades/). The mt genome assembling and annotation of F. elongatus were performed online using the DOGMA tool and MITOS [10, 11]. The circular mt genome of the F. elongatus genomic map was drawn via OGDraw v1.2 [12].

2.5. Nucleotide Variation Analysis. The nucleotide variation of F. elongatus between the Tianmen isolate (KM397348.1) and the current isolate was analyzed by employing DnaSp 5.0.

2.6. Phylogenetic Analysis. The phylogenetic relationships of F. elongatus and other available trematodes were based on mt genome using the neighbor-joining method with Kimura two-parameter analysis and bootstrap analysis of 100 replicates (MEGA 6.0). The available trematodes were F. elongatus (KM397348.1), F. cobboldi (KX169164.1), Gastrothylax crumenifer (KM400624.1), Paramphistomum cervi (KT199887.1, KF475773.1), Calicophoron microbothrioides (KR337555.1), Orthocoelium streptocoelium (KM659777.1), Explanatum explanatum (KT198989.1), Homalagaster paloniae (KT266674.1, KX169165.1), and Ogmocotyle sp. (KR006935.1). The numbers on the branches indicate the percentage of replicates that reproduced the topology for each clad.

3. Results and Discussion

In our study, the complete mt genome of the F. elongatus isolate was 14,288 bp long (Figure 1), which is longer (by 168 bp) than that of the F. elongatus isolated from Tianmen, China (14,120 bp) [3]. The difference may be because of employing different techniques and possibly the genetic prediction error; however, the gene and length are in line with each other. The present sequence of the mt genome has been submitted to the GenBank with the Accession number: MN537973. The circular mt genome of F. elongatus contains 12 protein-coding genes (cox1-3, nad1-6, nad4L, atp6, and cytb), 22 transfer RNA genes, and two ribosomal RNA genes (l-rRNA and s-rRNA) (Figure 1, Table 1); however, it lacks atp8, which is in line with F. elongatus of the Tianmen isolate and mt genomes of other trematodes, such as Gastrothylax crumenifer and Paramphistomum cervi [3, 13, 14]. The protein-coding genes of current F. elongatus isolates were transcribed in the same direction, and those genes were assembled in line of cox3, cytb, nd4L, nd4, atp6, nd2, nd1, nd3, cox1, l-rRNA, s-rRNA, cox2, nad6, and nad5 which was in accordance with previously reported results [3, 13, 14] (Figure 1; Table 1).

The overall A+T content of the mt genome of the current F. elongatus isolate was found to be 63.83%, and the nucleotide composition was A (19.83%), C (9.75%), G (26.43%), and T (44.00%). Moreover, T was the most favored nucleotide, while C was the least common one. These findings are also in accordance with the isolate results of Tianmen [3].

Among the 12 protein genes of the present F. elongatus isolate, ATG (5/12) and GTG (5/12) were the most common start codons and TAA (9/12) was the predominant stop codon (Table 1). In current results herein, the 3′-end of genes of nd1, nd3, nd4, nd5, dn6, atp6, cox3, and cox1 was found immediately adjacent to a downstream tRNA gene (Table 1), which was in parallel arrangement with F. elongatus Tianmen isolates of Gastrothylax crumenifer and Paramphistomum cervi [3, 13, 14].

In our study, a total of 3284 amino acids were encoded from the F. elongatus isolate mt genome excluding the termination codons. All of the 63 possible codons except CGC were found in the F. elongatus isolate. TTT (Phe) (9.84%) and TTG (Leu) (7.73%) codon were the most frequent amino acids found, whereas the ACC (Thr) (0.06%), GCC (Ala) (0.09%), CTC (Leu) (0.09%), and AAC (Asn) (0.09%) codon were the least frequent ones uncovered in F. elongatus (Table 2). Preferable codons were commonly uncovered with important functional gene regions, as those bias codons with silent sites were found to be related to maximize the translation efficiency [15, 16]. At the third codon position of the current F. elongatus mt protein genes, T (50.82%) was the most frequently observed and C (5.85%) was used least.
**Table 1:** The *mt* genome of *F. elongatus* isolated from cows using MITOS.

| Gene    | Position | Length (bp) | Start/stop codon of PCGs | Anticodons |
|---------|----------|-------------|--------------------------|------------|
| COX3    | 1–645    | 645         | ATG/TAG                  |            |
| tRNA-His| 646–713  | 68          |                          | GTC        |
| CYTB    | 714–1829 | 1116        | ATT/TAA                  |            |
| AT-loop | 1830–1892| 63          |                          |            |
| ND4L    | 1893–2156| 264         | ATG/TAG                  |            |
| ND4     | 2117–3397| 1281        | GTG/TAA                  |            |
| tRNA-Gln| 3409–3471| 63          |                          | GTT        |
| tRNA-Phe| 3485–3549| 65          |                          | TTC        |
| tRNA-Met| 3549–3612| 64          |                          | AGA        |
| ATP6    | 3613–4128| 516         | ATG/TAG                  |            |
| ND2     | 4133–5008| 876         | GTG/TAG                  |            |
| tRNA-Val| 5041–5101| 61          |                          | AAG        |
| tRNA-Ala| 5109–5179| 71          |                          | AAC        |
| tRNA-Asp| 5431–5500| 70          |                          |            |
| ND1     | 5530–6399| 870         | TTG/TAG                  |            |
| tRNA-Asn| 6419–6484| 66          |                          | TGG        |
| tRNA-Pro| 6489–6552| 64          |                          | CAG        |
| tRNA-Ile| 6554–6616| 63          |                          | CCG        |
| tRNA-Lys| 6623–6687| 65          |                          | CTG        |
| ND3     | 6701–7048| 348         | GTG/TAG                  |            |
| tRNA-Ser| 7060–7119| 60          |                          | GAG        |
| tRNA-Trp| 7132–7196| 65          |                          | AGT        |
| COX1    | 7200–8741| 1542        | GTG/TAA                  |            |
| l-rRNA  | 8575–9865| 1291        |                          |            |
| tRNA-Thr| 8751–8814| 64          |                          | GAA        |
| s-rRNA  | 9815–10,603| 789      |                          |            |
| COX2    | 10,624–11,205| 582    | ATG/TAG                  |            |
| ND5     | 11,244–11,651| 408    | ATG/TAG                  |            |
| tRNA-Tyr| 11,673–11,737| 65     |                          |            |
| tRNA-Leu| 11,757–11,820| 64     |                          | TAA        |
| tRNA-Ser| 11,822–11,890| 69     |                          | GTG        |
| tRNA-Leu| 11,897–11,961| 65     |                          | ATA        |
| tRNA-Arg| 11,965–12,030| 66     |                          | TGA        |
| ND5     | 12,031–13,611| 1581   | GTG/TAG                  |            |
| tRNA-Gly| 13,615–13,679| 65     |                          | ACG        |
| tRNA-Glu| 13,692–13,755| 64     |                          | TTG        |
| AT-loop | 13,756–14,228| 473     |                          |            |

**Figure 1:** Arrangement of the *mt* genome of *F. elongatus*.
Table 2: Codons usage of *F. elongatus* mt DNA-encoded proteins.

| Amino acid | Codon | Number | Frequency (%) | Amino acid | Codon | Number | Frequency (%) |
|------------|-------|--------|---------------|------------|-------|--------|---------------|
| Phe        | TTT   | 323    | 9.84          | Pro        | CCT   | 34     | 0.80          |
| Phe        | TTC   | 27     | 0.82          | Pro        | CCC   | 4      | 0.12          |
| Leu        | TTA   | 163    | 4.96          | Pro        | CCA   | 11     | 0.26          |
| Leu        | TTG   | 254    | 7.73          | Pro        | CCG   | 8      | 0.19          |
| Leu        | CTT   | 41     | 1.25          | Thr        | ACT   | 52     | 1.58          |
| Leu        | CTC   | 3      | 0.09          | Thr        | ACC   | 2      | 0.06          |
| Leu        | CTA   | 17     | 0.52          | Thr        | ACA   | 18     | 0.55          |
| Leu        | CTG   | 25     | 0.76          | Thr        | ACG   | 16     | 0.49          |
| Ile        | ATT   | 128    | 3.90          | Ala        | GCT   | 98     | 2.98          |
| Ile        | ATA   | 73     | 2.22          | Ala        | GCA   | 14     | 0.43          |
| Val        | GTT   | 176    | 5.36          | Ala        | GCG   | 31     | 5.18          |
| Val        | GTC   | 13     | 0.40          | Tyr        | TAT   | 170    | 5.18          |
| Val        | GTA   | 56     | 1.71          | Tyr        | TAC   | 8      | 0.24          |
| Val        | GTG   | 167    | 5.09          | His        | CAT   | 42     | 1.28          |
| Ser        | TCT   | 116    | 3.53          | His        | CAC   | 6      | 0.18          |
| Ser        | TCC   | 8      | 0.24          | Gin        | CAA   | 13     | 0.40          |
| Ser        | TCA   | 23     | 0.70          | Gin        | CAG   | 25     | 0.76          |
| Ser        | TCG   | 32     | 0.97          | Asn        | AAT   | 55     | 1.67          |
| Ser        | AGT   | 80     | 2.44          | Asn        | AAC   | 3      | 0.09          |
| Ser        | AGC   | 13     | 0.40          | Arg        | GTG   | 45     | 1.37          |
| Trp        | TGG   | 74     | 2.25          | Arg        | CGC   | 0      | 0             |
| Lys        | AAA   | 20     | 0.61          | Arg        | CGA   | 6      | 0.18          |
| Lys        | AAG   | 52     | 1.58          | Arg        | CGG   | 11     | 0.33          |
| Asp        | GAT   | 61     | 1.86          | Arg        | AGA   | 30     | 0.91          |
| Asp        | GAC   | 4      | 0.12          | Arg        | AGG   | 36     | 1.10          |
| Glu        | GAA   | 20     | 0.61          | Gly        | GGT   | 160    | 4.87          |
| Glu        | GAG   | 64     | 1.85          | Gly        | GGC   | 20     | 0.61          |
| Cys        | TGT   | 116    | 3.53          | Gly        | GGA   | 24     | 0.73          |
| Cys        | TGC   | 7      | 0.21          | Gly        | GGG   | 50     | 1.52          |
| Met        | ATG   | 3.11   | 2.48          |            |       |        |               |

Figure 2: A sliding window analysis of the complete mt genome sequences of *F. elongatus* of the current isolate and Tianmen isolate (KM397348.1).

Table 3: Multidomain analysis of the complete mt genome sequences of *F. elongatus* of the current isolate and Tianmen isolate (KM397348.1).

| Region | n | Sites | Net sites | δ | Eta | Hap | Hd | VarHd | Pi |
|--------|---|-------|-----------|---|-----|-----|----|-------|----|
| 1–14,228 | 2 | 14,228 | 14,120 | 181 | 181 | 2 | 1.000 | 0.25000 | 0.01282 |
frequently (Figure S1). In codons with $\geq 2$ unique bias, the TT codons were noticed at the highest point while CC ones were least found (Figure S2). These interesting results may reveal that *F. elongatus* mt is biased toward utilizing T-rich amino acid codons which are suggestive of the nucleotide bias [17]. However, until now, it is still unclear whether this bias of codon usage contributes to parasite mt systems or not [18].

A total of 21 tRNA gene sequences (61–71 bp) and 2 noncoding regions (NCR) (L-RNA and s-RNA) were found in the *F. elongatus* mt genome (Figure 1, Table 1) depicting one undetected tRNA gene in the current isolate, as other trematodes contain 22 tRNA gene sequences [3, 13, 14]. If the current research was supported by the Wenzhou City Public Welfare Science and Technology Plan Projects (N20140041).

**Supplementary Materials**

Figure S1: statistics of the third position of codons bias usage of *F. elongatus* mt DNA-encoded proteins. Figure S2: codons bias usage of *F. elongatus* mt DNA-encoded proteins.

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