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Characterization of the complete mitochondrial genome of *Khawia sinensis* belongs among platyhelminths, cestodes

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HIGHLIGHTS

- This is the first time to report the full mitochondrial genome of *Khawia sinensis*.
- By genetic analysis, we found *Khawia sinensis* closely related to species from Diphyllobothriidae.
- The complete mitochondrial genome of *Khawia sinensis* will provide useful information for the study of species identification, molecular epidemiology and population genetics.

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ABSTRACT

Khawia sinensis is an important species in freshwater fish causing considerable economic losses to the breeding industry. This is the first mt genome of a caryophyllidean cestode characterised. The entire mt genome of *K. sinensis* is 13,759 bp in length. This mt genome contains 12 protein-coding genes, 22 transfer RNA genes, two ribosomal RNA genes and two non-coding regions. The arrangement of the *K. sinensis* mt genome is the same as other tapeworms, however, the incomplete stop codon (A) is more frequent that other species. Phylogenetic analyses based on concatenated amino-acid sequences of the 12 protein-coding genes of 17 tapeworms including *K. sinensis* were conducted to assess the relationship of *K. sinensis* with other species, the result indicated *K. sinensis* was closely related with cestode species. This complete mt genome of *K. sinensis* will enrich the mitochondrial genome databases of tapeworms and provide important molecular markers for ecology, diagnostics, population variation and evolution of *K. sinensis* and other species.

1. Introduction

Caryophyllidea is a large class of cestodes inhabiting in the gastrointestinal tract of cypriniform and siluriform fish (Brunanská, 2009; Králová-Hromadová et al., 2013; Mackiewicz, 2003), it's widely distributed in Africa, Asia, Australia, Europe and North America (Králová-Hromadová et al., 2013; Oros et al., 2009). As an important family, Lytocestidae plays a significant role in the study of phylogenetic analysis among the cestode (Bruňanská et al., 2012). Worms in Lytocestidae have a monozoic body which lacks proglottisation and segmentation, and only have a single set of reproductive organs (Wu, 2015). *Khawia sinensis* is one of the key pathogens causing khawiasis, and can cause considerable economic losses to the breeding of fishes under heavy burden. In general, tubifexes serve as intermediate host, and the definitive host freshwater fish can be infected by intaking tubifex infected with *K. sinensis*.

The accurate identification of *K. sinensis* is essential for preventing and controlling of this species. Although morphological methods have been widely used in species identification, these methods are time and labor-consuming and not accurate, thus DNA-based molecular methods are needed (Bazsalovicsová et al., 2012). As a significant and useful molecular marker, the mitochondrial genome has been widely used in the study of species identification, epidemiology, ecology, population diversity, and

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phylogenetic analysis (Gasser et al., 2012; Shekhovtsov et al., 2010). Knowledge of the mitochondrial genome of *K. sinensis* can provide useful marker for the study of species identification, epidemiology and population diversity of species in Lytocestidae.

This study aimed to obtain and analyze the mitochondrial genome of *Khawia sinensis*, then, to compare it with other selected cestodes for phylogenetic analysis. The *K. sinensis* mt genome will provide important basis for the study on species identification, epidemiology, population diversity and phylogenetic analysis.

2. Materials and methods

2.1. Parasites and DNA isolation

Adults of *K. sinensis* were obtained from the intestines of an infected *Carassius auratus* in Hubei province, China, according to the Animal Ethics Procedures and Guidelines of the Huazhong Agricultural University. The worms were washed in physiological saline (0.9% sodium chloride solution) and then determined to species level according to the existing morphological descriptions (Taylor et al., 2007; Wu, 2015). One representative specimen was stained and mounted, and the rest were fixed in 70% (v/v) ethanol and stored at − 20 °C until use (Li, 2011). Total genomic DNA were isolated from single adult worm) using E.Z.N.A.® Tissue DNA Kit (OMEGA, USA).

2.2. Amplification and sequencing of K. sinensis mt genome

Seven pairs of primers based on the conserved regions of mtDNA from *Diplogonoporus balaenopterae* (Yamasaki et al., 2012), *Diphyllobothrium latum* (Park et al., 2007) and *Spirometra decipiens* (Eom et al., 2015) were designed to amplify short fragments from *cox*3, *nad*4, *nad*2, *cox*1, 12s, *cox*2, and *nad*5 (Table 1). Then, specific primers listed in Table 1 were designed based on the obtained sequences to amplify the remaining sequence in seven PCR reactions. The PCR reactions (50 μ l) were performed in 1X LATaq BufferII (Mg2+Plus), 400 mM each of dNTP, 50 pmol of each primer, and 2.5 U TaKaRa LA Taq polymerase (Takara, Japan) in a thermocycler (Biometra) under the following conditions: 35 cycles at 94 °C for 30 s, and annealed at 50 °C for 30 s, followed by extension at 72 °C for 1 min per 1 kb. PCR products were cloned into pGEM-T vector (Promega, USA) and then sequenced using an ABI 3730 automatic sequencer.

Table 1
Primers for the present study.

Primers	Sequences(5′-3′)	Target gene
XCCOX3F2	AGYACDGTDGGDTTRCATTT	COX3
XCCOX3R1	CANAYATAATCMACARAATGNCA	COX3
TCND4F	GANTCYCCNTAYTCWGARCG	ND4
TCND4R	CAMCGCTTHCCRTCYAAYTC	ND4
TCND2F	GTKTTYGTWGCDNTRGTDCAYTG	ND2
TCND2R	GAWGHHARNGAWAYRTGRCAYCA	ND2
TCCOX1F	GAYCCDTTRGGWGGWGGDGATCC	COX1
TCCOX1R	ACAMACWCGACGWGGYAAHCC	COX1
Insect12SF	AAWAAYGAGAGYGACGGGCG	12S
Insect12SR	TARACTAGGATTAGATACCC	12S
TCCOX2F	AAGRTDRTDGGNCRBCARTGRTAYTG	COX2
TCCOX2R	CGWCCHGGDATWGCATCYATCTT	COX2
TCND5F	GARGCNATGCGDGCNCCHACNCC	ND5
TCND5R	CANGTDGAYARDGCHAYWATCTTCTT	ND5
2CF2	TCATGTTCTTATCGGTGTTG	COX3- ND4
2CR2	AACAACCACTATTAACGAAAG	COX3- ND4
2CF3	TCGGTTTTGTACTAAATTCCTC	ND4- ND2
2CR8	TGAAAATCCGGCTAGTCCC	ND4- ND2
2CF9	TGTGGAGATATTAATTGTGG	ND2-COX1
2CR4	TCTTATTAGCCCAAACCCCGG	ND2-COX1
2CF5	ACAAGTATTTACTTCAAGCTC	COX1-12S
2CR5	CGGATCGTCCTTTACGTAGC	COX1-12S
2CF6	AGGTGAAGAAGGTAGGAAGC	12S-COX2
2CR6	CGACACCCTCAAATAGCTG	12S-COX2
2CF7	CGTTTGTTGTAGATAAGCC	COX2- ND5
2CR7	ACAAACATTACTTTGCTCTCCC	COX2- ND5
2CF1	TCATTACGTTACGGCATTAGTG	ND5-COX3
2CR1	TCAAGCCAAAAGAGTACCAC	ND5-COX3

2.3. Sequence analyses

Sequences were manually assembled and aligned against the complete mt genome sequences of other published tapeworms using the program DNAstar to infer gene boundaries (Le et al., 2000). The open reading frames (ORFs) were predicated using ORF Finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html) employing the echinoderm mt genetic code. Protein-coding gene sequences were translated into amino acid sequences using the echinoderm and flatworm mitochondrial genetic code (translation table 9) by ExPASy (http://web.expasy.org/translate/), and amino acid sequences were concatenated and analyzed by MEGA v.6.0 for phylogenetic analysis (Tamura et al., 2013). Translation initiation and termination codons were identified by comparisons with those reported previously (Le et al., 2000). Twenty tRNA genes were identified using the online program DOGMA (http://dogma.ccbb.utexas.edu/) (Wyman et al., 2004), and the remaining two tRNA genes and two rRNA were predicted by comparison with *D. balaenopterae* (Yamasaki et al., 2012), *D. latum* (Park et al., 2007) and *S. decipiens* (Eom et al., 2015).

2.4. Phylogenetic analysis

All the 12 protein-coding genes of the mt genome of *K. sinensis* and other selected parasites were translated and concatenated for phylogenetic analysis. The selected species included *Anoplocephala perfoliata* (NC_028425) (Guo, 2015), *Dipylidium caninum* (NC_021145) (Nakao et al., 2013), *Diphyllobothrium latum* (DQ_985706) (Park et al., 2007), *Diphyllobothrium nihonkaiense* (NC_009463) (Nakao et al., 2007a,b), *Diplogonoporus balaenopterae* (NC_017613) (Yamasaki et al., 2012), *Diplogonoporus grandis* (NC_017615) (Yamasaki et al., 2012), *Echinococcus canadensis* (NC_011121) (Nakao et al., 2007a,b), *Echinococcus multilocularis* (NC_000928) (Nakao et al., 2002), *Hymenolepis diminuta* (NC_002767) (Von et al., 2001), *Hymenolepis nana* (KT_951722) (Cheng et al., 2016), *Pseudanoplocephala crawfordi* (KR_611041) (Zhao et al., 2015), *Schyzocotyle acheilognathi* (NC_030316) (Brabec et al., 2016), *Spirometra decipiens* (KJ_599679) (Eom et al., 2015), *Spirometra erinaceieuropaei* (KJ_599680) (Eom et al., 2015), *Taenia asiatica* (NC_004826) (Jeon and Eom, 2006), *Taenia solium* (NC_004022) (Jeon and Eom, 2006). And *Fasciola hepatica* (NC_002546) (Le et al., 2000) was included as an outgroup. Concatenated amino acid sequence data of 12 protein-coding genes were aligned by MEGA v.6.0. The phylogenetic analysis was performed by Maximum Likelihood (ML) Method based on the Jones-Taylor-Thornton (JTT) model with default settings (Tamura et al., 2013).

3. Results and discussion

3.1. Characteristics of the K. sinensis mt genome

The *K. sinensis* complete mitochondrial genomes is 13,759 bp in length (KR676560), and contains 12 protein-coding genes, 22 tRNA genes, two rRNA genes and two repeat regions (Fig. 1), which is the same as *Anoplocephala perfoliata* (Guo, 2015), *Diphyllobothrium latum* (Park et al., 2007) and *Echinococcus canadensis* (Nakao et al.,