Complete Mitochondrial Genome Sequence Analysis and Phylogenetic Location Determination of Hydropsyche Fryeri (Insecta: Trichoptera)

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Abstract

*Hydropsyche fryeri* belongs to the Trichopteridae family and builds nests in clean and unpolluted streams using stones. It also can be used as an indicator of water quality. Here, we describe the complete mitochondrial genome sequence of *Hydropsyche fryeri*. The mitochondrial genome is 15,676 bp long and contains 13 protein-coding genes, 22 tRNAs, 2 rRNAs and an AT-rich control region. Phylogenetic tree analysis shows that *Hydropsyche fryeri* is more closely related to the family Hydroptera than other Trichoptera.

Introduction

*Hydropsyche fryeri* is a member of the Trichopteridae family. It has a fusiform head with white stripes and a brown or light-green body of about 1.9 ± 0.4 cm (Fig. 1). *Hydropsyche fryeri* live in clear rivers and can be used to detect water quality [1] and heavy metal [2] pollution. *Hydropsyche fryeri* has extremely strict requirements for water temperature and cannot survive below 20°C. However, *Hydropsyche Fryeri* can tolerate a turbulent water environment.

Most of the previous classification methods for insects of Trichoptera are morphological classification [3] and the phylogenetic data at the molecular level of the caddisy still requires further research.

Mitochondrial DNA (mtDNA) is a circular structure DNA that exists in the mitochondria of eukaryotic cells [4]. Mitochondrial DNA is inherited by the maternal line and its primary structure shows significant inter- and intraspecies variation and thus can be used for molecular classification [5]. In general, metazoan mitochondrial genomes consist of a non-coding sequence called the control region (CR) and 37 genes, including 13 protein-coding genes (PCGs), 22 tRNAs and 2 rRNAs [6]. Mitochondrial cytochrome oxidase 1 (COX 1) and 2 (COX 2) [7] and the tRNA gene sequences [8] are used for the classification of species [9] and infraspecific category identification [10]. In this study, we determined the mitochondrial DNA sequence of *Hydropsyche fryeri* and analysed the phylogenetic relationships between this species and other related species.

Results

The complete mitochondrial genome is 15,676 bp long and includes 13 protein-coding genes, 2 rRNAs, 22 tRNAs and an AT-rich control region (D-loop). The genome consists of 42.29% A, 39.92% T, 11.16% C and 6.63% G bases. The control region, located between tRNA-Ile and 12S-rRNA, is 631 bp long and has an A + T content of 82.21% (Table 2). Of the PCGs and tRNAs, NAD5 (1720 bp) was the longest and tRNA-Ser (60 bp) was the shortest (Table 1). Thirteen protein-coding genes had ATN as the starting codon. NAD2 started with ATA, COX1, ATP6, COX3, NAD4, NAD4L and COB started with ATG, and COX2, ATP8, NAD3, NAD5 and NAD1 started with ATT. The stop codon of COX1 and NAD5 is the incomplete stop codon T, TAA is the stop codon for the remaining coding proteins (Table 1), and Mitochondrial genes are circular structures (Fig. 2). Based on the results obtained from the relative synonymous codon usage analysis
(RSCU), the values were higher for GCT (Ala), CGA (Arg), GGA (Gly), TTA (Teu), CCT (pro), TCA (Ser), AGA (Ser) and TCT (Ser) and lower for CTC (Leu), CTG (Leu), AGC (Ser), ACG (Thr) and TGG (Trp) (Table 3, Fig. 3).
| Gene     | Position (bp) | Size (bp) | A + T Percent | Intergenic nucleotide (bp) | Inferred Initiation Codon | Inferred Termination Codon |
|----------|---------------|-----------|---------------|----------------------------|---------------------------|-----------------------------|
| tRNA-Ile | 1–69          | 69        | 84.06%        | 10                         |                           |                             |
| tRNA-Gln | 80–147        | 68        | 85.29%        | 7                          |                           |                             |
| tRNA-Met | 155–222       | 68        | 82.35%        | 17                         |                           |                             |
| NAD2     | 240–1220      | 981       | 86.85%        | 3                          | ATA                       | TAA                         |
| tRNA-Trp | 1224–1291     | 68        | 89.71%        | -8                         |                           |                             |
| tRNA-Cys | 1284–1348     | 65        | 87.69%        | 0                          |                           |                             |
| tRNA-Tyr | 1349–1417     | 69        | 84.06%        | 7                          |                           |                             |
| COX1     | 1425–2961     | 1537      | 73.52%        | 0                          | ATG                       | T                           |
| tRNA-Leu | 2962–3030     | 69        | 81.16%        | 0                          |                           |                             |
| COX2     | 3031–3711     | 681       | 76.51%        | 6                          | ATT                       | TAA                         |
| tRNA-Lys | 3718–3787     | 70        | 72.86%        | 4                          |                           |                             |
| tRNA-Asp | 3792–3856     | 65        | 87.69%        | 0                          |                           |                             |
| ATP8     | 3857–4024     | 168       | 89.88%        | -7                         | ATT                       | TAA                         |
| ATP6     | 4018–4692     | 675       | 80.3%         | 13                         | ATG                       | TAA                         |
| COX3     | 4706–5497     | 792       | 76.01%        | -1                         | ATG                       | TAA                         |
| tRNA-Gly | 5497–5560     | 64        | 92.19%        | 0                          |                           |                             |
| NAD3     | 5561–5914     | 354       | 82.49%        | 13                         | ATT                       | TAA                         |
| Gene       | Position (bp) | Size (bp) | A + T Percent | Intergenic nucleotide (bp) | Inferred Initiation Codon | Inferred Termination Codon |
|------------|---------------|-----------|---------------|----------------------------|---------------------------|----------------------------|
| tRNA-Ala   | 5928–5992     | 65        | 89.23%        | -1                         |                           |                            |
| tRNA-Arg   | 5992–6055     | 64        | 82.81%        | -1                         |                           |                            |
| tRNA-Asn   | 6055–6123     | 69        | 78.26%        | 0                          |                           |                            |
| tRNA-Ser   | 6124–6183     | 60        | 85.00%        | 26                         |                           |                            |
| tRNA-Glu   | 6210–6275     | 66        | 86.36%        | 1                          |                           |                            |
| tRNA-Phe   | 6277–6342     | 66        | 84.85%        | 0                          |                           |                            |
| NAD5       | 6343–8062     | 1720      | 81.22%        | 1                          | ATT                       | T                          |
| tRNA-His   | 8064–8126     | 63        | 88.89%        | -1                         |                           |                            |
| NAD4       | 8126–9451     | 1326      | 80.77%        | -7                         | ATG                       | TAA                        |
| NAD4L      | 9445–9735     | 291       | 87.97%        | 2                          | ATG                       | TAA                        |
| tRNA-Thr   | 9738–9803     | 66        | 87.88%        | 30                         |                           |                            |
| NAD6       | 9834–10343    | 510       | 88.24%        | 3                          | ATT                       | TAA                        |
| COB        | 10347–11477   | 1131      | 77.01%        | -2                         | ATG                       | TAA                        |
| tRNA-Ser   | 11476–11542   | 67        | 82.09%        | 145                        |                           |                            |
| tRNA-Pro   | 11688–11752   | 65        | 83.08%        | 4                          |                           |                            |
| NAD1       | 11757–12692   | 936       | 79.38%        | 1                          | ATT                       | TAA                        |
| tRNA-Leu   | 12694–12760   | 67        | 86.57%        | 0                          |                           |                            |
| 16S-rRNA   | 12761–14165   | 1405      | 85.55%        | 0                          |                           |                            |
| Gene       | Position (bp) | Size (bp) | A + T Percent | Intergenic nucleotide (bp) | Inferred Initiation Codon | Inferred Termination Codon |
|------------|---------------|-----------|---------------|-----------------------------|---------------------------|-----------------------------|
| tRNA-Val   | 14166–14231   | 66        | 86.36%        | 0                           |                           |                             |
| 12S-rRNA   | 14232–15045   | 814       | 88.21%        | 0                           |                           |                             |
| D-loop     | 15046–15676   | 631       | 97.78%        | 0                           |                           |                             |

Table 2

Total gene and base content of *Hydropsyche fryeri*.

| species       | Total gene size (bp) | AT content(%) | A Size (bp) | T Size (bp) | C Size (bp) | G Size (bp) |
|---------------|----------------------|---------------|-------------|-------------|-------------|-------------|
| Hydropsyche fryeri | 15676               | 82.21%        | 6629        | 6258        | 1750        | 1039        |

Genome annotation showed that tRNA-Trp, ATP8, COX3, tRNA-Ala, tRNA-Arg, tRNA-His, NAD4 and COB overlapped with their adjacent genes, and tRNA-Trp and tRNA-Cys had the highest degree of overlap. There were intervals between tRNA-Ile, tRNA-Gln, tRNA-Met, NAD2, tRNA-Tyr, COX2, tRNA-Lys, ATP6, NAD3, tRNA-Ser, tRNA-Glu, NAD5, NAD4L, tRNA-Thr, NAD6, tRNA-Ser, tRNA-Pro, and NAD1, and the interval between tRNA-Ser and the adjacent tRNA-Pro was the largest (Table 1).

Of the 22 tRNAs, only tRNA-Ser (AGA) at position 6124–6183 had a structure without a TWC arm, and an A + T content of 85.0%, while the other 21 tRNAs had typical clover structures with an A + T content of 72.9–92.2% (Table 1). Eight of the
Table 3
Protein coding gene codons and relative synonymous codon usage (RSCU) of *Hydropsyche Fryeri*.

| Amino acid | Codon | Number | RSCU | Amino acid | Codon | Number | RSCU |
|------------|-------|--------|------|------------|-------|--------|------|
| Ala        | GCT   | 54     | 2.51 | Lys        | AAA   | 113    | 1.77 |
| Ala        | GCA   | 21     | 0.98 | Lys        | AAG   | 15     | 0.23 |
| Ala        | GCC   | 8      | 0.37 | Met        | ATA   | 270    | 1.84 |
| Ala        | GCG   | 3      | 0.14 | Met        | ATG   | 24     | 0.16 |
| Arg        | CGA   | 31     | 2.75 | Phe        | TTT   | 369    | 1.79 |
| Arg        | CGT   | 14     | 1.24 | Phe        | TTC   | 43     | 0.21 |
| Arg        | CGC   | 0      | 0.00 | Pro        | CCT   | 70     | 2.46 |
| Arg        | CGG   | 0      | 0.00 | Pro        | CCA   | 29     | 1.02 |
| Asn        | AAT   | 248    | 1.84 | Pro        | CCC   | 15     | 0.53 |
| Asn        | AAC   | 21     | 0.16 | Pro        | CCG   | 0      | 0.00 |
| Asp        | GAT   | 50     | 1.75 | Ser        | TCA   | 108    | 2.55 |
| Asp        | GAC   | 7      | 0.25 | Ser        | AGA   | 91     | 2.15 |
| Cys        | TGT   | 20     | 1.60 | Ser        | TCT   | 88     | 2.08 |
| Cys        | TGC   | 5      | 0.40 | Ser        | AGT   | 24     | 0.57 |
| Gln        | CAA   | 49     | 1.85 | Ser        | TCC   | 22     | 0.52 |
| Gln        | CAG   | 4      | 0.15 | Ser        | AGG   | 5      | 0.12 |
| Glu        | GAA   | 66     | 1.81 | Ser        | AGC   | 1      | 0.02 |
| Glu        | GAG   | 7      | 0.19 | Ser        | TCG   | 0      | 0.00 |
| Gly        | GGA   | 100    | 2.26 | Stp        | TAA   | 11     | 2.00 |
| Gly        | GGT   | 49     | 1.11 | Stp        | TAG   | 0      | 0.00 |
| Gly        | GGG   | 22     | 0.50 | Thr        | ACA   | 61     | 1.89 |
| Gly        | GGC   | 6      | 0.16 | Thr        | ACT   | 58     | 1.80 |
| His        | CAT   | 46     | 1.56 | Thr        | ACC   | 8      | 0.25 |
| His        | CAC   | 13     | 0.44 | Thr        | ACG   | 2      | 0.06 |
| Ile        | ATT   | 418    | 1.81 | Trp        | TGA   | 86     | 1.95 |
| Ile        | ATC   | 44     | 0.19 | Trp        | TGG   | 2      | 0.04 |
Amino acid  | Codon | Number | RSCU  | Amino acid  | Codon | Number | RSCU  
--- | --- | --- | --- | --- | --- | --- | ---
Leu  | TTA  | 468  | 4.81 | Tyr  | TAT  | 147  | 1.83 |
Leu  | CTT  | 42   | 0.43 | Tyr  | TAC  | 14   | 0.17 |
Leu  | CTA  | 41   | 0.42 | Val  | GTT  | 71   | 2.12 |
Leu  | TTG  | 27   | 0.28 | Val  | GTA  | 53   | 1.58 |
Leu  | CTC  | 5    | 0.05 | Val  | GTC  | 5    | 0.15 |
Leu  | CTG  | 1    | 0.01 | Val  | GTG  | 5    | 0.15 |

22 tRNAs had base mismatches: the D-arm of tRNA-Gln (TTG) had a T-G mismatch of two bases; the D-arm of tRNA-Tyr (GTA) had a G-A mismatch; a pair of T-T mismatches occurred on the forearm of tRNA-Leu (TAA); a pair of T-G base mismatches occurred on the D-arm of tRNA-Gly (TCC); a pair of T-T mismatches occurred on the anticodon arm of tRNA-Lys (AGA); a pair of T-T mismatches occurred on the D-arm of tRNA-His and tRNA-Val (TAC) (Fig. 4).

We compared other families of Trichoptera with Lepidoptera, which are closely related to Trichoptera. We selected eight families (Pryganeidae, Limnephilidae, Apataniidae, Uenoidae, Pryganopsychidae, Sericostomatidae, Leptoceridae and Hydropsychidae) from Trichoptera and 22 species of Hepialidae from Lepidoptera for the construction of an evolutionary tree. *Hydropsyche fryeri* was most closely related to *Hydropsyche orris, Hydropsyche simulans* and *Hydropsyche pellucidula* of the genus Arctopsyche of Hepialidae and was relatively closely related to *Potamyia flava* and *Hydromanicus wulaianus* of Hydropsychidae. However, *Hydropsyche fryeri* was distantly separated from *Sericostoma personatum* of Sericostomatidae and *Triaenodes tardus* of Leptoceridae.

**Discussion**

The basic composition of the *Hydropsyche fryeri* mitochondrial genome is consistent with the common composition of metazoans. The entire genome is 15,676 bp long and contains both overlapping and spaced gene segments. There is one structurally abnormal tRNA of 22 tRNAs while the remaining 21 have normal clover structures.

The evolutionary tree constructed by the maximum likelihood method showed that the genomic sequence and the protein-coding sequences were consistent (Fig. 5). Therefore, *Hydropsyche fryeri* was identified as being a member of Hydropsychidae, which forms a sister population with Pryganeidae and Limnephilidae.

**Materials And Methods**

**Samples**
This study was conducted without harming protected or endangered species, and all research activities were authorized. Samples were collected from of Shiwandashan River system (21°49' 33" N, 107°59' 119" E) in Fangchenggang City, Guangxi Zhuang Autonomous Region (China). The collected samples were transported through a cold chain to the Key Laboratory of Biodiversity, College of Marine Sciences, Beibu Gulf University (China). The samples were dissected in vivo under normal saline to remove the intestinal tract and head. The specimen has been deposited in Ocean college marine specimen showroom of Beibu Gulf University (Voucher No. BBGC 00014).

**Mitochondrial DNA Extraction**

Total mitochondrial DNA was extracted according to the method of Roehrdanz (1997) with partial modifications. Cells were disrupted, proteinase K and RNase were added for enzymatic digestion in a water bath for 5 h at 56 °C. DNA was extracted in a phenol: chloroform: isoamyl alcohol (25:24:1) solution and then centrifuged with a cold isopropanol precipitate and 70% ethanol wash with a dissolved TE buffer.

**Gel Electrophoresis**

The mitochondrial genome was obtained by gel electrophoresis and sequenced by high-throughput sequencing \[11\]. A 1.0% agarose gel was prepared to separate the total mitochondrial genes, and the electrophoresis conditions were a voltage of 120 V and current of 40 Ma for 20 min. The gel was observed on a Tanon3500 gel imaging system (Shanghai Tianneng Technology Co., Ltd, China) and compared with a 10,000 bp DNA marker to preliminarily identify whether the DNA size was in the insect mtDNA size range.

**DNA Recovery And Purification**

DNA was cut from agarose gels, weighed in 2 ml centrifuge tubes, and the DNA was recovered according to the Tiangen universal DNA purification recovery kit protocol (Tiangen, Beijing, China).
Table 4
22 mitochondrial genome sequences downloaded from the NCBI.

| ID        | ORGANISM                | ORDER      | FAMILY         | GENUS      |
|-----------|-------------------------|------------|----------------|------------|
| KF717094  | *Eubasilissa regina*    | Trichoptera| Phryganeidae   | *Eubasilissa* |
| NC_023374 | *Eubasilissa regina*    | Trichoptera| Phryganeidae   | *Eubasilissa* |
| NC_039714 | *Phryganea cinerea*     | Trichoptera| Phryganeidae   | *Phryganea* |
| NC_044710 | *Limnephilus hyalinus*  | Trichoptera| Limnephilidae  | *Limnephilus* |
| NC_026219 | *Limnephilus decipiens* | Trichoptera| Limnephilidae  | *Limnephilus* |
| NC_036004 | *Anabolia bimaculata*   | Trichoptera| Limnephilidae  | *Anabolia*  |
| NC_043770 | *Hydatophylax nigrovittatus* | Trichoptera| Limnephilidae  | *Hydatophylax* |
| KF756944  | *Apatania sp. YW-2014*  | Trichoptera| Apataniidae    | *Apatania*  |
| KP455291  | *Threma gallicum*       | Trichoptera| Uenoidae       | *Threma*    |
| NC_043771 | *Phryganopsyche latipennis* | Trichoptera| Phryganopsychidae | *Phryganopsyche* |
| KP455290  | *Sericostoma personatum*| Trichoptera| Sericostomatidae| *Sericostoma* |
| NC_039659 | *Triaenodes tardus*     | Trichoptera| Leptoceridae   | *Triaenodes* |
| NC_036951 | *Hydropsyche orris*     | Trichoptera| Hydropsychidae | *Hydropsyche* |
| NC_036950 | *Hydropsyche simulans*  | Trichoptera| Hydropsychidae | *Hydropsyche* |
| NC_029246 | *Hydropsyche pellucidula* | Trichoptera| Hydropsychidae | *Hydropsyche* |
| NC_036955 | *Cheumatopsyche analis* | Trichoptera| Hydropsychidae | *Cheumatopsyche* |
| NC_036954 | *Cheumatopsyche campyla*| Trichoptera| Hydropsychidae | *Cheumatopsyche* |
| NC_036952 | *Cheumatopsyche speciosa* | Trichoptera| Hydropsychidae | *Cheumatopsyche* |
| NC_043769 | *Cheumatopsyche brevilineata* | Trichoptera| Hydropsychidae | *Cheumatopsyche* |
| NC_036953 | *Potamyia flava*        | Trichoptera| Hydropsychidae | *Potamyia*  |
| NC_036156 | *Hydromanicus wulaianus*| Trichoptera| Hydropsychidae | *Hydromanicus* |
| NC_044770 | *Thitarodes damxungensis*| Lepidoptera| Hepialidae      | *Thitarodes* |

Phylogenetic trees

The mitochondrial genome of *Hydropsyche fryeri* was sequenced and assembled using Illumina high-throughput sequencing technology and Spades v.3.5.0 software.
The newly sequenced genomes and 22 complete mitochondrial genome sequences close to the *hydropsyche fryer* BLAST results were downloaded from the National Center for Biotechnology Information (Table 4). We used jModelTest2.1.7 (https://code.google.com/p/jmodeltest2/) for the selected sequences of DNA nucleic acid model test \(^{[12]}\) and Prottest3.2 (https://code.google.com/p/prottest3/) for the amino acid model test \(^{[13]}\). We selected the AIC (Akaike Information Criterion) *Broussonetia papyrifera* minimum value as the best model and used RAXML 8.1.5 (https://sco.H–its.org/exelixis/web/software/raxml/index.html) and the Maximum Likelihood (ML) method to construct the phylogenetic tree with a bootstrap value set to 1000 \(^{[14]}\).

**Declarations**

**Data availability statement**

The data that support the findings of this study are openly available in GenBank of NCBI at https://www.ncbi.nlm.nih.gov/, reference number MW413803.

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**Author contributions**

Y. J. W presented the experimental protocols, J. C. H, X. F. Z performed the experimental work, Y. L processed the experimental data, H. L. Q prepared the picture, and Y. M. L, H. W, Y. L, R. X. Z wrote and revised the article, all authors reviewed the manuscript.

**Competing interests**

The authors declare no competing interests.

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**Figures**
Figure 1

Morphology of Hydropsyche fryeri, 0.25 cm.

Figure 2

The mitochondrial ring structure of Hydropteryx fryeri.
Figure 3

Relative synonymous codon usage analysis of Hydropsyche Fryeri.
**Figure 4**

The clover structure of 22 tRNA.
**Figure 5**

Hydropsyche Fryeri phylogenetic tree.