

## Characterization of the complete mitochondrial genome of *Haemadipsa tianmushana* Song 1977 (Hirudiniformes, Haemadipsidae) and its phylogenetic analysis

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### ABSTRACT

The complete mitochondrial genome of *Haemadipsa tianmushana* Song 1977 from China has been determined and reported for the first time in this study. It was 14,625 bp in length and consisted of 22 tRNA genes, 2 rRNA genes, 13 protein-coding genes (PCGs), and 3 control regions. The nucleotide base content of the complete mitogenome for this species was 35.1% A, 10.5% C, 11.6% G, and 42.8% T. The tRNA genes were ranged from 57 bp (Ser<sup>TCT</sup>) to 66 bp (Gln<sup>TTG</sup>) in length. The phylogenetic analyses indicated that Hirudinea is a mono-phyletic clade. And it includes *Whitmania acranulata*, *Whitmania pigra*, *Whitmania laevis*, *Zeylanicobdella arugamensis*, *Ozobranchus jantseanus* and *Placobdella lamothei*. In Hirudiniformes, *H. tianmushana* and three species of Haemopidae were obviously clustered into two independent branches. This result is consistent with a taxonomy that they all belong to the same sub-order. This study adds to the genetic resources currently available for the species.

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*Haemadipsa tianmushana* Song 1977, belongs to the family Haemadipsidae, suborder Hirudiniformes (Schoch et al. 2020). Land leeches in Haemadipsidae are mostly from the humid tropical rainforest habitats and habitually take blood from humans and other animals (Won et al. 2014; Huang et al. 2019). Compared with most blood-feeding arthropods, they have the larger gut capacity, being highly abundant and easy to collect, therefore haemadipsid leeches may be particularly useful as iDNA sources for biodiversity monitoring. Drinkwater identified sequences from 14 mammalian genera, spanning nine families and five orders using *Haemadipsa picta* and *Haemadipsa sumatrana*. (Drinkwater et al. 2019). *H. tianmushana* is endemic leech species only distributed in China, which might serve as suitable and useful samples of biodiversity research. In addition, compared with nuclear DNA, the mitochondrial DNA of many animals tends to relatively higher mutation rate (Brown et al. 1979; Duda 2021). Almost all the mitogenome sequence datasets included the Mitochondrial Cytochrome C Oxidase I (COI) sequence. COI sequences variation among the clariid fishes of India (*Clarias magur*, *Clarias dussumieri*, and *Clarias gariepinus*) and their relationship with other representative clariids (Devassy et al. 2016). Therefore mitochondrial DNA is considered one of the most reliable molecular markers for phylogenetic studies

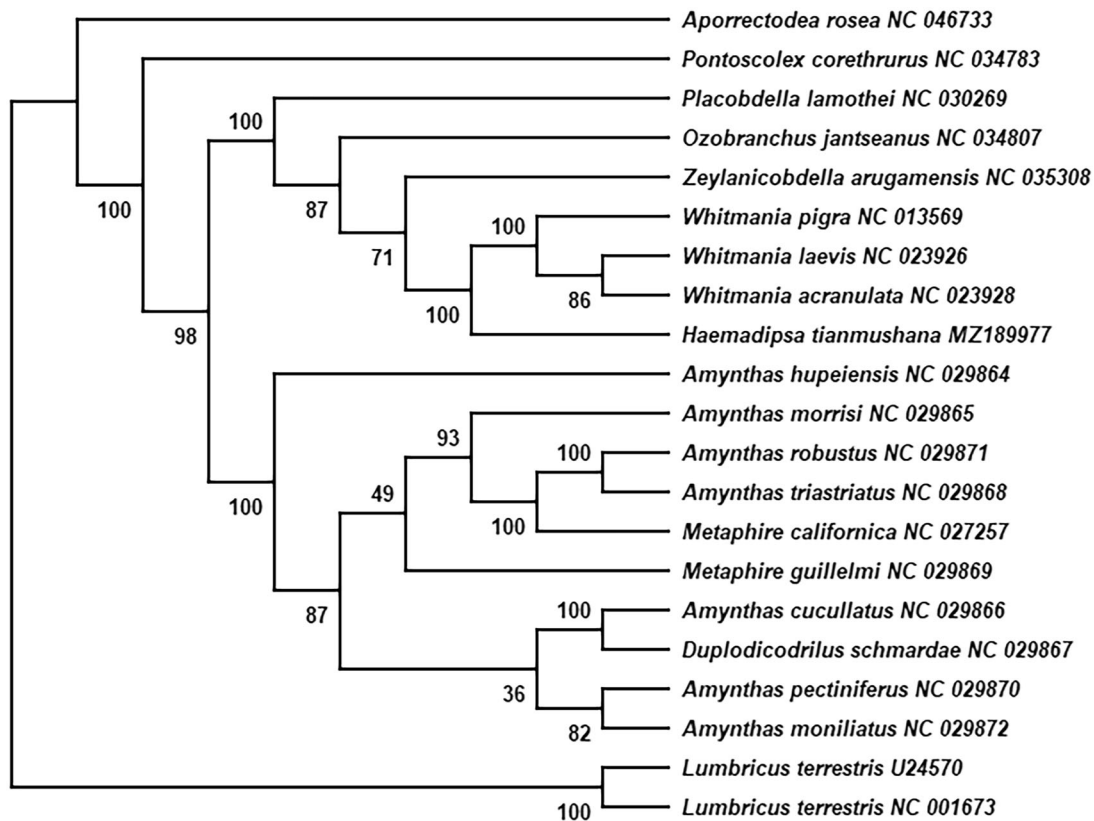
(Shen et al. 2011; Chen et al. 2012; Xu et al. 2014; Vella and Vella 2021).

Specimen samples were obtained from Mount Huangshan, Anhui Province, China (118°1'E, 30°1'N). A specimen of *H. tianmushana* was deposited at the Institute of Medicinal Plant Development(Linchun Shi, [lcshi@implad.ac.cn](mailto:lcshi@implad.ac.cn)) under the voucher number HS22. The genomic DNA was extracted from the tissue sample using TIANamp Genomic DNA Kit (Tiangen, Beijing, China) following the manufacturer's protocol. A NanoDrop 2000 ultra-micro spectrophotometer (Thermo Scientific, USA) was used to measure the yield and purity of genomic DNA, and next quantified using Qubit 4.0 (Thermo Scientific, USA). This process was followed by creating PCR-free libraries from the extracted genomic DNA (Liu et al. 2021). Next-generation sequencing was performed using the Illumina Novaseq platform. Trimmomatic v0.38 was employed to filter the low-quality reads and the sequencing adapter (Bolger et al. 2014). The complete circular mitogenome was *de novo* assembled using GetOrganelle v1.7.4 (Li et al. 2020). The mitogenome was annotated using MITOS (Bernt et al., 2013). To confirm the exactness of the mitochondrial genome, the manual correction was performed by annelid multiple sequence alignment. The mitogenomic sequencing data were individually mapped to the COI databases using BOWTIE2 v2.4.4 (Langmead and Salzberg 2012).

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**Figure 1.** The phylogenetic position for *H. tianmushana* according to the ML phylogenetic tree. The bootstrap support values are shown on the branches.

PCGs were identified by ORF Finder (<https://www.ncbi.nlm.nih.gov/orffinder/>) using mitochondrial genetic codes.

The whole mitochondrial genome of *H. tianmushana* is a circular molecule and 14,625 bp in length. It was deposited in GenBank under accession no. MZ189977. It consists of 22 tRNA genes, 2 rRNA genes, 13 protein-coding genes (PCGs), and 3 control regions (Bronstein et al. 2018). The nucleotide base content of *H. tianmushana* mitogenome was 35.1% A, 10.5% C, 11.6% G, and 42.8% T. The 22.1% of (G + C) showed great preference to AT. It follows the typical gene order and composition of fish species (Satoh et al. 2016). The mitogenome of *H. tianmushana* consists of 13 PCGs, PCGs' lengths range between 171 bp (*atp8*) and 1722 bp (*nad5*). Most of the PCGs utilize ATG as their termination codon except *nad5*, *nad6*, *cox1*, and *nad1* similar to the structure and variability of most other fish mitogenomes (Satoh et al. 2016). The mitogenome of *H. tianmushana* contains 22 tRNA genes varying from 57 bp (Ser<sup>TCT</sup>) to 66 bp (Gln<sup>TTG</sup>) in length, and all produced the expected typical cloverleaf structure. There are three control regions. The first control region is 49 bp in length located between tRNA-Trp and tRNA-Arg. The second is 85 bp in length located between tRNA-Arg and *atp6*. And the last is 124 bp in length located between *nad4* and tRNA-Met.

The mitogenome of *H. tianmushana* was aligned with other 18 Clitellata species whose mitogenomic protein sequences have more than 60% sequence homology with that of *H. tianmushana* using ClustalW (Thompson et al. 1994). The phylogenetic position of *H. tianmushana* was confirmed using RAxML v8.0.0 (Stamatakis 2014) to create a Maximum-Likelihood (ML) tree with GTRGAMMA model. *Lumbricus*

*terrestris* (U24570 and NC001673) was used as an outgroup (Liu et al. 2017). The result (Figure 1) suggests that Hirudinea is a mono-phyletic group. And it includes *Whitmania acranulata*, *Whitmania pigra*, *Whitmania laevis*, *Zeylanicobdella arugamensis*, *Ozobranchus jantseanus*, and *Placobdella lamothei*. In Hirudiniformes, *H. tianmushana* and three species of Haemopidae were obviously clustered into two independent branches. The phylogenetic analysis results were consistent with the morphological observations. This study provides reliable mitogenome information from *H. tianmushana* that could facilitate the species identification and phylogenetic study of *H. tianmushana*.

### Disclosure statement

No potential conflict of interest was reported by the authors(s).

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### Data availability statement

The genome sequence data that support the findings of this study are openly available in Genbank of NCBI at <https://www.ncbi.nlm.nih.gov/> under the accession no. MZ189977. The associated BioProject, SRA,

and Bio-Sample numbers are PRJNA743159, SRR15020542, and SAMN20000658, respectively.

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