



The complete mitochondrial genome of major malaria vector *Anopheles anthropophagus* (Diptera: Culicidae) in China

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ABSTRACT

Anopheles anthropophagus (Xu and Feng 1975) is the major vector of malaria in Eastern and Southern China. The species *An. anthropophagus* is considered a synonym of *An. lesteri* (Baisas & Hu, 1936), although they differ in several key biological characteristics. Here, we report the complete mitochondrial genome of *An. anthropophagus* for the first time. The mitogenome of *An. anthropophagus* is a typical circular, double-stranded molecule with a total length of 15,413 base pairs, and contains 13 protein-coding genes, 22 transfer RNA genes, two ribosomal RNA genes, and an AT-rich control region. A phylogenetic analysis of the complete mitogenomes of 16 species of *Anopheles* (Culicidae) revealed that *An. anthropophagus* is closely related to *An. sinensis* (Wiedemann 1828), in the family Culicidae. The *An. anthropophagus* mitogenome provides new data for further taxonomic and phylogenetic studies of the genus *Anopheles*.

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Anopheles anthropophagus belongs to the *Anopheles hyrcanus* group (Xu and Feng 1975), which is widely distributed in Oriental and Palearctic regions. The species in the *hyrcanus* group are difficult to distinguish morphologically, especially *An. lesteri* and *An. sinensis* (Gao et al. 2004). The species *An. anthropophagus* is considered a synonym of *An. lesteri* (Wilkerson et al. 2003; Rueda et al. 2005; Qu 2008), and has been identified as the main vector of malaria and Malayan filariasis in China (Zhu et al. 2013). After decades of effort to control malaria, especially vigorous vector control measures, the population of *An. anthropophagus* has been drastically reduced and has even disappeared in some areas, including provinces in Eastern and Southwestern China (Xu et al. 2001; Pan et al. 2012). On the contrary, the population of *An. sinensis*, which is sympatric with *An. anthropophagus*, has not declined to the same extent. Analysis of the mitochondrial genome of *An. anthropophagus* could provide insights that extend our understanding of the genetic characteristics and structure of the genus *Anopheles* (Cameron 2014).


In this study, we determined the complete mitochondrial genome of *An. anthropophagus* (Genbank: MW279150) for the first time and analyzed it phylogenetically. The specimens of *An. anthropophagus* was originally collected in the north of Jiangsu Province, reared in the laboratory of Jiangsu

Institute of Parasitic Diseases, Wuxi, Jiangsu, China (31°56'N, 120°23'E), and were deposited at Anhui Provincial Key Laboratory of Infection and Immunology, Bengbu Medical College, Bengbu, Anhui, China (voucher Mos-2020-An-001, Baiqing Li, baiqingli@bbmc.edu.cn). DNA was extracted from the mosquito tissue with the AxyPrep Plasmid Miniprep Kit (Axygen, China). To amplify the entire mitochondrial genome of *An. anthropophagus*, 11 pairs of primers (Supplementary Table S1) were designed based on the known mitogenomes of mosquito species. The PCR products were sequenced using the Sanger sequencing method (Sangon Biotech, China). The raw nucleotide sequences were trimmed to remove low-quality ends and assembled with DNASTAR V11.0 Lasergene (DNA Star). MITOS (<http://mitos2.bioinf.uni-leipzig.de>) (Bernt et al. 2013) was used to annotate the mitogenome.

The mitochondrial genome of *An. anthropophagus* is a typical circular, double-stranded molecule with a total length of 15,413 base pairs, and contains 13 protein-coding genes, 22 transfer RNA genes, two ribosomal RNA genes, and a control region. The nucleotide composition of the mitochondrial genome is 40.21% A, 38.03% T, 9.20% G, and 12.56% C. The A+T base composition (78.24%) of the complete mitogenome is higher than the G+C base composition (21.76%),

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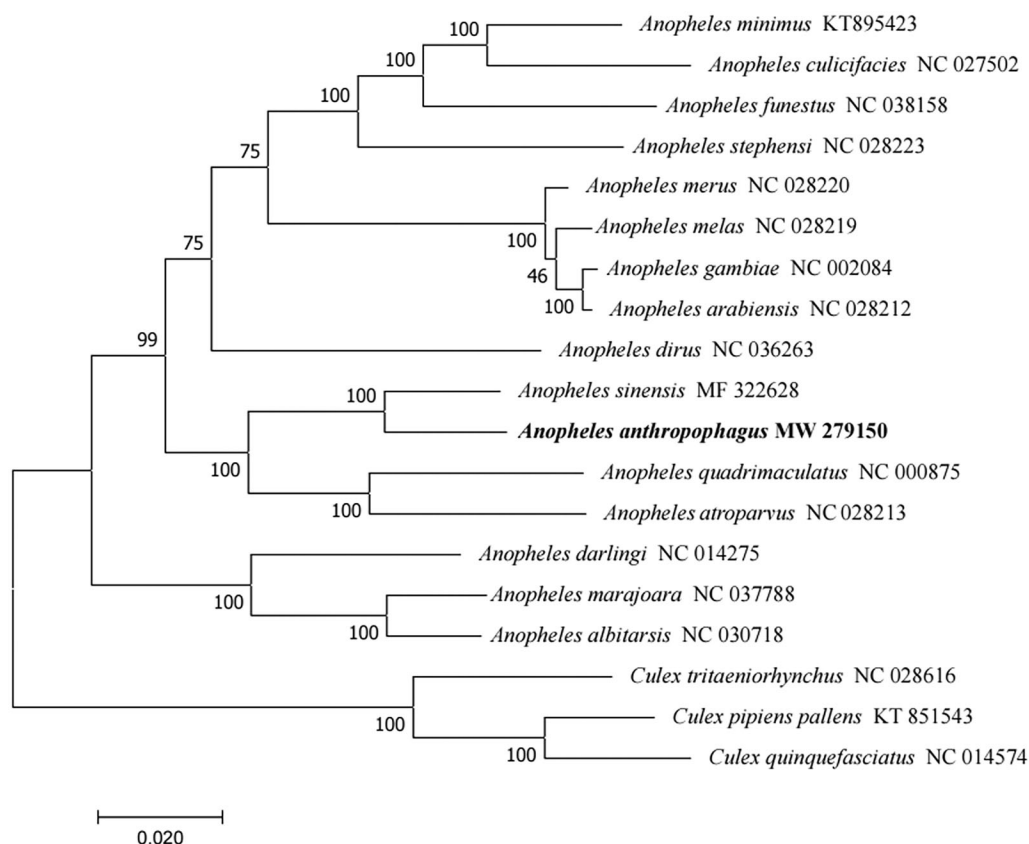


Figure 1. Phylogenetic tree of 16 *Anopheles* species based on their mitochondrial genomes. Numbers near the nodes represent bootstrap values. The GenBank accession number is listed next to each species within the tree. *Culex* was used as the outgroup. The branch length scale bar indicates relative differences (0.020 = 2.0% nucleotide difference).

and thus, the nucleotide composition is highly A+T biased. The locations and order of all genes are similar to those in other *Anopheles* species (Chen et al. 2017). The start codon ATG is used by six protein-coding genes (*COII*, *ATP6*, *COIII*, *ND4*, *ND4L*, and *CYT2B*), ATT by four protein-coding genes (*ND2*, *ATP8*, *ND6*, and *ND1*), TCG by *COI*, ATA by *ND3*, and GTG by *ND5*. All 13 protein-coding genes use either TAA (*ND2*, *ATP8*, *ATP6*, *ND3*, *ND4L*, *ND5*, *ND6*, *Cyt B*, *ND1*) or T (*CO1*, *CO2*, *CO3*, *ND4*) as the termination codon. The 22 tRNAs range in length from 62 to 72 bp with a typical cloverleaf structure. The mitogenome of *An. anthropophagus* contains two rRNA genes, *16S rRNA* and *12S rRNA*, they are all located in the N chain. The *16S rRNA* is 1,335 in length and is located between *tRNA^{Leu}* and *tRNA^{Val}*. The *12S rRNA* is 794 bp in length and is located between *tRNA^{Val}* and the control region. The length of the control region is 566 bp with high A+T content (94.17%), and is located between *12S rRNA* and *tRNA^{Ile}*. 9 intergenic spacer regions are ranging in length from 1 to 20 bp, and the largest interval appears between *tRNA^{Ser}* and *ND1*. Thirteen intergenic overlapping regions are between 1 and 7 bp in size (Supplementary Table S2).

The phylogenetic analysis of the complete mitochondrial genomes of *Anopheles* species was performed with the genus *Culex* as the outgroup. A phylogenetic tree was constructed using the maximum likelihood (ML) method in MEGA 7.0 (Sudhir et al. 2016) under the Tamura-Nei model, with 1000 bootstrap replicates. The results showed that

An. anthropophagus is closely related to *An. sinensis* (Figure 1), which are sympatric species and have both been confirmed as the major vectors of *Plasmodium vivax* in China. In conclusion, the complete mitochondrial genome of *An. anthropophagus* reported in this study provides useful information for further taxonomic, phylogenetic, and evolutionary analyses of the genus *Anopheles*.

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Disclosure statement

No potential conflict of interest was reported by the author(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. MW279150.

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