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MITOGENOME REPORT

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The characterization of the mitochondrial genome of *Graptemys ouachitensis*

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ABSTRACT

Graptemys ouachitensis (CAGLE, 1953) belongs to the *Graptemys* genus, *the* Emydidae family, and the Testudines order. This study involved sequencing the complete mitochondrial genome (mitogenome) of *G. ouachitensis* using next-generation sequencing, and analyzing the essential characteristics, and phylogenetic relationship. The results revealed that the *G. ouachitensis* mitogenome was 16,674 bp in length (A: 34.1%, C: 26.0%, G: 13.0%, T: 26.9%) and included 22 *tRNAs*, 13 protein-coding genes, two ribosomal RNA genes, and a non-coding control region (GenBank accession: NC071766). The genome composition of *G. ouachitensis* presented a slight A + T bias (61.0%) and exhibited a positive AT skew (0.118) and a negative GC skew (–0.333). A phylogenetic analysis based on the complete mitogenome indicated that the *G. ouachitensis* was more closely associated with *Malaclemys terrapin* than the other eight known Emydidae species. Thus, our findings present a novel mitogenome at the species level. This study introduces the first complete mitogenome of *G. ouachitensis*, providing valuable molecular information for phylogenetic and conservation genetics analyses of *G. ouachitensis*.

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KEYWORDS
Graptemys ouachitensis; mitogenome; phylogenetic analysis

1. Introduction

Graptemys ouachitensis (CAGLE, 1953) belongs to the *Graptemys* genus, *the* Emydidae family, and the Testudines order. *Graptemys* is one of the most diverse genera in the Emydidae with 14 species. *G. ouachitensis* is strictly distributed in river systems and large creeks of the eastern USA (such as Alabama, Texas, Iowa, Minnesota, Wisconsin, and Mississippi) and southeastern Canada (Lovich and Gibbons 2021). *G. ouachitensis*' taxonomic status is debatable, with some taxonomists arguing that it should be reinstated as a subspecies of *Graptemys pseudogeographica* (Praschag et al. 2017). Although 14 species fall under the genus *Graptemys*, no complete mitogenome of any species among them has been reported. Given this gap, we aimed to sequence and obtain the entire mitogenome of *G. ouachitensis* using next-generation sequencing (NGS). As this is the first mitogenome of *G. ouachitensis*, the findings will enrich the *Graptemys* molecular database. Furthermore, we analyzed the phylogenetic relationships among nine known complete Emydidae mitogenomes, which is critical for future phylogenetic studies and will improve our understanding of the genetic biodiversity of Emydidae species.

2. Materials and methods

2.1. Ethical approval

This work was approved by the Animal Research Ethics Committee of Hainan Provincial Education Center for Ecology and Environment, Hainan Normal University, China, under the research permit (HNECEE2022-006).

2.2. Sample collection and DNA extraction

In this study, a deceased *G. ouachitensis* was obtained from a local turtle farm named Hong Wang (longitude: 110.23E, latitude: 19.76 N) in Dongshan Town, Haikou City, Hainan Province, China. The Hong Wang turtle farm has ***a domestication and breeding license*** issued by the Department of Wildlife Administration under the State Council. The deceased specimen (Figure 1, taken by the author Xiaofei Zhai) was preserved under voucher number HNSD-HN2018N02 at the Turtles Research and Conservation Center of Hainan Normal University (longitude: 110.35E, latitude: 20.00 N; Guangwei Ma, email: maguangwei77@163.com). *G. ouachitensis* can be identified by its morphology, which includes a rectangular yellow mark behind the eyes, unlike other *Graptemys* species

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(Haitao 2011). A muscle sample was extracted from the preserved specimen, and the total genomic DNA was isolated from the muscle tissue using a standard phenol-chloroform extraction protocol (Sambrook et al. 1989). The DNA's degrees of concentration and purity were evaluated using a Thermo Scientific NanoDrop 2000. The integrity was assessed using an Agilent 2100 Bioanalyzer and electrophoresis on a 1% agarose gel. The genomic DNA was stored at −80 °C, until preparation for NGS.

Figure 1. Species reference image. The image was taken by the author (Xiaofei Zhai).

2.3. NGS, mitochondrial genome assembly and annotation

The genomic DNA was sent to Huitong Biotechnology Co., Ltd. (Shenzhen, China) for library construction and sequencing on Illumina NovaSeq 6000 using dry ice and DNase-free tubes with 150 bp paired-end reads. Then, 8.18 Gb of raw reads were obtained, and 8.11 Gb of clean reads were obtained from the raw reads after removing adaptor sequences using AdapterRemoval v2 (Schubert et al. 2016) and filtering low-quality sequences using SOAPdenovo2 (Luo et al. 2012). Subsequently, the clean data were assembled using MIRA (version V5rc1) to construct contigs and scaffolds

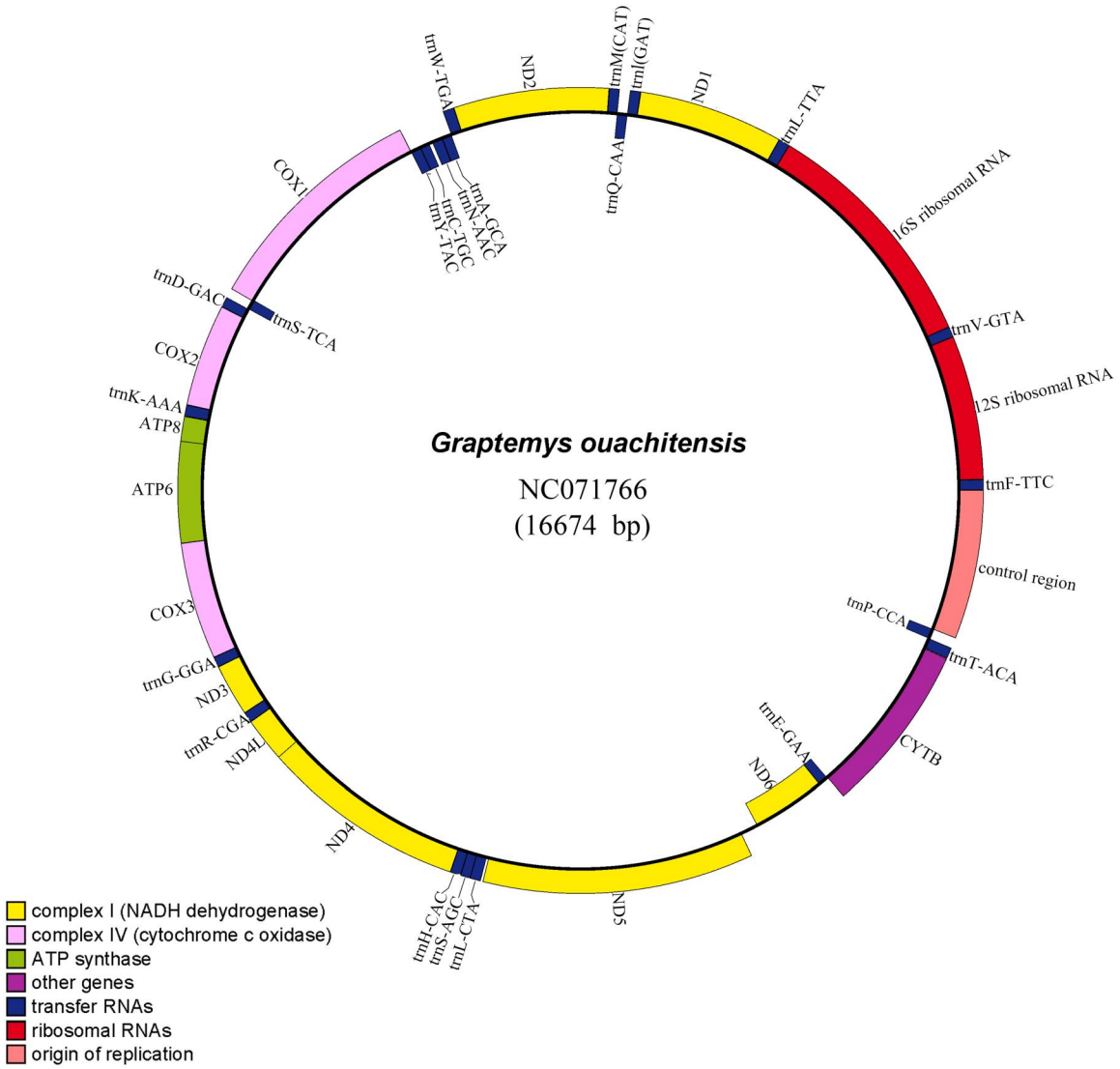

Figure 2. Graphical map of the mitogenome of *Graptemys ouachitensis*.
The yellow, pink, and green segments represent protein-coding genes; the blue and red segments represent tRNA genes and rRNA genes, respectively. Genes for tRNAs are abbreviated using a single letter (F = Phe; V = Val; L2 = Leu; I = Ile; Q = Gln; M = Met; W = Trp; A = Ala; N = Asn; C = Cys; Y = Tyr; S2 = Ser; D = Asp; K = Lys; G = Gly; R = Arg; H = His; S1 = Ser; L1 = Leu; E = Glu; T = Thr; P = Pro). The inside line and outside line indicate the L-strand and H-strand, respectively.

following the mitogenome of *Chrysemys picta* (NCBI accession number: NC002073) as reference sequences.

The MITOS model was used to predict the locations of protein-coding genes (PCGs) and rRNA genes (Bernt et al. 2013). The initiation and termination codons were identified using ORF finder and Blastn of NCBI, according to their alignment with other related species. Moreover, MITOS was utilized to predict and annotate the locations of *tRNA*. AT skew = (A − T)/(A + T) and GC skew = (G − C)/(G + C) were analyzed to describe base composition (Perna and Kocher 1995). OGDRAW (version 1.3.1), an online mitochondrial visualization tool, generated a graphical diagram of the complete mitogenome (Greiner et al. 2019). The control region sequences of *G. ouachitensis* and eight other species of Emydidae (Supplementary Material, Table S1) were examined using the Tandem Repeat Finder with the default settings to detect repetitive tandem sequences (Benson 1999). Finally, the complete mitogenome sequence was uploaded to the GenBank database with accession number NC071766.

2.4. Phylogenetic analysis

MEGA-X (Kumar et al. 2018) was used to construct a phylogenetic tree based on *G. ouachitensis*' complete mitogenome and eight other Emydidae species (Supplementary Material, Table S1). The phylogenetic analyses were performed using the maximum likelihood (ML) method, with 1000 bootstrap replications. *Cuora flavomarginata* (EU708434) and *Cuora aurocapitata* (AY874540) served as out-groups.

3. Results

Following morphological identification (a rectangular yellow mark behind the eyes, Figure 1), we conclude that the sample in this study is *G. ouachitensis*. After NGS and assembly, a circular mitogenome with a length of 16,674 bp was ultimately generated (Figure 2), the shortest of the known Emydidae mitogenomes (16,674–17,258 bp, Supplementary Material, Table S1). The genome coverage across the reference figure is detailed in the Supplementary Material (Figure S1). The *G. ouachitensis* mitogenome contained 13 PCGs, 22 tRNAs, two rRNAs, and a non-coding control region (Figure 2 and Supplementary Material, Table S2), similar to all known Emydidae. As with all known Emydidae, nine of the 37 genes were coded on the light strand (L-strand) and 28 on the heavy strand (H-strand). Furthermore, like in other Emydidae species, the mitogenome of *G. ouachitensis* was closely aligned, with only a small number of bases overlapping adjacent genes, indicating efficient RNA transcription and protein translation (Supplementary Material, Table S2).

The genome composition of *G. ouachitensis* (A: 34.1%; C: 26.0%; G: 13.0%; T: 26.9%) presented a slight A + T bias (61.0%), a positive AT skew (0.118), and a negative GC skew (–0.333). In this study, the AT skew was higher than that of *Malaclemys terrapin* (0.115) but lower than that of most Emydidae mitogenomes (Supplementary Material, Table S1). Moreover, the GC skew was higher than that of most of the other previously sequenced species of Emydidae (Supplementary Material, Table S1). Different regions of the *G. ouachitensis* mitogenome had similar A + T contents, ranging from 60.5% to 61.6%, except for the control region (66.2%; Supplementary Material, Table S3).

The PCG region was 11,405 bp long and comprised 68.40% of the *G. ouachitensis* mitogenome. The size of the 13 PCGs ranged from 168 bp to 1830 bp (Supplementary Material, Table S2). The number of bases in the 13 PCGs followed the pattern A > C > T > G, with a slight A + T bias (60.5%; Supplementary Material, Table S3). The AT and GC skews were 0.098 and −0.401, respectively (Supplementary Material, Table S3). The PCG region of *G. ouachitensis*

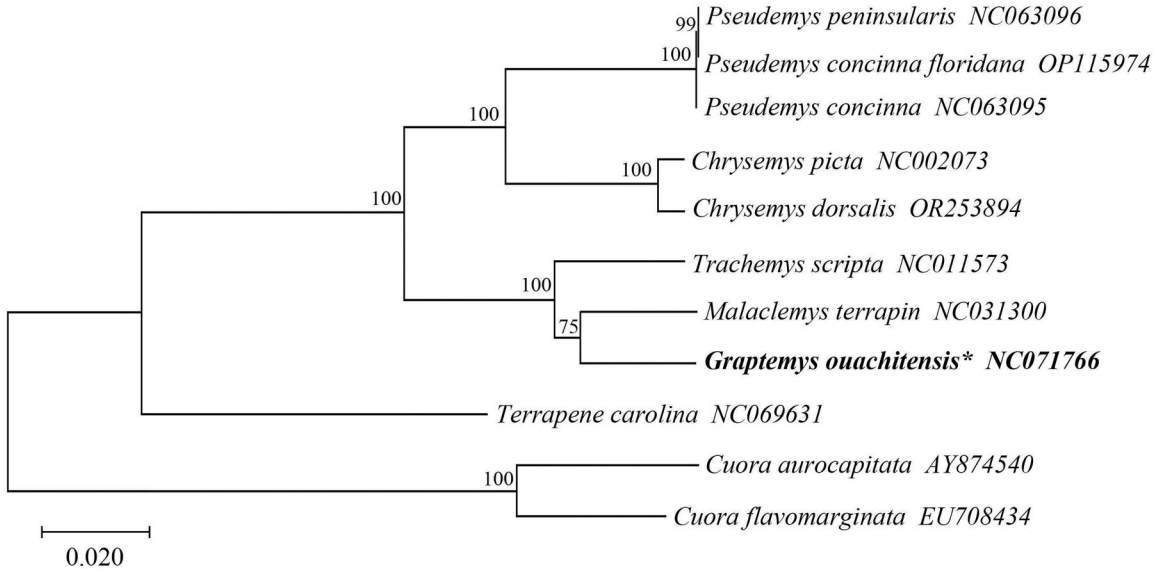

Figure 3. A phylogenetic tree of nine emydidae species based on the complete mitogenome.
The phylogenetic tree was constructed based on the complete mitogenome of *G. ouachitensis* (NC071766) and eight other species of the family *Emydidae* (*Chrysemys dorsalis*, OR253894; *Chrysemys picta*, NC002073; *Malaclemys terrapin*, NC031300; *Pseudemys concinna*, NC063095; *Pseudemys floridana*, OP115974; *Pseudemys peninsularis*, NC063096; *Terrapene carolina*, NC069631; and *Trachemys scripta*, NC011573), using the ML methods, inferred from 1000 replicates, in MEGA-X. *Cuora flavomarginata* (EU708434) and *Cuora aurocapitata* (AY874540) were set as out-groups. Scientific names and GenBank accessions numbers are labeled at the tips. The mitochondrial genome sequenced in this study is shown in bold and marked with an asterisk. Nodal support indicated by bootstrap.

exhibited a slightly positive AT skew, indicating a higher incidence of the A nucleotide than the T one, a feature observed in other mitogenomes of Emydidae species. Additionally, the PCG region of *G. ouachitensis* mitogenome had higher GC skew and lower AT skew values than most other known Emydidae species (Supplementary Material, Table S3).

The *G. ouachitensis* mitogenome contained 22 *tRNA* genes: 14 *tRNAs* were encoded by the H-strand and eight *tRNAs* by the L-strand (Figure 2 and Supplementary Material, Table S2). The *tRNA* sequences ranged between 66 bp and 75 bp, with moderate A + T bias (61.6%) and positive AT skew (0.117; Supplementary Material, Table S3).

The *12S rRNA* was located between *tRNA-Phe* and *tRNA-Val*, and *16S rRNA* between *tRNA-Val* and *tRNA-Leu* (Figure 2 and Supplementary Material, Table S2). The two *rRNAs* were 966 bp and 1617 bp long, respectively. The A + T content of the *rRNA* region was 60.9% and showed a positive AT skew (0.274) and a negative GC skew (–0.148; Supplementary Material, Table S3).

The control region (CR) was 997 bp, situated between *tRNA-Pro* and *tRNA-Phe*, and had negative AT (–0.030) and GC skews (–0.243; Supplementary Material, Table S3). The CR length of *G. ouachitensis* was the shortest of the known Emydidae mitogenomes (997–1584 bp, Supplementary Material, Table S4). Moreover, tandem repetitions were observed in the CR of all known Emydidae species (Supplementary Material, Table S4). Notably, a tandem repeat sequence [5′-$(TA)_{20}$-3′ motif] was found in the *G. ouachitensis* mitogenome, which was also identified in *Chrysemys dorsalis*, *Pseudemys concinna*, *Pseudemys floridana*, *Pseudemys peninsularis*, and *Terrapene carolina* (Supplementary Material, Table S4).

To identify the phylogenetic relationships of *G. ouachitensis* with other Emydidae species, a phylogenetic tree was constructed based on the complete mitogenome of *G. ouachitensis* and eight other species of the family Emydidae, with *C. flavomarginata* and *C. aurocapitata* (family *Geoemydidae*) as out-groups, using the ML methods, inferred from 1000 replicates, in MEGA-X. Due to the absence of other *Graptemys* species mitogenome, the result demonstrated that *G. ouachitensis* was more closely related to *Malaclemys terrapin*, followed by *Trachemys scripta*, and most distantly related to *Terrapene carolina*, compared to the other eight known Emydidae species (Figure 3).

4. Discussion and conclusion

This research describes a novel mitogenome at the species level. The first complete mitogenome of *G. ouachitensis* was assembled and characterized. The CR length of *G. ouachitensis* was the shortest of the known Emydidae mitogenomes (997–1584 bp, Supplementary Material, Table S4), which is a common phenomenon, as the CR is generally considered to have the most significant length variation in the mitogenome (Boore 1999).

Traditional taxonomy is based primarily on the external morphology of the species, which is inaccurate. Phylogenetic analyses can provide more precise information about the relationships between species. A previous study revealed that the *G. ouachitensis* and *M. terrapin* were on one branch by the ML phylogeny analysis of the mitochondrial *cytochrome b* gene (Spinks et al. 2009). In this study, our result also showed that *G. ouachitensis* was more closely related to *M. terrapin* compared to the other eight known Emydidae species (Figure 3). This suggests that the developmental processes of *G. ouachitensis* and *M. terrapin* may be more similar, and the origins may be more consistent. However, more evidence is needed to confirm this due to the paucity of data on *Graptemys*. Meanwhile, the complete mitogenome of *G. pseudogeographica* should also be established to determine the taxonomic status of *G. ouachitensis* (Praschag et al. 2017).

In summary, the study of the complete *G. ouachitensis* mitogenome could help us understand this species' basic mitogenome characteristics. Furthermore, it provided valuable molecular information for phylogenetic and conservation genetics analyses of *G. ouachitensis*.

Author contributions

GM conceived the original idea. YR, ZZ, and TW carried out the experiment. ZZ, XX, and XZ analyzed the data. YR and GM wrote and proofread the manuscript. All authors agree to be accountable for all aspects of the work.

Disclosure statement

The authors report that there are no competing interests to declare.

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Data availability statement

The genome sequence data supporting this study's findings are openly available in GenBank of NCBI at https://www.ncbi.nlm.nih.gov with the accession number NC071766. The associated BioProject, SRA, and BioSample numbers are PRJNA861665, SRP387857, and SAMN29921211, respectively.

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