


The complete mitochondrial genome of the red-footed tortoise (*Chelonoidis carbonarius* Spix 1824)

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ABSTRACT

In this study, we report the complete mitochondrial genome of the red-footed tortoise (*Chelonoidis carbonarius*). The complete mitochondrial genome of *C. carbonarius* is 16,639 bp in length, including 13 protein-coding genes, 22 tRNA genes, and two rRNA genes. The mitogenome was deposited in NCBI GenBank under the accession number OQ789392. Furthermore, we also constructed a phylogenetic tree of *Chelonoidis* using eight species. These results will aid the conservation of *Chelonoidis* from the perspective of genetic evolution.

ARTICLE HISTORY

Received 22 August 2023
Accepted 18 December 2023

KEYWORDS

mtDNA genome;
phylogenetic status;
Chelonoidis; red-footed
tortoise

Introduction

Although tortoises have several ecological functions, over 20% of chelonian species globally have become extinct (Le Balle et al. 2021). Fitzinger (1835) divided the tortoises into three genera, namely *Testudo* Linnaeus 1758, *Cinixys* Wagler 1830, and *Geochelone* Fitzinger 1835. *Chelonoidis* Fitzinger 1835 is a subgenus of *Geochelone*. According to Olson and David (2015), there are six living species in *Chelonoidis*, namely *Chelonoidis carbonarius* Spix 1824, *Chelonoidis chilensis* Gray 1870, *Chelonoidis denticulatus* Linnaeus 1766, *Chelonoidis niger* Quoy & Gaimard 1824, *Chelonoidis phantasticus* Van Denburgh 1907, and *Chelonoidis vicina* Günther 1874. Two other species, *Chelonoidis abingdonii* Günther 1877 and *Chelonoidis alburyorum* Franz & Franz 2009, have already gone extinct. Furthermore, *C. vicina*, *C. phantasticus*, and *C. abingdonii* are currently considered the subspecies of *C. niger* by most, namely *Chelonoidis niger vicina*, *Chelonoidis niger phantasticus*, and *Chelonoidis niger abingdonii*, respectively (The Reptile Database, www.reptile-database.org). Although the populations of several of these species of giant tortoises are increasing according to the International Union for Conservation of Nature (IUCN), the survival of many wild populations is still under threat due to habitat loss and overhunting (Cayot 2008; Edwards et al. 2014). Revealing the genetic characteristics and phylogenetic relationships can provide a scientific basis for *Chelonoidis* species conservation.


Chelonoidis carbonarius Spix 1824 (Testudines, Testudinoidea, Testudinidae) (Figure 1), also referred to as

the red-footed tortoise, has a broad geographic distribution in South America (Fuente et al. 2018; Trevizan-Baú et al. 2018). A previous study indicated that the red-footed tortoise may be an important seed dispersal agent for tropical plants (Strong and Fragoso 2006). The conservation status of *C. carbonarius* has not been evaluated by IUCN. To investigate the genetic characteristics of *C. carbonarius* and elucidate the evolutionary relationship among *Chelonoidis* species, we sequenced the complete mitochondrial genome of *C. carbonarius* and constructed a phylogenetic tree of *Chelonoidis* species.



Figure 1. Reference image of the red-footed tortoise (*C. carbonarius*) from Echeverry-Alcendra, 2019.

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 Supplemental data for this article can be accessed online at <https://doi.org/10.1080/23802359.2023.2298081>.

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Figure 2. Photograph of the sampled red-footed tortoise (*C. carbonarius*) under voucher number GWRC-202100310128. This photograph was taken by Guangda Yang in the Guangzhou Wildlife Rescue Monitoring Center, Guangdong Province, China.

Materials and methods

One living individual of *C. carbonarius* was obtained during a rescue operation in Guangdong Province, China (Figure 2). Species identification was performed according to morphological characteristics (Vitt and Caldwell 2014). After the rescue, this individual was separate from other turtles in a shelter. For sequencing, we collected the fresh fecal tissue of this individual in its shelter. Feces is not the best tissue to use, but it works and does not disturb live animals. Since *C. carbonarius* is not distributed in China, the geographical source of the sampled individual is unknown. However, having the mitochondrial genome of this species is still of value. After the sampled individual died of an unknown reason, the specimen was deposited at Guangzhou Wildlife Rescue Monitoring Center, Guangdong Province, China (collector: Guangda Yang, 84165137@qq.com) under the voucher number GWRC-202100310128.

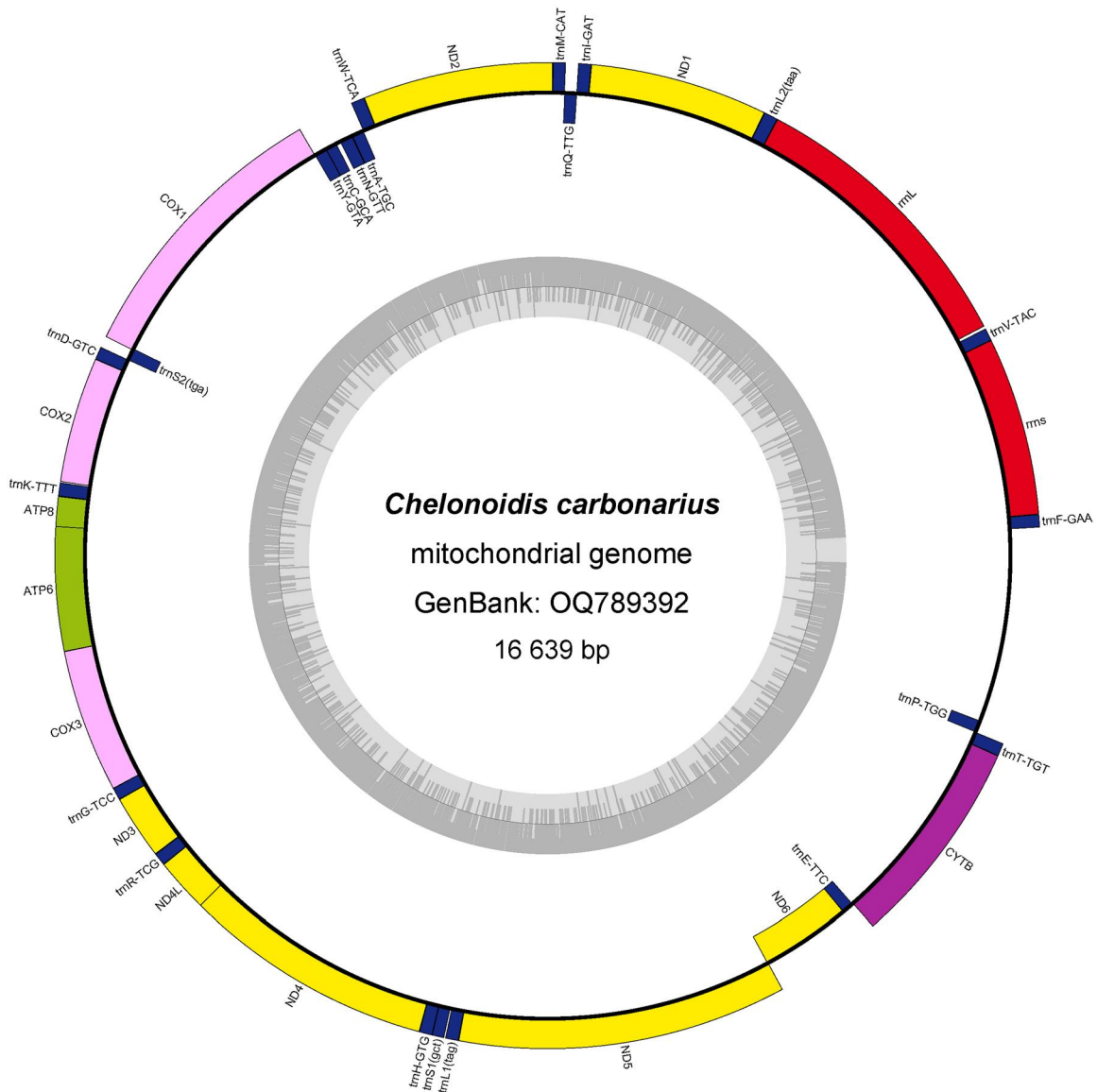


Figure 3. Circular map of the *Chelonoidis carbonarius* mitochondrial genome (GenBank accession: OQ789392), including 13 protein-coding genes, 22 tRNAs, and two rRNAs.

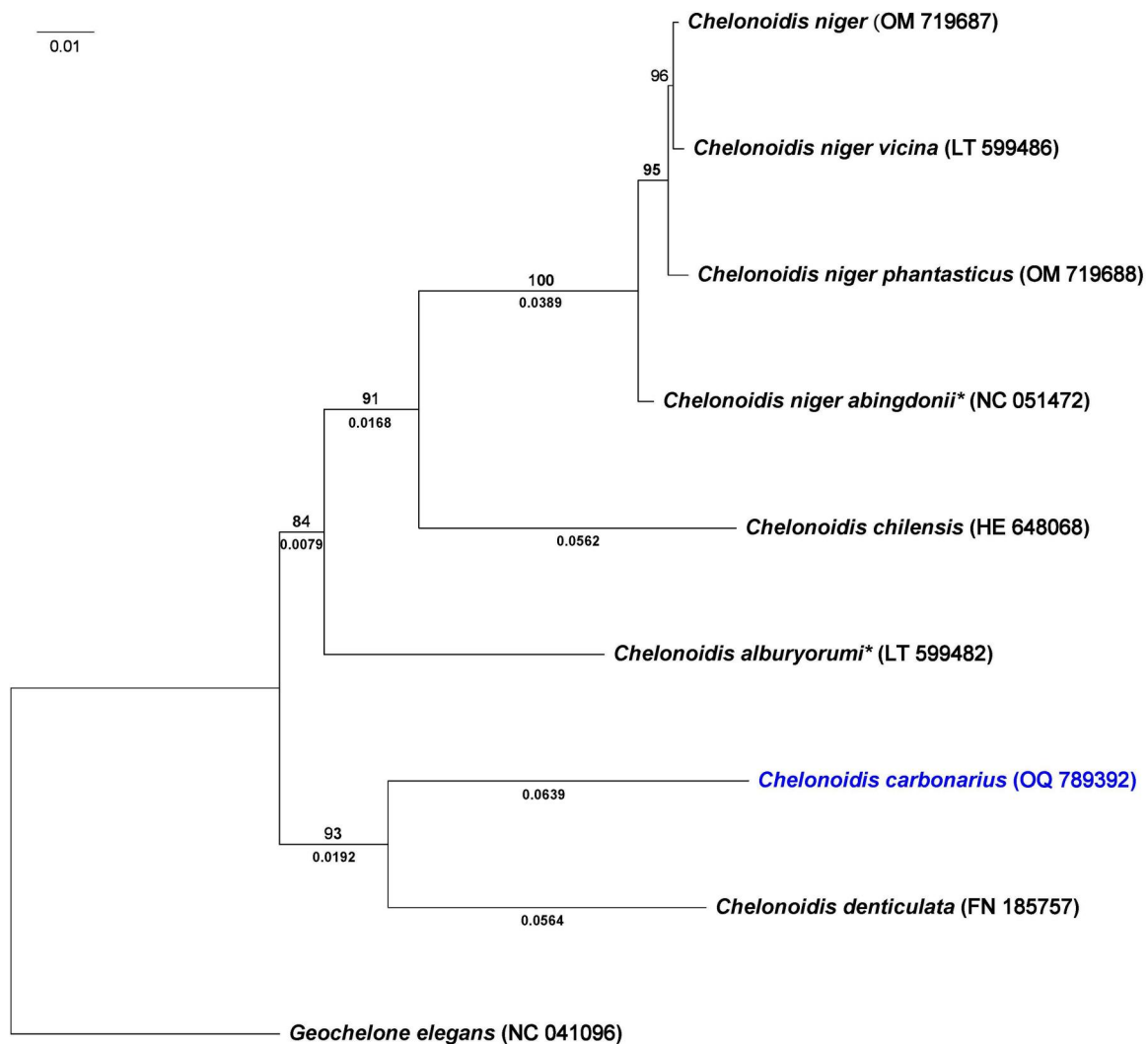


Figure 4. Maximum-likelihood phylogenetic tree of eight species in *Chelonoidis* based on the *Cytochrome b* (*Cytb*) gene with *Geochelone elegans* as the outgroup. The sequence of *C. carbonarius* was sequenced in this study (highlighted in blue), and the other seven sequences were downloaded from NCBI. The accession numbers are given in the brackets. The asterisk (*) indicates that the species is extinct.

Total DNA was extracted using the EasyPure Kit of Genomic DNA (Transgen Biotech, Beijing, China). After checking the quality of DNA using agarose gel electrophoresis (AGE), sequencing libraries were generated using the NEBNext Ultra DNA Library Prep Kit for Illumina. Next-generation sequencing was performed using the NovaSeq 6000 platform. The raw reads were quality-trimmed to remove adapter sequences, low-quality reads, reads with >5% unknown bases, and ambiguous bases using SOAPnuke v 1.3.0. We obtained 35,145,906 filtered reads as clean reads from 35,232,734 raw reads. We obtained clean reads and performed de novo assembly using SPAdes v.3.13.0. MITOS (<http://mitos2.bioinf.uni-leipzig.de/index.py>) was used for sequence annotation. After obtaining the mitochondrial sequence, we utilized BLAST in GenBank to prove the confirmability of our sequence from *C. carbonarius*, based on different mitochondrial genes, including *Cytb*, *COX1*, and *ND1* (>99.00% identity). A maximum-likelihood tree of eight species in *Chelonoidis*, namely *C. niger* (OM 719687), *C. n. vicina* (LT 599486), *C. n. phantasticus* (OM 719688), *C. n. alburyorumi* (LT 599482), *C. chilensis* (HE 648068), *C. abingdonii* (NC

051472), *C. denticulatus* (FN 185757), and *C. carbonarius* (OQ 789392), was constructed with branch-lengths based on the *Cytochrome b* (*Cytb*) gene. *Geochelone elegans* (NC 041096) was used as the outgroup. Before constructing the phylogenetic tree, the sequences of eight species were aligned using muscle v5. The maximum-likelihood phylogenetic tree was constructed using IQ-Tree v.16.12 with 1000 bootstrap replicates to recover the phylogenetic relationship between *C. carbonarius* and its sister clade (Poulakakis et al. 2020).

Results

The complete mitochondrial genome sequence of *C. carbonarius* was submitted to GenBank under accession number OQ789392 (BioProject, PRJNA981663; BioSample, SAMN35682342; SRA, SRR24880930). Regarding the sequencing results, up to 99.99% of the raw reads exhibited high quality (>Q40), and a total of approximately 5.27 billion clean reads for approximately 5.30 Gb were generated. The full length of the mitochondrial genome of *C. carbonarius* was 16,639 bp. The read coverage depth map

of *C. carbonarius* is shown in Figure S1. The GC content was approximately 39.23%, and the complete mitochondrial genome included 13 protein-coding genes, 22 tRNA genes, and two rRNA genes (Figure 3). Additionally, the duplicate control-region, which was known in some tortoises, was not found for the mitochondrial genome of *C. carbonarius*. The maximum-likelihood tree illustrating the phylogenetic relationship among species in *Chelonoidis* is shown in Figure 4. *C. niger*, *C. n. vicina*, *C. n. phantasticus*, and *C. n. abingdonii* were clustered as one evolutionary branch and further clustered with *C. chilensis*, *C. alburyorumi*, and *C. denticulatus* and *C. carbonarius*, respectively.

Discussion and conclusion

The complete mitogenome of *C. carbonarius* is 16,639 bp in length. The maximum-likelihood tree based on the *Cytb* gene showed the phylogenetic relationship among eight species in *Chelonoidis*. The phylogeny in our study agrees with other phylogenies based on mitochondrial data, with *C. carbonarius* placed sister to *C. denticulata*, and is identical for *Cytb* to other published, near complete mitochondrial genomes of *C. carbonarius* (Poulakakis et al. 2020). The inclusion of more related taxa on future phylogenetic analyses may help to further understand the phylogeny of chelonian species. Furthermore, human activities have led to the extinction of many tortoise species (Kehlmaier et al. 2021). The high-quality mitochondrial genome of *C. carbonarius* and the phylogenetic tree can provide insight into *Chelonoidis* species conservation from the perspective of genetic evolution.

Acknowledgments

We thank LetPub (www.letpub.com) for its linguistic assistance during the preparation of this manuscript.

Authors contributions

Hongliang Dou collected the sample. Guangda Yang, Hongliang Dou, and Jun Li designed the study. Guangda Yang, Yin Mei, Yingjin Mao, Chunhe Lyu, He Wang, and Jun Li analyzed the data and prepared the manuscript draft. All authors participated in the discussion and editing of the manuscript. All authors read, edited, and approved the final manuscript.

Ethical approval

All sampling procedures were approved by Guangdong Wildlife Rescue Monitoring Center, China (approval number: GWRC-20220516).

Disclosure statement

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

Funding

The author(s) reported there is no funding associated with the work featured in this article.

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Data availability statement

The complete mitochondrial genome sequenced in this study is available in NCBI (<https://www.ncbi.nlm.nih.gov/>) under accession number OQ789392. The associated BioProject, BioSample, and SRA numbers are PRJNA981663, SAMN35682342, and SRR24880930, respectively.

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