The complete mitochondrial genome of De Brazza’s monkey (Cercopithecus neglectus)

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ABSTRACT

The complete mitochondrial genome of Cercopithecus neglectus was described. The mitogenome is 16,490 bp in length and consists of 13 protein-coding genes (PCGs), 22 transfer-RNA genes, and one non-coding region. All the 13 PCGs were 11,398 bp in length with most common start codon of ATG and termination codon of TAA. The overall GC content was 42.5%. The result of phylogenetic analysis showed that the relationship of C. neglectus was close to C. mona, C. pogonias, C. wolfi and C. denti.

Background

De Brazza’s monkey (Cercopithecus neglectus Schlegel, 1876) was assessed to be Least Concern (LC) by IUCN (2016) and was listed in Appendix II of CITES. This species distributes widely in Angola, Cameroon, Equatorial Guinea, Gabon, Congo, Central African Republic, Uganda, Kenya, Sudan and Ethiopia (Mwenja et al. 2019). Deforestation and habitat loss were dominant factors causing the C. neglectus population to decline (Brennan 1985). The classification of the has been a major challenge for primate taxonomists (Groves 2001; Butynski 2002; Grubb et al. 2003), and the phylogenetic status of C. neglectus with other guenons species, should be concentrated. In this study, we sequenced the complete mitochondrial genome of C. neglectus and estimated the phylogenetic status of 17 Cercopithecus species. We hope to provide valuable information for the classification of Cercopithecus species (Ayoola et al. 2019).

Materials and methods

The blood sample was collected from a female C. neglectus in Shanghai Zoological Park (longitude-121.36319, latitude-31.192682), Shanghai, China in March 2020. The sampling process was approved by the welfare and ethical committee in Shanghai Zoological Park. A specimen was deposited at Endangered Species Conservation and Research Center of Shanghai Zoological Park (Liu Qunxiu, liuqunxiu2019@163.com) under the voucher number SH0008.

Total genomic DNA was extracted from the blood sample by Ezup blood kit (developed by Shanghai Sango Biotech Company, No.BS18253). Based on closely related species, degenerate primers were designed for PCR-amplification.

The nucleotide sequences and relative PCR primers are shown in Table 1.

PCR was carried out under the following conditions: (1) 95 °C for 5 min (initial denaturation), (2) 94 °C for 30 sec

Table 1. Sequences of primers used in amplification of the complete mitochondrial genome in C. neglectus.

| Number | Primer sequences (5’-3’) | Gene region |
|--------|-------------------------|-------------|
| 1      | 1-F 5’-GCAAGACAGCCTGAAGATGCTCAGTA-3’ | 25-1236 = 1212 |
| 2      | 1-R 5’-GGTGGCATCCTGATAGGCTCAGTA-3’ | 1047-2293 = 1247 |
| 3      | 2-F 5’-GACCTACATCGGAAGATCCTCAATA-3’ | 2092-3402 = 1311 |
| 4      | 2-R 5’-TGTTATGTTGGAATTCTACCTCAGTA-3’ | 3195-4488 = 1294 |
| 5      | 3-F 5’-ATGAGGCTGCTGGAAGATAATGCTCAGTA-3’ | 4288-5630 = 1343 |
| 6      | 3-R 5’-GGCTACCTGCGATGCTCAGTA-3’ | 5444-6716 = 1273 |
| 7      | 4-F 5’-GCCGCTACCTGCGATGCTCAGTA-3’ | 6549-7721 = 1123 |
| 8      | 4-R 5’-CTGCGCTACCTGCGATGCTCAGTA-3’ | 7543-8454 = 912 |
| 9      | 5-F 5’-GGTGGCATCCTGATAGGCTCAGTA-3’ | 8208-9547 = 1340 |
| 10     | 5-R 5’-GGTGGCATCCTGATAGGCTCAGTA-3’ | 9328-10,552 = 1225 |
| 11     | 6-F 5’-CCAGCAATATCACCTACCTTACAGTA-3’ | 10,405-11,710 = 1306 |
| 12     | 6-R 5’-CTAAGCAGCCGATGCTCAGTA-3’ | 11,490-12,703 = 1214 |
| 13     | 7-F 5’-GCCGCTACCTGCGATGCTCAGTA-3’ | 12,511-13,878 = 1368 |
| 14     | 7-R 5’-CTGCGCTACCTGCGATGCTCAGTA-3’ | 13,179 |
| 15     | 8-F 5’-GGTGGCATCCTGATAGGCTCAGTA-3’ | 13,697-14,978 = 1282 |
| 16     | 8-R 5’-CTGCGCTACCTGCGATGCTCAGTA-3’ | 14,798-15,900 = 1102 |
| 17     | 9-F 5’-GGTGGCATCCTGATAGGCTCAGTA-3’ | 15,738-223 |

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(denaturation), (3) 58 °C for 30 sec (annealing), (4) 72 °C for 60 sec (extension), and then repeat (2)–(4) 38 cycles and a final extension at 72 °C for 10 min. Amplified PCR products were examined, purified and sequenced on ABI DNA sequencer (3730XL, America). Annotation was carried out by mitochondrial genome annotation (MITOS) (Bernt et al. 2013), tRNAscan-SE (Chan and Lowe 2019) and GeSeq – Annotation of Organellar Genomes web server (Tillich et al. 2017). Base composition was analyzed by MEGA 7.0 (Kumar et al. 2016).

The phylogenetic position of C. neglectus was assessed by comparison with the mtDNA sequences of different species from the NCBI database. Evolutionary phylogeny analysis was performed by MEGA 7.0 based on Maximum likelihood (ML) method because ML picks out the topology with the maximum likelihood based on good theoretical basis of statistics (Kumar et al. 2016). The phylogeny trees of 14 primates and 17 Cercopithecus species were set up by bootstrap of 500 iterations.

Results

We obtained the complete mtDNA genome of an individual of C. neglectus. The mitogenome was submitted to NCBI GenBank and is available with accession number MW160353.

The complete mitogenome of C. neglectus is 16,490 bp in length. The base composition is A (31.6%), C (29.7%), T (25.8%) and G (12.8%) with 42.5% GC content. The mitogenome is consists of 37 genes [13 protein-coding genes (PCGs), two ribosomal RNAs (rRNA), 22 transfer RNAs (tRNA)]. All tRNAs have the typical cloverleaf structure.

All the 13 protein-coding genes (11,398 bp in length) were predicted by comparing with the previously published

| Gene       | Position | Codons | Start | End   | Anti-condon | Strand | Space/Overlap |
|------------|----------|--------|-------|-------|-------------|--------|---------------|
| tRNA-Phe   | 1        | 70     | 70    | GAA   | +           | 0      |               |
| rrnS       | 135      | 377    | 949   | TAC   | +           | 0      |               |
| rrnL       | 1089     | 2647   | 1559  | TAG   | +           | 0      |               |
| rrnL       | 2648     | 2722   | 75    | TAA   | +           | 1      |               |
| nad1       | 2725     | 3679   | 955   | ATG   | +           | 0      |               |
| trRNA-Ile  | 3680     | 3748   | 69    | CAT   | +           | 3      |               |
| trRNA-Gln  | 3746     | 3817   | 72    | TTG   | +           | 1      |               |
| trRNA-Met  | 3819     | 3886   | 68    | CAT   | +           | 0      |               |
| nad2       | 3887     | 4928   | 1042  | ATC   | TAG         | 0      |               |
| rrnTrp     | 4929     | 4994   | 66    | TCA   | +           | 7      |               |
| trRNA-Ala  | 5002     | 5069   | 68    | TGC   | –           | 1      |               |
| trRNA-Asn  | 5071     | 5143   | 73    | GCT   | +           | 32     |               |
| Tma-Cys    | 5176     | 5242   | 67    | GCA   | –           | 1      |               |
| Tma-Tyr    | 5242     | 5307   | 66    | GTA   | –           | 12     |               |
| cox1       | 5320     | 6888   | 1569  | ATG   | TAA         | –28    |               |
| Tma-Ser    | 6861     | 6929   | 69    | TGA   | –           | 3      |               |
| Tma-Asp    | 6933     | 7000   | 68    | GTC   | +           | 1      |               |
| cox2       | 7002     | 7071   | 64    | TAG   | +           | 23     |               |
| Tma-Lys    | 7709     | 7774   | 66    | TTT   | +           | 2      |               |
| atp8       | 7777     | 7977   | 201   | ATG   | TAA         | –41    |               |
| atp6       | 7937     | 817    | 681   | ATG   | TAA         | +      | –1             |
| cox3       | 8617     | 9400   | 784   | ATG   | T(A)        | +      | 0              |
| Tma-Gly    | 9401     | 9468   | 68    | TCC   | +           | 0      |               |
| nad3       | 9469     | 9812   | 344   | ATT   | T(A)        | +      | 2              |
| Tma-Arg    | 9815     | 9879   | 65    | TCA   | –           | 0      |               |
| nad4       | 9880     | 10,176 | 297   | ATG   | TAA         | +      | 7              |
| nad4       | 10,170   | 11,547 | 1378  | ATG   | T(A)        | +      | 0              |
| Tma-His    | 11,548   | 11,616 | 69    | GTG   | +           | 0      |               |
| Tma-Ser    | 11,617   | 11,675 | 59    | GCT   | +           | 0      |               |
| Tma-Leu    | 11,676   | 11,746 | 71    | TAG   | +           | 6      |               |
| nad5       | 11,753   | 13,555 | 1803  | ATG   | TAA         | +      | 0              |
| nad6       | 13,556   | 14,074 | 519   | ATG   | AGG         | –      | 0              |
| Tma-Glu    | 14,075   | 14,143 | 69    | TTC   | –           | 4      |               |
| cob        | 14,148   | 15,288 | 1141  | ATG   | T(A)        | +      | 0              |
| Tma-Thr    | 15,289   | 15,356 | 68    | GTA   | +           | 2      |               |
| Tma-Pro    | 15,359   | 15,426 | 68    | TGG   | –           | 375    | 509            |
| D-loop     | 15,802   | 15,981 | 180   |       | +           | 509    |               |

Figure 1. Phylogenetic relationship of 13 primate species based on Maximum likelihood model. Genbank accession Number: Homo sapiens (DQ246832.1), Gorilla gorilla (NC001645.1), Hylabates moloch (CM020645.1), Varecia variegata variegata (NC012737.1), Presbytis melalophos mitrata (KY117601.1), Rhinopithecus bieti (HM125579.1), Papio anubis (MG787545.1), Macaca mulatta (NC005943.1), Cercopithecus diana (NC023963.1), Leontopithecus chrysopygus (NC037878.1), Callimico goeldii (NC024628.1), Callithrix jacchus (CM021961.1), Trachypithecus poliocephalus (NC034795.1). Mus musculus (MT410886.1) was set as outgroup taxon.
monkey mitochondrial genome sequences. We found similar gene arrangement and codon usage with other Cercopithecidae mitochondrial genome (Lei et al. 2010; Chang et al. 2016). A total of 10 of them use ATG as the start codon, while ND2, ND3 and ND5 use ATC, ATT and ATA separately. For the stop codon, there are ten protein-coding genes terminated with the typical stop condon of TAA, whereas ND1, ND2 and ND6 terminated with TAG, TAG and AGG (Table 2).

For primates, *C. neglectus* was clustered together with *Papio Anubis* and *Macaca mulatta* with high bootstrap values (Figure 1). Among the Cercopithecus species, *C. neglectus* was close to *C. mona*, *C. pogonias*, *C. wolfi* and *C. denti* (Figure 2). In the phylogeny analyses of guenons species, Chatterjee et al. (2009) reported that *C. neglectus* forms a clade with *C. mona*, *C. hamlyni*, *C. solatus* and several Chlorocebus species. Disotell and Raum (2004) found that *C. neglectus*, *C. pogonias* and *C. mona* were clustered together. Katerina et al. (2013) inferred the evolutionary relationships of all guenon taxa and reported that *C. neglectus* was close to *C. Diana*, *C. mona* and *C. pogonias wolfi* (Katerina et al. 2013). However, all the analyses on *C. neglectus* were based on sub-region or partial mitochondrial genome sequence. In our study, we determined the first complete mitochondrial genome sequence of *C. neglectus* and estimated the phylogeny position of *C. neglectus* with other species. This study will help to better understand the features of *C. neglectus* mitochondrial genome and provide more potential information for further evolutionary relationships within Cercopithecus.

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Disclosure statement

There was no potential conflict of interest in the authors, and the authors alone are responsible for the content of the paper.

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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. MW160353 and available at: https://www.ncbi.nlm.nih.gov/nuccore/MW160353.1/.

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