

## Determination and phylogenetic analysis of the complete mitochondrial genome of *Bubalus bubalis* Linnaeus, 1758 breed Nili-Ravi (Artiodactyla: Bovidae)

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### ABSTRACT

Nili-Ravi buffalo (*Bubalus bubalis* Linnaeus, 1758 breed Nili-Ravi, NRB) is a famous water buffalo breed in the world. It is the first time that the complete mitochondrial genome sequence of the NRB was reported. The total length of the mtDNA is 16,356 bp. It contains the typical structure, including 22 transfer RNA genes, 2 ribosomal RNA genes, 13 protein-coding genes, and 1 non-coding control region (D-loop region). The overall composition of the mtDNA was estimated to be 33.11% for A, 26.45% for T, 26.55% for C, and 13.89% for G. Phylogenetic analyses using neighbor-joining (N-J) computational algorithms showed that the analyzed 18 Ruminantia species are divided into four major clades: Bovidae, Cervidae, Giraffidae, and Atilocapridae. In addition, our work confirmed that NRB has a close genetic relationship with *B. bubalis* isolate India 4.

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Nili-Ravi buffaloes (*Bubalus bubalis* Linnaeus, 1758 breed Nili-Ravi, NRB) are characterized as a tropical/subtropical breed with crude feed tolerance and excellent milk production performance (Hifzulrahman et al. 2019). In this study, we newly determined the complete mitochondrial genome of NRB, and the adult individuals of NRB were collected at its culturing farm in Nanning city (22°90'29.27"N and 108°36'07.38"E), Guangxi Zhuang Nationality Autonomous Region, China on May 2019. And the specimens were stored at -80 °C in the National Buffalo Resources Specimen Library of China (Buffalo Research Institute, Chinese Academy of Agricultural Sciences, and Guangxi Zhuang Nationality Autonomous Region, Nanning, China) with a catalog number of NRB20190501. Total genomic DNA was extracted from the whole blood specimen of a single individual using the EasyPure Kit of Genomic DNA (Transgen Biotech, Beijing, China). The whole mitochondrial genome was amplified with 11 pairs of primers and sequenced by BioSune Biotech (Shanghai, China). The DNA sequence was analyzed using DNASTar.Lasergene version 7.1 software (Madison, WI), tRNA Scan-SE1 Version 21 software (Lowe and Eddy 1997), and DOGMA software (Wyman et al. 2004).

The total length of the mtDNA is 16,356 bp. It contains the typical structure, including 22 transfer RNA genes, 2 ribosomal RNA genes, 13 protein-coding genes, and 1 D-loop region (GenBank accession No. MN481528). The overall composition of the mtDNA was estimated to be 33.11% for A, 26.45% for T, 26.55% for C, and 13.89% for G, and the percentage of A and T (59.56%) was higher than that of G and C

(40.44%). Besides the ND4L initiation codon is GTG, and ND2, ND3, and ND5 are ATA, the rest of the proteins are ATG. All these genes have 14 spaces and 12 overlaps both in the length of 1–40 bp. These genes had four types of termination codons, including TAA, TAG, AGA, and an incomplete termination codon 'T--'. 'T--' is the 5' terminal of the adjacent gene (Anderson et al. 1981). Among 13 protein-coding genes, the longest one was the ND5 gene (1830 bp), which was located between the tRNA<sup>Leu</sup> and ND6, and the shortest one was ATPase8 gene (201 bp), which was located between the tRNA<sup>Lys</sup> and ATPase6. The lengths of 12S rRNA and the 16S rRNA were 957 and 1568 bp. And deduced 22 tRNA genes were distributed in rRNA and protein-coding genes, ranging from 60 to 75 bp in size. The mitochondrial DNA D-loop region of the NRB was located between tRNA<sup>Pro</sup> and tRNA<sup>Phe</sup> with a length of 925 bp.

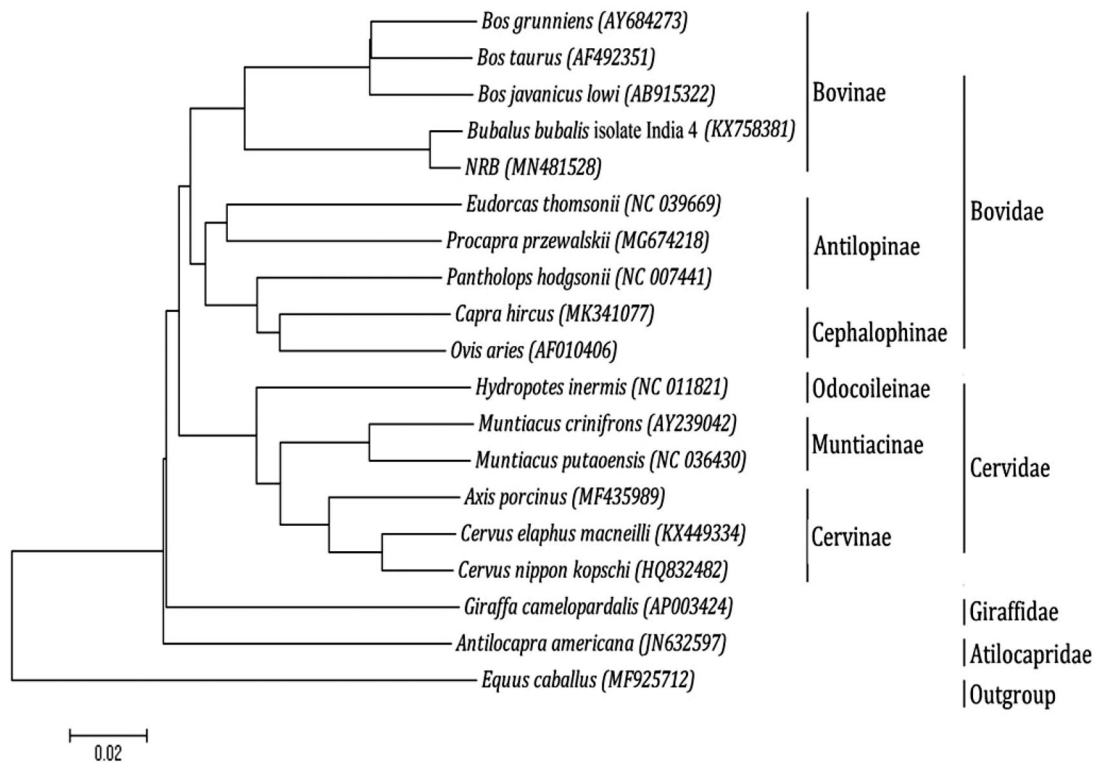
Phylogenetic analysis was performed using the complete mitochondrial DNA sequences of 18 Ruminantia species. Each of the sequence datasets was aligned by ClustalX (Thompson et al. 1997) and analyzed by neighbor-joining (N-J) in MEGA version 4.0 (Tempe, USA) (Tamura et al. 2007), and bootstrap analysis was performed with 100 replications. An N-J tree showed that the analyzed species are divided into four major clades (Figure 1). Bovidae makes up the first lineage, which is sister to the second group, Cervidae; Giraffidae forms the third group and is sister to Bovidae and Cervidae. The lineage consisting of these three groups, in turn, is sister to the fourth clade, Atilocapridae. In addition, our work confirmed that NRB has a close genetic relationship with *B. bubalis*

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**Figure 1.** Phylogenetic analysis based on complete mitochondrial genome sequences. An N-J tree was built based on the phylogenetic analysis of 18 Ruminantia species' complete mitochondrial genomes. The mitochondrial genome sequences of the Ruminantia species were obtained from the GenBank databases (Accession numbers have marked on the figure). Abbreviation of species indicates: NRB: Nili-Ravi buffalo.

isolate India 4 which is consistent with those reported previously (Hussain et al. 2017).

## Disclosure statement

No potential conflict of interest was reported by the authors.

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