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MITOGENOME ANNOUNCEMENT

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

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ABSTRACT

Murrah buffalo (*Bubalus bubalis* Linnaeus, 1758 breed Murrah, MB) is a most productive water buffalo breed. It is the first time that the complete mitochondrial genome sequence of the MB was reported. The total length of the mtDNA is 16,359 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 33.00% for A, 26.32% for T, 26.71% for C and 13.97% for G. Phylogenetic analyses using 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 MB and *Bubalus bubalis* isolate India 4 have a close genetic relationship.

Murrah buffaloes (*Bubalus bubalis* Linnaeus, 1758 breed Murrah, MB) are characterized as a tropical/subtropical breed with excellent milk production performance, heat tolerance and diseases resistance (Shaari et al. 2019). In this study, we newly determined the complete mitochondrial genome of MB, and the specimens were collected from the adult individuals of MB 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 MB20190501. 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). Whole mitochondrial genome was amplified with 11 pairs of primers and sequenced by BioSune Biotech (Shanghai, China). DNA sequence was analyzed using DNAStar.Lasergene.v7.1 software (Madison, WI), tRNA Scan-SE1.21 software (Lowe and Eddy 1997) and DOGMA software (Wyman et al. 2004).

The total length of the mtDNA is 16,359 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) (GenBank accession No. MN488587). The overall composition of the mtDNA was estimated to be 33.00% for A, 26.32% for T, 26.71% for C and 13.97% for G, and the percentage of A and T (59.32%) was higher than that of G and C (40.68%). All the protein initiation codons are ATG, except for ND2, ND3 and ND5 are ATA. All these genes have 13 spaces and 11 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 ND5 gene (1821 bp), which was located between the tRNA^{Leu} and ND6, and the shortest one was ATPase8 gene (201 bp), which was located between the tRNA^{Lys} and ATPase6. The lengths of 12S rRNA and the 16S rRNA were 957 bp and 1569 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 MB was located between tRNA^{Pro} and tRNA^{Phe} with a length of 928 bp.

Phylogenetic analysis was performed using the complete mitochondrial DNA sequences of 18 Ruminantia species. Each of the sequence dataset was aligned by ClustalX (Thompson et al. 1997) and analyzed by neighbor-joining (N-J) in MEGA 4.0 (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
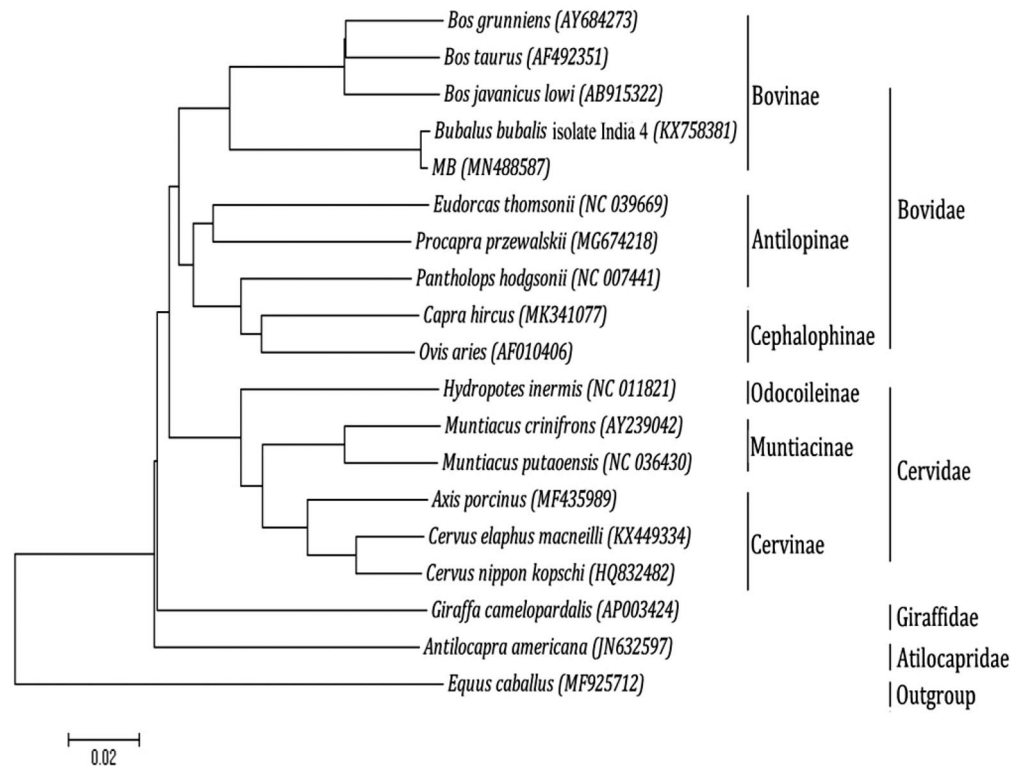
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Figure 1. Phylogenetic analysis based on complete mitochondrial genome sequences. An N-J tree was built based on the phylogenetic analysis of 18 Ruminantia species' complete mitochondrial genomes. The mitochondrial genome sequences of the Ruminantia species were obtained from the GenBank databases (Accession numbers have marked on the figure). Abbreviation of species indicates: MB: Murrah buffalo.

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 MB have a close genetic relationship with *Bubalus bubalis* isolate India 4.

Disclosure statement

No potential conflict of interest was reported by the authors.

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