



Mitogenome Analyses of Water Buffalo: Closeness of the Genetic Architecture of River Buffalo and Wild Buffalo (*Bubalus arnee*) Excludes Swamp Buffalo



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WATER buffalo species belongs to genus *Bubalus* and involves the wild and the domestic types. Domestic water buffaloes are reclassified into river and swamp types. Here, we have reconstructed the phylogenetic relationship between members of water buffalo species based on mitogenome sequences. The phylogenetic tree included lowland anoa, mountain anoa in addition to wild, river and swamp water buffalo types. Results indicated the paraphyly of water buffalo species since lowland anoa and mountain anoa were clustered with river and wild water buffalo in a separate clade, leaving swamp buffalo at the outside. Results also indicated close genetic architecture between the mitogenomes of river and wild water buffalo types. These findings may indicate that river buffalo and swamp buffalo belong to two distinct species and that river buffalo and not swamp buffalo is a descendant of wild water buffalo. Mitogenome analysis provided more information about the taxonomic status of water buffalo types and verified their relationship with the members of genus *Bubalus*.

Keywords: Wild buffalo, River buffalo, Swamp buffalo, Mitogenome sequence, Phylogeny.

Introduction

Water buffalo species belongs to genus *Bubalus* and involves the wild water buffalo type (*Bubalus arnee*) and the domestic water buffalo type (*Bubalus bubalis*). The domestic water buffaloes are reclassified into river and swamp types [1]. It was assumed that both river and swamp types descended from divergent wild water buffalo populations and then evolved in separate geographical regions [2]. After domestication in the western region of the Indian subcontinent, the river buffalo has spread west as far as Egypt, the Balkans, Greece and Italy. Swamp buffalo was domesticated in the China/ Indochina border region and dispersed through south-east Asia and

China as far as the Yangtze River valley [2].

River and swamp buffaloes differ in morphology, behavior and number of chromosomes [3]. It was also proven that the two types are also genetically distinct, using autosomal and sex-linked DNA markers [4,5,6,7,8,9], partial and complete mitochondrial (mt) DNA sequences [9, 10,11,12,13] and SNP markers [14]. Therefore, the taxonomic status of the domestic water buffalo types is still debated and they were considered as two subspecies by some authors [10,11] or different species by others [9,13-15].

With the recent availability of the wild water buffalo (*Bubalus arnee*) mitogenome

sequence [16], we reconstructed the phylogenetic relationship between the three water buffalo types and the other members of genus *Bubalus* based on the whole mitogenome sequence. The phylogenetic tree included: lowland anoa (*Bubalus depressicornis*), mountain anoa (*Bubalus quarlesi*) in addition to the wild, river and swamp water buffalo types.

Materials and Methods

The present study was based on 10 Egyptian river buffalo mitogenome sequences that we have submitted previously to GenBank[13] in addition to 21 mitogenomes selected from the databases and representing all the species of the genus *bubalus* except tamaraw species (*Bubalus mindorensis*). The 21 mitogenomes included 11 swamp buffaloes, 5 river buffaloes, 3 wild buffaloes, 1 lowland anoa and 1 mountain anoa.

Alignment of the mitogenome sequences and maximum-likelihood (ML) phylogenetic tree

reconstruction were performed using the function “build” of ETE3 v3.1.1 [17] as implemented on the GenomeNet (<https://www.genome.jp/tools/ete/>). The tree was constructed using FastTree with slow NNI and MLACC=3 (to make the maximum-likelihood NNIs more exhaustive) [18]. African buffalo (*Syncerus caffer*) mitogenome sequence was used as an outgroup. GenBank accession numbers of all mitogenomes are in the Fig.1.

Results

The ML phylogenetic tree that based on the mitogenomes of *Bubalus* members and was rooted with the African buffalo is shown in Fig.1. The phylogenetic analysis showed two main clades. The swamp buffalo samples were segregated independently in a separate clade. The second clade was rebranched into two subclades: one subclade included the wild water buffalo and the river buffalo samples. The second subclade

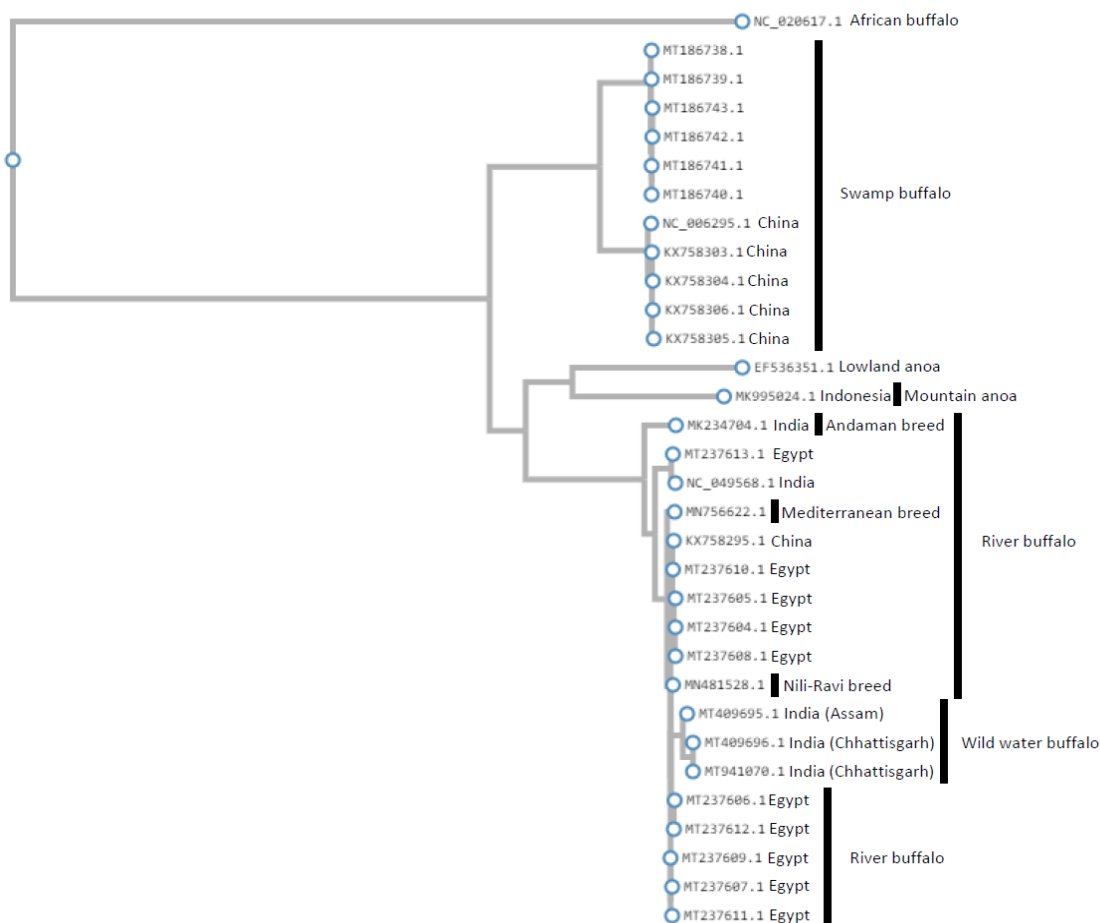


Fig. 1. Phylogeny of genus *Bubalus* based on complete mitochondrial genomes. The tree was rooted with African buffalo.

included the two species, lowland anoa and mountain anoa.

Discussion

Interest in the taxonomic status of the water buffalo types, and their relationship with other members of genus *Bubalus*, except for tamaraw species (*Bubalus mindorensis*) since no mitogenome is yet available, has increased with the recent availability of the wild water buffalo mitogenome sequence.

Results of the mitogenome-based phylogenetic study indicated the paraphyly of the water buffalo species since lowland anoa and mountain anoa were clustered with river and wild water buffalo in a separate clade, leaving the swamp buffalo at the outside. Results also indicated close genetic architecture between the mitogenomes of river and wild water buffalo types as they segregated together in a separate subclade. These findings may indicate that river buffalo and swamp buffalo belong to two distinct species and that river buffalo and not the swamp buffalo is a descendant of wild water buffalo (*Bubalus arnae*). They also confirm previous reports which suggested that the river buffalo and swamp buffalo belong to distinct species [9,13-15]. However, additional investigations are needed including nuclear markers to validate the hypothesis of the origination of river buffalo and not swamp buffalo from wild water buffalo (*Bubalus arnae*).

Acknowledgment

None

Funding statement

This research was supported by PHC IMHOTEP 2018 (project No. 39515UM).

Conflict of interest

Authors declare no conflict of interest

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تحليلات تسلسل الجينوم الميتوكوندري للجاموس المائي: تقارب البناء الوراثي في الجاموس النهري والجاموس البري (*Bubalus arnee*) استبعد جاموس المستنقعات.

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تنتمي أنواع جاموس الماء إلى جنس بوبالس (*Bubalus*) وتشمل نوع جاموس الماء البري ونوع جاموس الماء المستأنس (الجاموس النهري وجاموس المستنقعات). وفي هذه الدراسة تمت إعادة بناء العلاقة التطورية بين أعضاء أنواع جاموس الماء (أنواع جاموس الماء البرية والنهرية والمستنقعات) بناءً على تسلسل جينوم الميتوكوندريا. تضمنت شجرة النشوء والتطور أنواع جاموس المياه البرية والنهرية والمستنقعات بالإضافة إلى نوعين آخرين من نفس الجنس (أنواع الأراضي المنخفضة وأنواع الجبال). دلت النتائج على تشابه أنواع جاموس الماء البرية والنهرية مع أنواع الأراضي المنخفضة وأنواع الجبال في كلد منفصل، تاركين جاموس المستنقعات في الخارج. أشارت النتائج أيضاً إلى بنية وراثية وثيقة بين جينوم الميتوكوندريا في جاموس المياه البري والنهري. قد تشير هذه النتائج إلى أن جاموس الأنهار وجاموس المستنقعات ينتميان إلى نوعين مختلفين وأن جاموس الأنهار وليس جاموس المستنقعات هو سليل لجاموس الماء البري. قدمت الدراسة مزيداً من المعلومات حول الحالة التصنيفية لأنواع جاموس الماء والتحقق من علاقتها بأعضاء جنس *Bubalus*.