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The Phylogenetic Relationships within the Tribe Bovini (Bovidae: Bovinae) Using Mitochondrial Genome

Taghi Ghassemi-Khademi*[1], Seyed Massoud Madjdzadeh[2], Rasoul Khosravi[3] and Asadollah Asadi[4]

[1] Department of Biology, Faculty of Sciences, Shiraz University, Shiraz, Iran
[2] Department of Biology, Faculty of Sciences, Shahid Bahonar University of Kerman, Kerman, Iran
[3] Department of Natural Resources and Environmental Engineering, School of Agriculture, Shiraz University, Shiraz, Iran
[4] Department of Biology, Faculty of Science, University of Mohaghegh Ardabili, Ardabil, Iran

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Corresponding authors:
✉ T. Ghassemi-Khademi
t.ghassemi@shirazu.ac.ir

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ABSTRACT

Molecular data are powerful tools to resolve taxonomic problems. Each gene in each taxon shows a degree of variation through which we can understand phylogenetic relationships among different taxa. In this survey, the phylogenetic relationships within the tribe Bovini were reevaluated using 24 mitogenomes and cytochrome b (*cytb*), cytochrome *c* oxidase subunit 1 (*cox1*), 16S ribosomal RNA (*16S rRNA*), and NADH dehydrogenase subunit I (*ND1*) mitochondrial markers. We used all the gene sequences of extinct, domesticated, and wild species within the tribe Bovini. The phylogenetic trees were reconstructed using the maximum likelihood (ML) method. Based on the mitogenomes, the average base composition of mtDNA sequences was 27.1% T, 26% C, 33.5% A, and 13.4% G, showing a strong AT bias (60.6%). Our results revealed that the genus *bison* is not an independent taxon in the taxonomic rank of the genus and it is completely a paraphyletic taxon. Saola (*Pseudoryx nghetinhensis*) showed a sister relationship with other species belonging to the subtribe Bovina and it might be better to place this species within the subtribe Bovina. Also, in the mentioned subtribe, we distinguished three distinct monophyletic groups. In all of the phylogenetic trees, the subtribe Bubalina was a monophyletic taxon, and *Syncerus caffer* had a sister group relationship with other species belonging to the genus *Bubalus*. The obtained data should be taken into consideration in future conservation efforts for this tribe.

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Introduction

The animal cells have an organelle named "mitochondria", which, with minor exceptions contains the same genes. There are 13 protein-coding genes, 22 transfer RNA or *tRNA* genes, two ribosomal RNA or rRNA genes, and one noncoding control region (Li *et al*., 2020; Lin *et al*., 2020) within the mitochondria. Therefore, the comparison of the same genes carries out across many different animal taxa. A comparison of the mtDNA genes can provide valuable information on the species evolution and their phylogenetic relationships. Another advantage of mtDNA is the compilation of a large comparative database, built up as researchers have sequenced mitochondrial genes in a variety of organisms (Smith, 2011; Colagar *et al*., 2013; Colagar and Karimi, 2014; Abdilzadeh *et al.,* 2019).

The subfamily Bovinae includes three tribes including Bovini (cattle and Buffalo), Tragelaphini (spiral-horned antelopes), and Boselaphini (Nowak, 1999; Castelló, 2016). Among these tribes, the tribe Bovini has played an important role in human cultural evolution

(Castelló, 2016). At least, five species of this tribe have been domesticated for livelihood purposes during the Holocene (Hassanin, 2014). The tribe Bovini has evolved from a Boselaphine ancestral stock in the Indian subcontinent, and then they distributed to Africa and Eurasia, then to North America. Three divergent lineages have been identified within this tribe including Bovina (Cattle and Bison), Bubalina (Buffalo), and Pseudorygina (only represented by the recently discovered Saola) which may have been diversified approximately 13 My, during the late Middle Miocene (Castelló, 2016).

The genus *Bos* consists of the following species: Aurochs (*Bos primigenius*), Gaur (*Bos gaurus*), Banteng (*Bos javanicus*), Kouprey (*Bos sauveli*), European bison (*Bos bonasus*), Caucasian bison (*Bos caucasicus*), American bison (*Bos bison*), and Yak (*Bos mutus*; Groves and Grubb, 2011). This genus also has four domesticated species including Gayal (*Bos frontalis*), Domestic Yak (*Bos grunniens*), European Cattle (*Bos taurus*), Sanga Cattle (*Bos taurus africanus*), and Zebu (*Bos indicus*; Castelló, 2016).

The genus *Bubalus* consists of the following species: Asian wild buffalo (*Bubalus arnee*), tamaraw (*Bubalus mindorensis*), lowland anoa (*Bubalus depressicornis*), mountain anoa (*Bubalus quarlesi*; Groves and Grubb, 2011), and two domesticated species including domestic swamp buffalo (*Bubalus bubalis kerabau)* and domesticated river buffalo (*Bubalus bubalis* bubalis; Castelló, 2016). The genus *Syncerus* consists of Cape buffalo (*Syncerus caffer*), Lake Chad buffalo (*Syncerus brachyceros*), Virunga buffalo (*Syncerus mathewsi*), and forest buffalo (*Syncerus nanus*; Groves and Grubb, 2011). Moreover, the saola (*Pseudoryx nghetinhensis*) is another species within the tribe Bovini (Groves and Grubb, 2011). Several lines of evidence suggested the incorporation of the genera *Bos* and *Bison* into a single genus, *Bos* (Yang *et al*., 2013; Fernández and Vrba, 2005), and some valid studies have followed this suggestion (Groves and Grubb, 2011; Castelló, 2016).

Although in recent years several studies have investigated the phylogenetic relationships within the tribe Bovini using different molecular markers (e.g. Groves, 1981; Hartl *et al*., 1988; Miyamoto *et al*., 1989; Janecek *et al*., 1996; Ritz *et al*., 2000; Geraads, 1992; Matthee and Davis, 2001; Buntjer *et al*., 2002; Wall *et al*., 1992; Prusak *et al*., 2004; Hassanin and Ropiquet, 2004; Li *et al*., 2008; MacEachern *et al*., 2009a, b; Xuan *et al*., 2010; Yang *et al*., 2013; Bibi, 2013; Hassanin *et al*., 2012, 2013), a comprehensive re-evaluation study has not been carried out on the phylogenetic relationships within this tribe using different mitochondrial genes. In the above-mentioned studies (e.g. Hassanin *et al*., 2012; Yang *et al*., 2013) limited numbers of mitogenomes have been used to study the phylogenetic relationships within this tribe. However, today there are a fairly complete collection of whole-genome sequences of the mitochondrial DNA and multiple sequences (especially complete genome of mtDNA) which can provide sufficient information about the evolution and evolutionary process reconstruction (Ghassemi-Khademi, 2017; Ghassemi-Khademi and Hamidi, 2019; Ghassemi-Khademi and Madjdzadeh, 2019) and their results are very close to the reality.

Materials and Methods

All gene sequences including complete mitochondrial genome (n=24; Table 1), cytochrome b (*cytb*; n=49; Table 2), cytochrome *c* oxidase subunit 1 (*cox1*; n=43; Table 3), 16S ribosomal RNA (or *16S rRNA*; n=36; Table 4), NADH dehydrogenase subunit I (or *ND1*; n=23; Table 5) belonging to the tribe Bovini were downloaded from NCBI.

The titles of the received sequences were edited by ExcaliBAR (Aliabadian *et al*., 2014). BioEdit 7.0.5.3 (Hall, 1999) was used to create a DNA sequence alignment using the Clustal W algorithm (Thompson *et al*., 1994) in all of the received sequences. The corresponding gene sequences of *Gazella subgutturosa* [JN376044.1 (*16S rRNA*); KX859267.1 (*cox1*); JN632643.1 (mitogenomes)], *Pelea capreolus* [AF022055.1 (*cytb*)], *Tragelaphus oryx* [JN632704.1 (*ND1*)], and *Oreotragus oreotragus* [JN645583.1 (*cox1*)] were used as outgroups in the analyses.

The best-fit nucleotide substitution model for phylogenetic analysis was determined by Bayesian Information Criterion (BIC; Schwarz, 1978) using ModelTest (Posada and Crandall, 1998) in the MEGA6.06 (Tamura *et al*., 2013). The model with the lowest BIC was considered

to best describe the substitution pattern. The evolutionary history was inferred using the Maximum Likelihood method "ML" for each gene, separately. The trees were reconstructed using the highest log-likelihood. In all of the phylogenetic trees, the percentage of replicate trees, in which the associated taxa are clustered together in the bootstrap test (10,000 replicates for mitogenomes and 1000 replicates for other genes), shown next to the branches (Felsenstein, 1985). In all analyses, all positions containing gaps and missing data were eliminated using RAxML-7.0.4 (Stamatakis, 2006) for mitogenomes and MEGA6 (Tamura *et al*., 2013) for other genes. The robustness of clades was calculated by the bootstrap method, such that 50-60% was considered as weak support (as bootstrap values), 61-75% as moderate support, 76-88% as good support, and ≥89% as strong support (see Win *et al*., 2017; with minor modification). In addition to MEGA6, phylogenetic trees were constructed using Bayesian inference in MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003) under the most generalizing model (GTR+ G+ I). The Bayesian inference of phylogeny was conducted for 8×10^6 generations. The obtained phylogenetic trees were visualized and edited by FigTree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

Since all of the species belonging to a single genus were considered as a separate group, five groups were determined and pair fixation indices (F_{ST}) among these groups were calculated based on mitogenomes using Arlequin 3.5 (Excoffier *et al*., 2005). Referring to the criterion for genetic differentiation by Wright (1984), genetic differentiation was defined as low for $F_{ST}<0.05$, moderate for $0.05<F_{ST}<0.15$, high for $0.15<F_{ST}<0.25$, and very high for $F_{ST}>0.25$.

Moreover, evolutionary divergence over sequence pairs between groups (different genera) also was calculated in MEGA6 (Tamura *et al*., 2013). The variance estimation method was bootstrap with 1000 replications. The rate of variation among sites was modeled with a gamma distribution (shape parameter = 1). Codon positions included were 1^{st}+ 2^{nd}+ 3^{rd}+ Noncoding.

Table 1. The list of samples (in alphabetical order) with GenBank accession number. The nucleotide composition of the complete mitochondrial genome is given (n=24).

Taxon	GenBank No.	T	C	A	G	Total	Ref.
Bison bison	EU177871.1	27.2	26.0	33.7	13.2	16319.0	Achilli *et al*. (2008)
Bison bonasus	KY055664.1	27.2	25.9	33.7	13.2	16326.0	Węcek *et al*. (2016)
†***Bison priscus***	KM593920.1	27.2	25.9	33.7	13.2	16318.0	Marsolier-Kergoat *et al*. (2015)
†***Bison schoetensacki***	KU886087.1	27.1	26.0	33.6	13.3	16325.0	Palacio *et al*. (2017)
Bos frontalis	MF614103.1	27.1	26.0	33.6	13.3	16346.0	Wang and Yan, 2017
Bos gaurus	NC_024818.1	27.1	26.0	33.5	13.3	16345.0	Hassanin *et al*. (2012)
	JN632604.1	27.1	26.0	33.5	13.3	16345.0	Hassanin *et al*. (2012)
Bos grunniens	JQ692071.1	27.2	25.8	33.7	13.2	16323.0	Qiu *et al*. (2012)
Bos indicus	MF667932.1	27.1	26.0	33.5	13.4	16320.0	Meethal *et al*. (2017)
	MF667931.1	27.2	26.0	33.5	13.4	16320.0	Meethal *et al*. (2017)
	KX575711.1	27.1	26.0	33.4	13.4	16339.0	Srirattana *et al*. (2017)
	AF492350.1	27.1	26.0	33.4	13.4	16339.0	Hiendleder *et al*. (2008)
	MF667930.1	27.1	26.0	33.5	13.4	16320.0	Meethal *et al*. (2017)
	AY126697.1	27.1	26.0	33.5	13.4	16341.0	Miretti *et al*. (2002)
	MF667929.1	27.1	26.0	33.5	13.4	16333.0	Meethal *et al*. (2017)
Bos javanicus	JN632605.1	27.0	26.1	33.5	13.4	16345.0	Hassanin *et al*. (2012)
Bos mutus	KR106993.1	27.3	25.8	33.7	13.2	16322.0	Chunnian *et al*. (2016)
†***Bos primigenius***	NC_013996.1	27.2	26.0	33.4	13.4	16337.0	Edwards *et al*. (2010)
	JQ437479.1	27.2	26.0	33.4	13.5	16338.0	Lipinski *et al*. (2012)
Bos taurus	V00654.1	27.2	25.9	33.4	13.5	16338.0	Anderson *et al*. (1982)
Bubalus bubalis	NC_006295.1	26.3	26.7	33.0	14.0	16359.0	Qian *et al*. (2004)
Bubalus depressicornis	EF536351.1	26.4	26.6	33.1	13.9	16355.0	Hassanin *et al*. (2012)
Pseudoryx nghetinhensis	EF536352.1	27.6	25.4	34.1	12.9	16358.0	Hassanin *et al*. (2012)
Syncerus caffer	EF536353.1	26.7	26.3	33.5	13.5	16359.0	Hassanin *et al*. (2012)
Avg.		27.1	26.0	33.5	13.4	16336.3	

† extinct taxa

Table 2. The list of cytochrome b (*cytb*) gene samples (in alphabetical order) with GenBank accession number (n=49).

Taxon	GenBank No.	Taxon	GenBank No.
Bison bison	AF036273.1	*Bos grunniens*	AY374124.1
Bison bonasus	KP866277.1		AF091631.1
Bison bonasus	KP866276.1		AB542192.1
	KP866275.1	*Bos indicus*	EF061244.1
	KP866273.1		EF061242.1
	KP866272.1		EF061241.1
	KP866269.1		EF693799.1
	KP866265.1		JN117615.1
	KP866261.1		JN117611.1
	KP866260.1	*Bos mutus*	KM280688.1
	KP866259.1		KM280687.1
Bison priscus	KM593920.1		KM280686.1
	NC_027233.1	*Bos primigenius*	NC_013996.1
	KX269111.1	*Bos sauveli*	AY689189.1
	KX269110.1	*Bos Taurus*	GU249570.1
Bos frontalis	EF061233.1		GU249569.1
	EF061229.1		GU249566.1
	EF061227.1	*Bubalus arnee*	D32193.1
	EU807956.1	*Bubalus depressicornis*	AF091632.1
	JQ404407.1	*Bubalus mindorensis*	D82895.1
	AY689187.1	*Bubalus quarlesi*	D82891.1
	EF685911.1	*Pseudoryx nghetinhensis*	AF091635.1
Bos gaurus	DQ459331.1		NC_020616.1
	DQ459330.1		EF536352.1
		Syncerus caffer	AF036275.1

Table 3. The list of cytochrome c oxidase I (*cox1*) gene samples (in alphabetical order) with GenBank accession number (n=43).

Taxon	GenBank No.	Taxon	GenBank No.
Bison bison	JF443195.1	*Bos javanicus*	JN632606.1
	JF443194.1	*Bos mutus*	KY829451.1
	JF443193.1	*Bos primigenius*	NC_013996.1
	JF443192.1	*Bos taurus*	HQ860420.1
	JF443191.1		HM102289.1
	JF443190.1		JF700141.1
Bison bonasus	JF444283.1		JF700140.1
	EU623450.1		KX859287.1
Bison priscus	KM593920.1		GU130590.1
Bos frontalis	HQ269429.1		GU130589.1
Bos gaurus	KF808255.1		FJ958336.1
Bos grunniens	HQ269465.1		FJ958334.1
	HQ269463.1	*Bubalus bubalis*	NC_006295.1
	HQ269462.1	*Bubalus depressicornis*	NC_020615.1
			EF536351.1
	HQ269433.1	*Pseudoryx nghetinhensis*	NC_020616.1
	HQ269432.1		EF536352.1
Bos indicus	KF952284.2	*Syncerus caffer*	JN082178.1
	KF952282.2		KF482455.1
	KF952281.2		KJ192911.1
	KF952278.2		
	KF952277.2		
	KF952276.2		
	KF952273.2		

Table 4. The list of *16S rRNA* gene samples (in alphabetical order) with GenBank accession number (n=36).

Taxon	GenBank AC No.	Taxon	GenBank No.
Bison bison	DQ318383.1	*Bubalus bubalis*	FJ748607.1
Bison priscus	KM593920.1		FJ748605.1
	NC_027233.1		FJ748601.1
Bos frontalis	MF959941.1		KT375471.1
Bos gaurus	JN714142.1		KT375478.1
	JN714141.1		KT375493.1
	JN714140.1		KT375466.1
Bos grunniens	KR677383.1		KT375463.1
	KT827215.1		DQ867009.1
	KT827193.1		DQ904379.1
	EU910139.1		NC_006295.1
Bos javanicus	JN632606.1	*Bubalus depressicornis*	U87062.1
Bos mutus	NC_025563.1	*Pseudoryx nghetinhensis*	NC_020616.1
	KM233417.1		EF536352.1
Bos primigenius	JQ437479.1	*Syncerus caffer*	KJ193206.1
	GU985279.1		U87061.1
	NC_013996.1		JQ235547.1
Bos Taurus	KF163089.1		JQ235546.1

Table 5. The list of NADH dehydrogenase subunit I (*ND1*) gene samples (in alphabetical order) with GenBank accession number (n=23).

Taxon	GenBank AC No.	Taxon	GenBank No.
Bison bison	GU947006.1	*Bos primigenius*	GU985279.1
	GU947002.1		MF169212.1
Bison priscus	KM593920.1	*Bos taurus*	AF493542.1
	KX269144.1	*Bubalus bubalis*	KY607431.1
Bos frontalis	MF959941.1	*Bubalus depressicornis*	NC_020615.1
Bos gaurus	NC_024818.1		EF536351.1
	JN632604.1	*Pseudoryx nghetinhensis*	AY576932.1
Bos grunniens	KR011113.1		NC_020616.1
Bos indicus	MF667929.1	*Syncerus caffer*	JQ235505.1
Bos javanicus	AB915322.1		EF536353.1
Bos mutus	KM233417.1		
	NC_025563.1		
	KR106993.1		

Results

In most of the phylogenetic trees, all species in the analysis were clustered together and outgroups were separated from the ingroup members (Figs. 1-5) implying the presence of relatively close genetic distances within the tribe. The average length of the mitochondrial genome was calculated as 16336.3 bp. In 16336.3, the average base composition of mtDNA sequences was 27.1% T, 26% C, 33.5% A, and 13.4% G, showing a strong AT bias (60.6%).

Besides, in all of the phylogenetic trees, the BI posterior probability values of the taxa were equal to 100; thus, we can infer that the tribe Bovini is a monophyletic group with the highest BI posterior probability value. Also, based on the lowest BIC, the Tamura 3-parameter model (Tamura, 1992) was chosen as the best nucleotide substitution model for ML trees of *16S rRNA* genes. Hasegawa-Kishino-Yano (HKY+G; Hasegawa *et al*., 1985) was the best model for cytochrome b and NADH dehydrogenase subunit I (*ND1*) genes, and (HKY+G+I) for cytochrome c oxidase I genes. Finally, the GTR+G model was the best model for complete mitochondrial genomes.

Based on the topology of the ML phylogenetic tree of *16S rRNA* sequences, the relationship of different species belonging to the tribe Bovini is shown in Fig. 1.

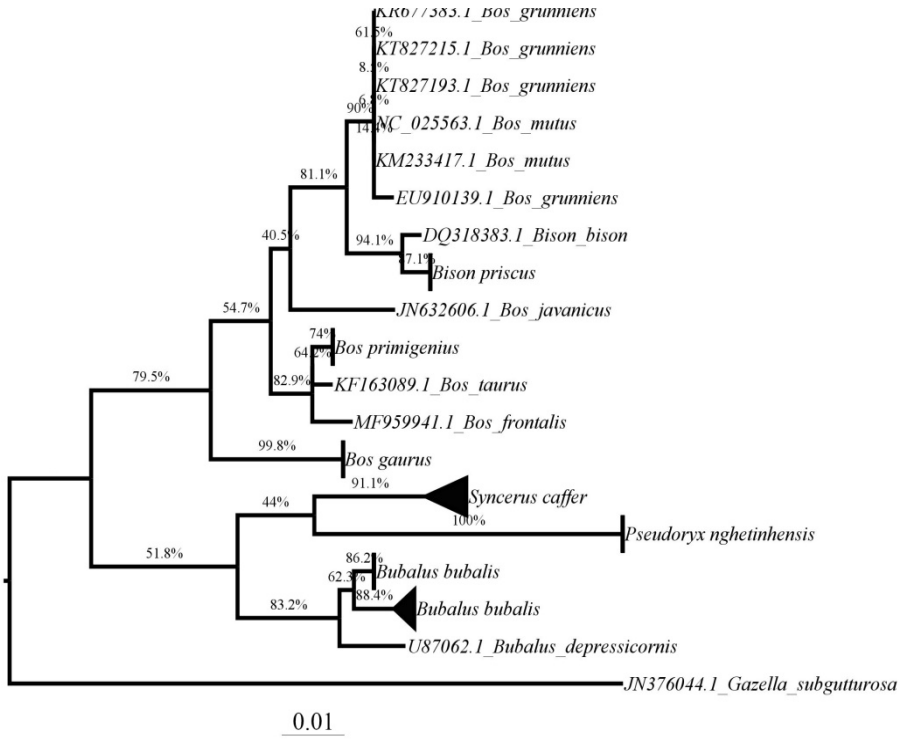

Fig. 1. Maximum Likelihood tree based on the Tamura 3-parameter distance using 16S ribosomal RNA (*16S rRNA*) sequences; the numbers on each branch correspond to the bootstrap support. (percentages lower than 50 are not shown). The tree was rooted with *G. subgutturosa*; [{(*Bubalus bubalis*+*Bu. depressicornis*)+ (*Pseudoryx nghetinhensis*+ *Syncerus caffer*)}+ {*Bos gaurus*+ ((*B. primigenius*+*B. taurus*+*B. frontalis*)+ ((*B. javanicus*)+ (*Bison priscus*+ *Bi. bison*)+ *B. grunniens*+ *B. mutus*))}].

In the obtained tree, we can distinguish two distinct major clusters. The results showed that all species belonging to the genera *Syncerus*, *Pseudoryx*, and *Bubalus* have weak (but acceptable) bootstrap support (=51.8) and moderate posterior probability (=73.32) values. In other clusters, the species within *Bos* and *Bison* showed a monophyletic group with a very strong posterior probability (=99.99) and good bootstrap support (=79.5) value.

Furthermore, based on the topology of the ML phylogenetic tree of *ND1* sequences, the relationship of different genera belonging to the tribe Bovini is shown in Fig. 2.

In this phylogenetic tree, we can distinguish two distinct major clusters, where the sequences of *Syncerus* and *Bubalus* species showed moderate bootstrap support (74.3) and very high BI posterior probability (99.51). In another cluster, all species of two genera, *Bos* and *Bison* form a monophyletic group with strong ML bootstrap (99.1). However, the topology of the Bayesian phylogenetic tree was different from that of the topology of the ML phylogenetic tree. In the ML tree, *P. nghetinhensis* nested as a sister taxon of the two mentioned clusters, while in the Bayesian tree, this species is the sister with a cluster including two genera *Bos* and *Bison*; with moderate BI posterior probability (61.76).

The relationship of the ML phylogenetic tree of cytochrome b (*cytb*) is shown in Fig. 3.

The tree obtained from this method formed two distinct major clusters. All species of *Bos*, *Bison*, and *P. nghetinhensis* formed a single monophyletic group with moderate bootstrap support (71.7) and strong BI posterior probability (91.2). In other cluster, the sequences belonging to the species of *Syncerus* and *Bubalus* constructed a single cluster with well supported ML bootstrap (79.33) and the highest BI posterior probability (100).

The relationship of species based on the ML method with cytochrome c oxidase I (*cox1*) sequences is shown in Fig. 4.

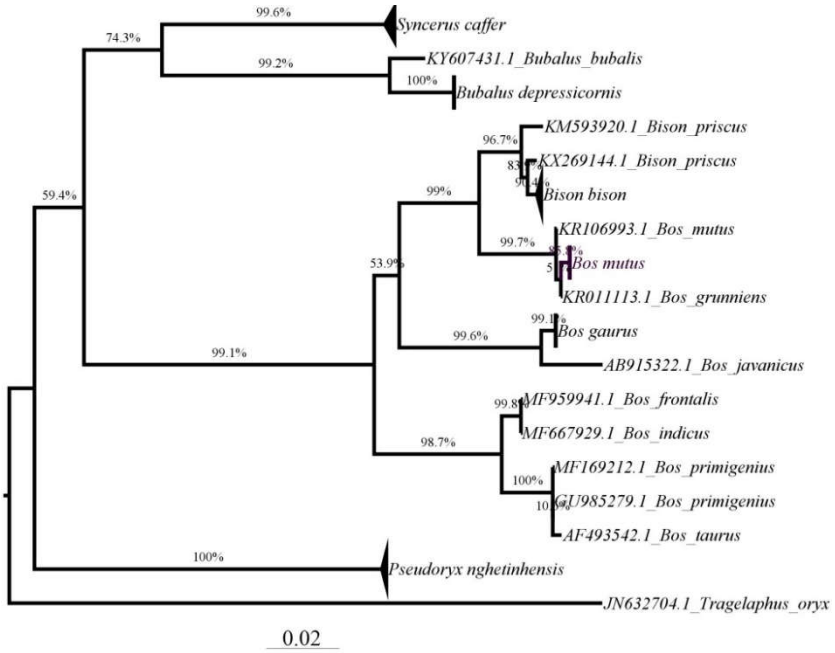

Fig. 2. Maximum Likelihood tree based on the Hasegawa-Kishino-Yano (HKY+G) distance using NADH dehydrogenase subunit I (*ND1*) sequences; the numbers on each branch correspond to the bootstrap support values. The tree was rooted with *T. oryx*; [{*Syncerus caffer*+ (*Bubalus bubalis*+ *Bu. depressicornis*)}+ {{((*Bi. priscus*+ *Bi. bison*)+ (*B. mutus*+ (*B. mutus*+ *B. grunniens*)+ (*B. gaurus*+ *B. javanicus*)}+ {((*B. frontalis*+*B. indicus*)+ (*B. primigenius*+ *B. taurus*}+ *Pseudoryx nghetinhensis*].

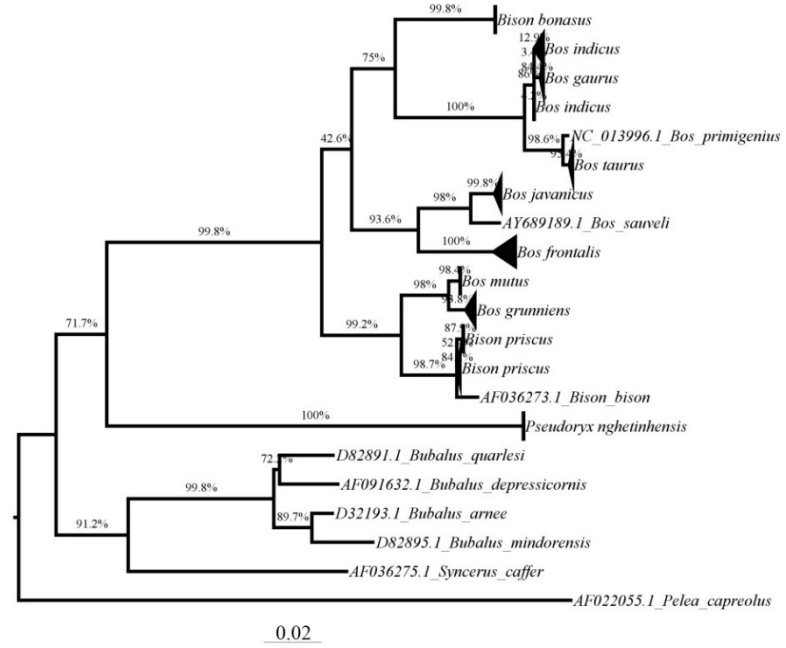

Fig. 3. Maximum Likelihood tree based on the Hasegawa-Kishino-Yano (HKY+G) distance using cytochrome b (*cytb*) sequences; the numbers on each branch correspond to the bootstrap support values. The tree was rooted with *P. capreolus*; [{(*Bi. bonasus*)+ (*B. indicus*+ *B. gaurus*+ *B. indicus*+ *B. primigenius*+ *B. taurus*)+ ((*B. javanicus*+ *B. sauveli*)+ *B. frontalis*)+ ((*B. mutus*+ *B. grunniens*)+ (*Bi. priscus*+ *Bi. bison*))+ *Pseudoryx nghetinhensis*}+ {((*Bu. quarlesi*+ *Bu. depressicornis*)+ (*Bu. arnee*+ *Bu. mindorensis*))+ *Syncerus caffer*}].

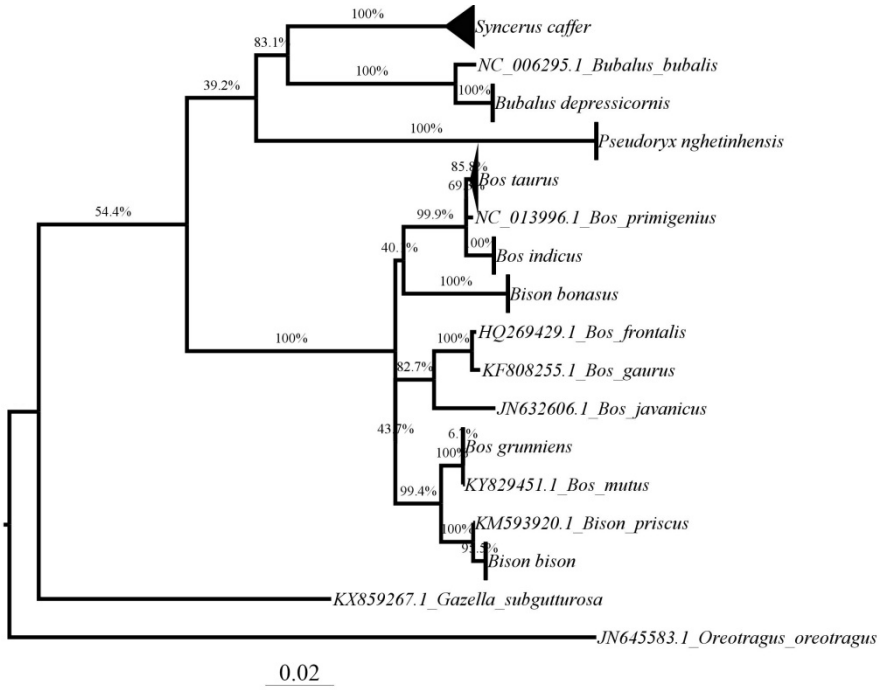

Fig. 4. Maximum Likelihood tree based on the Hasegawa-Kishino-Yano (HKY+G+I) distance using cytochrome c oxidase I (*cox1*) sequences. The numbers on each branch correspond to the bootstrap support values. The tree was rooted with *G. subgutturosa* and *O. oreotragus*; [{(*Sy. Caffer*+ (*Bu. bubalis*+ *Bu. depressicornis*))+ {((*B. taurus*+ *B. primigenius*+ *B. indicus*)+ *Bi. bonasus*+ ((*B. frontalis*+ *B. gaurus*)+ *B. javanicus*)+ ((*B. grunniens*+ *B. mutus*)+ (*Bi. priscus*+ *Bi. bison*))].

In this tree, all of the species *Syncerus*, *Bubalus*, and *Pseudoryx* formed a single monophyletic group with weak bootstrap support (39.2) and high BI posterior probability (100). In another main cluster, all species of *Bos* and *Bison* formed a single monophyletic cluster with a strongly ML bootstrap (98.7) and high BI posterior probability (100).

Finally, the topology of the ML method with complete mitochondrial genome sequences is shown in Fig. 5.

Based on the topology of this tree, there are probably two distinct major clusters. All species of *Bos*, *Bison*, and *Pseudoryx* constructed a single monophyletic group with strong bootstrap support (90). In another major cluster, three species belonging to the subtribe Bubalina constructed a monophyletic cluster with the highest bootstrap (100).

Using complete mitochondrial genomes, all of the species belonging to a single genus were considered as a separate group, so considering the outgroup, six groups were determined and phylogenetic distances between these groups were calculated. As expected, the outgroup was at a distance far from the tribe members and ingroup, the shortest distance was obtained between the genera *Bos* and *Bison* (803.6) and the longest distance between the genera *Bos* and *Pseudoryx* (1896.3; Table 6). As shown in Table 2, the shortest genetic differentiation between different groups using pairwise F_{ST} values was between the genera *Bos* and *Bison* (0.25) but the highest F_{ST} was detected between *Pseudoryx* and *syncerus* (1.00; Table 7).

Discussion

In the current study, four mtDNA gene fragments with a complete mitochondrial genome were used to re-evaluate the phylogenetic relationships within the tribe Bovini. In a comprehensive phylogenetic research, Hassanin *et al*. (2012) evaluated the pattern and divergence time of Cetartiodactyla using complete mitochondrial genomes. They used 12 complete mtDNA sequences belonging to the tribe Bovini, and their maximum likelihood phylogenetic tree supported a monophyletic cluster for the tribe Bovini with strong support value (89%) and the sequences belonging to the subtribes Bubalina and Bovina formed a monophyletic cluster with the highest

bootstrap value (100%). The standing of *P. nghetinhensis* as a sister taxon of Bovina was weak (38). In our ML phylogenetic tree, *P. nghetinhensis* showed a sister relationship with the subtribe Bovina with strong bootstrap (90%), similar to the cytochrome b phylogenetic tree with bootstrap 71.7. Also, *P. nghetinhensis* had low genetic differentiation (using pairwise F_{ST} values) with the genera *Bos* and *Bison* compared with *S. caffer* and *Bubalus*.

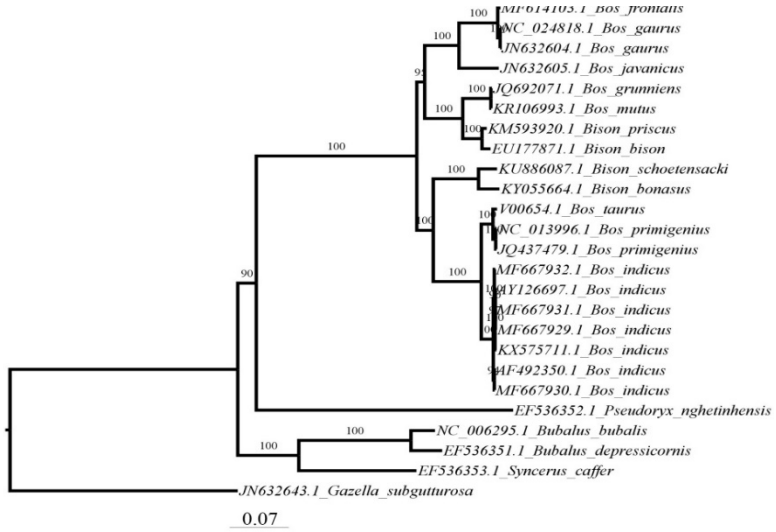

Fig. 5. Maximum Likelihood tree based on the GTR+G+I distance using complete mitochondrial genome sequences. The numbers on each branch correspond to the bootstrap support values. The tree was rooted with *T. oryx*; [{((*Bu. depressicornis*+ *Bu. bubalis*)+ *Syncerus caffer*)+ {{(*B. frontalis*+ *B. javanicus*)+ ((*Bi. priscus*+ *Bi. bison*)+ (*B. grunniens*+ *B. mutus*))}+ {(*Bi*+*schoetensacki*+ *Bi. bonasus*)+ (*B. primigenius*+ *B. taurus*)+ (*B. indicus*)}+ *P. nghetinhensis*].

Table 6. Genetic distances between genera of the tribe Bovini based on complete mitochondrial sequences.

Taxa	*Bos*	*Bison*	*Bubalus*	*Pseudoryx*	*Syncerus*	*Gazella subgutturosa*
Bos	0					
Bison	803.625	0				
Bubalus	1808.844	1780.5	0			
Pseudoryx	1896.313	1868.25	1835	0		
Syncerus	1736.438	1693.25	1308.5	1768	0	
Gazella subgutturosa	2045	2046.25	2092.5	2059	1971	0

Table 7. Pairwise F_{ST} based on the complete mitochondrial genomes between different genera belonging to the tribe Bovini.

Taxa	*Bos*	*Bison*	*Bubalus*	*Pseudoryx*	*Syncerus*	*Gazella subgutturosa*
Bos	0					
Bison	0.258	0				
Bubalus	0.699	0.68	0			
Pseudoryx	0.699	0.653	0.799	0		
Syncerus	0.672	0.618	0.718	1	0	
Gazella subgutturosa	0.721	0.683	0.823	1	1	0

Based on these results, we can conclude that *P. nghetinhensis* has a close phylogenetic relationship with Bovini. However, in other phylogenetic trees in the current study, its phylogenetic position was variable. In other studies (Yang *et al*., 2013; Mckenna and Bell, 1997; Hassanin *et al*., 2013), *P. nghetinhensis* showed a sister relationship with all species belonging to the tribe Bovini. Overall, saola has a robust and reliable relationship with Bovini and this has been supported by both mitochondrial and nuclear genomes (Hassanin, 2014). Identification of the sister-group of *P. nghetinhensis* was a problematic issue (Hassanin, 2014), but our results (Fig. 5) showed that Bovina is the sister-group of saola.

Previous studies have shown the presence of three divergent lineages within the tribe Bovini including Bovina, Bubalina, and Pseudorygina (Gatesy and Arctander, 2000; Hassanin and Ropiquet, 2004; Hassanin *et al*., 2012, 2013; Yang *et al*., 2013; Bibi, 2013; Hassanin, 2014). Our results confirmed the previous findings and all phylogenetic trees, except for the 16s RNA, confirmed the presence of these three lineages within the tribe Bovini.

Among these three different subtribes, we found a subtribe that consists of two genera, *Bos* and *Bison*. There has been some confusion about the scientific name of *bison*. Linnaeus initially classified *Bison* as *Bos* (1758) and later, other zoologists elevated it to a level of a separate genus, *Bison*, but the comprehensive studies about the phylogeny of ruminants showed that species had been named under *Bos* were paraphyletic and interestingly, the yak (*B. grunniens*) was more closely related to *Bison* species than that of other *Bos* species (Miyamoto *et al*., 1989; Groves and Grubb, 2011; Hassanin *et al*., 2012). To maintain monophyly in the taxonomy of Bovini, the phylogenies were faced with three options: 1) classifying *Bison* as *Bos*, 2) changing the genus of the yak to *Bison*, or a separate genus 3) maintaining paraphyly within the tribe Bovini. However, the scientists concluded that the genus *Bison* should be regarded as a synonym of *Bos* (Hassanin *et al*., 2013).

In most of the previous studies, two species of the genus *Bison* (American bison (*Bi. bison*) and European bison (*Bi. bonasus*)) have been studied. In the current study, in addition to this species, we studied two extinct *Bison*, the steppe wisent (*Bi. priscus*; Bojanus, 1827) and the woodland bison (*Bi. schoetensacki*; Freudenberg, 1910). It should be noted that the phylogeny of the woodland bison had not been studied among all of the species belonging to the tribe Bovini. The mitogenome of *Bi. schoetensacki* was sequenced by Palacio *et al*. (2017) and *Bi. priscus* by Marsolier-Kergoat *et al*. (2015), but in these valuable works, they studied their phylogenetic position among the species belonging to the genera *Bos* and *Bison* only, not among all of the species of the tribe Bovini.

Based on the complete mitochondrial phylogenetic tree, four species belonging to the genus *Bison* did not form a monophyletic cluster together. This result indicated that the genus *Bison* is not a monophyletic taxon at all. Of course, the monophyly of *Bison* has been confirmed by the study of the Y-chromosome (Verkaar *et al*., 2004; Nijman *et al*., 2008) and 18 autosomal genes (Hassanin *et al*., 2013). The monophyly of *Bison* is in agreement with the morphology and the fact that hybrids between *Bi. bison* and *Bi. bonasus* are fertile (Hassanin 2014), but it is completely in disagreement with the results of mtDNA data. According to Hassanin *et al*., (2013) and Hassanin, (2014), this conflict is because of mtDNA introgression and higher levels of homoplasy. We have to emphasize that in the previous studies monophyly of two species, *Bi. bison* and *Bi. bonasus* have been studied. Since the mitogenomes provide sufficient information about the phylogeny of related taxa (Hassanin *et al*., 2012; Yang *et al*., 2013; Ghassemi-Khademi, 2017), probably our results are close to reality.

According to the mitogenomes, we found that the genus *Bison* is completely paraphyletic. Our results showed that all species belonging to the genus *Bison*, fall into two different clusters including *Bi. priscus* and *Bi. bison* as a monophyletic cluster with the highest bootstrap. Also *B. grunniens*, *B. mutus, Bi. Schoetensacki,* and *Bi. bonasus* showed a sister relationship with *B. taurus*, *B. primigenius*, and *B. indicus* with the highest bootstrap value. The phylogenetic similarity between *Bi. priscus* and *Bi. bison* (Marsolier-Kergoat *et al*., 2015) and that between *Bi. schoetensacki* and *Bi. bonasus* (Palacio *et al*., 2017) has been shown previously. In this study, we showed *Bi. schoetensacki* in addition to having a close phylogenetic relationship with *Bi. bonasus* as sister taxon, phylogenetically had a close relationship with *B. primigenius* and two domesticated species of *B. taurus* and *B. indicus*, while in the study of Palacio *et al*. (2017) close phylogenetic relationship of extinct *Bi. schoetensacki* with *B. taurus* and *B. indicus* has not been shown.

Moreover, *Bi. priscus* had a close phylogenetic relationship with *Bi. bison* as sister taxon and *B. mutus* and domesticated species of *B. grunniens* with the highest bootstrap value, while in the study of Marsolier-Kergoat *et al*. (2015) close phylogenetic relationship of *Bi. priscus* with *B.*

mutus has not been shown. However, at the moment, we cannot argue with certainty about the reasons for conflict between the results of mitogenomes and other studies (morphology and nuclear genes) which need to be further explored.

Among the different subtribes belonging to the tribe Bovini, Bubalina was the only subtribe whose phylogenetic topology is almost unchanged in all of the trees. In all of the phylogenetic trees, all species belonging to the genus *Bubalus* formed a single monophyletic group and *S. caffer* had a sister relationship with this group. Therefore, there is no doubt about the monophyly of Bubalina and *S. caffer* as its sister taxon. Now, we have not completed mitochondrial DNA sequences of all species of Bubalina, but based on the cytochrome b phylogenetic tree, there were two distinct groups in this subtribe where *Bu. quarlesi* and *Bu. depressicornis* as well as *Bu. arnee* and *Bu. mindorensis* clustered together separately with a strong support value (99.8%).

In the present study, for the first time, twenty-four complete mitochondrial genomes and four distinct genes were used to reconstruct the phylogenetic relationships within the tribe Bovini. We also used all the gene sequences of extinct, domesticated, and wild species of Bovini. In general, we can conclude that there are not two different distinct genera: *Bos* and *Bison* and the genus *Bison* is not an independent taxon in the taxonomic rank of the genus. Probably, the genus *Bison* is a subgenus within the genus *Bos*. Moreover, all species belonging to *Bison*, probably are paraphyletic, but making a definitive decision, in this case, requires further researches. In an overview, Bovina is a monophyletic group; but within this subtribe, there are three distinct monophyletic subgroups. We also found that determining the phylogenetic position of the Saola (*P. nghetinhensis*) needs to be re-evaluated comprehensively because in some studies, this species showed a sister relationship with the tribe Bovini and in other studies similar to the present study, has located as a sister taxon of the Bovina. Therefore it is better to classify this species within the subtribe Bovina, not into a single subtribe, Pseudoryina. Undoubtedly, the subtribe Bubalina is a monophyletic group, and *S. caffer* is a sister taxon of other species belonging to the genus *Bubalus*. Anyway, if we had the complete mitochondrial sequences of all species, we could be more confident about phylogenetic relationships within this subtribe. Overall, the tribe Bovini is a distinct and monophyletic group within bovids and they are separated from other members of this group.

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Conflicts of interest

The authors have no conflict of interest to declare.

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