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Taghi Ghassemi Khademi
Iranian Academic Center for
Education, Culture and Research
(ACECR), Ardabil branch,
Ardabil, Iran

A re-evaluation of phylogenetic relationships within the tribe Tragelaphini (Bovinae: Bovidae), based on complete mitochondrial genomes

Taghi Ghassemi Khademi

Abstract
In this research, phylogenetic relationships of eight species from the tribe Tragelaphini were evaluated using complete mitochondrial genomes. Today, the most of the species belonging to the tribe Tragelaphini, face the risk of declining generation and extinction and they are on the red list of IUCN. Therefore, recognizing the evolutionary biology of them is a very positive step in preventing their extinction. In present research, the average base composition of mtDNA sequences was 26.9% T, 25.9% C, 33.6% A, and 13.6% G, showing a strong AT bias (60.5%). The phylogenetic trees were investigated using the ML, ME and NJ methods and found that they have very identical topologies. In general, the results of this study were similar to the other molecular phylogenetic studies and in obvious contradiction with the morphological results. For example, Derby eland (*Taurotragus derbianus*) shows a very close phylogenetic relationship with other members of the tribe Tragelaphini and should not be in a separate genus. Also, in all of the phylogenetic trees, *T. imberbis* completely was separated from other species and was not located into a single cluster with them. Undoubtedly, the phylogenetic position of this species should be re-examined. In total, the results of current study showed that the complete mitochondrial genomes are very useful, powerful, and accurate tools for evaluating the phylogenetic relationships of the members of the tribe Tragelaphini. Besides, using these genomes, we can meticulously reconstruct and modify their classification.

Keywords: Tragelaphini, phylogeny, mtDNA, biosystematics, IUCN

1. Introduction

Taxonomically, spiral-horned antelopes belonging to Kingdom: Animalia (metazoan), Phylum: Chordata, Subphylum: Vertebrata, Class: Mammalia, Sub-class: Theria, Super- order: Eutheria (Placenta), Order: Artiodactyla, Suborder: Ruminantia, Family: Bovidae, Sub-family: Bovinae, and tribe: Tragelaphini [1, 2, 6, 52]. The family Bovidae is a highly heterogeneous clade comprising approximately 49 recent genera and 140 species [3] whose evolutionary relationships are often obscure, in large part owing to morphological convergence among species [2]. Systematic work on Bovidae has been difficult, as it is one of the most troublesome groups of mammals to classify [4].

The family Bovidae includes three tribes: Bovini (cattle and buffalo), Tragelaphini (spiral-horned antelopes) and Boselaphini (Nilgai, Four-horned Antelope) [4]. Sexual dimorphism is highly prevalent in this subfamily, with the males of some species weighing nearly twice as much as their female counterparts. Bovines have played an important role in the cultural evolution of humans, as numerous species within this subfamily have been domesticated for subsistence purposes [4].

The tribe Tragelaphini is a group of medium-sized to large antelopes, with spiral or twisted horns, white vertical stripes, and significant sexual dimorphism. Horns are found in the males of all species, while most females are hornless, except in the larger forms: Elands and Bongo [4]. The tribe Tragelaphini is represented by a group of medium to large-bodied species that are widely distributed through forested and bush-savannah regions of Africa south of the Sahara [2]. This group tends to large size, a lighter build, longer necks and considerable sexual dimorphism [1]. The nine extant species are assigned to two genera [5]: The *Tragelaphus* genus containing the Greater kudu: *T. strepsiceros*, Lesser kudu: *T. imberbis*, Nyala: *T. angasii*, Mountain nyala: *T. buxtoni*, Sitatunga: *T. spekei*, Bushbuck: *T. scriptus* and Bongo: *T. eurycerus*. Also, the *Taurotragus* genus containing two species with names of common eland: *Taurotragus oryx* and Derby eland: *Taurotragus derbianus* [2].

Correspondence
Taghi Ghassemi Khademi
Iranian Academic Center for
Education, Culture and Research
(ACECR), Ardabil branch,
Ardabil, Iran

These two genera were identified based on morphology and fossil evidence, conventionally [7]. However, there has been considerable disagreement regarding their classification (see reviews by Nowak, 1999; Skinner and Chimimba 2005). Cranial similarities suggest a sister relationship between *T. spekei* and *T. angasi* and between *T. imberbis* and *T. strepsiceros* respectively [9, 10], while the presence of horns in both sexes of *T. euryceros* and the two species belonging to *Taurotragus* genus, *Taurotragus oryx* and *Taurotragus derbianus*, are thought to indicate a close evolutionary affinity among these species [11].

In sharp contradiction, previous studies using different genes of mtDNA, or combined nuclear intron and them, regarding comprehensive taxon representation failed to provide genetic support for the recognition of three tragelaphid genera [2], or for any of the sister species relationships outlined above [12, 13, 14, 15, 16].

a) Most of members of the tribe Tragelaphini are on the red list of IUCN

Bovids are ecologically, economically, and biologically important animals in the world. Thus, determining the phylogenetic relationships (especially based on the complete mitochondrial genomes that can provide more accurate outcomes) between them, can be an effective step in planning for the conservation and enhancement of multiplication of these animals in the world. Particularly, most animal species belonging to this family, today are in serious danger of extinction and declining of the population all over the world [6, 17]. Meanwhile, all of the species belonging to the tribe Tragelaphini (Except: *Taurotragus derbianus* and *T. oryx*) face the risk of declining generation and extinction and they are on the red list of IUCN (See the website: www.iucnredlist.org).

For example, the bongo (*T. eurycerus*) is a near threatened species on the red list of IUCN [40]. The IUCN Antelope specialist group, considers the western or lowland bongo, to be lower risk (Near Threatened), and the eastern or mountain bongo of Kenya, to be critically Endangered [40]. The Lowland Bongo faces an ongoing population decline as habitat destruction and hunting for meat (mainly through snares), and Bongo trophies increase with the relentless expansion of commercial forestry exploitation and human settlement. Despite its large size Bongo is a shy and reclusive species, attributes that may offer some degree of protection from hunting [41, 42].

Threats to Mountain Bongo include hunting with dogs and loss of habitat in the Mau and Eburu forests for illegal logging. The decline of Mountain Bongo populations in the Aberdares in recent years has been attributed to increased hunting by local people and habitat loss, and even with the increased numbers of Lion in the area [41]. Although these factors have no doubt contributed to the decline of Mountain Bongo, the impact of disease has probably been underestimated. The grazing of cattle in the forest reserves of Mount Kenya and the Aberdares as high as the Hagenia forest on the Aberdares plateau may have greater implications for Bongo conservation than hunting pressure in terms of disease transmission [40, 41].

Also, nyala (*T. angasii*) is in danger of extinction today and the major threats to the population of it are poaching, habitat loss, agriculture and cattle grazing [33]. Fortunately, This species is currently of Least Concern, and the population is considered stable by both the IUCN (International Union for Conservation of Nature) and CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) [33, 34].

Moreover, the giant eland (*T. derbianus*) is in red list of IUCN (Least Concern), and the major threats to the western giant eland population are overhunting for its rich meat and habitat destruction caused by the expansion of human and livestock populations [35]. Besides, The kéwel or harnessed bushbuck (*T. scriptus*) have disappeared from some areas in the drier parts of its former range because of habitat destruction and increasing aridity, but it is expanding its distribution within the equatorial forest zone as this is opened up by human activities. There do not seem to be any major threats to its long-term conservation, although numbers may be gradually decreasing locally as hunting pressures increase in parts of its range. However, this species is also on the red list of IUCN (Least Concern) [36].

Loss of habitat is the main threat to the future persistence of the sitatunga or marshbuck (*T. spekii*). The ever-increasing loss of wetlands throughout their range has cut off former routes of dispersal and many populations are becoming isolated. Sitatunga are vulnerable to long-term changes in water level because it alters vegetation structure, which in turn largely determines their distribution and abundance. Habitat fragmentation, and both lower and higher water levels make them more vulnerable to predation and meat hunting in many parts of its range [37]. These animals are extremely vulnerable to fire; at the same time, vast areas of Bangweulu and Busanga are burnt each year [37]. Today, this species is on the red list of IUCN (Least Concern) [38].

Besides, the greater kudu (*T. strepsiceros*) status is less satisfactory in the northern parts of its range, due to over-hunting and habitat loss [39]. Today, this species is on the red list of IUCN (Least Concern) [39]. However, these animals seem to be quite resilient to hunting pressure and remain abundant and well managed in other parts of its range [39].

The lesser kudu (*T. imberbis*) is one of the other specie from the tribe Tragelaphini on red list of IUCN (Near Threatened) [43]. Its shyness and preference for thick cover enable it to withstand considerable hunting pressure. This species is relatively plentiful throughout the Ogaden region wherever there is a sufficient dense bush, despite widespread, uncontrolled hunting by local people [42, 43]. On the other hand, its susceptibility to rinderpest, resulted in a substantial decrease in its numbers in eastern regions of Kenya during the mid-1990s [42, 43].

Also, The common eland (*T. oryx*) is on the red list of IUCN (Least Concern) (44). Habitat loss (due to expanding human settlements) and poaching for its superior meat have resulted in considerable reductions of range and populations. The Common Eland's habit of wandering over large areas may affect its future in ways that cannot be fully predicted, for example it may make the species more vulnerable to poaching or disease [44, 45].

Eventually, the bushbuck (*T. scriptus*) also is on the red list of IUCN (Least Concern) [46]. This valuable species have disappeared from some areas in the drier parts of its former range because of habitat destruction and increasing aridity, but it is expanding its distribution within the equatorial forest zone as this is opened up by human activities. There do not seem to be any major threats to its long-term conservation, although numbers may be gradually decreasing locally as hunting pressures increase in parts of its range [42, 46].

b) Importance of mitochondrial genome in phylogenetic studies

Mitochondrial DNA (mtDNA) is a circular DNA of 15000-20000 bp and is conserved in vertebrate animals. mtDNA is

highly variable in structure, content, organization, and quality of genes expression in the different of animals [18, 54]. The mitochondrial genome is popular for evolutionary and phylogenetic studies because of the relative simplicity of extraction and simple sequence organization, maternal inheritance, free of recombination in most cases and rapid rate of sequence divergence [17].

Several mitochondrial genomes (mtDNA) are used for estimating the phylogenetic relationships among animal taxa and molecular phylogenetic evolution analysis. Overall, using several genomes of mtDNA is better than using the single gene for phylogenetic analysis of animals, because multiple sequences (especially complete genome of mtDNA) provide sufficient information about evolution and of evolutionary process reconstruction [18].

Although several researchers [16, 17, 19, 20, 21, 22, 23, 24, 25, 26, 53] have studied the phylogenetic relationship among species belonged to Bovidae, there is not an independent and comprehensive research article about phylogenetic relationships within the tribe Tragelaphini (Bovinae: Bovidae) exclusively, based on complete mitochondrial genomes.

2. Materials and Methods

All complete mitochondrial genome sequences belonging to the tribe Tragelaphini were downloaded (n=9) from NCBI (Table: 1). All of the complete mitogenome sequences, belong to the study of Hassanin and his colleagues (2012) [54]. Sequences were aligned with Mega6 [27] using the Clustral W alignment method. Also, the corresponding gene sequences of *Bos grunniens* and *Bos mutus* were used as outgroups in the analysis. Nucleotid composition of mtDNA of studied species (n=9) and their accession numbers (n=11) have been shown in Table 1. The evolutionary history was inferred using the Neighbor-Joining method [28]. The optimal tree was identified with the sum of branch length = 0.486. Moreover, the evolutionary history was inferred using the Minimum Evolution method [30]. The optimal tree was identified with the sum of branch length = 0.486. Also, the evolutionary history was inferred by using the Maximum Likelihood method. The tree with the highest log likelihood (-52342.7) was shown. In all of the three phylogenetic trees, the percentage of replicate trees, in which the associated taxa are clustered together in the bootstrap test (1000 replicates), are shown next to the branches [29].

In this study, each species were considered as a separate group; therefore, considering the outgroups (into one group) we had nine groups (Table 2). So, evaluation of evolutionary divergence over sequence pairs between the mentioned groups (different species) was calculated and variance estimation method was bootstrap with 1000 replications. The rate variation among sites was modeled with a gamma distribution (shape parameter=1). Codon positions included were 1st+2nd+3rd+Noncoding.

In all of the above analyzes, it involved 11 mitogenome sequences, and all positions containing gaps and missing data were eliminated. Finally, there were a total of 15381 positions in the final dataset. All of the evolutionary analyzes were computed using the Kimura 2-parameter method [31] and were conducted in MEGA6 [27].

Also, the robustness of clades were calculated by bootstrap method and thus, in this study, was considered 50-60% as weak support (as bootstrap values), 64-75% as moderate support, 76-88% as good support and strong support as values ≥89% [32].

3. Results and Discussion

Phylogenetic analysis of eight species belonging to the tribe Tragelaphini (n=9) was performed using complete sequences of the mtDNA. Average length of the mitochondrial genome was calculated 16391.2 bp. In 16391.2 bp, the average base composition of mtDNA sequences was: 26.9 T, 25.9% C, 33.6% A, and 13.6% G, showing a strong AT bias (60.5%). Since each genus was considered as a separate group, in addition to the outgroups, nine groups were determined and phylogenetic distances between these groups were calculated. As the results indicated (Table 2), the outgroups were at a distance far from the tribe members, implying the presence of relative close genetic distances among them.

The short distances were obtained between three group 1, 2, and 3 (Group 1: *T. scriptus* (two sequences), Group 2: *T. spekii*, Group 3: *T. eurycerus*). Thus, it can be inferred that these three groups or these three species are phylogenetically the closest species. As mentioned earlier, the longest distance was obtained between outgroup (*B. grunniens* and *B. mutus*) and other groups.

Molecular phylogenetic trees for mitogenomes were constructed using the NJ, ME, and ML methods, which all provided the completely same topologies (Figs: 1, 2, and 3). Moreover, *Boss* pp. used as the outgroups, were completely separated from other groups. Three phylogenetic trees revealed a great and main clade; all the species belonging to Tragelaphini tribe (except *T. imberbis*) constructed a major and great clade with very strong support (as values ≥99%).

Within this clade, we can distinguish three distinct groups: within group 1, three species including *T. scriptus*, *T. spekii*, and *T. eurycerus* showed the highest supported monophyly values (=100) in all three NJ, ME, and ML phylogenetic trees. Also, within group 2, three species including *T. strepsiceros*, *T. oryx*, and *Taurotragus derbianus* constructed a monophyletic clade with the very weak support values (≤49) in all three NJ, ME, and ML phylogenetic trees. Both group 1 and 2 constructed a monophyletic clade with the highest supported values (=100) and T. angasii sistered to this monophyletic clade with the highest supported values (≥99). In all three NJ, ME, and ML phylogenetic trees, *T. imberbis* was not located within great and main clade of Tragelaphini.

Based on the topology of NJ, ME, and ML phylogenetic trees, in group 1, the relationship of species are as follows: [(*T. scriptus* + *T. scriptus*) + (*T. spekii* + *T. eurycerus*)]. On the other hand, in group 2, the relationship of species are as follows: [*T. strepsiceros* + (*T. oryx* + *Taurotragus derbianus*)] and *T. angasii* are located next to this group. Respectively, *T. imberbis* and *T. angasii* were the most basal taxa within phylogenetic trees.

In a study, Rubes and colleagues [2], evaluvated phylogenomic relationships of spiral-horned antelops by cross-species chromosome painting. Their results supported the monophyly of Tragelaphini and a clade comprising *T. scriptus*, *T. spekei*, *T. euryceros*, *T. oryx* and *Taurotragus derbianus* (Fig. 4). This clade includes the so-called 'Closed forest' group of species [2] and with the exception of *T. strepsiceros*, the members of the 'Closed forest' group were clustered next together in present study.

If you note, In both current and mentioned studies, two species of *T. oryx* and *T. derbianus* have been located into one cluster and this is an evidence of close phylogenetic relationships between them. However, *T. oryx* and *Taurotragus derbianus* are shown as a recently derived arid group within mentioned clade [2], suggesting that the ability to survive in extreme arid habitats has been a recently derived trait [2].

The depicted phylogenetic tree based on cytochrome b by Matthee and Robinson [12], for Tragelaphini has been shown on Fig. 5. It is almost similar to the phylogenetic study of current research. Of course, the results of present study are more accurate and reliable than mentioned study, because using the complete mitochondrial genome gives us more accurate results than using a single gene. Nevertheless, the results of Matthee and Robinson's study are closer to reality and contrasts with the results of morphological studies [9]. As the present study, the results of Matthee and Robinson's research revealed a close evolutionary association between the *T. euryceros* (Bongo) and *T. spekii* (Sitatunga) with *T. scriptus* (Bushbuck). The molecular results are thus supportive of the suggestions of Wilson and Reeder's (1993) and Tijskens (1968) [47, 48], for the ability of the Bongo and Sitatunga to hybridize.

Likewise, respectively the failure of the Lesser Kudu (*T. imberbis*) and Greater Kudu (*T. strepsiceros*), and the Common Eland (*T. oryx*) and Derby Eland (*T. derbianus*), to cluster [12], as sister taxa would be consistent with the previous findings [49].

The above results [12] and the results of the current study, are in contrast to morphological studies [9], because the Sitatunga and Bongo are two species which differ noticely in morphology [12]. Additionally, the Lesser Kudu and Grater Kudu are two species which have similarities in morphological traits and dietary [12, 9]. Also, Nyala (*T. angasii*) and sitatunga which have approximately similar skeletal features [9, 50], were not clustered together in phylogenetic trees. Also, Matthee and Robinson's study [12] revealed estimatedly, Nyala and Sitatunga diverged from each other 9.1 Mya and the Lesser and Greater Kudu separated from each other 11.2 Mya. In addition, the diversification probably started approximately 14 Mya but speciation was more gradual occurring up till 5 million years ago, which provided more time for the accumulation of synapomorphies, and less time for the mtDNA signal to be erased [13].

Probably, the mentioned phylogenetic relationships reflect the effects of variance [12]. Because Bongo, Sitatunga, and Bushbuck, all three live in a thick forest habitat with heavy rains [12]. In return, the Lesser Kudu, Nyala, Derby eland, also the Greater Kudu and Common eland live in arid regions [12]. Therefore, the tribe Tragelaphini may have evolved in the dry bushlands and savannas of Africa, and the pathway of advancement has tended toward moist woodlands and forest habitats [12]. Strikingly, this theory is in contrast to that of Kingdon (1982), who designated the swamp-dwelling

Sitatunga as the least specialized species in the Tragelaphini radiation [12, 9].

In another study, Willows-Munro and colleague [13] used Four nuclear DNA markers (thyrotropin-THY, protein-kinase CI-PRKCI, Bspectrin non-erythrocytic SPTBN, and stem cell factor-MGF) for the phylogenetic resolution among nine *Tragelaphus* spp. The retrieved tree by the combined analysis of all molecular data (Four nuclear DNA markers) has been shown in Fig. 5. If you note, the topology of the tree drawn in the study of Willows-Munro *et al.*, is nearly different from that of the current study (Figs: 1, 2, and 3). It seems the best genes in determining the phylogenetic relationships of the tribe Tragelaphini are mitochondrial genes [13], especially complete mitochondrial genomes. In the mentioned study, Wilson and colleagues have concluded that the mitochondrial gene (cytochromr b) is very useful in resolving phylogenetic relationships within the spiral-horned antelope [13] and nuclear DNA markers are not powerful tools for detecting phylogenetic relationships among them.

In all of the Nj, ML, and ME phylogenetic trees, *T. imberbis* completely was separated from other species and it was not located in a single cluster with them. This species has distinct cytogenetic position than other tragelaphini because it has 38 diploid chromosomes and unlike others in the tibe Tragelaphini, the X and Y chromosomes are compound and each is fused with one of two identical autosomes (51). Undoubtedly, the phylogenetic position of this species should be re-examined and probably must be located in a new and separate tribe.

The most primitive members of the Tragelaphini, *T. imberbis* and *T. angasii* both occur in dense woodland, with the former generally found at higher altitudes [3]. It seems probable that the fragmentation of dense forest habitats resulted in the isolation of *T. imberbis* and *T. angasii* [13]. These two species were basal species in phylogenetic trees, and despite being separated from each other completely, they are adjacent to each other. The close but old evolutionary relationship of these taxa is further supported by the lack of a unique 31 bp deletion in the SPTBN DNA region which is a synapomorphy uniting the other species of the tribe Tragelaphini [13].

In general, the results of this study indicated that using the complete mitochondrial genomes is a very useful, powerful, and accurate tool for evaluating of the phylogenetic relationships of animals and can be effective in the reconstruction and modification of existing animal classification.

Table 1: Nucleotide composition of mitogenomes of studied species from the tribe Tragelaphini (n=9) and their accession numbers in addition to outgroups that have been received from GenBank (www.ncbi.nlm.nih.gov) (n=11).

| Scientific Name	T(U)	C	A	G	Total	Accession Number	References
Tragelaphus scriptus	26.8	26.1	33.8	13.4	16402.0	JN632707	(Hassanin et al. 2012)
Tragelaphus scriptus	26.8	26.0	33.6	13.5	16384.0	JN632706	(Hassanin et al. 2012)
Tragelaphus spekii	27.1	25.7	33.8	13.4	16408.0	NC_020620	(Hassanin et al. 2012)
Tragelaphus eurycerus	26.8	26.0	33.9	13.3	16348.0	JN632703	(Hassanin et al. 2012)
Tragelaphus oryx	26.9	26.0	33.7	13.5	16396.0	JN632704	(Hassanin et al. 2012)
Tragelaphus strepsiceros	26.9	25.9	33.6	13.6	16381.0	JN632708	(Hassanin et al. 2012)
Tragelaphus angasii	27.1	25.5	33.4	14.0	16398.0	JN632702	(Hassanin et al. 2012)
Tragelaphus imberbis	27.0	25.6	33.3	14.0	16405.0	NC_020619	(Hassanin et al. 2012)
Taurotragus derbianus	26.9	26.0	33.5	13.6	16399.0	NC_020618	(Hassanin et al. 2012)
Avg.	26.9	25.9	33.6	13.6	16391.2		
Bos grunniens						KR011113	(Guo et al. 2015)
Bos mutus						KR106993	(Chunnian et al. 2016)

Table 2: Kimura-2 parameter distance between the species belonging to the tribe Tragelaphini, based on complete mitochondrial sequences.

|	Gp_1	Gp_2	Gp_3	Gp_4	Gp_5	Gp_6	Gp_7	Gp_8
Gp_1								
Gp_2	0.050							
Gp_3	0.047	0.050						
Gp_4	0.070	0.082	0.080					
Gp_5	0.075	0.088	0.087	0.086				
Gp_6	0.104	0.117	0.116	0.116	0.121			
Gp_7	0.114	0.130	0.128	0.128	0.134	0.135		
Gp_8	0.069	0.084	0.080	0.054	0.089	0.115	0.130	
Gp_9	0.152	0.163	0.162	0.170	0.175	0.173	0.173	0.167

Group 1) *Tragelaphus scriptus* (two sequences), Group 2) *T. spekii*, Group 3) *T. eurycerus*, Group 4) *T. oryx*, Group 5) *T. strepsiceros*, Group 6) *T. angasii*, Group 7) *T. imberbis*, Group 8) *Taurotragus derbianus*, Group 9) *Bos grunniens* and *B. mutus*.
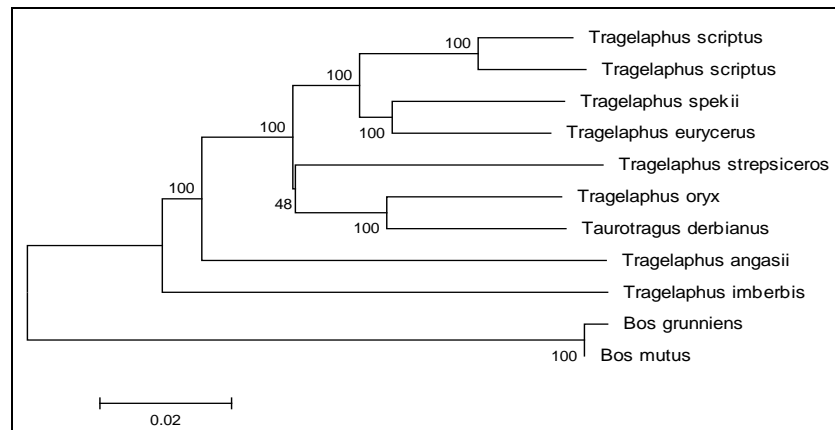

Fig 1: Neighbor-joining tree based on Kimura 2- parameter distance using complete mitochondrial genome sequences. The numbers on each branch correspond to bootstrap support values. The tree was rooted with two *Bos* spp. sequences.
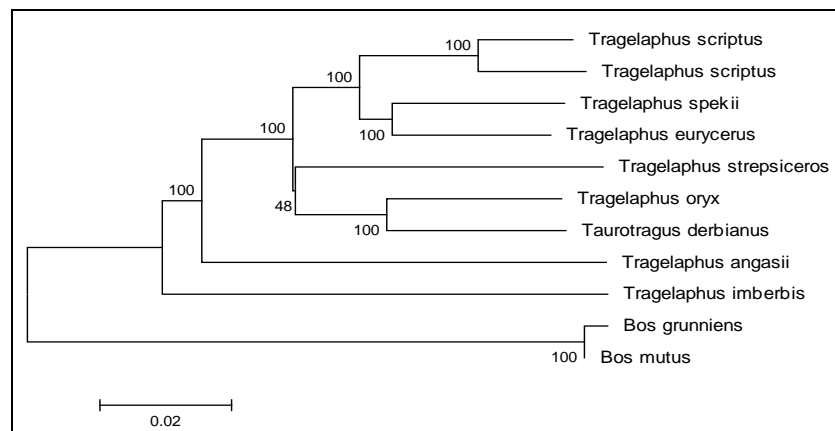
Fig 2: Minimum evulution tree based on Kimura 2- parameter distance using complete mitochondrial genome sequences. The numbers on each branch correspond to bootstrap support values. The tree was rooted with two *Bos* spp. sequences.
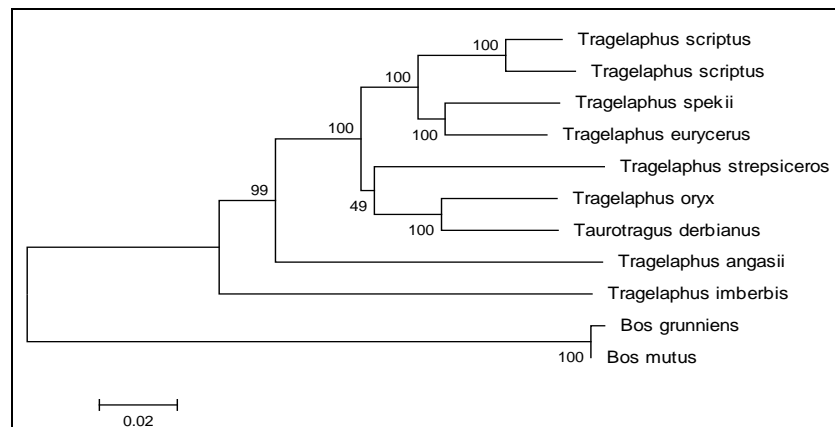

Fig 3: Maximum Likelihood tree based on Kimura 2- parameter distance using complete mitochondrial genome sequences. The numbers on each branch correspond to bootstrap support values. The tree was rooted with two *Bos* spp. sequences.
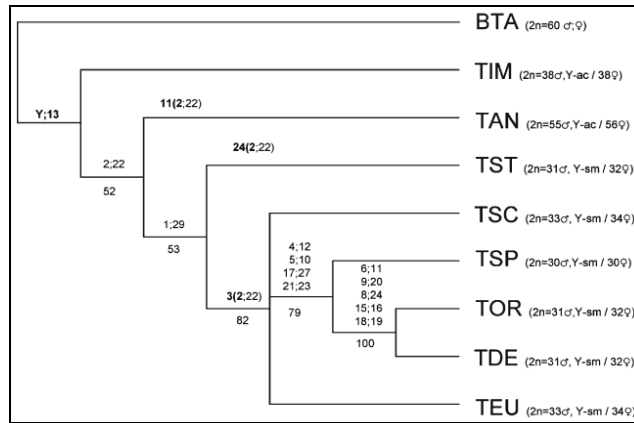
Fig 4: Phylogenetic reconstruction of the Tragelaphini by cross-species chromosome painting. Greater kudu (*T. strepsiceros*, TST), Lesser kudu (*T. imberbis*, TIM), Nyala (*T. angasii*, TAN), Sitatunga (*T. spekei*, TSP), Bushbuck (*T. scriptus*, TSC), Bongo (*T. eurycerus*, TEU), Common eland (*T. oryx*, TOR) and Derby eland (*Taurotragus derbianus*, TDE). Synapomorphies are shown above branches and percentage bootstrap values below branches (Rubes *et al*., 2008).
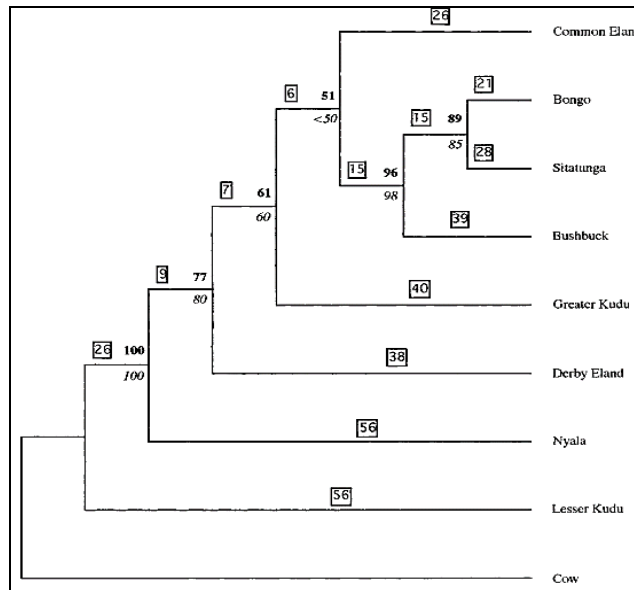
Fig 5: Tragelaphini evolutionary tree reflecting the results obtained from parsimony (equal weighting) and maximum likelihood analyses based on cytochrome b gene. The number of changes along each branch is indicated in the boxes, also shown are parsimony (above branches) and maximum likelihood (below branches) bootstrap values (Matthee and Robinson, 1999). [*T. oryx* (Common eland), *Taurotragus derbianus* (Derby eland), *T. strepsiceros* (Greater kudu), *T. imberbis* (Lesser kudu), *T. euryceros* (Bongo), *T. angasii* (Nyala), *T. scriptus* (Bushbuck), *T. spekii* (Sitatunga)].
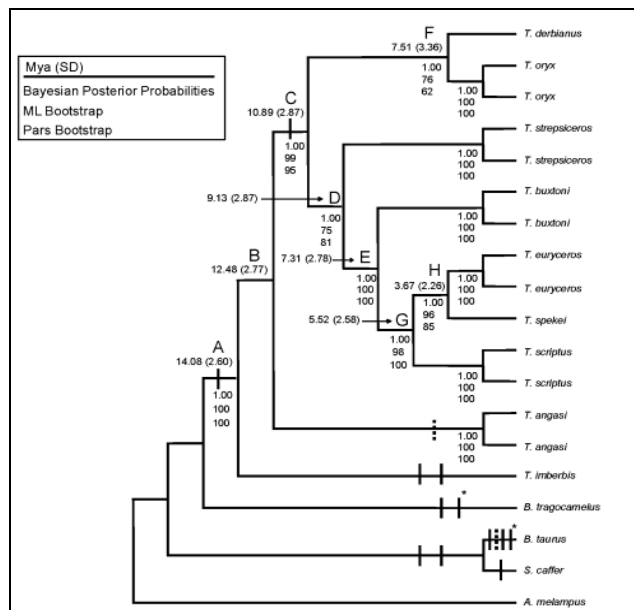
Fig 6: Topology retrieved by the combined analysis of all molecular data (Four nuclear DNA markers). Values below the branches represent Bayesian posterior probabilities, maximum likelihood bootstrap values, parsimony bootstrap values (Willows-Munro *et al*., 2005).

4. Conclusion

In total, the results of the current study were similar to the other molecular phylogenetic studies and in obvious contradiction with the morphological results. Undoubtedly, the results of the molecular phylogenetic studies, especially comparative studies based on complete mitochondrial genomes, are more accurate and reliable than the results of the morphological studies and using these genomes, we can meticulously reconstruct and modify the phylogenetic relationships of the members of the tribe Tragelaphini.

5. Acknowledgement

6. References

1. Wilson DE, Reeder DM. Antilopinae, in Mammal Species of the World: A Taxonomic and Geographic Reference. Johns Hopkins University Press, 3ª ed. 2005. ISBN: 0-8018-8221-4.
2. Rubes j, Kubickova s, Pagacova E, Cernohorska H, Di-Berardino D, Antoninova M *et al*. Phylogenomic study of spiral-horned antelope by cross-species chromosome painting. Chromosome Research, 2008. DOI: 10.1007/s10577-008-1250-6.
3. Nowak RM. Walker's Mammals of the World. Baltimore: The Johns Hopkins University Press, 1999, 2.
4. Castelló JR. Bovids of the world: Antelopes, Gazelles, Cattle, Goats, Sheep, and Relatives. Princeton University Press, Princeton, New Jersey, USA, 2016, 664.
5. Grubb P. Order Artiodactyla. In: Wilson, DE, Reeder, DM, eds. Mammal Species of the World, A Taxonomic and Geographic Reference. Baltimore: Johns Hopkins University Press, 2005; 637-722.
6. Ghassemi-Khademi T. Iranian gazelles. Journal of Middle East Applied Science and Technology (JMEAST), 2014; 13(3): 385-390.
7. Ansell WFH. Order Artiodactyla. In: Meester J, Setzer HW, eds. The Mammals of Africa: An Identification Manual. Washington: Smithsonian Institute Press, 1971; part 15, pp. 1-84.
8. Skinner JD, Chimimba CHT. The Mammals of the Southern African Subregion. Cambridge: Cambridge University Press, 2005.
9. Kingdon J. East African Mammals: An Atlas of Evolution in Africa, vols 3C and 3D. London: Academic Press, 1982.
10. Alden PC, Estes RD, Schlitter D, McBride B. National Audubon Society Field Guide to African Wildlife, New York: Knopf, 1995.
11. Gentry AW. The subfamilies and tribes of the family Bovidae. Mammal Review, 1992; 22: 1-32.
12. Matthee CA, Robinson TJ. Cytochrome b phylogeny of family Bovidae: resolution within the Alcephini, Antilopini, Neotragini and Tragelaphini. Molecular Phylogenetics and Evolution, 1999; 12: 31-46.
13. Willows-Munro S, Robinson TJ, Matthee CA. Utility of nuclear DNA intron markers at lower taxonomic levels: Phylogenetic resolution among nine *Tragelaphus* spp. Molecular Phylogenetics and Evolution, 2005; 35: 624-636.
14. Georgiadis NJ, Kat PW, Oketch H, Patton J. Allozyme divergence within the Bovidae. Evolution, 1990; 44: 2135-2149.
15. Essop MF, Harley EH, Baumgarten I. A molecular phylogeny of some Bovidae based on restriction-site mapping of mitochondrial DNA. Journal of Mammalogy, 1997; 78: 377-386.
16. Gatesy J, Amato G, Vrba ES, Schaller G, DeSalle R. A cladistic analysis of mitochondrial ribosomal DNA from the Bovidae. Molecular Phylogenetics and Evolution, 1997; 7: 303-319.
17. Ghassemi-Khademi T. Evaluation of phylogenetic relationships of Antilopini and Oreotragini tribes (Bovidae: Artiodactyla) based on complete mitochondrial genomes. Journal of Wildlife and Biodiversity, 2017; 1: 1-11.
18. Zhang WQ, Zhang MH. Complete mitochondrial genomes revealphylogeny relationship and evolutionaryhistory of the family Felidae. Genetics and Molecular Research, 2013; 12(3): 3256-3262.
19. Hassanin A, Douzery EJP. The tribal radiation of the family Bovidae (Artiodactyla) and the evolution of the mitochondrial cytochrome b gene. Molecular Phylogenetics and Evolution, 1999; 13:227.
20. Rebholz W, Harley E. Phylogenetic relationships in the bovid subfamily Antilopinae based on mitochondrial DNA sequences. Molecular Phylogenetics and Evolution, 1999; 12: 87–94.
21. Wronski T, Wacher T, Hammond RL, Winney B, Hundertmark JK, Blacket MJ, Mohammed OB, Flores B, Omer SA, Macasero W, Plath M, Tiedemann R, Bleidorn C. Two reciprocally monophyletic mtDNA lineages elucidate the taxonomic status of mountain gazelles (*Gazella gazella*). Systematics and Biodiversity, 2010; 8: 119–129.
22. Manuel H, Ferna N, Elisabeth S. A complete estimate of the phylogenetic relationships in Ruminantia: A dated species level supertree of the extant ruminants. Biological Reviews, 2005; 269–302.
23. Groves CP. Phylogenetic relationships within recent Antilopini (Bovidae). In:Vrba, ES., Schaller, George B. (Eds.), Antelopes, Deer, and Relatives: Fossil Record, Behavioral Ecology, Systematics, and Conservation. Yale University Press, New Haven and London, 2000; 223–233.
24. Kuznetsova MV, Kholodova MV. Revision of phylogenetic relationships inthe Antilopinae subfamily on the basis of the mitochondrial rRNA and b-spectrinnuclear gene sequences. Doklady Biological Sciences, 2003; 391: 333–336.
25. Marcot JD. Molecular phylogeny of terrestrial artiodactyls. In: Prothero, DR, Foss, SE. (Eds.), The Evolution of Artiodactyls. The Johns Hopkins UniversityPress, Baltimore, 2007.
26. Bärmann EV, Rössner GF, Wörheide G. A revised phylogeny of Antilopini (Bovidae, Artiodactyla) using combined mitochondrial and nuclear genes. Molecular Phylogenetics and Evolution, 2013; 67(2): 484-493.
27. Tamura K, Stecher G, Peterson D, Filipski A, and Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution, 2013; 30: 2725-2729.
28. Saitou N, Nei M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution, 1987; 4: 406-425.
29. Felsenstein J. Confidence limits on phylogenies: An approach using the bootstrap. Evolution, 1985; 39: 783-

791.

30. Rzhetsky A, Nei M. A simple method for estimating and testing minimum evolution trees. Molecular Biology and Evolution, 1992; 9: 945-967.

31. Kimura M. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution, 1980; 16: 111-120.

32. Win NZ, Choi EY, Park J, Park JK. Molecular phylogenetic relationship of the subfamily Nymphalinae (Lepidoptera: Nymphalidae) in Myanmar, inferred from mitochondrialgene sequences. Journal of Asia-Pacific Biodiversity, 2017; 10: 86-90.

33. IUCN SSC Antelope Specialist Group. *Tragelaphus angasii*. (errata version published in 2017). The IUCN Red List of Threatened Species 2016; e.T22052A115165681.

34. Ichards M, Shurter S. "Nyala antelope (*Tragelaphus angasi*). Retrieved 1 August 2012.

35. IUCN SSC Antelope Specialist Group. *Tragelaphus derbianus*. The IUCN Red List of Threatened Species 2008; e.T44172A10867579.

36. IUCN SSC Antelope Specialist Group. *Tragelaphus scriptus*. (errata version published in 2017), The IUCN Red List of Threatened Species 2016; e.T22051A115165242.

37. May J, Lindholm R. *Tragelaphus spekii* - Sitatunga. Mammals of Africa: Volume VI: Pigs, Hippopotamuses, Chevrotain, Giraffes, Deer and Bovids (Eds J. KINGDON, D. HAPPOLD, T. BUTYNSKI, M. HOFFMANN, M. HAPPOLD and J. KALINA). Bloomsbury Publishing, 2013, London, U.K.

38. IUCN SSC Antelope Specialist Group. *Tragelaphus spekii*. (errata version published in 2017), The IUCN Red List of Threatened Species 2016; e.T22050A115164901.

39. IUCN SSC Antelope Specialist Group. *Tragelaphus strepsiceros*. The IUCN Red List of Threatened Species 2016; e.T22054A50196734.

40. IUCN SSC Antelope Specialist Group. *Tragelaphus eurycerus*. (errata version published in 2017), The IUCN Red List of Threatened Species 2016; e. e.T22047A115164600.

41. Elkan PW, Smith JLD. *Tragelaphus eurycerus*. In: J. S. Kingdon and M. Hoffmann (eds), The Mammals of Africa. VI. Pigs, Hippopotamuses, Chevrotain, Giraffes, Deer, and Bovids, 2013; Bloomsbury Publishing, London, UK.
    42. East R. African Antelope Database 1998. IUCN, Gland, Switzerland and Cambridge, 1999; UK.

42. IUCN SSC Antelope Specialist Group. *Tragelaphus imberbis*. (errata version published in 2017), The IUCN Red List of Threatened Species 2016; e.T22053A115165887.

43. IUCN SSC Antelope Specialist Group. *Tragelaphus oryx*. (errata version published in 2017), The IUCN Red List of Threatened Species 2016; e.T22055A115166135.

44. Thouless CR. *Tragelaphus oryx* Common Eland. Pages 191–198 in Kingdon JS, Hoffmann M, editors. Mammals of Africa. Volume VI: Pigs, Hippopotamuses, Chevrotain, Giraffes, Deer and Bovids. Bloomsbury Publishing, London, 2013; UK.

45. IUCN SSC Antelope Specialist Group. *Tragelaphus scriptus*. (errata version published in 2017), The IUCN Red List of Threatened Species 2016; e.T22051A115165242.

46. Wilson DE, Reeder DM. Mammal Species of the World: A Taxonomic and Geographic Reference. Smithsonian Institution Scholarly Press, 1993; London, UK.

47. Tijskens J. Preliminary notes on the F1 Bongo antelope X Sitatunga hybrids, *Taurotragus eurycerus* X *Tragelaphus spekei* at Antwerp zoo. International Zoo Yearbook, 1968; 8: 137–139.

48. Georgiadis N, Kat P, Oketch H. Allozyme divergence within the Bovidae. Evolution, 1990; 44: 2135–2149.

49. Roberts A. The Mammals of South Africa, Trustees of 'The Mammals of South Africa' book fund, Johannesburg, 1951.

50. Benirschke K, Ruedi D, Muller H, Kumamoto AT, Wagner KL, Downes HS. The unusual karyotype of the lesser kudu, *Tragelaphus imberbis*. Cytogenetic and Genome Research, 1980; 26(2–4): 85–92.

51. Ghassemi-khademi M, Ghassemi-Khademi T, Javadi SS, Ghassemi-Khademi A. The extincted gazelle of Moghan plain and feasibility of revival of this species on taht region. International Conference on Environmental Planning and Management (ICEPM), 2013; Tehran University, Tehran, Iran.

52. Lei R, Jiang Z, Hu Z, Yang W. Phylogenetic relationships of Chinese antelopes (subfamily Antilopinae) based on mitochondrial ribosomal RNA genesequences. Journal of Zoology, 2003; 261: 227–237.

53. Ghassemi-Khademi. Evaluation of genetic diversity of common honey bees (*Apis mellifera* L.) in selected region of Azerbaijan of Iran. Ms.C Thesis of Animal Biosystematic, 2011; Shahid Beheshti University, Tehran, Iran.

54. Hassanin A, Delsuc F, Ropiquet A, Hammer C, Jansen van Vuuren B, Matthee C *et al*. Pattern and timing of diversification of Cetartiodactyla (Mammalia, Laurasiatheria), as revealed by a comprehensive analysis of mitochondrial genomes, Comptes Rendus Biologies. 2012; 335(1):32-50.

55. Chunnian L, Wu X, Ding X, Wang H, Guo X, Chu M, Bao P, Yan P. Characterization of the complete mitochondrial genome sequence of wild yak (*Bos mutus*), Mitochondrial DNA Part A, 2016; 27(6): 4266-4267.

56. Guo X, Pei J, Bao P, Chu M, Wu X, Ding X, Yan P. The complete mitochondrial genome of the Qinghai Plateau yak *Bos grunniens* (Cetartiodactyla: Bovidae), Mitochondrial DNA Part A. 2015; 27(4):2889-2890.