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Phylogenetic Relationships within the Tribe Hippotragini (Antilopinae: Bovidae) Based on Mitochondrial Genome

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Abstract

In this survey, phylogenetic relationships within the tribe Hippotragini were re-evaluated using 132 gene sequences and ten complete mitogenomes. Based on the complete mitochondrial genomes, the average base composition of mtDNA sequences was 26.9 % T, 26.3 % C, 33.4 % A, and 13.4 % G, showing a strong AT bias (60.3 %). The phylogenetic trees were constructed using maximum likelihood and Bayesian inference methods, and were found to have very identical topologies. The results of this study revealed that this tribe is a strongly supported monophyletic group, and in all of the phylogenetic trees, except for COXI, *Addax nasomaculatus* is considered as a sister group for the monophyletic group of the genus *Oryx*. All of the species belonging to the genus *Hippotragus* constructed another monophyletic clade. Besides, within the monophyletic group of the genus *Oryx,* there are probably two distinct evolutionary pathways, i.e. *O. gazella* was located as a sister taxon of other species. Also, based on the whole mitogenomes, the extinct blaubok (*H. leucophaeus*), the endangered giant sable (*H. niger variani*), and the southern sable (*H. niger niger*), showed very close phylogenetic relationships and the roan antelope (*H. equinus*) is nested as their sister taxon.

KeyWords: Hippotragini, Phylogeny, Re-evaluation, mtDNA, Bovidae, Antilopinae.

1. Introduction

Taxonomically, the tribe Hippotragini belongs to the Family Bovidae, Subfamily Antilopinae (Wilson and Reeder, 2005; Castelló, 2016). Historically, these antelopes were considered to be a separate subfamily within the family Bovidae, but current molecular evidence suggests that they are best relegated to a tribal level within the subfamily Antilopinae (one of two subfamilies within the Family Bovidae) (Holland, 2015; Mathee and Davis, 2001; Kuznetsova *et al*., 2002; Hassanin and Douzery, 2003; Ropiquet, 2006; Hassanin *et al*., 2012).

These “horse-like” antelopes consist primarily of large grazing antelopes with large horns: oryxes, addax, sable, and roan antelopes. Hippotragini species are restricted to Africa and the Arabian Peninsula, and are primarily grazers. Most species live in arid habitats and have an erect mane along the nape of the neck (Castelló, 2016; Holland, 2015). The females are almost or quite as well-horned as the males. The horns, which are ringed, are generally more or less parallel and the frontal sinuses extend into the horn pedicels (Groves and Grubb, 2011).

As antelopes specialized in harsh arid conditions, all species have very high-crowned hypsodont teeth and a bending braincase to maximize the grazing efficiency (Prothero and Foss, 2007). Also, they employ a highly efficient thermoregulation mechanism (“nasal sweating”) to effectively cool their bodies and prevent water loss (Kingdon and Hoffman, 2013; Holland, 2015). All species are gregarious and form mostly small herds (Holland, 2015).

The tribe Hippotragini consists of three genera: *Addax*, *Oryx,* and *Hippotragus.* The genus *Hippotragus* consists of three species: *H. leucophaeus* (blaubok) (extint), *H. equinus* (roan antelope), and *H. niger* (sable) (Groves and Grubb, 2011). Four subspecies are usually recognized: *H. niger niger, H. niger kirkii, H. niger anselli, H. niger roosevelti,* and the isolated Giant Sable (*H. niger variani*) from Angola (Pitra *et al*. 2002). In some references, *H. niger roosevelti* and *H. niger variani* have been elevated to full species (Groves and Grubb, 2011). The genus *Addax* consists of a single species: *A. nasomaculatus* (Groves and Grubb, 2011). The genus *Oryx* consists of six species: *O. beisa* (Beisa oryx), *O. callotis* (fringe-eared oryx), *O. gazelle* (gemsbok), *O. dammah* (scimitar- horned oryx), *O. leucoryx* (Arabian oryx), and *O. gallarum* (Groves and Grubb, 2011).

The first ancestors of the Hippotragini, Alcephalini, and Caprini tribes evolved in Eurasia during the early Miocene (Turner and Anton, 2004; Prothero and Foss, 2007; Kingdon and Hoffman, 2013; Geraads *et al*., 2008; Holland, 2015). Fossil genera including *Palaeoryx*, *Tragoreas*, and *Damalavus*, were intermediates between Hippotragini, Alcephalini, and Caprini (Prothero and Foss, 2007; Holland, 2015). Late Miocene crown members of Hippotragini are found in Northern Africa and include *Tchadotragus* and *Saheloryx* (Geraads *et al.*, 2008). It was not until the Pliocene that the Hippotragini diversity exploded in Eurasia as a response to the cooling

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temperatures and the advancement of grasslands (Holland, 2015).

There has been a considerable advancement in sequencing mammalian mtDNA molecules and their analysis. Mitochondrial DNA (mtDNA) possesses several favourable characteristics, including large quantity in the cell simplicity of extraction, small genome size, haploid, maternal inheritance with extremely low probability of paternal leakage, higher mutation rate than nuclear DNA, and the amenability to change mainly through mutation rather than recombination. All these features make mtDNA a useful and one of the most frequently used markers for evolutionary and phylogenetic studies (Ghassemi-Khademi, 2017).

Several researchers (Amir and Shobrak, 2011; Hassanin *et al*., 2012; Matthee and Davis, 2001; Elmeer *et al*., 2012; Iyengar *et al*., 2006; Khan *et al*., 2011; Kuznetsova *et al*., 2002; Masembe *et al*., 2006; Matthee and Robinson, 1999; Osmers *et al*., 2012; Khan *et al*., 2008; Arif *et al*., 2009; Themudo *et al*., 2015; Yang *et al*., 2013) have studied the phylogenetic relationships of species belonging to the tribe Hippotragini using different genes, but there is no comprehensive re-evaluation study of the phylogenetic relationships within the tribe of Hippotragini using different mitochondrial genes. Determining the phylogenetic relationships between the genera belonging to this subfamily can be an effective step in planning for the conservation of these animals in the wild. Most species belonging to this subfamily are in serious danger of extinction and their populations are clearly declining (Holland, 2015).

2. Materials and Methods

Gene sequences including cytochrome b (n=54), 12S ribosomal RNA (or 12S rRNA) (n=37), 16S ribosomal RNA (or 16S rRNA) (n=29), and cytochrome c oxidase I (n=12), and complete mitochondrial genome sequences (n=10) (a total of 142 sequences) belonging to the tribe Hippotragini were downloaded from NCBI (Tables 1, 2, 3, 4 and 5). The titles of the received sequences were edited by ExcaliBAR (Aliabadian *et al*., 2014); and BioEdit 7.0.5.3 software (Hall 1999) was used to create a DNA sequence alignment using Clustal W algorithm (Thompson *et al*., 1994) in all of the received sequences. In all of the studied genes, the corresponding gene sequences of *Pelea capreolus* were used as an outgroup in the analyses.

The most appropriate sequence evolution model for the given data was determined with Model test (Posada and Crandall, 1998) as implemented in the MEGA6 software (Tamura *et al*., 2013). The model with the lowest BIC (Bayesian Information Criterion) scores is considered to best describe the substitution pattern. The evolutionary history was inferred using the Maximum Likelihood method "ML" for each of the studied genes separately. The trees were calculated with 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 (1000 replicates), were shown next to the branches (Felsenstein 1985). In all of the above-mentioned analyses, all positions containing gaps and missing data were eliminated, and were conducted in MEGA6 (Tamura *et al*., 2013). Also, the robustness of clades was calculated by the bootstrap method. In this study, 50-60 % was considered as a weak support (as bootstrap values), 61-75 % as a moderate support, 76-88 % as a good support, and ≥89 % as strong support values (Retrieved from: Win *et al*., 2017; with minor modification).

Table 1. The accession numbers of cytochrome b genes and scientific names of studied species from the tribe Hippotragini (n=54) received from GenBank (www.ncbi.nlm.nih.gov)(n=55).

Scientific Names	Accession Numbers	Scientific Names	Accession Numbers
Hippotragus equinus	AF022060	*Oryx leucoryx*	FJ937683
	HQ641316		JN632679
	JF728769		NC_020732
	JN632647	*Oryx gazella*	JN869312
	NC_020712		KC282640
Addax nasomaculatus	AF034722		NC_016422
	JN632591		JN632678
	KM582122		JF728779
	NC_020674	*Oryx dammah*	JF728778
Oryx beisa	DQ138192		JN632677
	DQ138193	*Pelea capreolus*	AF022055
	DQ138194	*Oryx leucoryx*	FJ937660
	DQ138195		FJ937661
	DQ138196		FJ937664
	DQ138197		FJ937665
	DQ138198		FJ937670
	DQ138199		FJ937676
	DQ138200		FJ937679
	DQ138201		FJ937680
	DQ138202		FJ937681
	DQ138203		FJ937682
	DQ138204		
	DQ138205		
	DQ138206		
	DQ138207		
	DQ138208		
	DQ138209		
	DQ138210		
	HM209249		
	JN632676		
	NC_020793		

In addition, the Bayesian analyses of the studied gene sequences were run with the parallel version of MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003) on a cluster with one processor assigned to each Markov chain under the most generalizing model (GTR+G+I) because over parametrization apparently does not negatively affect Bayesian analyses (Huelsenbeck and Ranala, 2004). Each Bayesian analysis comprised two simultaneous runs of four Metropolis-coupled Markov-chains at the default temperature (0.2). Analyses were terminated after the chains converged significantly, as indicated by the average standard deviation of split frequencies<0.01. The Bayesian inference of phylogeny was conducted for 8,000,000 generations. Seven hundred bootstrap replicates were used as ML branch support values. The posterior probabilities equal/higher than 0.95 and bootstrap supports equal/higher than 70 % were considered as strong support values (Ghassemi-Khademi, 2018). The obtained phylogenetic trees were visualized and edited by FigTree software 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, three groups were determined and the pair fixation indices (F_{ST}) among these groups was calculated based on mitogenomes using Arlequin 3.5 (Excoffier *et al*., 2005). Referring to the criterion for genetic differentiation by Wright (1978), 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 $F_{ST}>0.25$.

Table 2: The accession numbers of 12S ribosomal RNA (or 12S rRNA) genes and scientific names of studied species from the tribe Hippotragini (n=37) received from GenBank (www.ncbi.nlm.nih.gov)(n=38).

Scientific Names	Accession Numbers	Scientific Names	Accession Numbers
Hippotragus niger	AY670653	*Oryx dammah*	U86970
	KM245339		JN632677
	JN632648	*Oryx beisa*	JN632676
	NC_020713		NC_020793
	U86974	*Pelea capreolus*	U86994
Hippotragus leucophaeus	AF091709	*Oryx gazella*	M86500
	NC_035309		JN869312
	MF043256		NC_016422
Addax nasomaculatus	U86973		JN632678
	JN632591		KC282640
	NC_020674	*Oryx leucoryx*	FJ914296
Oryx leucoryx	FJ914314		FJ914294
	FJ914312		FJ914293
	FJ914311		FJ914292
	FJ914308		FJ914291
	FJ914306		U86971
	FJ914305		JN632679
	FJ914302		NC_020732
	FJ914299		
	FJ914297		

Table 3. The accession numbers of 16S ribosomal RNA (or 16S rRNA) genes and scientific names of studied species from the tribe Hippotragini (n=29) received from GenBank (www.ncbi.nlm.nih.gov)(n=30).

Scientific Names	Accession Numbers	Scientific Names	Accession Numbers
Addax nasomaculatus	U87023	*Oryx leucoryx*	FJ914269
	JN632591		FJ914268
	NC_020674		FJ914267
	AY122049		U87021
Hippotragus niger	U87024		JN632679
	KM245339		NC_020732
	JN632648	*Oryx gazella*	M86500
	NC_020713		JN869312
Hippotragus leucophaeus	NC_035309		NC_016422
	MF043256		JN632678
Hippotragus equinus	U87025		KC282640
	JN632647	*Oryx dammah*	U87020
	NC_020712		JN632677
Pelea capreolus	U87044	*Oryx beisa*	JN632676
			NC_020793

Table 4. The accession numbers of cytochrome c oxidase I (COX1) genes and scientific names of studied species from the tribe Hippotragini (n=12) received from GenBank (www.ncbi.nlm.nih.gov)(n=13).

Scientific Names	Accession Numbers	Scientific Names	Accession Numbers
Oryx dammah	JF444371	*Addax nasomaculatus*	JN632591
Hippotragus equinus	HQ603146		NC_020674
	HQ603145	*Hippotragus niger*	JN632648
	HQ603144		NC_020713
Oryx gazella	JF444372	*Oryx beisa*	JN632676
	JX436995	*Pelea capreolus*	JN632684
Hippotragus leucophaeus	MF043256		

Table 5. Nucleotid composition of mtDNA of studied species from the tribe Hippotragini (n=10) received from GenBank (www.ncbi.nlm.nih.gov).

Scientific name	**Accession Number**	**T**	**C**	**A**	**G**	**Total**	**References**
Addax nasomaculatus	JN632591	27.0	26.1	33.6	13.3	16751.0	(Hassanin *et al*. 2012)
Oryx gazella	JN869312	26.9	26.1	33.4	13.5	16661.0	Direct Submission
Oryx beisa	JN632676	27.0	26.1	33.5	13.3	16518.0	(Hassanin *et al*. 2012)
Oryx dammah	JN632677	27.1	26.1	33.4	13.5	16688.0	(Hassanin *et al*. 2012)
Oryx leucoryx	JN632679	27.2	26.0	33.5	13.3	16680.0	(Hassanin *et al*. 2012)
Oryx gazella	JN632678	27.1	26.0	33.5	13.4	16667.0	(Hassanin *et al*. 2012)
Hippotragus equinus	JN632647	26.5	26.6	33.6	13.3	16436.0	(Hassanin *et al*. 2012)
Hippotragus leucophaeus	MF043256	26.5	26.6	33.4	13.5	16468.0	(Themudo and Campos 2017)
Hippotragus niger	JN632648	26.6	26.5	33.3	13.6	16506.0	(Hassanin *et al*. 2012)
Hippotragus niger	KM245339	26.8	26.6	33.1	13.4	16160.0	(Themudo *et al*. 2015)
Avg.		26.9	26.3	33.4	13.4	16553.5	************
Pelea capreolus	JN632684	*	*	*	*	*	(Hassanin *et al*. 2012)

3. Results

As the results indicated, outgroups were separated from the tribe members in all of the phylogenetic trees (Figures 1-10), implying the presence of relatively close genetic distances among tribe members. Also, in most of the phylogenetic trees, all species belonging to a single genus were clustered together. In addition, in order to show the accuracy of the phylogenetic analyses, different genera of this tribe were demarcated precisely. The average length of mitochondrial genome was calculated 16553.5 bp. In 16553.5, the average base composition of mtDNA sequences was 26.9 % T, 26.3 % C, 33.4 % A, and 13.4 % G, showing a strong AT bias (60.3 %).

In addition, in all of the phylogenetic trees, the BI posterior probability values of the tribe members were equal to 100; thus, it can be inferred that the tribe Hippotragini is a monophyletic group with the highest BI posterior probability value. Also, based on lowest BIC, Tamura 3-parameter model (Tamura, 1992) was chosen as the best model for ML trees of 16S rRNA, COX1, and 12S rRNA genes. Hasegawa-Kishino-Yano (Hasegawa *et al*., 1985) and Kimura 2-parameter (Kimura, 1980) were the best models for complete mitochondrial genomes and cytochrome b genes, respectively.

Based on the topology of the ML phylogenetic tree of 12S rRNA sequences, the relationship of different genera belonging to the tribe Hippotragini is as follows: [{(*O. leucoryx*) + (*O. dammah*) + (*O. gazella*) + (*O. beisa*) + *A. nasomaculatus*)} + {(*H. leucophaeus*) + (*H. niger*)}].

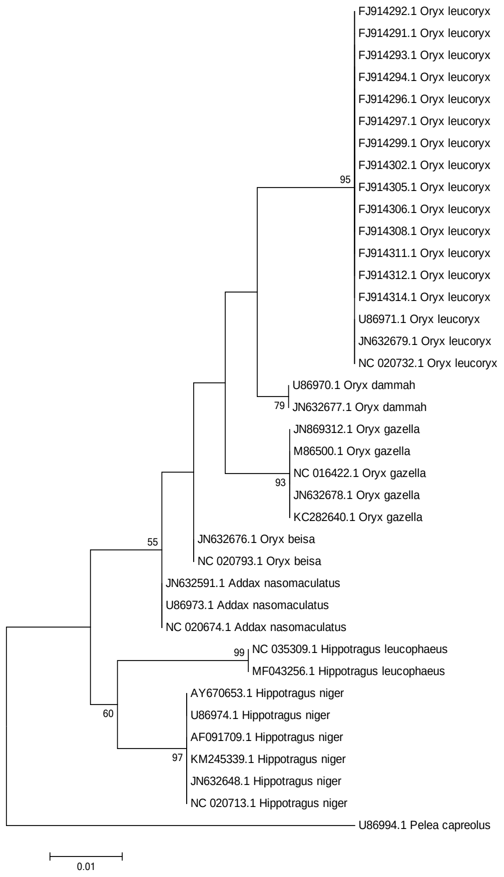

Figure 1. Maximum Likelihood tree based on Tamura 3-parameter distance using 12S ribosomal RNA (12S rRNA) sequences; the numbers on each branch correspond to bootstrap support values (percentages lower than 50 are not shown). The tree was rooted with a single *Pelea capreolus* sequences.

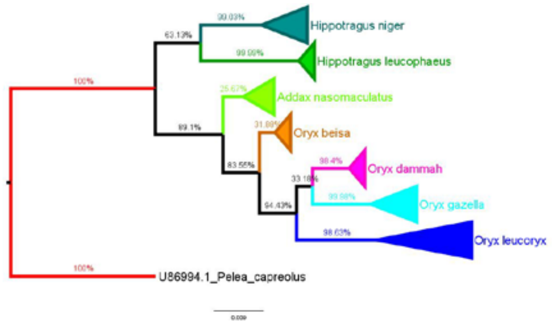

Figure 2. Bayesian phylogeny reconstructed based on 12S ribosomal RNA (12S rRNA) sequences. The values beside the branches are BI posterior probability values. The tree was rooted with a single *Pelea capreolus* sequence.

In this tree, two distinct major clusters can be distinguished. The results showed that all species belonging to the genera *Addax* and *Oryx*, have the weak (but acceptable) supported ML bootstrap (=55) and high BI posterior probability (=89.1) values. In another cluster, two species including *H. niger* and *H. leucophaeus* constructed a monophyletic group with moderate BI posterior probability (=63.13) and weak (but acceptable) supported ML bootstrap (=60) values.

Moreover, based on the topology of ML phylogenetic tree of 16S ribosomal RNA (16S rRNA) sequences, the relationship of different genera belonging to the tribe Hippotragini is as follows:

[{(*O. leucoryx* + *O.dammah*) + (*O. beisa* + *O. gazelle*) + *A. nasomaculatus*} + {(*H. niger*) + (*H. leucophaeus* + *H. equinus*)}].

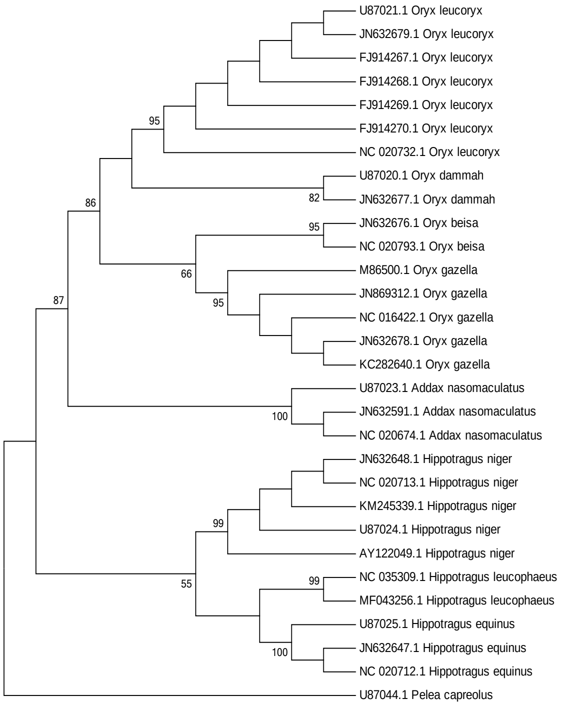

Figure 3. Maximum Likelihood tree based on Tamura 3-parameter distance using 16S ribosomal RNA (16S rRNA) sequences; the numbers on each branch correspond to bootstrap support values. (percentages lower than 50 are not shown). The tree was rooted with a single *Pelea capreolus* sequence.

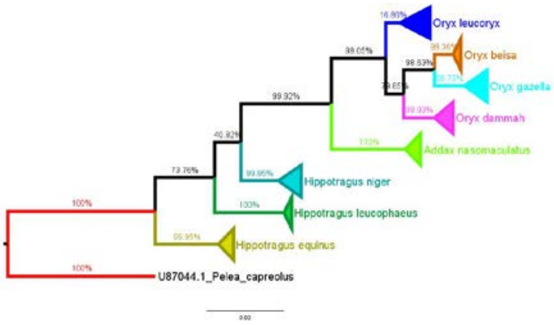

Figure 4. Bayesian phylogeny reconstructed based onbased on 16S ribosomal RNA (16S rRNA) sequences. The values beside the branches are BI posterior probability values. The tree was rooted with a single *Pelea capreolus* sequence.

In this phylogenetic tree also, two distinct major clusters can be distinguished. Where all of the sequences belonging to the genera *Oryx* and *Addax* showed good supported ML bootstrap (=87) and very high BI posterior probability (=99.92) values. In another cluster, three species including *H. niger*, *H. equines*, and *H. leucophaeus* built a monophyletic group with weak (but acceptable) supported ML bootstrap (=55). Of course in this case, the topology of the Bayesian phylogenetic tree was slightly different from the topology of ML phylogenetic tree; because in this tree, three species belonging to the genus *Hippotragus* did not construct a single monophyletic group.

Furthermore, based on the topology of ML phylogenetic tree of Cytochrome b sequences, the relationship of different genera belonging to the tribe Hippotragini is as follows:

[{((*O. beisa*)+(O.*gazella*+*O. leucoryx*)+*O.dammah*)+*A. nasomaculatus*}+{*H. equinus*}].

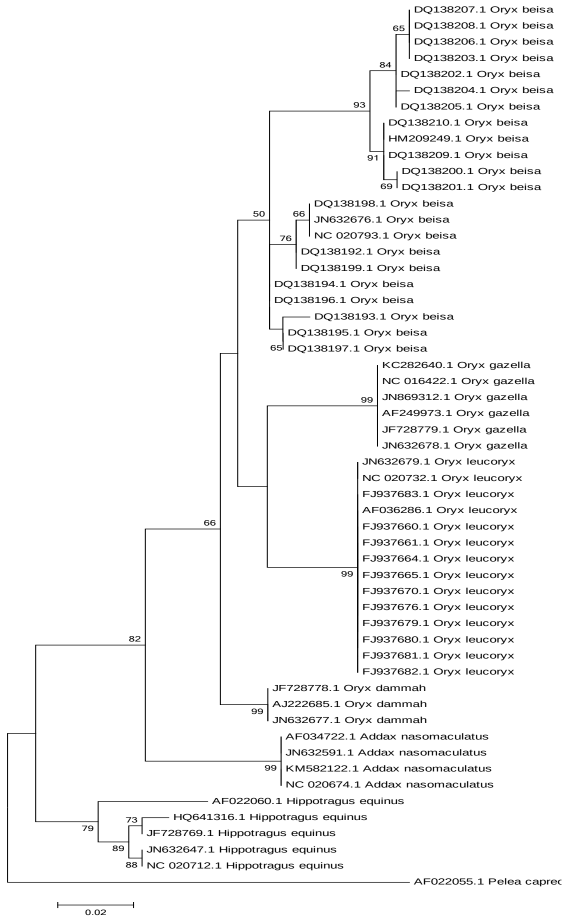

Figure 5. Maximum Likelihood tree based on Kimura 2-parameter distance using Cytochrome b sequences; the numbers on each branch correspond to bootstrap support values. (percentages lower than 50 are not shown). The tree was rooted with a single *Pelea capreolus* sequence.

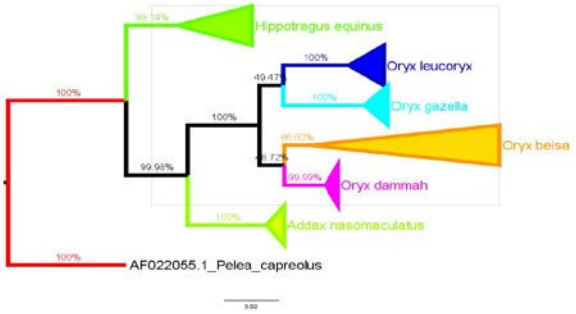

Figure 6. Bayesian phylogeny reconstructed based on Cytochrome b sequences. The values beside the branches are BI posterior probability values. The tree was rooted with a single *Pelea capreolus* sequence.

In this phylogenetic tree, there are, also, two distinct major clusters. All of the species belonging to the genera *Oryx* and *Addax* constructed a single monophyletic group with good supported ML bootstrap (=82) and very high BI posterior probability (=99.98) values. In another cluster, all of the sequences belonging to the species *H. equinus* constructed a single cluster with good supported ML bootstrap (=79) and very high BI posterior probability (=99.74) values.

Moreover, based on the topology of ML phylogenetic tree of Cytochrome c oxidase I (COXI) sequences, the relationship of different genera belonging to the tribe Hippotragini is as follows:

[{((*O. dammah* + *O. beisa*) + *A. nasomaculatus*) + *O. gazella*} +{(*H. equinus*) +((*H. leucophaeus*)+(*H. niger*))}].

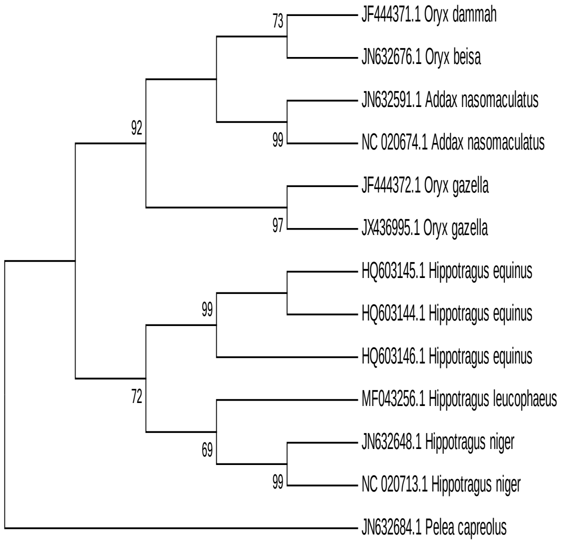

Figure 7: Maximum Likelihood tree based on Tamura 3-parameter distance using Cytochrome c oxidase I (COX1) sequences; the numbers on each branch correspond to bootstrap support values. (Percentages lower than 50 are not shown). The tree was rooted with a single *Pelea capreolus* sequence.

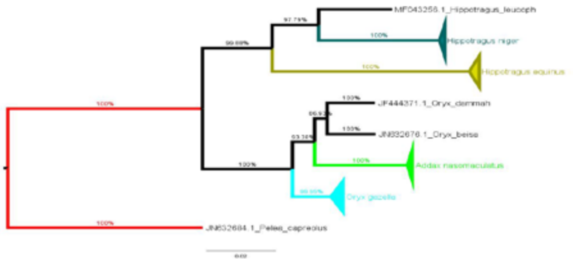

Figure 8: Bayesian phylogeny reconstructed based on Cytochrome c oxidase I (COX1) sequences. The values beside the branches are BI posterior probability values. The tree was rooted with a single *Pelea capreolus* sequence.

In this tree, there are two distinct major clusters too. All of the species belonging to the genera *Oryx* and *Addax* constructed a single monophyletic group with strong supported ML bootstrap (=92) and highest BI posterior probability (=100) values. In another major cluster, three species belonging to the genus *Hippotragus* constructed a single monophyletic cluster with moderate supported ML bootstrap (=72) and very high BI posterior probability (=99.88) values.

Eventually, based on the topology of ML phylogenetic tree of complete mitochondrial genome sequences, the relationship of different genera belonging to the tribe Hippotragini is as follows:

[{((*O. dammah* + *O. leucoryx*) + *O. beisa*) + (*O. gazella*) + *A. nasomaculatus*}+{*H. equinus* + (*H. leucophaeus* + (*H. niger*))}].

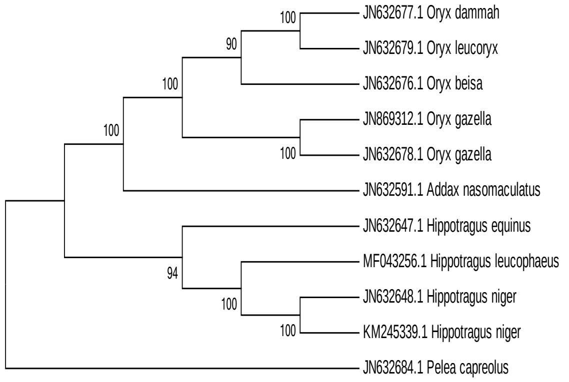

Figure 9. Maximum Likelihood tree based on Hasegawa-Kishino-Yano distance using complete mitochondrial genome sequences. The numbers on each branch correspond to bootstrap support values. The tree was rooted with a single *Pelea capreolus* sequence.

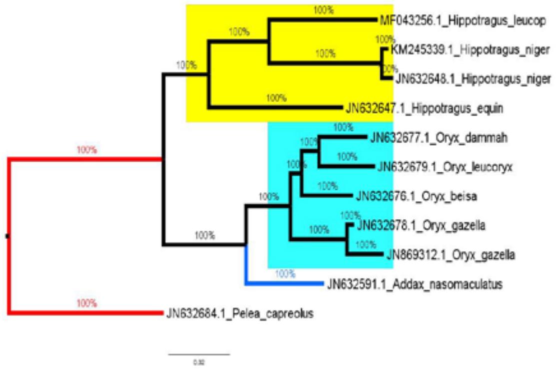

Figure 10. Bayesian phylogeny reconstructed based on complete mitochondrial genome sequences. The values beside the branches are BI posterior probability values. The tree was rooted with a single *Pelea capreolus* sequence.

Based on the topology of this tree that is probably close to reality, and similar to previous phylogenetic trees, there are two completely distinct major clusters. All of the species belonging to the genera *Oryx* and *Addax* constructed a single monophyletic group with strongest supported ML bootstrap (=100) and highest BI posterior probability (=100) values. In another major cluster, three species belonging to the genus *Hippotragus* constructed a single monophyletic cluster with strong supported ML bootstrap (=94) and highest BI posterior probability (=100) values.

Using complete mitochondrial genomes, all of the species belonging to a single genus were considered as a separate group. In addition to the outgroup, four 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 the ingroup. The shortest distance was obtained between the genera *Addax* and *Oryx* (=841), and the longest distance was found between the genera *Oryx* and *Hippotragus* (=1529). Also, the genetic distance between the genera *Addax* and *Hippotragus* was equal to 1486. Furthermore, genetic differentiation between populations using pairwise F_{ST} values were very high ($F_{ST}>0.25$). According to the results of genetic distances, the highest F_{ST} was detected between *Oryx* and *Hippotragus* (=0.484), and lowest F_{ST} was detected between the genera *Addax* and *Oryx* (0.326). The genetic differentiation between the genera *Oryx* and *Hippotragus* was significant (F_{ST} P-value=0.027) (Table 6).

Table 6. Pairwise F_{ST} based on complete mitochondrial genomes between different genera belonging the tribe Hippotragini.

Name	*Oryx*	*Addax*	*Hippotragus*
Oryx	0.00		
Addax	0.326	0.00	
Hippotragus	0.484	0.354	0.00

4. Discussion

In this study, four gene fragments in addition to complete mitochondrial genomes were used to evaluate the phylogenetic relationships within the tribe of Hippotragini. Interrelationships among Hippotragini have been largely overlooked (Holland, 2015).

In a comprehensive phylogenetic study using supertree methodology consisting of 201 source trees, Price *et al.* (2005) introduced *Hippotragus* as a sister group to *Addax*/*Oryx* clade. The results of the present study are exactly consistent with these results. In all of the phylogenetic trees, *Hippotragus* was located as a sister group to *Addax*/*Oryx* monophyletic cluster.

In another comprehensive and interesting phylogenetic research, Hassanin *et al*. (2012) evaluated the pattern and timing of diversification of Cetartiodactyla, using complete mitochondrial genomes (Hassanin *et al*., 2012). They used seven complete mtDNA sequences belonging to *H. equinus*, *H. niger*, *A. nasomaculatus*, *O. beisa*, *O. gazella*, *O. leucoryx*, and *O. dammah* for the tribe Hippotragini. The phylogenetic relationships of different species of Hippotragini were as follows:

[{*H. equinus* + *H. niger*} + {*A. nasomaculatus* + ((*O. beisa*) + (*O. gazella*) + (*O. leucoryx* + *O. dammah*))}]; and the bootstrap percentages of maximum likelihood and Bayesian trees supported a monophyletic cluster for the tribe Hippotragini (100 %) with the genus *Hippotragus* (82 %) as a sister group to *Addax*/*Oryx* (100 %) (Hassanin *et al*., 2012). Hassanin *et al*., (2012) used seven complete sequences belonging to the tribe Hippotragini, but in the present study, we added three other sequences (received from NCBI) to this seven mentioned sequences. For this reason, the phylogenetic relationships within the tribe Hippotragini in the tree in this study is slightly different than those based on seven complete mitochondrial genomes (specially in the case of the species belonging to the genus *Hippotragus*). The obtained topologies from complete mitochondrial genome provide a higher level of support than those based on individual or partial mitochondrial genes (Krzywinski *et al*., 2006) and probably the topology presented in this paper, is likely to be closer to reality.

In all of the obtained phylogenetic trees (except for cytochrome oxidase I), the genus *Addax* was a sister group of all species belonging to the genus *Oryx*. Interestingly, in addition to the complete mitochondrial genomes, this theory is also proven using other gene fragments including: 12S ribosomal RNA, 16S ribosomal RNA and Cytochrome b. Accordingly, these two genera are sister groups. This theory has also been confirmed using control region (D-loop) gene fragments (Khan *et al*., 2008b).

In another study, Yang *et al*. (2013) analyzed phylogenetic relationships within the family Bovidae based on complete mitochondrial genomes. The relationship of the species belonging to the tribe Hippotragini was as follows:

[{*H. equinus* + *H. niger*}+ {(*O. gazelle* + (*O. beisa* + O. *leucoryx*)) + (*O. dammah* + *A. nasomaculatus*)}].

In the mentioned tree, *A. nasomaculatus* was clustered with *O. dammah* and this cluster was a sister group of other species belonging to the genus *Oryx*. Moreover, Themudo *et al*., (2015) presented a similar topology; as a result, the genus *Oryx* would be considered as polyphyletic. However, all of the studied genes (except COXI) in the present study constructed a single clade for this genus, so all species belonging to the genus *Oryx* may be form a single monophyletic group, and *A. nasomaculatus* is considered as its sister group. Yang *et al.* (2013) considered Hippotragines as a subfamily (Hippotraginae), but current molecular evidences suggest that they are best relegated to a tribal level within the subfamily Antilopinae (Groves and Grubb, 2011).

Among the different species of the genus *Oryx*, the scimitar-horned oryx (*O. dammah*) and the Arabian oryx (*O. leucoryx*) showed very close phylogenetic relationships. These two species constructed a single monophyletic cluster as shown by the maximum likelihood (ML) trees of 12S rRNA and 16S rRNA, especially by the maximum likelihood and Bayesian phylogenetic trees of complete mitochondrial genomes (Ghassemi-Khademi 2017). Other studies have shown similar results (Khan *et al*., 2008c; Arif *et al*., 2009; Hassanin *et al*., 2012). These two species are probably sister species, although cytochrome b and Cytochrome c oxidase I (COX I) failed to cluster them in the ML trees.

Except for complete mitochondrial genomes, Bayesian phylogenetic trees did not cluster the scimitar-horned oryx and Arabian oryx; therefore, the maximum likelihood methods may be more superior to the Bayesian method in inferring phylogenetic relationships using gene fragments in this tribe. Likelihood-based approaches have proven to be especially powerful for inferring phylogenetic trees, but they tend to be prohibitively slow due to the requirement of multidimensional space for possible outcomes (optimal trees), in addition to the computational complexity of bootstrap repetitions (Khan *et al*., 2008c).

The genus *Oryx* is a monophyletic taxon. In the study of Hassanin *et al*. (2012), all of the species belonging to this genus have constructed a single cluster with strong bootstrap percentages of maximum likelihood (=94), but in this tree, two different clades can be distinguished where three species of *O. gazella*, *O. leucoryx*, and *O. dammah* constructed a monophyletic group, but the node of this clade has not a strong bootstrap support value (=56) and *O. beisa* is the sister taxon. In the present study, the topology of phylogenetic relationships within *Oryx* genus was different from that in Hassanin *et al*. (2012).

Based on the results of present study, Bayesian phylogenetic trees based on complete mitochondrial genomes, in addition to cytochrome b, 16S rRNA, and 12S rRNA genes, reconstructed a monophyletic group of the genus *Oryx* with high BI posterior probability values of 100, 100, 98.05, 83.55, respectively. Also, maximum likelihood phylogenetic trees based on complete mitochondrial genomes, in addition to cytochrome b and 16S rRNA genes reconstructed a monophyletic group with strong (100), moderate (66) and good (86) bootstrap support values, respectively. The monophylethic clade of *Oryx* in cytochrome b gene was not supported by acceptable bootstrap support values (percentage was lower than 50).

Based on complete mitochondrial genomes, two distinct clades have been distinguished within the genus *Oryx*, where three species including: *O. dammah*, *O. leucoryx*, and *O. beisa* form a monophyletic group with a strong support bootstrap value (=90), and two sequences belonging to *O. gazella* were located as a sister group for this monophyletic group with the highest support value (=100). This result was not illustrated in the works of Hassanin *et al*. (2012) and Yang *et al*. (2013). This result was also confirmed using cytochrome c oxidase I (COXI) genes, even though in the mentioned phylogenetic tree, *A. nasomaculatus* nested within the clade of the genus *Oryx*, but *O. gazella* was distinguished from other species.

In the present paper, two different clades were introduced within the genus *Oryx*; this topology is proposed as follows:

[{(*O. dammah* +*O. leucoryx*)+*O. beisa*}+{*O. gazella*}]; and *A. nasomaculatus* as a sister group for this monophyletic group.

The genus *Hippotragus* consists of three species: *H. leucophaeus* (blaubok) (extinct), *H. equinus* (roan antelope), *H. niger* (sable). *H. n. roosevelti* (Roosevelt's sable) and *H. n. variani* are sometimes considered as full species (Groves and Grubb 2011). In the previous studies (Hassanin *et al*. 2012, Yang *et al*. 2013, Themudo *et al*. 2015), only complete sequences of mtDNA of *H. niger* and *H. equinus* were used, while in this study, complete mitochondrial genomes of Blaubok (*H. leucophaeus*) were also compared. As a result, the topology of the phylogenetic relationships within this genus may be different.

Relatively, only very little has been published on the relationship between this extinct species (*H. leucophaeus*) and other species of the genus *Hippotragus*. The results of this study indicate that *H. n. niger* and *H. n. variani* have a very close phylogenetic relationship with the blaubok (*H. leucophaeus*) forming a single monophyletic group with highest bootstrap support value (=100). The roan antelope (*H. equinus*) is nested as their sister group with a high bootstrap value (=94). There is no complete mitochondrial sequence of *H. n. roosevelti or H. n. variani* Cytochrome c oxidase I phylogenetic tree also confirmed this topology, where two sequences of *H. niger* constructed a single monophyletic group with *H. leucophaeus* with a very high BI posterior probability value (=97.79), and *H. equinus* is the sister group of this clade. This topology has been also depicted by Themudo and Campos (2018).

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