

Evaluation of phylogenetic relationships of Antilopini and Oreotragini tribes (Bovidae: Artiodactyla) based on complete mitochondrial genomes

Taghi Ghassemi-Khademi

Iranian academic center for education, culture and research (ACECR), Ardabil, Iran.
e-mail: t.ghasemi@acecr.ac.ir

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Abstract

In this research, phylogenetic relationships of 24 species from the subfamily Antilopinae were evaluated using complete mitochondrial genomes. The average base composition of mtDNA sequences was 27.8% T, 25.2% C, 33.7% A, and 13.3% G, showing a strong AT bias (61.5%). The phylogenetic trees were investigated using the NJ, ME and UPGMA methods and found that they have very identical topologies. Overall, consistent with findings of previous studies, the results revealed that the Antilopini tribe has been correctly demarcated. Also, it was found that the Oreotragini tribe, which is represented by a single species (*Oreotragus oreotragus*), is completely separated from the Antilopini tribe and thus its taxonomic position must be reviewed again. In general, the results of this study indicated that the complete mitochondrial genomes are very useful, powerful, and accurate tools for evaluating the phylogenetic relationships of animals and biosystematics studies. Besides,

using these genomes, we can meticulously reconstruct and modify the animal classification.

Keywords: Antilopinae, phylogeny, mtDNA, biosystematics, taxonomy.

Introduction

Taxonomically, Antilopes belongs to Kingdom: Animalia (metazoan), Phylum: Chordata, Subphylum: Vertebrata, Class: Mammalia, Subclass: Theria, Super- order: Eutheria (Placenta), Order: Artiodactyla, Suborder: Ruminantia, Family: Bovidae, Sub-family: Antilopinae, and tribe: Antilopini (Wilson and Reeder 2005).

Based on the traditional and previous classifications, the subfamily of Antilopinae is comprised of three different tribe and sixteen live genera: the tribe Antilopini has eight genera including Genus *Gazella*, Genus *Antilope*, Genus *Ammodorcas*, Genus *Antidorcas*, Genus *Litocranius*, Genus *Eudorcas*, Genus *Nanger*, and Genus *Procapra*. The tribe Saigini has two genera including Genus *Saiga* and Genus *Pantholops*. This genus sometimes is classified in Caprinae Subfamily. The tribe Neotragini also has six genera including Genus *Dorcatragus*, Genus *Madoqua*, Genus *Neotragus*, Genus *Oreotragus*, Genus *Ourebia*, and Genus *Raphicerus* (Malcolm *et al.* 1997, Wilson and Reeder 2005). The sister taxon of Antilopinae subfamily is Bovinae subfamily and both groups comprise the artiodactyl family Bovidae (Bärmann *et al.* 2013).

Nowadays, Antilopinae subfamily includes nine tribes, which are very distinct from each other: Hippotragini, Alcelaphini, Reduncini, Cephalophini, Neotragini, Oreotragini, Aepycerotini, Antilopini, and Caprini (Castelló 2016). Antilopini tribe often referred to as true antelopes and consists true Gazelles (genera *Eudorcas*, *Gazella*, and *Nanger*), *Procapra* (which appear to be as different from the Gazelles as they are from the dwarf antelopes), *Saiga*, and dwarf antelopes (*Dorcatragus*, *Madoqua*, *Ourebia*, and *Raphicerus*). Also, the Oreotragini tribe, represented by one living genus, the klipspringer (*Oreotragus oreotragus*), are small, stocky antelopes, with females weighing more than and being slightly longer than males (Castelló 2016).

Recently Hassanin *et al.* (2012) have introduced new names for four subtribes for Antilopini (gazelles and their allies) tribe: Antilopina, based on the genus *Antilope*, consists of *Antilope cervicapra*, *Gazella* spp., *Nanger* spp., *Eudorcas* spp., *Antidorcas marsupialis*, *Ammodorcas clarkei*, *Litocranius walleri*, and *Saiga tatarica*; Procaprina, based on the genus *Procapra*, includes the three living *Procapra* species; Ourebina, based on the genus *Ourebia*, is monotypic and includes only *O. ourebi*; and Raphicerina, based on the genus *Raphicerus*, includes *Raphicerus* spp., *Dorcatragus megalotis*, and *Madoqua* spp. (Bärmann *et al.* 2013). Therefore, based on new classifications, the Antilopinae subfamily has several tribes that one of them is Antilopini tribe with four subtribes and Oreotragini is a separate tribe in this subfamily (Bärmann *et al.* 2013). The tribe Neotragini is presently known to be polyphyletic, as *Neotragus* and *Oreotragus* are not closely related to Antelopes (Bärmann *et al.* 2013).

In the hunting and itineraries literature, antilopes have been listed as two different animals; in the old classifications, the gazelles are considered as

antilopes relatives but the new classification calls all of them as true Antilopes. The body of true antilopes is not very large. Anatomically, they are significantly different from the rams, ewes, wild goats, and goats. They have narrow body and legs with a flat back or slightly raised bump. In some species, only the males have antlers while in some others the females also have very short and fine antlers. The antilopes are limited in semi-arid and arid regions, herbaceous steppes, or areas with trees and shrubs. Their food includes forage, trees leaves and shrubs, buds and young seedlings. Many differences are seen in antilopes regarding appearance as well as the habitat selection (Ghassemi-Khademi 2014).

In total, the gazelles, blackbucks, springboks, gerenuks, dibatags, and Central Asian gazelles are often referred to as 'true Antilopes', and are usually classified as the only representatives of the Antilopinae. True antelopes occur in much of Asia and Africa, with the highest concentration of species occurring in East Africa in Sudan, Eritrea, Ethiopia, Somalia, Kenya, and Tanzania (Wilson and Reeder 2005).

Gazelles are medium-sized ungulates with a fine body that mostly live in the deserts. They have big eyes and their snouts are hairy up to their nostrils. In the animal face, there is a middle dark band from the snout to the forehead and a smaller dark bar extends in each side of the face from the snout to the eyes front. The body color in the back is generally brownish-gray or reddish and white in the ventral part (Ghassemi-Khademi 2013).

Regarding apparent shape, the antilopes are divided into four groups:

1. Gazelle-like antilopes: Most of the antilopes species are in this group, and the studied species in this paper are considered as members of this group.

2. Bovid-like antilopes: They are similar to bovine in appearance, and mostly live in Africa.

3. Horse-like antilopes: They are like a horse in appearance, all living in arid and desert areas of Africa.

4. Goat-like antilopes: The group members are like goats and ibexes in appearance, and live in relatively rocky areas (Sheikhjabbari 2003).

Importance of mitochondrial genome in phylogenetic studies:

Mitochondrial DNA (mtDNA) is a circular DNA of 15000-20000 bp and is conserved in vertebrate animals. This genome is highly variable in structure, content, organization, and quality of genes expression in the different of animals (Zhang and Zhang 2013). 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 (Katouzian and Rajabi-Maham 2013).

Several mitochondrial genomes 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 (Zhang and Zhang 2013).

Although several researchers (Hassanin and Douzery 1999, Rebholz and Harley 1999, Wronski *et al.* 2010, Manuel *et al.* 2005, Gatesy *et al.* 1997, Groves 2000, Kuznetsova and Kholodova 2003, Lei *et al.* 2003, Marcot 2007, Bärermann *et al.* 2013), have studied the phylogenetic relationship among species

belonged to Antilopes, there is not an article about phylogenetic relationship of the subfamily of Antilopinae based on complete mitochondrial genomes. The Antilopes are ecologically, economically, and biologically important animals in the world. Thus, determining the phylogenetic relationships (especially based on the complete mitochondrial genome that can provide most accurate outcomes) between the genera belonging to this subfamily 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 subfamily today are in serious danger of extinction and declining of the population all over the world (Ghassemi-Khademi 2014).

Material and methods

All complete mitochondrial genome sequences belonged to Antilopinae subfamily were downloaded (n=24) from NCBI (Table: 1).

Sequences were aligned with Mega.6 (Tamura *et al.* 2013) using the Clustral W alignment method. Also, the corresponding gene sequences of *Tragelaphus strepsiceros* were used as an outgroup in this analysis.

The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei 1987). The optimal tree was identified with the sum of branch length = 1.16304401. 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 (Felsenstein 1985). The evolutionary distances, which were computed using the Kimura 2-parameter method (Kimura 1980). The rate variation among sites was modeled with a gamma distribution (shape parameter = 1). Evolutionary analyses were conducted in MEGA6 (Tamura *et al.* 2013).

Table 1. The sequence's GenBank accession numbers the species belonging to the Antilopini and Oreotragini tribes used for phylogenetic analysis.

| No. | Scientific name | Accession Number |
|-----|---------------------------------|------------------|
| 1 | <i>Gazella erlangeri</i> | JN632639 |
| 2 | <i>Gazella gazelle</i> | JN632640 |
| 3 | <i>Gazella dorcas</i> | JN632638 |
| 4 | <i>Gazella spekei</i> | JN632642 |
| 5 | <i>Gazella cuvieri</i> | JN632636 |
| 6 | <i>Gazella leptoceros</i> | JN632641 |
| 7 | <i>Gazella subgutturosa</i> | JN632643 |
| 8 | <i>Gazella bennettii</i> | JN632635 |
| 9 | <i>Eudorcas rufifrons</i> | JN632633 |
| 10 | <i>Nanger soemmerringii</i> | JN632667 |
| 11 | <i>Nanger dama</i> | JN632665 |
| 12 | <i>Nanger granti</i> | JN632666 |
| 13 | <i>Raphicerus campestris</i> | JN632693 |
| 14 | <i>Ourebia ourebi</i> | NC_020733 |
| 15 | <i>Antilope cervicapra</i> | AP003422 |
| 16 | <i>Antidorcas marsupialis</i> | JN632596 |
| 17 | <i>Procapra gutturosa</i> | JN632689 |
| 18 | <i>Procapra przewalskii</i> | GU386355 |
| 19 | <i>Oreotragus oreotragus</i> | JN632675 |
| 20 | <i>Madoqua kirkii</i> | JN632654 |
| 21 | <i>Madoqua saltiana</i> | JN632655 |
| 22 | <i>Saiga tatarica</i> | JN632700 |
| 23 | <i>Dorcatragus megalotis</i> | NC_020701 |
| 24 | <i>Litocranius walleri</i> | JN632653 |
| 25 | <i>Tragelaphus strepsiceros</i> | NC_020752 |

The evolutionary history was inferred using the Minimum Evolution method (Rzhetsky and Nei 1992). The evolutionary distances, which were computed using the Kimura 2-parameter method (Kimura 1980). Evolutionary analyses were conducted in MEGA6 (Tamura *et al.* 2013).

The evolutionary history was inferred using the UPGMA method (Sneath and Sokal 1973), where it was found that optimal tree has a sum of branch length = 1.04367943. 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 (Felsenstein 1985). The evolutionary distances,

computed using the Kimura 2-parameter method (Kimura 1980).

Evaluation of evolutionary divergence over sequence pairs between groups (different genera) also was calculated. Variance estimation method was bootstrap with 1000 replications. In this study, each genus was considered as a separate group; therefore, considering the outgroup we had 14 groups (Table 3). The rate variation among sites was modeled with a gamma distribution (shape parameter = 1). The analysis involved 25 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. Evolutionary analyses and nucleotide composition were calculated in MEGA6 (Tamura *et al.* 2013).

In all of the above, the analyzes involved 25 nucleotide sequences, wherein all positions containing gaps and missing data were eliminated. There was a total of 16016 positions in the final dataset.

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), 64-75% as a moderate support, 76-88% as a good support, and $\geq 89\%$ as a strong support as values (Win *et al.* 2017).

Results

Phylogenetic analysis of 24 species belonging to the subfamily Antilopinae was performed using complete sequences of the mtDNA. The average length of mitochondrial genome was calculated 16416.8 bp. In 16416.8, the average base composition of mtDNA sequences was 27.8% T, 25.2% C, 33.7% A, and 13.3% G, showing a strong AT bias (61.5%). Since each genus was considered as a separate group, in addition to the outgroup, 14 groups were determined and phylogenetic distances between these groups were calculated. As the results indicated (Table 3), the outgroup was at a distance far from

subfamily members, implying the presence of relative close genetic distances among subfamily members.

The shortest distances were obtained between three group 1, 2, and 3 (Group 1: *Gazella erlangeri*, *G. gazella*, *G. dorcas*, *G. spekei*, *G. cuvieri*, *G. leptoceros*, *G. subgutturosa*, *G. bennettii*; Group 2: *Eudorcas rufifrons*; Group 3: *Nanger soemmerringii*, *N. dama*, *N. granti*). Thus, it can be inferred that these three groups or these three genera (*Gazella*, *Eudorcas*, and *Nanger*) are phylogenetically the closest genera. As mentioned earlier, the longest distance was obtained between outgroup (*T. strepsiceros*) and other groups.

Molecular phylogenetic trees for complete mtDNA genomes were constructed using the NJ, ME, and UPGMA methods, which all provided the same topologies (Fig. 1, 2, and 3). Moreover, *T. strepsiceros*, used as the outgroup, was completely separated from other groups. Three phylogenetic trees revealed a great and main clade; all the species belonging to Antilopini tribe constructed a monophyletic clade with very strong support (as values $\geq 89\%$). Within this monophyletic clade, we can distinguish three distinct groups: Four genera including *Gazella*, *Eudorcas*, *Nanger*, and *Antilope* showed the highest supported monophyly values (=100) in all three NJ, ME, and UPGMA phylogenetic trees. Also, three genera including *Saiga*, *Antidorcas*, and *Litocranius*, with the highest supported values (=100) were placed next to this group. The second group consisted of two genera (*Ourebia* and *Procapra*) with a weak support value (=60) in both NJ and ME trees and the third group consisted of three genera (*Raphicerus*, *Dorcatragus*, and *Madoqua*) with a good support value (≥ 77) in both NJ and ME trees. The NJ, ME, and UPGMA phylogenetic trees showed that *Oreotragus oreotragus* was not included in any of these groups.

Table 2. Nucleotid composition of mtDNA of 24 species of Antilopinae subfamily.

| Scientific Name | T(U) | C | A | G | Total |
|-------------------------------|------|------|------|------|---------|
| <i>Gazella erlangeri</i> | 27.8 | 25.1 | 33.8 | 13.3 | 16432.0 |
| <i>Gazella gazella</i> | 27.8 | 25.1 | 33.8 | 13.4 | 16434.0 |
| <i>Gazella dorcas</i> | 27.6 | 25.4 | 33.8 | 13.2 | 16430.0 |
| <i>Gazella spekei</i> | 27.4 | 25.5 | 33.8 | 13.3 | 16434.0 |
| <i>Gazella cuvieri</i> | 27.3 | 25.5 | 33.9 | 13.2 | 16427.0 |
| <i>Gazella leptoceros</i> | 27.3 | 25.6 | 33.9 | 13.3 | 16434.0 |
| <i>Gazella subgutturosa</i> | 27.2 | 25.6 | 34.0 | 13.1 | 16434.0 |
| <i>Gazella bennettii</i> | 27.5 | 25.4 | 34.0 | 13.2 | 16431.0 |
| <i>Eudorcas rufifrons</i> | 27.7 | 25.1 | 33.8 | 13.3 | 16420.0 |
| <i>Nanger soemmerringii</i> | 27.6 | 25.4 | 33.5 | 13.5 | 16379.0 |
| <i>Nanger dama</i> | 27.6 | 25.4 | 33.5 | 13.5 | 16379.0 |
| <i>Nanger granti</i> | 27.7 | 25.3 | 33.5 | 13.5 | 16382.0 |
| <i>Raphicerus campestris</i> | 28.3 | 24.9 | 33.4 | 13.4 | 16394.0 |
| <i>Ourebia ourebi</i> | 26.6 | 26.3 | 33.4 | 13.7 | 16394.0 |
| <i>Antilope cervicapra</i> | 27.0 | 25.9 | 33.7 | 13.4 | 16431.0 |
| <i>Antidorcas marsupialis</i> | 28.8 | 24.3 | 33.8 | 13.1 | 16434.0 |
| <i>Procapra gutturosa</i> | 28.3 | 24.7 | 34.0 | 13.0 | 16434.0 |
| <i>Procapra przewalskii</i> | 28.3 | 24.7 | 34.0 | 13.0 | 16434.0 |
| <i>Oreotragus oreotragus</i> | 27.8 | 25.2 | 33.7 | 13.4 | 16434.0 |
| <i>Madoqua kirkii</i> | 27.4 | 25.7 | 33.0 | 13.9 | 16434.0 |
| <i>Madoqua saltiana</i> | 28.0 | 25.2 | 33.1 | 13.7 | 16406.0 |
| <i>Saiga tatarica</i> | 28.3 | 24.9 | 33.8 | 13.0 | 16377.0 |
| <i>Dorcatragus megalotis</i> | 29.3 | 23.9 | 33.9 | 12.9 | 16380.0 |
| <i>Litocranius walleri</i> | 28.1 | 24.9 | 34.1 | 12.9 | 16434.0 |
| Average | 27.8 | 25.2 | 33.7 | 13.3 | 16416.8 |

Based on the topology of NJ and ME phylogenetic trees, in group 1, the relationship of a genus of *Gazella* is as follows: [(*G. erlangeri* + *G. gazella*) + (*G. dorcas* + *G. spekei*)] + [*G. bennettii* + ((*G. subgutturosa*) + (*G. cuvieri* + *G. leptoceros*))]. On the other hand, the relationship of genera of *Eudorcas* and *Nanger* are as follows: [*E. rufifrons* + ((*N. granti*) + (*N. dama* + *N. soemmerringii*))]. The Nj and UPGMA trees showed that the species belonging to *Gazella* genus and genera of *Eudorcas* and *Nanger* are as sister groups together. Also, the relationship of three genera of *Saiga*, *Antidorcas*, and *Litocranius* was identified as follows: [*S. tatarica* + (*A. marsupialis* + *L. walleri*)].

In group 2, the relationship of *Ourebia* and *Procapra* genera are as follows: [*O. ourebi* + (*P. gutturosa* + *P. przewalskii*)] and the relationship of three genera of *Raphicerus*, *Dorcatragus*, and

Madoqua are as follows: [*R. campestris* + (*D. megalotis* + (*M. kirkii* + *M. saltiana*))]. In addition, *O. oreotragus*, do not form a monophyletic group with any of groups. In the UPGMA tree, the relationship of three genera of *Raphicerus*, *Dorcatragus*, and *Madoqua* is as follows: [(*D. megalotis* + (*M. kirkii* + *M. saltiana*))] + *R. campestris*. However, generally, in all three NJ, ME, and UPGMA trees, these four species are located in very close phylogenetic distances together.

Discussion

Antilopinae subfamily includes nine tribes, which are very distinct from each other (Castelló 2016). Antilopini tribe, often referred to as true antelopes, includes true Gazelles (genera *Eudorcas*, *Gazella*, and *Nanger*), *Procapra* (which appear to be as different from the Gazelles as they are from the dwarf antelopes), *Saiga*, and dwarf antelopes (*Dorcatragus*, *Madoqua*, *Ourebia* and *Raphicerus*) (Castelló 2016).

As shown in NJ, ME, and UPGMA trees in this study (Figs. 1, 2, and 3), the true Gazelles (genera: *Eudorcas*, *Gazella*, and *Nanger*) have formed a single cluster with a good support value (≥ 83) and species belonging to *Gazella* genus and (*Eudorcas* and *Nanger* genera) are sister groups together. Also, as can be seen from Figs. 1 and 3, this result is consistent with previous results and the new morphological classification of Antilopini (Castelló 2016), because these three genera are considered as true gazelles. In this regard, the results of this research and previous phylogenetic studies show close phylogenetic relationships among these three genera (Hassanin *et al.* 2012; Bärmann *et al.* 2013). Thus, the morphological classification of true gazelles is consistent with molecular information (Castelló 2016).

Furthermore, it was seen that the species belonging to *Gazella* genus are in very close phylogenetic distances to each other, suggesting that gazelles as a completely monophyletic group have originated from the single common ancestor.

This tribe (Antilopini) includes true gazelles (genera: *Eudorcas*, *Gazella*, and *Nanger*), *Procapra* (which appear to be as different from the Gazelles as they are from the dwarf antelopes), *Saiga*, and dwarf antelopes (*Dorcatragus*, *Madoqua*, *Ourebia*, and *Raphicerus*) (Castelló 2016).

The results of this study showed that the dwarf antelopes (*Dorcatragus*, *Madoqua*, *Ourebia*, and *Raphicerus*) did not fall in a single clade and, contrary to Castelló's view, *Procapra* genus is located in close phylogenetic distances with dwarf antelopes, especially *Ourebia* genus. This result is consistent with the results of previous studies (Ropiquet *et al.* 2009, Agnarsson and May-Collado 2008, Kuznetsova and Kholodova 2003).

Therefore, it is possible that to claim this genus (*Procapra*) should be placed in a group of dwarf antelopes. The results of this study showed that the dwarf antelopes are divided into two separate clades: [*R. campestris* + (*D. megalotis* + (*M. kirkii* + *M. saltiana*))] and [*O. ourebi* + (*P. gutturosa* + *P. przewalskii*)]. These results are approximately in agreement with previous findings (Ropiquet *et al.* 2009, Agnarsson and May-Collado 2008, Bärmann *et al.* 2013). In a comprehensive study, Bärmann *et al.* (2013) reconstructed the phylogenetic relationships of Antilopini from simultaneous maximum parsimony analyses using nine genes, four mitochondrial markers (*cytb*, *COIII*, *12S*, *16S*), and six nuclear genes (*KCAS*, *SPTBN1*, *PRKCI*, *MC1R*, *THYR*).

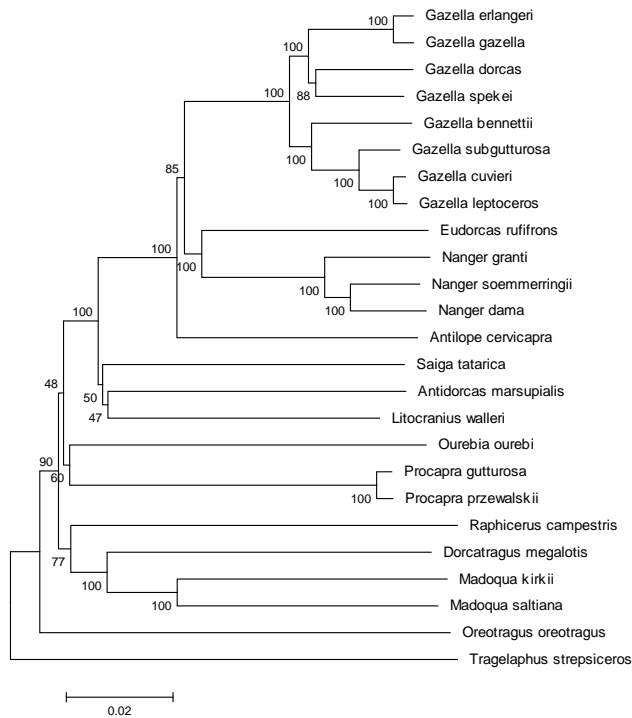


Figure 1. Neighbor-joining tree based on Kimura 2-parameter distance using complete mitochondrial genome sequences. The numbers on each branch correspond to bootstrap support values. The rate variation among sites was modeled with a gamma distribution (shape parameter = 1). The tree was rooted with a *T. strepsiceros* sequence.

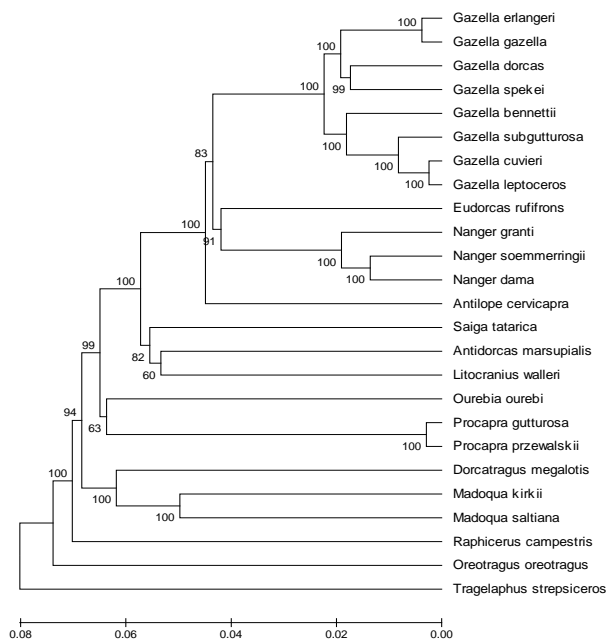


Figure 2. UPGMA tree based on Kimura 2-parameter distance using complete mitochondrial genome sequences. The numbers on each branch correspond to bootstrap support values. The tree was rooted with a *T. strepsiceros* sequence.



Figure 3. Minimum evolution 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 a *T. strepsiceros* sequence.

They constructed a single most parsimonious tree (MPT) with 7878 steps from analysis of the full-taxon (Fig. 5) with a topology similar to those of NJ, ME, and UPGMA trees in the present study. Therefore, the results of this study showed that dwarf antelopes obviously are divided into two distinct groups. Also, in this study, three genera from Antilopini (*Saiga*, *Antidorcas*, and *Litocranius*) showed the close phylogenetic relationships with each other [*S. tatarica* + (*A. marsupialis* + *L. walleri*)]. It is noteworthy that these results are very similar to the result of Bärmann et al. (2013) shown in Fig. 5. Therefore, these three genera are located in a separate group into Antilopina subtribe but with a very strong support value (=100) and formed a distinct clade with other Antilopes (True Antilopes and *Antilope cervicapra*); thus, they are considered as sister group with the mentioned group.

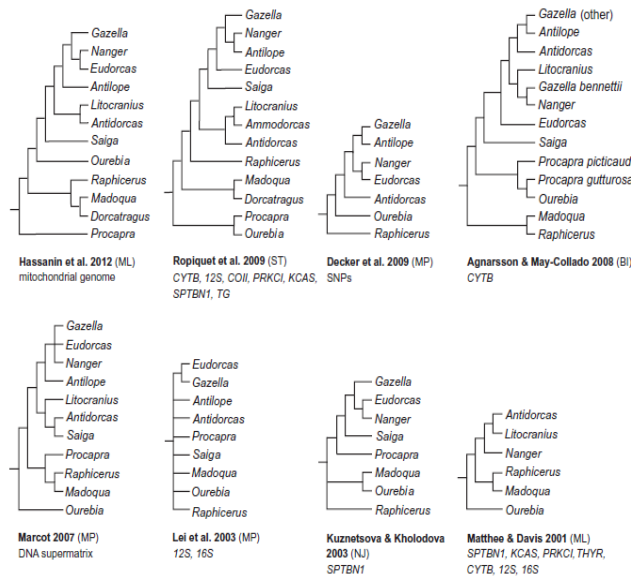


Figure 4. Phylogenetic relationships of Antilopini recovered in previous molecular studies since 2001 (retrieved from Bärmann *et al.* 2013). If you note carefully, the results of this study, are consistent with the results of the previous studies.

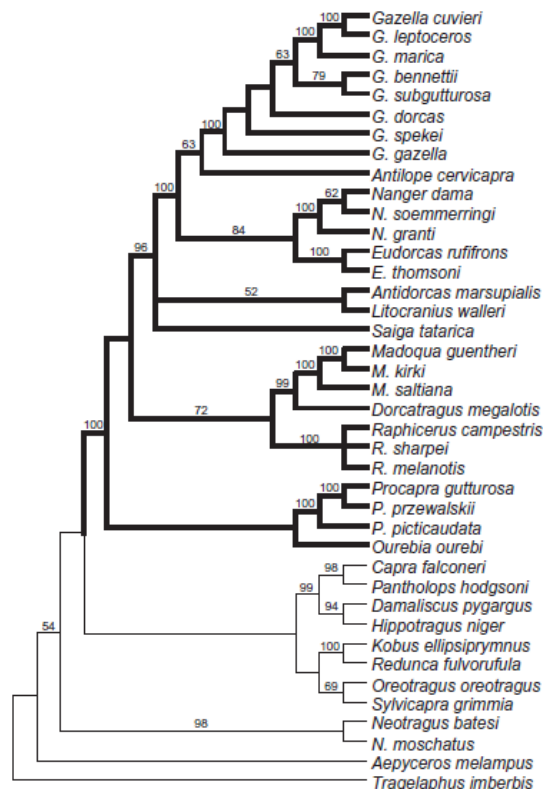


Figure 5. Phylogenetic relationships of Antilopini reconstructed from simultaneous maximum parsimony analyses of nine genes (CYTB, COIII, 12S, 16S, KCAS, SPTBN1, PRKCI, MC1R, and THYR). This tree is single most parsimonious tree (MPT) with 7878 steps from analysis of the full-taxon set (Bärmann *et al.* 2013).

Overall, a great clade was detected in NJ, ME, and UPGMA trees, with a very strong support value (≥ 89); which constitutes the Antilopini tribe, suggesting that this tribe has been correctly demarcated based on the morphological findings (Castelló 2016, Wilson and Reeder 2005, Bärmann *et al.* 2013, Ghassemi-Khademi 2014).

Also, the Oreotragini tribe that is presented by one living genus, the klipspringer (*O. oreotragus*), consists of small, stocky antelopes, with females slightly heavier and longer than the males (Castelló 2016). Nowadays, the tribe Neotragini is known to be polyphyletic, as *Neotragus* and *Oreotragus* are not closely related to Antilopes (Bärmann *et al.* 2013). The obtained NJ, ME, and UPGMA trees in this study revealed that Oreotragini tribe, which is represented by a single species (*O. oreotragus*), is completely separated from Antilopini tribe and is not located in a great cluster of Antilopini. As can be seen from Table 3, after the outgroup, the tribe Oreotragini on average has relatively large distances with members of the Antilopini tribe. Undoubtedly, the taxonomic position of this tribe must be reviewed again.

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 reconstruction and modification of existing animal classification.

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Table 3. Phylogenetic distances between genera belonging to the subfamily Antilopinae based on complete mitochondrial sequences.

| Name | Gp_1 | Gp_2 | Gp_3 | Gp_4 | Gp_5 | Gp_6 | Gp_7 | Gp_8 | Gp_9 | Gp_10 | Gp_11 | Gp_12 | Gp_13 | Gp_14 |
|-------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|-------|
| Gp_1 | | | | | | | | | | | | | | |
| Gp_2 | 1015.8 | | | | | | | | | | | | | |
| Gp_3 | 723.3 | 977.3 | | | | | | | | | | | | |
| Gp_4 | 1682.2 | 2047.0 | 1793.0 | | | | | | | | | | | |
| Gp_5 | 1610.7 | 1918.0 | 1648.7 | 2064.0 | | | | | | | | | | |
| Gp_6 | 1031.7 | 1348.0 | 1062.7 | 2060.0 | 1879.0 | | | | | | | | | |
| Gp_7 | 1371.6 | 1713.0 | 1428.3 | 2005.0 | 1891.0 | 1708.0 | | | | | | | | |
| Gp_8 | 1500.2 | 1852.0 | 1591.5 | 1903.0 | 1785.5 | 1789.0 | 1759.0 | | | | | | | |
| Gp_9 | 1788.8 | 2126.0 | 1881.0 | 2161.0 | 2127.0 | 2118.0 | 2059.0 | 1989.5 | | | | | | |
| Gp_10 | 952.6 | 1294.5 | 998.7 | 1293.0 | 1297.0 | 1236.0 | 1211.0 | 1146.3 | 1414.5 | | | | | |
| Gp_11 | 1394.6 | 1737.0 | 1450.7 | 1972.0 | 1919.0 | 1720.0 | 1651.0 | 1758.5 | 2079.0 | 1220.5 | | | | |
| Gp_12 | 1631.2 | 1979.0 | 1698.3 | 1999.0 | 1988.0 | 1942.0 | 1894.0 | 1865.0 | 2156.0 | 1051.5 | 1886.0 | | | |
| Gp_13 | 1299.7 | 1654.0 | 1348.3 | 1945.0 | 1792.0 | 1608.0 | 1554.0 | 1750.0 | 2067.0 | 1171.0 | 1577.0 | 1841.0 | | |
| Gp_14 | 1965.7 | 2264.0 | 2002.3 | 2376.0 | 2258.0 | 2258.0 | 2284.0 | 2162.5 | 2337.0 | 1582.5 | 2229.0 | 2341.0 | 2148.0 | |

Group 1) *Gazella erlangeri*, *Gazella gazella*, *Gazella dorcas*, *Gazella spekei*, *Gazella cuvieri*, *Gazella leptoceros*, *Gazella subgutturosa*, *Gazella bennettii*, group 2) *Eudorcas rufifrons*, group 3) *Nanger soemmerringii*, *Nanger dama*, *Nanger granti*, group 4) *Raphicerus campestris*, group 5) *Ourebia ourebi*, group 6) *Antelope cervicapra*, group 7) *Antidorcas marsupialis*, group 8) *Procapra gutturosa*, *Procapra przewalskii*, group 9) *Oreotragus oreotragus*, group 10) *Madoqua kirkii*, *Madoqua saltiana*, group 11) *Saiga tatarica*, group 12) *Dorcatragus megalotis*, group 13) *Litocranius walleri*, group 14) *Tragelaphus strepsiceros*.

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