

# Re-evaluation of Molecular Phylogeny of the Subfamily Cephalophinae (Bovidae: Artiodactyla); with Notes on Diversification of Body Size

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## Abstract

Duikers from the subfamily Cephalophinae are small to medium-sized antelope native to sub-Saharan Africa. Since most of the species belonging to this group are considered at-risk due to the process of population decline and probable extinction, recognizing their evolutionary biology will be useful toward preventing their extinction. Herein, molecular phylogeny of fourteen species of duikers were re-evaluated using complete mitochondrial genome sequences (n=17), cytochrome *b* (n=64), cytochrome *c* oxidase I (n=81), as well as the tRNA-proline gene and D-loop sequences. The analysis of a total of 225 gene sequences showed the average base composition of mtDNA sequences, based on mitogenome sequences, as follows: 26.8 % T, 26.5 % C, 33.3 % A, and 13.4 % G, with a great AT bias (60.1 %). According to the phylogenetic trees which resulted from maximum likelihood analysis, five distinct groups were indicated as West African red (*Cephalophus callipygus*, *C. ogilbyi*), East African red (*C. leucogaster*, *C. natalensis*, *C. rufilatus*, *C. nigrifrons*), savanna (*Sylvicapra grimmia*), giant (*C. dorsalis*, *C. jentinki*, *C. silvicultor*, *C. spadix*), and dwarf (*Philantomba maxwellii*, *P. monticola*) duikers. Furthermore, the current study showed that results will be similar using limited mitochondrial genes or either mitogenomes. Giving a careful attention to the phylogeny of duikers, two distinct evolutionary lineages were observed which could be defined as two distinct tribes (i.e. dwarf duikers and other species) within this subfamily. It means that their phylogeny is in accordance with their body-size classes. Therefore, similar approaches would be useful for those animals in which phylogeny is in accordance with their geographic position and body size, as can be seen in duikers.

**Keywords:** Duikers, Molecular phylogeny, Body size, Geographical location, Bovidae.

## 1. Introduction

The subfamily, Cephalophinae (Bovidae), consists of several artiodactyl species, commonly named as duikers, which are widely distributed in sub-Saharan Africa, but mainly inhabit tropical forests of Africa (Wilson and Reeder, 2005). Although they can be easily recognized due to their typical body form, they differ significantly in size. Unlike most bovids, females are slightly larger than males in this subfamily. Moreover, in contrast to most other bovids, duikers are primarily frugivorous (Castelló, 2016). However, they also feed on foliage, leaves of bushes, vines, and lower branches of trees. Sometimes they eat meat and may take nestlings, bird eggs, insects, and carrion. In captivity, they are sometimes fed dog food as a diet supplement (Burton and Burton, 2002). Duikers are very shy, elusive, and highly active creatures which need to eat large amounts of food (fruits) to maintain their energy levels (Burton and Burton, 2002). In most species, both sexes bear short pointed horns facing back over the neck with a tuft of hair between them, with the exception of the females of common duikers (*Sylvicapra grimmia*) which have only stunted horns or none at all. Just beneath the eyes, there are crescent-shaped glandular openings that

secrete a kind of scent. Duikers rub this scent on each other for future recognition, and also use it to mark out their territories (Burton and Burton, 2002; Castelló, 2016).

Duikers in the subfamily Cephalophinae contain three genera (Vuuren and Robinson, 2001; Grubb and Groves, 2005; Johnston and Anthony, 2012). The monotypic savanna specialist *Sylvicapra* genus contains a single savannah dwelling species, *S. grimmia*, and several subspecies widely distributed across the area from the Sahel to Austral Africa. The recently derived, species-rich, forest dwelling, genus *Cephalophus*, has several species and numerous subspecies inhabiting humid African tropical lowlands and mountain forests (Johnston and Anthony, 2012). Finally, the dwarf *Philantomba* contains the two smallest and most widely-distributed duiker species, namely *P. maxwellii* and *P. monticola* (Colyn *et al.*, 2010; Johnston and Anthony, 2012). They both occupy a range of natural habitats, including mosaic shrubby and wooded savannah habitats near human settlements. These two species are considered as the most frequently hunted antelopes in western and central Africa, and thus, represent a significant proportion of the local bushmeat market (Colyn *et al.*, 2010).

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Today, the most significant issues about duikers are the declining of their populations and the risk of extinction in Africa. Most species of duikers are common and widespread across sub-Saharan Africa (Burton and Burton, 2002). However, the destruction of fruit trees and the development of land in their place for settlement and agriculture (Burton and Burton, 2002), in addition to several other factors, have severely reduced the population of duikers to the point that, presently, all the members of the subfamily are mentioned on the red list of IUCN (Table 1).

**Table 1.** Scientific names and the IUCN red list categories for the duiker species of the subfamily Cephalophinae.

Scientific Name	Red List Category	Reference
<i>C. adersi</i>	Critically Endangered	(Finnie, 2008)
<i>C. callipygus</i>	Least Concern	IUCN
<i>C. dorsalis</i>	Near Threatened	IUCN
<i>C. harveyi</i>	Least Concern	IUCN
<i>C. jentinki</i>	Endangered	IUCN
<i>C. leucogaster</i>	Near Threatened	IUCN
<i>C. natalensis</i>	Least Concern	IUCN
<i>C. niger</i>	Least Concern	IUCN
<i>C. nigrifrons</i>	Least Concern	IUCN
<i>C. ogilbyi</i>	Least Concern	IUCN
<i>C. rufilatus</i>	Least Concern	IUCN
<i>C. silvicultor</i>	Near Threatened	IUCN
<i>C. spadix</i>	Endangered	(Moyer <i>et al.</i> , 2016)
<i>C. weynsi</i>	Least Concern	IUCN
<i>C. zebra</i>	Vulnerable	IUCN
<i>P. maxwellii</i>	Least Concern	IUCN
<i>P. monticola</i>	Least Concern	IUCN
<i>Sylvicapra grimmia</i>	Least Concern	IUCN

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; Marcot, 2007; Bärmann *et al.*, 2013; Ghassemi-Khademi, 2017a,b; Ghassemi-Khademi and Madjzadeh, 2019; Hassanin *et al.*, 2012) have studied the phylogenetic relationship among the species belonging to Bovidae, there are few studies which are specifically focused on phylogenetic relationships within the subfamily, Cephalophinae (Johnston and Anthony, 2012; Jansen-van Vuuren and Robinson, 2001). Furthermore, based on previous studies, four major lineages for duikers have been introduced (Johnston and Anthony, 2012; Johnston, 2011; Jansen-van Vuuren and Robinson, 2001).

On the whole, since these animals and also all bovines are ecologically, economically, and biologically important animals in the world, the determination of phylogenetic relationships among them is an effective step toward planning for the conservation and enhancement of multiplication of these animals in the world. In this regard, phylogenetic analyses based on the complete mitochondrial genomes can provide most accurate inferences (Ghassemi-Khademi, 2017a).

In the present study, the taxonomical validation of the existent evolutionary lineages in Cephalophinae has been evaluated based on different genes and complete genomes of mitochondria.

## 2. Materials and Methods

Totally, 225 gene sequences belonging to the Cephalophinae were obtained from NCBI, including all

complete mitochondrial genome sequences (n=17), as well as sequences of cytochrome *b* (cyt *b*) (n=64), cytochrome *c* oxidase I (COI) (n=81), and tRNA-proline-D-loop (n=63). Sequences were aligned with Mega6 (Tamura *et al.*, 2013) using the clustal W alignment method. In all analyses, the corresponding gene sequences of *Tragelaphus speikii* and *T. eurycerus* were used as outgroups.

The evolutionary history was inferred using the Maximum Likelihood (ML) method for each of the studied genes separately. Evolutionary divergence over sequence pairs between groups (different species) was also evaluated based on mitogenomes. The variance estimation method was bootstrap with 1000 replications. Herein, each species belonging to the subfamily Cephalophinae was considered as a separate group. Considering the outgroups, there were a total of fifteen groups (Table 2). Those positions containing gaps and missing data were eliminated. All of the evolutionary analyses were computed using the Kimura 2-parameter method (Kimura, 1980) in Mega6 (Tamura *et al.*, 2013). Moreover, the robustness of clades was calculated by the bootstrap method. In this study, a bootstrap value of 50-60 % was considered as weak, 64-75 % as moderate, 76-88 % as good, and  $\geq 89$  % as a strong support (Win *et al.*, 2017).

## 3. Results

The phylogenetic analysis of fourteen species belonging to Cephalophinae was carried out using mitogenomes, cyt *b*, COI, and tRNA-proline-D-loop genes. The average length of complete mitochondrial genomes was calculated to be 16,427.9 base pairs. In 16427.9, the average base composition of mtDNA sequences was 26.8 % T, 26.5 % C, 33.3 % A, and 13.4 % G, showing a strong AT bias (60.1 %).

The molecular phylogenetic trees for the complete mtDNA genomes and also for the cyt *b*, COI, and the tRNA-proline-D-loop genes were constructed using the ML method, which all provided nearly the same topologies (Figures 1-4). In all of the trees, five different groups of duikers, namely dwarf, giant, savanna, east African and west African duikers, were clearly separated from each other. Four phylogenetic trees based on four different genes, revealed a great main clade; all of the species belonging to the subfamily Cephalophinae, with the exception of the two species of *P. maxwellii* and *P. Monticola*, constructed a great monophyletic clade.

Moreover, as the results indicate, *T. speikii* and *T. eurycerus* (used as outgroups) were completely separated from other species. The outgroups were at a far distance (Table 3) and separated from the members of Cephalophinae in all of the constructed phylogenetic trees (Figures 1-4), which implies the presence of relative close genetic distances among duikers. Based on mitogenomes, the shortest phylogenetic distances were obtained between the two species of *C. silvicultor* and *C. spadix* (= 0.25), and also *C. callipygus* and *C. ogilbyi* (= 0.24). Furthermore, in all phylogenetic trees, the two species of *C. silvicultor* and *C. spadix*, as well as the two species of *C. callipygus* and *C. ogilbyi* were located close to each other. Thus, it can be inferred that these species are phylogenetically the closest species within the Cephalophinae.

As mentioned earlier, based on mitogenomes, the longest distance was obtained between the outgroups (*T.*

*spekii* and *T. eurycerus*) and other species. Besides, after the outgroups, dwarf duikers (*P. maxwellii* and *P. monticola*) had the most phylogenetic distances with other members of the subfamily, and in all phylogenetic trees, the two species of *P. maxwellii* and *P. monticola* were located separately from other species and did not construct a single cluster with them. Moreover, based on the mitogenomes, among the five groups of duikers (Giant, Dwarf, East African, West African, and Savanna duikers) and *C. adersi*, the shortest phylogenetic distances were obtained between west African red and giant duikers (= 0.89), savanna and giant duikers (= 0.85), east and west African red duikers (= 0.82), and *C. adersi* and west African red duikers (= 0.89) (Table 3).

Using complete mitochondrial genome sequences, two distinct groups were distinguished within the great monophyletic clade of the constructed phylogenetic tree; in these distinct groups, six species including *C. callipygous*, *C. ogilbyi*, *C. leucogaster*, *C. natalensis*, *C. rufilatus*, and *C. nigrifrons* showed the highest supported

**Table 2.** Phylogenetic distances between species belonging to the subfamily Cephalophinae based on complete mitochondrial sequences. Gp refers to Group.

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	0.025													
Gp_3	0.053	0.053												
Gp_4	0.056	0.057	0.059											
Gp_5	0.086	0.088	0.087	0.091										
Gp_6	0.085	0.085	0.083	0.089	0.090									
Gp_7	0.090	0.090	0.091	0.095	0.088	0.098								
Gp_8	0.089	0.091	0.089	0.094	0.024	0.094	0.091							
Gp_9	0.090	0.090	0.092	0.094	0.082	0.099	0.096	0.087						
Gp_10	0.093	0.093	0.092	0.098	0.082	0.100	0.096	0.086	0.061					
Gp_11	0.091	0.090	0.090	0.094	0.080	0.097	0.092	0.083	0.030	0.061				
Gp_12	0.090	0.091	0.091	0.095	0.081	0.099	0.096	0.084	0.007	0.060	0.029			
Gp_13	0.110	0.115	0.111	0.115	0.110	0.115	0.113	0.112	0.114	0.112	0.111	0.114		
Gp_14	0.111	0.113	0.110	0.113	0.105	0.114	0.110	0.108	0.114	0.111	0.112	0.114	0.067	
Gp_15	0.167	0.168	0.167	0.166	0.168	0.168	0.164	0.170	0.171	0.166	0.170	0.170	0.168	0.163

**Note:** Group 1: *Cephalophus silvicultor*, Group 2: *C. spadix*, Group 3: *C. dorsalis*, Group 4: *C. jentinki*, Group 5: *C. callipygus* (three sequences), Group 6: *C. grimmia*, Group 7: *C. adersi*, Group 8: *C. ogilbyi*, Group 9: *C. rufilatus*, Group 10: *C. leucogaster*, Group 11: *C. natalensis*, Group 12: *C. nigrifrons*, Group 13: *Philantomba maxwellii*, Group 14: *P. monticola* (two sequences), Group 15: *Tragelaphus spekii*, *T. eurycerus*.

**Table 3.** Phylogenetic distances between different groups belonging to the subfamily Cephalophinae based on complete mitochondrial sequences.

	Giant duiker	West African red duiker	Savanna duiker	Aders's duiker	East African red duiker	Dwarf duiker
Giant duiker						
West African red duiker	0.089					
Savanna duiker	0.085	0.091				
Aders's duiker	0.092	0.089	0.098			
East African red duiker	0.092	0.082	0.099	0.095		
Dwarf duiker	0.112	0.107	0.114	0.111	0.113	
Outgroups	0.167	0.168	0.168	0.164	0.169	0.165

**Table 4.** Nucleotide composition of mtDNA of the studied species of the Cephalophinae (n=17) and their accession numbers obtained from GenBank (www.ncbi.nlm.nih.gov) (n=19).

Scientific Name	T(U)	C	A	G	Total	Accession Number	Reference
<i>Cephalophus silvicultor</i>	27.0	26.3	33.4	13.4	16425.0	JN632622	(Hassanin <i>et al.</i> , 2012)
<i>Cephalophus spadix</i>	27.0	26.2	33.3	13.5	16430.0	JN632623	(Hassanin <i>et al.</i> , 2012)
<i>Cephalophus dorsalis</i>	26.7	26.6	33.4	13.4	16425.0	JN632615	(Hassanin <i>et al.</i> , 2012)
<i>Cephalophus jentinki</i>	27.0	26.1	33.5	13.3	16391.0	NC_020688	(Hassanin <i>et al.</i> , 2012)
<i>Cephalophus callipygus</i>	26.5	26.7	33.3	13.5	16427.0	JN632613	(Hassanin <i>et al.</i> , 2012)
<i>Cephalophus callipygus</i>	26.5	26.6	33.3	13.5	16427.0	JN632614	(Hassanin <i>et al.</i> , 2012)
<i>Sylvicapra grimmia</i>	26.7	26.6	33.4	13.3	16437.0	JN632701	(Hassanin <i>et al.</i> , 2012)
<i>Cephalophus callipygus</i>	26.4	26.8	33.3	13.5	16422.0	JN632612	(Hassanin <i>et al.</i> , 2012)
<i>Cephalophus adersi</i>	26.7	26.5	33.6	13.2	16435.0	JN632611	(Hassanin <i>et al.</i> , 2012)
<i>Cephalophus ogilbyi</i>	26.4	26.7	33.3	13.6	16374.0	JN632620	(Hassanin <i>et al.</i> , 2012)
<i>Cephalophus rufilatus</i>	26.3	27.0	33.0	13.7	16429.0	JN632621	(Hassanin <i>et al.</i> , 2012)
<i>Cephalophus leucogaster</i>	26.8	26.5	33.2	13.5	16426.0	JN632617	(Hassanin <i>et al.</i> , 2012)
<i>Cephalophus natalensis</i>	26.5	26.8	33.1	13.6	16429.0	JN632618	(Hassanin <i>et al.</i> , 2012)
<i>Cephalophus nigrifrons</i>	26.4	26.9	33.0	13.7	16406.0	JN632619	(Hassanin <i>et al.</i> , 2012)
<i>Philantomba maxwellii</i>	27.7	25.7	33.4	13.2	16440.0	JN632685	(Hassanin <i>et al.</i> , 2012)
<i>Philantomba monticola</i>	27.4	26.0	33.7	13.0	16501.0	JN632686	(Hassanin <i>et al.</i> , 2012)
<i>Philantomba monticola</i>	27.5	25.9	33.6	13.0	16451.0	JN632687	(Hassanin <i>et al.</i> , 2012)
<b>Avg.</b>	26.8	26.5	33.3	13.4	16427.9		
<i>Tragelaphus spekii</i>						NC_020620	(Hassanin <i>et al.</i> , 2012)
<i>Tragelaphus eurycerus</i>						JN632703	(Hassanin <i>et al.</i> , 2012)

**Table 5:** Accession numbers of cytochrome *b* genes for the studied species belong to the subfamily Cephalophinae (n=64) obtained from GenBank (www.ncbi.nlm.nih.gov) (n=66).

Scientific Name	Accession Number	Scientific Name	Accession Number
	AF153886		AF153884
	AF153885		AF153884
<i>Cephalophus callipygus</i>	FJ807612		AF091634
	FJ807575		FJ807596
	FJ807574		FJ807595
	FJ807573	<i>Cephalophus dorsalis</i>	FJ807590
	JN632614		FJ807589
<i>Cephalophus leucogaster</i>	AF153889		FJ807588
	FJ807606		FJ807577
	JN632617		FJ807576
	AF153890		JN632615
<i>Cephalophus natalensis</i>	FJ807611	<i>Cephalophus jentinki</i>	AF153888
	FJ807610		JN645578
	JN632618		NC_020688
	AF153890		AF153898
<i>Cephalophus rufilatus</i>	FJ807626		FJ807622
	FJ807625	<i>Cephalophus silvicultor</i>	FJ807587
	JN632621		FJ807579
	AF153896		FJ807571
<i>Cephalophus nigrifrons</i>	FJ807627		JN632622
	FJ807609		AF153883
	FJ807572	<i>Cephalophus adersi</i>	FJ807617
	JN632619		FJ807616
	AF153897		JN632611
<i>Cephalophus ogilbyi</i>	FJ888512	<i>Philantomba maxwellii</i>	JF728780
	FJ807628		JN632685
	FJ807618		JF728781
	JN632620	<i>Philantomba monticola</i>	JN632687
	FJ807613		JN632686
<i>Sylvicapra grimmia</i>	FJ807591	<i>Tragelaphus spekii</i>	JF728788
	FJ807592	<i>Tragelaphus euryceros</i>	AF036276
	FJ807593		

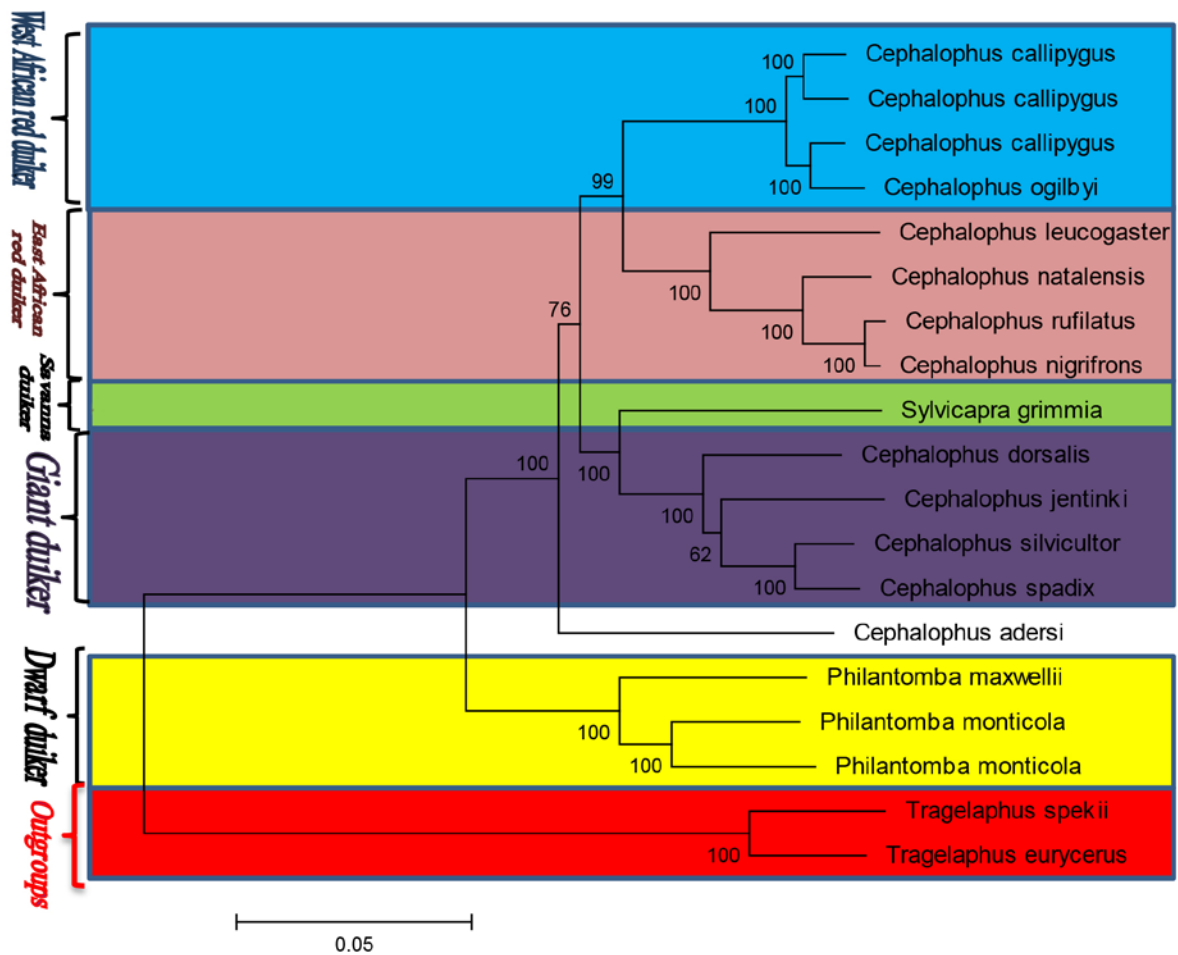
**Table 6.** Accession numbers of Cytochrome *c* oxidase I (COX1) genes for the studied species from the subfamily Cephalophinae (n=81) obtained from GenBank (www.ncbi.nlm.nih.gov) (n=85).

Scientific Name	Accession Number	Scientific Name	Accession Number
<i>Cephalophus ogilbyi</i>	KJ192792		HQ644091
	KJ192791		GQ144514
	KJ192789		GQ144513
	KJ192790		KJ192767
<i>Cephalophus leucogaster</i>	HQ644098	<i>Cephalophus dorsalis</i>	KJ192768
	HQ644097		KJ192965
	GQ144515		KJ192975
	GQ144516		KJ192982
	GQ144517		GQ144511
	HQ644096		GQ144507
	HQ644095		KJ192980
	GQ144521		GQ144509
		<i>Cephalophus jentinki</i>	HQ644094
<i>Cephalophus natalensis</i>	HQ644104	<i>Cephalophus silvicultor</i>	HQ644113
	HQ644103		HQ644112
<i>Cephalophus rufilatus</i>	HQ644111	<i>Cephalophus spadix</i>	KJ192969
	HQ644110		HQ644115
	HQ644108		HQ644114
<i>Cephalophus nigrifrons</i>	HQ644107	<i>Cephalophus adersi</i>	HQ644087
	GQ144550		HQ644086
	GQ144549		KJ192888
	GQ144548		KJ192889
	GQ144547		KJ192890
	GQ144546		KJ192891
	HQ644102		KJ192892
		<i>Philantomba maxwellii</i>	KJ192893
<i>Philantomba monticola</i>	KJ192774		KJ192974
	HM144016		HM144021
	HM144015		HQ644099
	HM144026		KJ192976
	HM144022		HQ644090
	HM144020		HQ644089
	KJ192775		HQ644088
			GQ144491
<i>Tragelaphus spekkii</i>	HQ644120		GQ144492
	KJ192918		GQ144494
<i>Tragelaphus eurycerus</i>	EU623454		GQ144498
	LC143641		GQ144504
<i>Sylvicapra grimmia</i>	HQ644119	<i>Cephalophus callipygus</i>	HM144025
	HQ644118		GQ144499
	KX012658		GQ144500
			HM144023
			GQ144502
			GQ144493
			GQ144505

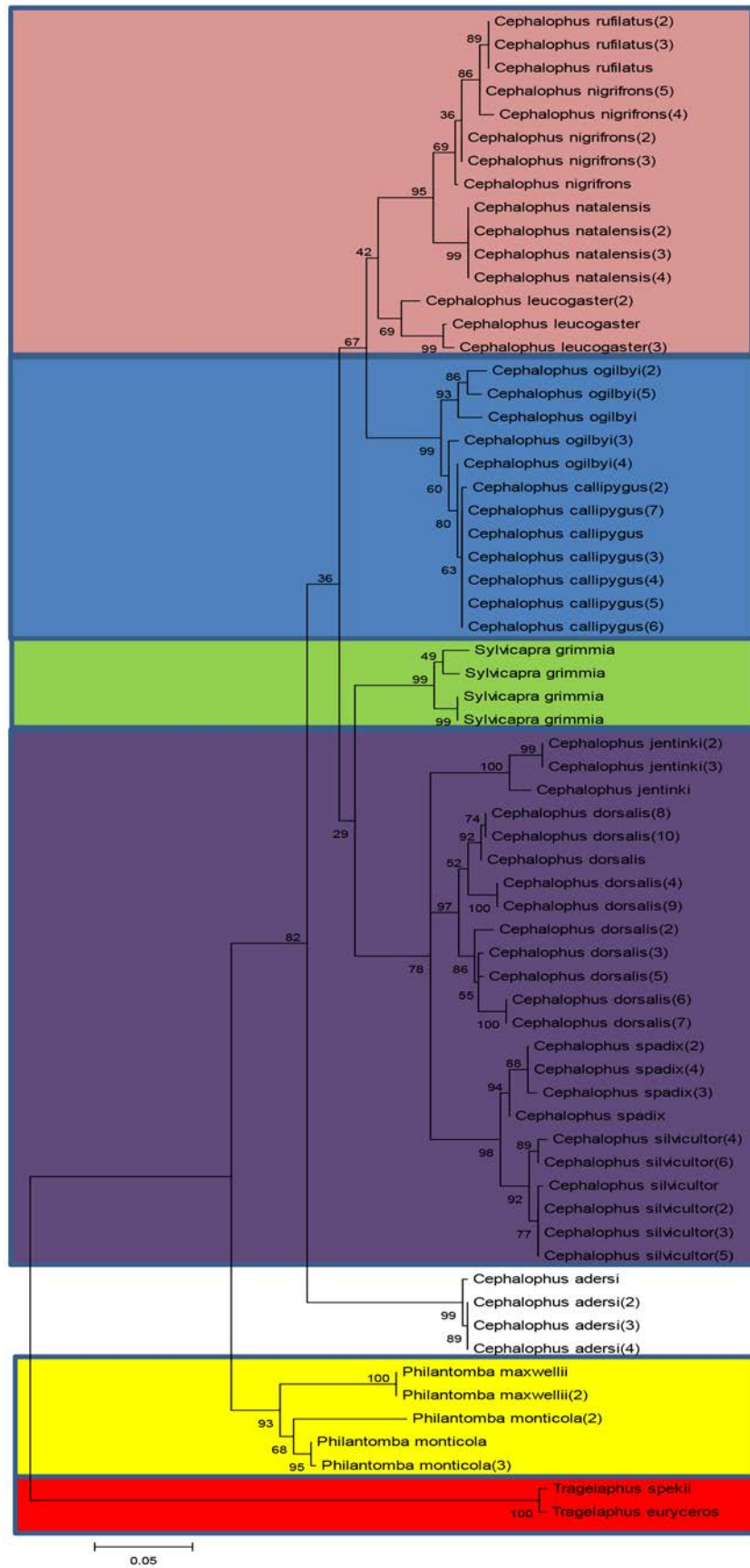
**Table 7.** Accession numbers of tRNA-proline gene and D-loop sequences for the studied species from the subfamily Cephalophinae (n=63) obtained from GenBank (www.ncbi.nlm.nih.gov)(n=67).

Scientific Name	Accession Number	Scientific Name	Accession Number
<i>Cephalophus callipygus</i>	FJ823345		FJ823384
	FJ823338		FJ823383
	FJ823339		FJ823379
	FJ823340		FJ823375
	FJ823342		FJ823371
	FJ823341		FJ823367
<i>Cephalophus ogilbyi</i>	FJ823363	<i>Cephalophus dorsalis</i>	FJ823372
	FJ823362		FJ823381
	FJ823360		FJ823376
	FJ823333		FJ823373
<i>Cephalophus leucogaster</i>	FJ823337	<i>Cephalophus jentinki</i>	FJ823382
	FJ823335		NC_020688
	FJ823334		FJ823359
	FJ823336		FJ823358
<i>Cephalophus natalensis</i>	FJ823314	<i>Cephalophus silvicultor</i>	FJ823357

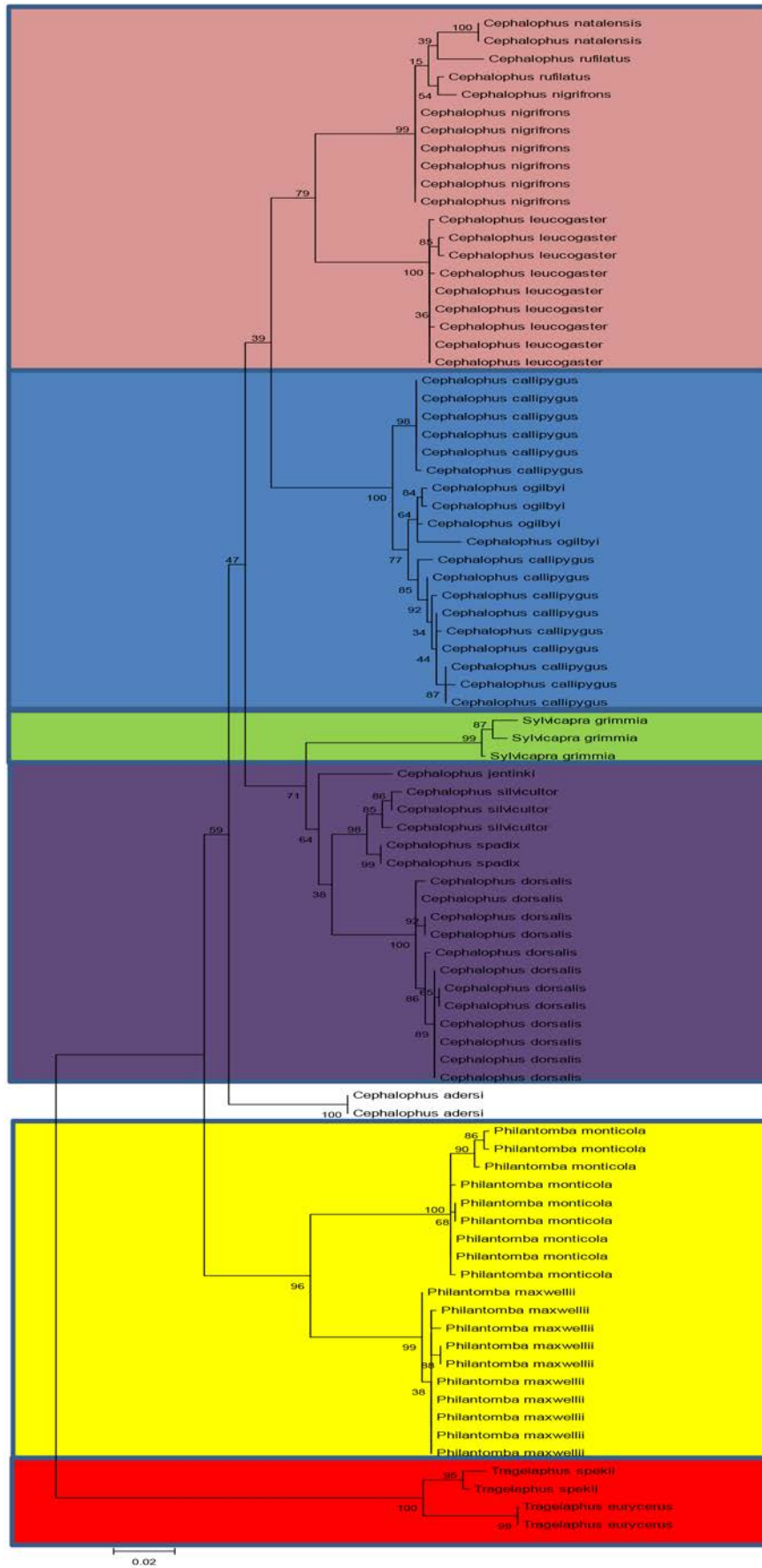
	FJ823315		FJ823353
	FJ823325		FJ823356
<i>Cephalophus rufilatus</i>	FJ823326		FJ823354
	FJ823323		AM903086
	FJ823324		HG323850
<i>Cephalophus nigrifrons</i>	FJ823331		AM903085
	FJ823328		AM903084
<i>Cephalophus adersi</i>	FJ823312	<i>Cephalophus spadix</i>	HG323849
	FJ823313		FJ823351
	FJ823311		FJ823352
<i>Philantomba maxwellii</i>	FJ823310		HG323852
	FJ823309		FJ823349
	FJ823283		FJ823297
<i>Tragelaphus spekii</i>	FJ823286	<i>Sylvicapra grimmia</i>	FJ823296
	FJ823282		FJ823295
<i>Tragelaphus eurycerus</i>	JN632703		FJ823294
			FJ823304
			FJ823305
		<i>Philantomba monticola</i>	FJ823306
			FJ823308
			FJ823303



**Figure 1.** 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 *Tragelaphus* species sequences.

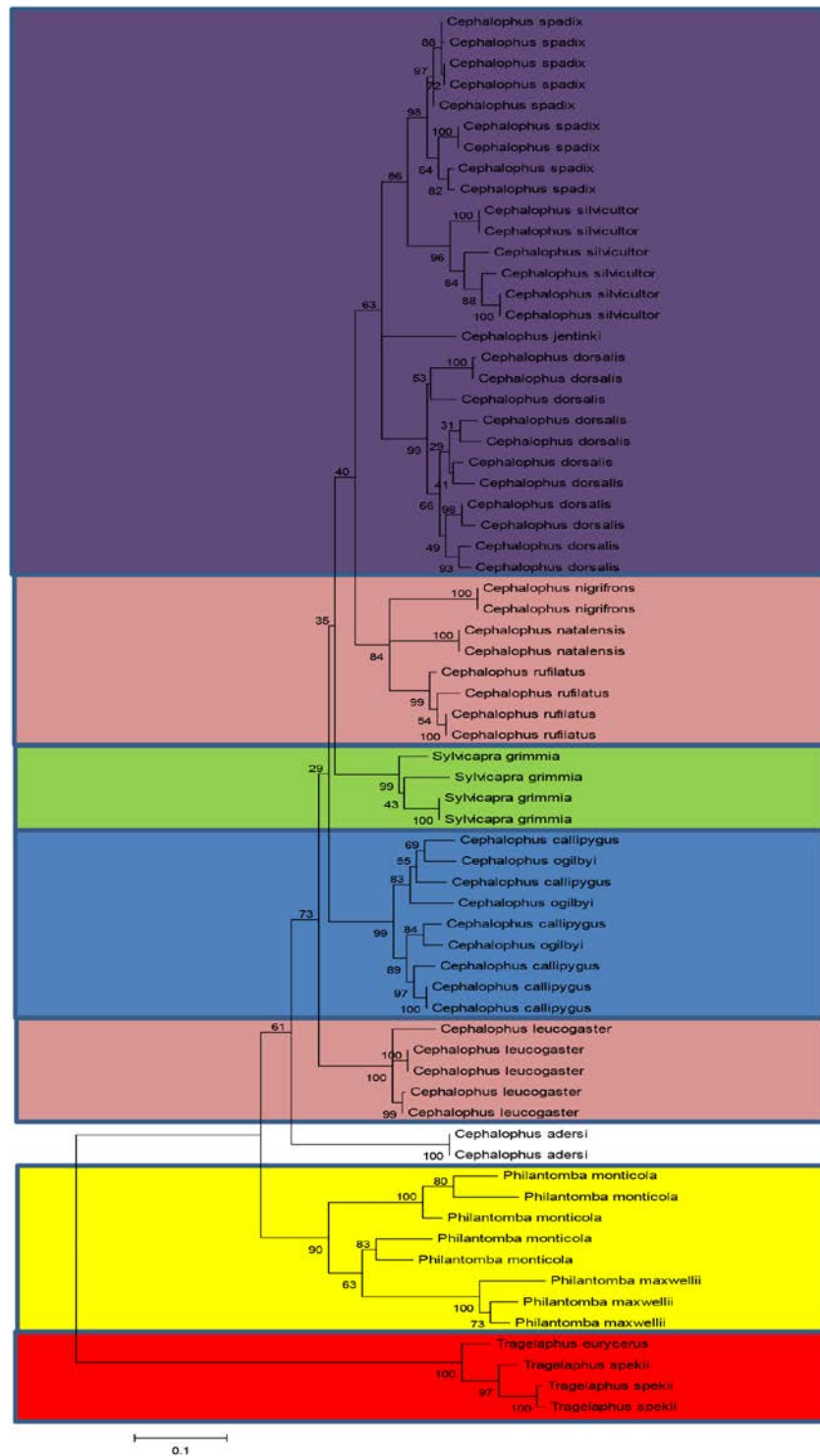


**Figure 2.** Maximum Likelihood tree based on Kimura 2- parameter distance using Cytochrome *b* sequences. The numbers on each branch correspond to bootstrap support values. The tree was rooted with two *Tragelaphus* species sequences.



**Figure 3.** Maximum Likelihood tree based on Kimura 2- parameter distance using Cytochrome c oxidase I (COX1) sequences. The numbers on each branch correspond to bootstrap support values. The tree was rooted with two *Tragelaphus* species sequences.





**Figure 4.** Maximum Likelihood tree based on Kimura 2- parameter distance using tRNA-proline gene and D-loop sequences. The numbers on each branch correspond to bootstrap support values. The tree was rooted with two *Tragelaphus* species sequences.

#### 4. Discussion

Today, the subfamily Cephalophinae is introduced with three duiker genera: 1) the recently derived, species-rich, and forest-dwelling *Cephalophus*, 2) the dwarf *Philantomba*, and 3) the monotypic savanna specialist *Sylvicapra* (Johnston and Anthony, 2012). Using two mitochondrial genes (cyt *b* and 12S rRNA) and fluorescence *in situ* hybridization, Jansen-van Vuuren and Robinson (2001) retrieved four adaptive lineages in dwarf duikers (as the basal lineage), giant duikers (*C. silvicultor*,

*C. spadix*, *C. dorsalis*, and *C. jentinki*), the red duikers with a subdivision comprising an East African red duikers group (including *C. leucogaster*, *C. rufilatus*, *C. nigrifrons*, *C. natalensis*, *C. rubidus*, and *C. harveyi*), a West African red duiker group (including *C. callipygus*, *C. weynsi*, *C. ogilbyi*, and *C. niger*), and a savanna specialist (*S. grimmia*) (Jansen-van Vuuren and Robinson, 2001).

Moreover, Johnston and Anthony (2012) used portions of two coding mDNA genes including 514 base pairs of the cyt *b* gene, 658 base pairs of COXI, and four nuclear

DNA markers including mechano growth factor (MGF), protein kinase CI (PRKCI), spectrin beta chain, non-erythrocytic (SPTBN1), and thyrotropin (THY) for evaluating the phylogenetic relationships within the subfamily Cephalophinae. The results of this study confirmed Jansen-van Vuuren and Robinson's statements and also confirmed four adaptive lineages of duikers completely separated from each other (Johnston and Anthony, 2012) (Figures 1- 4).

Therefore, using the complete mitochondrial genomes and three different genes of mtDNA, in addition to the confirmation of the presence of four phylogenetic lineages of duikers, resulted in inferences that are nearly compatible with those achieved when using limited mitochondrial genes. Accordingly, in the case of the phylogenetic studies of duikers, the fastest and economic method is to use limited mitochondrial genes, as it does not require using complete mitochondrial genomes. However, using mitogenomes in phylogenetic studies resulted in more accurate and reliable inferences in the world of animal diversification (Ghassemi-Khademi, 2017a, b).

Herein, similar to previous studies (Jansen-van Vuuren and Robinson, 2001; Johnston, 2011; Johnston and Anthony, 2012) the position of the genus *Philantomba* as a sister clade to the remaining Cephalophinae was confirmed considering it as a separate genus. Relations between West African red and giant duikers, savanna and giant duikers, east and West African red duikers, and finally, *C. adersi* and West African red duikers showed the shortest phylogenetic distances based on mitogenomes (Table 2). However, in all phylogenetic trees except those of the tRNA-proline-D-loop genes, savanna and giant duikers, and also East and West African red duikers were located close to each other. These observations are similar to results obtained by previous studies (Johnston, 2011; Johnston and Anthony, 2012). The East and West African red duiker lineages which are monophyletic and are considered as sister taxa (Johnston, 2011), sistered with each other in all of the resulted phylogenetic trees except in the tRNA-proline-D-loop genes tree. This result implies that they belong to a single lineage. Based on mitogenomes, the savanna duiker (*Sylvicapra grimmia*) and giant duikers had one of the shortest phylogenetic distances together, and were located as the sister group of giant duikers which leave the genus *Cephalophus* as a paraphyletic group (Johnston and Anthony 2012). Hence, this genus (*Cephalophus*) cannot be considered as a monophyletic group.

In all of the phylogenetic trees, *C. adersi* was located separately from other species belonging to a paraphyletic group of the genus *Cephalophus*, and constructed a sister taxon. Similar to the previous results (Johnston, 2011), in all of the phylogenetic trees, this species was basal to East and West African red, savanna, and giant duikers. Although it seems necessary to re-evaluate the phylogenetic position of this species carefully (Johnston and Anthony, 2012), this taxon is, undoubtedly, a member of the paraphyletic group of the genus *Cephalophus*. Furthermore, two species of dwarf duikers, *P. maxwellii* and *P. monticola* were located separately from other species, and did not construct a single cluster with them. Besides, after the outgroups, these species showed the greatest phylogenetic distances with other members of

subfamily Cephalophinae. Given this evidence, one can probably identify two distinct tribes within the subfamily; one for dwarf duikers and the other for the rest of the species.

On the other hand, the correlation of phylogeny with animal body size and ecological traits (e.g. geographical locality) has been evaluated in several studies (Barnagaud *et al.*, 2014; Leonhardt *et al.*, 2013; Abellán and Ribera, 2011; Diniz-filho and d-sant Ana, 2000; Diniz-filho and Torres, 2002; Wollenberg *et al.*, 2011; Carrascal *et al.*, 2008). Meanwhile, the results of the current study and some earlier studies (Johnston, 2011; Johnston and Anthony, 2012) show that phylogenetic relationships of species belonging to the subfamily Cephalophinae correspond to their body size and geographic location. An example of such situation in the animal world belongs to the Apini tribe of honey bees. Based on body size, this kind of bees is divided into three giant, medium, and dwarf groups and their phylogenetic status correspond exactly to this morphological classification (Hepburn and Radloff, 2011). Geographic and phylogenetic relationships also shape the chemical profiles of stingless bees on a global scale (Leonhardt *et al.*, 2013). Furthermore, Barnagaud and his colleagues (2014) stated that ecological traits influence the phylogenetic structure of bird species (Barnagaud *et al.*, 2014). Given the above studies, and the results of the present study, it can be inferred that there is a strong correlation in some animal species between phylogeny with body size and ecological traits. It seems that the phylogeny of duikers is a specific example in this case, because these animals are divided into two different groups, phylogenetically.

In conclusion, the correlation between the phylogenetic situation and the morphological status (body size) as well as the geographical location in the world of invertebrates and vertebrates is, undoubtedly, an important and interesting subject for future studies.

#### 4. Conclusion

The analysis of a total of 225 gene sequences showed five distinct groups within the subfamily Cephalophinae. Based on the complete mitochondrial genome sequences, cytochrome *b*, cytochrome *c* oxidase I, as well as the tRNA-proline gene and the D-loop sequence phylogenetic trees, these five groups of duikers were divided into two different clusters, dwarf duikers and non-dwarf duikers. This means that their phylogeny corresponds to their body-size classes. Probably, differences in the body size may create distinctive phylogenetic paths for taxa within a group or those with a close relationship. Finding relationships between the body size and phylogeny in a group of animals, requires comprehensive studies including, above all, ecological, biological, and phylogenetic approaches which will, undoubtedly, reveal many hidden facts in the worlds of animals.

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