

## CHAPTER 1

### GENERAL INTRODUCTION

#### AIMS AND OBJECTIVES OF THE STUDY

In the past, studies of systematic relationships among duiker antelope comprising the tribe Cephalophini have focused almost exclusively on the morphological and phenotypic variation evident among representatives of the group (St. Leger 1936, Heyden 1968, Groves & Grubb 1974, 1981, Kingdon 1982, 1997). This approach has, however, proved to be limited in its usefulness and the taxonomic status and relationships of many species remain unresolved. In an attempt to redress these deficiencies, the phylogenetic history of the cephalophine species is examined using techniques and approaches which have hitherto not been applied to this specious and phenotypically divergent group of African antelope.

The objectives were:

- i) To assess the molecular phylogenetic relationships and taxonomic status of taxa comprising the Cephalophini based on cytochrome *b* and 12S rRNA nucleotide sequence data
- ii) To ascertain by fluorescence *in situ* hybridization (FISH) whether highly repeated satellite sequences differ between the species of duiker antelope
- iii) To determine the degree of concordance in the phylogenetic placement of taxa from three independent data sets (mitochondrial DNA nucleotide sequences, comparative cytogenetics, and the fluorescence *in situ* hybridization patterns of chromosomes)
- iv) To correlate the phylogeny of the taxa with available climatic data in an attempt to clarify the events that have shaped the speciation and evolutionary events within the group.

## INTRODUCTION

### Natural history of duiker antelope

Members of the tribe Cephalophini are, with the exception of a single species (*Sylvicapra grimmia*), adapted to an inconspicuous existence in forests. According to Kingdon (1982) there is not a single indigenous forest in Africa that does not provide habitat to at least one duiker species, a clear indication of their relative importance as the dominant medium-sized forest antelope. Several morphological and dietary adaptations enable them to successfully occupy this niche. All the *Cephalophus* taxa have a similar body form with short front legs and an arched back. Well developed hind quarters propel them through the thick undergrowth while the horns are short and project backwards from the skull (Heyden 1968, Bigalke 1972, Walker *et al.* 1975, Kingdon 1982).

Color variation within the tribe is unprecedented for antelope (for species descriptions see Happold 1973, Walker *et al.* 1975, Kingdon 1997) and there is speculation that this may account for species of similar size and habitat preference existing sympatrically (Kingdon 1982). Although the majority of species show quite considerable variation in coat and facial markings, Groves and Grubb (1974) recognize four independent trends towards melanism within the group with the completely black *C. niger* representing the extreme in phenotype.

The monotypic *Sylvicapra* is the only taxon within the tribe that is not a strict forest specialist. It prefers drier and relatively more exposed habitats, hence the more slender body form (Bigalke 1972, Walker *et al.* 1975, Groves & Grubb 1981). Moreover, this species has the widest distribution of all duiker, ranging from Senegal to east, central, and southern Africa (Walker *et al.* 1975). Apart from habitat differences distinguishing *Sylvicapra* from *Cephalophus*, Ansell (1971) delimits the taxa on the presence/absence of horns in the females (*Sylvicapra* females do not have horns), the plane of horn projection (in *Cephalophus* the horns project backwards while they are upright in *Sylvicapra*), as well as the length of the ears.

As is typical for the majority of small antelope, all duiker are thought to be territorial and, in most species there is a close association between the sexes with territories shared by both males and females; males are, however, more active in patrolling and defending them (Kingdon 1997). It is generally thought that the length of horns in females are correlated

with the degree of active territorial defense. *Sylvicapra* males have territories that encompass the home ranges of several females.

### Higher order taxonomy

The Cephalophini is one of 14 recognized tribes within the family Bovidae (Vrba 1985a). The higher order classification of bovids is problematic, in particular the intratribal, intertribal, and subfamilial relationships. Simpson (1945) noted that morphological convergence and rapid radiation, in conjunction with the questionable monophyly of some tribes/subfamilies, renders the classification of the Bovidae difficult, a point which is further underscored by the lack of agreement concerning the number of recognized species, tribes, and subfamilies within the family (Simpson 1945, Ansell 1971, Gentry 1978, Vrba 1985a, Gentry 1992).

Although the monophyly of the Cephalophini has never been questioned, the taxonomic placement of this tribe has proved problematic. Various authors argue that duiker antelope represent a distinct, early off-shoot within the bovid radiation (Bigalke 1972, Vrba 1985a, Georgiadis *et al.* 1990, Gentry 1992), while others propose a more recent origin based on the high degree of overall similarity between species (Kingdon 1982, 1997). Morphological data (Gentry 1992) indicate a close association between the Cephalophini and Tragelaphini, and between the Cephalophini and the Boselaphini/Bovini (the two latter tribes are closely related to each other). A basal placement of the Cephalophini is in agreement with allozyme data (Georgiadis *et al.* 1990), with the cephalophine representatives being only distantly related to all other tribes.

Phylogenetic trees constructed from mitochondrial DNA sequence data of both the large and small ribosomal subunits suggest a common ancestry for the tribes Cephalophini, Reduncini and Antelopini (Gatesy *et al.* 1992). In a similar study by Allard *et al.* (1992) using nucleotide sequences from the 12S rRNA gene, the minimum sequence divergence (uncorrected distances; Kraus & Miyamoto 1991) between any two taxa of these tribes (the Ovibovini, Peleini, and Rupicaprini were not available to these authors) was between *C. maxwellii* (Cephalophini) and *Kobus ellipsiprymnus* (Reduncini). Most recently, however, sequence data from the complete mitochondrial DNA cytochrome *b* gene (Matthee & Robinson 1999a) indicate a sister taxon relationship between the Cephalophini and a neotragine representative (*Oreotragus oreotragus*).

## Taxonomy of the Cephalophini

The taxonomy of the tribe Cephalophini has been complicated by the inconsistent treatment of some taxa and the questionable taxonomic status of others. Currently, two genera are recognized (Grubb 1993), the monotypic *Sylvicapra* Ogilby, 1837 and the specious *Cephalophus* H. Smith, 1827. However, the validity of *Sylvicapra* has been questioned (Lydekker & Blaine 1914, Haltenorth 1963, Van Gelder 1977; but see Heyden 1968, Ansell 1971, Meester *et al.* 1986, Grubb 1993, Kingdon 1997). Haltenorth (1963) followed Lydekker and Blaine (1914) in treating *Sylvicapra* as a subgenus, while Van Gelder (1977) recognized only *Cephalophus*, and regarded *Sylvicapra* as a synonym based on evidence of hybridization between *S. grimmia* and *Cephalophus* spp. (*C. nigrifrons*; Anon. 1965 in Van Gelder 1977 and *C. natalensis*; Van Gelder 1977).

A third genus, *Philantomba* Blyth, 1840 is sometimes recognized for *C. monticola* and *C. maxwellii* with the distinction resting on the absence of inguinal glands in these species Pocock (1910). This was followed by later authors (Hard 1969; Groves & Grubb 1981). In spite of these considerations the recognition of *Philantomba* is not universal (see Ralls 1973, Haltenorth & Diller 1986, Kingdon 1997) and Grubb's (1993) most recent revision of the tribe submerged *Philantomba* in *Cephalophus*, a treatment which is followed in the present study.

*Cephalophula* has been proposed as the genus name for *C. zebra*. This species is a distinct member of the forest group and is characterized by several unique features. These include an unique, heavily boned skull with the nasals of both sexes being unusually thick and reinforced (Heyden 1968, Kingdon 1982). Moreover, the attachment of the horns to the skull and their plane of projection distinguishes *C. zebra* from other representatives of the Bovidae (Heyden 1968).

Dobzhansky (1970) and Van Gelder (1977) state that the boundaries of a genus are arbitrary constructs since the criteria for delimiting them are subjective. A number of definitions and criteria have been proposed to delineate species. These include taxonomic/morphological species, biological species, and phylogenetic species. Given that convergent evolution and local adaptations pose serious problems when using phenotypic characters, it is not surprising that molecular results often contradict species descriptions based on morphological grounds (see O'Brien & Mayr 1991). Although criticized (Wiley 1978, Frost & Hillis 1990, Wheeler & Nixon 1990), the most widely accepted definition is that of the

biological species which remains the baseline for the taxonomic description of our fauna and flora. Under this concept, a species is defined as a group of naturally (or potentially) interbreeding populations that are reproductively isolated from other such groups (Mayr 1963). Notwithstanding difficulties involved with determining reproductive compatibilities between alleged species (specifically allopatric forms; see Avise & Ball 1990), the biological species concept has several advantages (e.g. the recognition of variation within species that is often partitioned geographically or temporally by population subdivision into subspecies; O'Brien & Mayr 1991), and this definition of a species is followed herein.

The deep divisions that characterize duiker taxonomy are reflected by the lack of consensus on the number of species. If one excludes the monotypic *S. grimmia* which appears to enjoy overwhelming support (Ansell 1971, Bigalke 1972, Happold 1973, Eltringham 1979, Groves & Grubb 1981, Grubb 1993, Kingdon 1997), 10 forest species are recognized by Walker *et al.* (1975), 11 - 13 species by Haltenorth and Diller (1986), 15 species by Ansell (1971), Bigalke (1972) and Groves and Grubb (1981), 17 species by Kingdon (1997) and finally, 18 species by Grubb (1993) and Nowak (1999). The various taxonomic classifications that have been suggested and their synonyms are summarized in Table 1.

St. Leger (1936) examined all the duiker specimens of the subgenus *Cephalophus* available to her in the British, Tervueren, Berlin and Leyden Museums. Based mainly on the size of the false hoof and texture of hair on the shoulders she proposed two species groups. The first consisted of only *C. nigrifrons* and *C. rufilatus* (including *C. n. rubidus*, which is currently recognized as a valid species; see Grubb 1993 and Table 1); the second comprised the remaining taxa: *C. jentinki*, *C. silvicultor*, *C. spadix*, *C. niger*, *C. weynsi*, *C. w. harveyi* (currently recognized as a valid species; see Grubb 1993 and Table 1), *C. adersi*, *C. natalensis*, *C. zebra*, *C. dorsalis*, *C. callipygus*, *C. leucogaster* and *C. ogilbyi*.

This approach was further extended by Groves and Grubb (1981) who, after an evaluation of external and skeletal features, proposed the possible existence of three species groups or subgenera in *Cephalophus*<sup>1</sup>. The first comprised the monotypic *C. (Cephalophula) zebra*.

<sup>1</sup> Note that these authors recognize *Philantomba* as the genus name for *P. monticola* and *P. maxwellii* and that they are therefore not included in *Cephalophus*.

Table 1 Taxonomic classification of the tribe Cephalophini (Bovidae) following Grubb (1993). The synonyms listed in Grubb's revision are presented under earlier classifications.

Species (Grubb 1993)	Earlier classifications	Reference
<i>C. adersi</i>	<i>C. harveyi adersi</i>	Holdenorth 1963
	<i>C. natalensis adersi</i>	Heyden 1968
<i>C. callipygus</i>	<i>C. ogilbyi callipygus</i>	Groves & Grubb 1981
	<i>C. harveyi callipygus</i>	Holdenorth 1963
	<i>C. natalensis callipygus</i>	Heyden 1968
<i>C. dorsalis</i>		
<i>C. harveyi</i>	<i>C. natalensis harveyi</i>	Ansell 1971, Heyden 1968, Ellerman <i>et al.</i> 1953, Groves & Grubb 1981
<i>C. jentinki</i>		
<i>C. leucogaster</i>	<i>C. dorsalis leucogaster</i>	Rode 1943
<i>C. maxwellii</i>	<i>C. monticola maxwellii</i>	Holdenorth & Diller 1986
	<i>P. maxwellii</i>	Pocock 1910, Groves & Grubb 1981
<i>C. monticola</i>	<i>P. monticola</i>	Pocock 1910, Groves & Grubb 1981
<i>C. natalensis</i>		
<i>C. niger</i>		
<i>C. nigrifrons</i>		
<i>C. ogilbyi</i>		
<i>C. rubidus</i>	<i>C. nigrifrons rubidus</i>	Ansell 1971, Groves & Grubb 1981
<i>C. rufilatus</i>		
<i>C. silvicultor</i>		
<i>C. spadix</i>	<i>C. silvicultor spadix</i>	Holdenorth 1963
<i>C. weynsi</i>	<i>C. natalensis weynsi</i>	Ansell 1971
	<i>C. callipygus weynsi</i>	Kingdon 1997
<i>C. zebra</i>	<i>Cephalophula zebra</i>	
	<i>C. doria</i>	Heyden 1968
<i>S. grimmia</i>	<i>C. grimmia</i>	Van Gelder 1977
	<i>C. (Sylvicapra) grimmia</i>	Holdenorth 1963

As emphasized previously, this species is characterized by many unique features. The second species group was thought to include four species of duiker *C. silvicultor*, *C. dorsalis*, *C. spadix* and *C. jentinki* and is currently regarded by Grubb (pers comm<sup>2</sup>) as paraphyletic. These duiker are generally larger, their horns are not thickened and, if present in females, are nearly as large as those of the males. Juveniles are characterized by a dark, speckled pelage. The third subgenus represents a monophyletic clade in which the front of the skull is thickened in males, the horns are thickened and present in females (although of smaller size than in the males), and the juvenile pelage is similar to that of the adults. This assemblage includes *C. niger*, *C. natalensis*, *C. nigrifrons*, *C. rufilatus*, *C. ogilbyi*, *C. leucogaster*, and *C. adersi* and is known as the *Cephalopia* species group.

Most recently, Kingdon (1997) recognized 17 species within a single genus, *Cephalophus*, which he subdivided into four adaptive lineages. Although the number of recognized taxa differ, there is good agreement with these four lineages and the three species-groups originally proposed by Groves and Grubb (1981). First, *C. monticola* and *C. maxwellii* are clustered as the conservative dwarfs. The second lineage, the so-called red duiker, are the most diverse in which the smaller sized duiker are seen as conservative (having retained some primitive adaptive traits), occupying peripheries or islands that are peripheral to the central habitat (e.g. *C. adersi* and *C. nigrifrons*), while others have adapted to marginal environments within the forests. Three species are placed within the fibre duiker lineage (*C. ogilbyi*, *C. dorsalis* and *C. jentinki*) which is believed to have originated in Upper Guinea; these species have largely retained a western geographic bias in their present distribution. Interestingly, some of the red duiker i.e. *C. leucogaster* are characterized by much coarser diets and are seen as closely related to the fibre diet group. Lastly, the giant duiker lineage comprising the two largest species (*C. silvicultor* and *C. spadix*), is regarded as being the evolutionary most derived.

## Speciation

Mammalogy provides exceptionally fertile grounds for advancing and testing evolutionary theory since, in many instances, it couples living forms to a particularly rich fossil record (Vrba 1992). Among the best studied groups in this regard is the family Bovidae, a large assemblage comprising African and Indian antelope, cattle sheep and goats which includes

<sup>2</sup> Peter Grubb. 35 Downhills Park Road, London N17 6PE. United Kingdom.

some 45 genera and 124 species, more than half of which are endemic to the grasslands and savannas of sub-Saharan Africa (Vrba 1985a).

The earliest known Eurasian bovid dates back approximately 20 million years ago (myr; Vrba 1985a). At much the same time the first sub-Saharan bovids also appeared, with subsequent early evolutionary activity restricted to 14 myr ago. Synchronous events among taxa presumed to be species are also concentrated near the Miocene-Pliocene border (5 myr ago), the late Pliocene (2.5 myr ago), as well as mid Pleistocene (0.9 and 0.7 myr ago; Vrba 1985b).

These temporally separated speciation events suggested by the fossil record are closely tied to glacial oscillations. Global temperature changes over the past 65 million years (based on deep sea records) show large fluctuations with a net cooling trend (Prentice & Matthews 1988). Smaller excursions in the form of periodic cycles, known as glacials and interglacials, are also evident. Evidence from the Pliocene and Pleistocene shows that the cycles were accompanied not only by large scale expansions and retractions of ice at the poles, but also by major climatic and vegetational changes in the tropics. During the downward extension of the snow caps on the mountains, the resulting precipitation extended the area of montane forest to almost a continuum (Van Zinderen Bakker 1962). During the interglacials, however, there was a southward shift in aridity which is thought to have caused a significant reduction in the size of both the Upper Guinea and the main Congo forest blocks (Moreau 1963), habitat to many of the duiker species. This change in habitat would result in severe fragmentation of tropical rainforest species possibly leading to speciation by distance (allopatric speciation), and to the high species diversity found in this biome (Grubb 1982, Myers 1982). Given the profound effect that climatic changes have had on the tropical forests, forest specialists such as the duiker antelope are useful models for furthering our understanding of the evolutionary events leading to speciation and diversity within this biome.

### **Conservation status**

The short to medium term survival of several duiker antelope species is threatened. Human invasion and deforestation are responsible for habitat loss. Additionally, hunting pressure on rare and endangered taxa is intensifying due to commercial bush-meat hunters (Kingdon 1982, Williams 1997). Moreover, several species show narrow endemism, for example *C. rubidus* which is found only at high altitudes on the Ruwenzori Mountains, *C. jentinki* and



*C. zebra* which are limited to the high primary forest zone between Sierra Leone and the Niouniourou river, and *C. adersi* which occurs only on the Island of Zanzibar and in Kenya's Sokoke coastal forests. These considerations have contributed, in part, to *C. adersi*, *C. dorsalis*, *C. jentinki*, *C. monticola*, *C. ogilbyi*, *C. rubidus*, *C. silvicultor*, *C. spadix* and *C. zebra* as being listed by CITES and IUCN as deserving of endangered and/or vulnerable status (Grubb 1993).

Given this background, the need for a comprehensive review of the duiker species and their phylogenetic relationships is evident. Not only will an understanding of the relationships within the group allow for the clarification of the taxonomic status of many taxa, but the data may provide wildlife managers with an evolutionary framework that could prove useful in reassigning conservation priorities to some of the species.

## INTRODUCTION

The mammalian order Artiodactyla is the second largest order of mammals, with over 200 families and 3000 genera. The order is characterized by a variety of adaptations to different environments, and is one of the most diverse groups of mammals. The order is divided into several suborders, including Suiformes, Cetartiodactyla, and Tylopoda. The suborder Suiformes includes the families Suidae and Tayassuidae, and is characterized by a variety of adaptations to different environments, including the ability to dig and to swim. The suborder Cetartiodactyla includes the families Bovidae, Cervidae, Giraffidae, and Hippidae, and is characterized by a variety of adaptations to different environments, including the ability to run and to swim. The suborder Tylopoda includes the family Camelidae, and is characterized by the ability to walk on humps. The order Artiodactyla is one of the most diverse groups of mammals, and is found in a wide range of environments, from the tundra to the desert. The order is also one of the most economically important groups of mammals, providing food and clothing for humans. The order is also one of the most interesting groups of mammals, with a variety of unique adaptations and behaviors. The order is a rich and diverse group of mammals, and is one of the most important groups of mammals in the world.

The discovery of the polymerase chain reaction (PCR) by Mullis & Faloona (1986) has had a profound effect on phylogenetic analysis, leading to the development of a number of techniques to describe diversity and evolutionary relationships in all taxonomic groups. The highly targeted sequences used to resolve phylogenetic relationships have been derived from the mitochondrion. The extranuclear mitochondrial molecule is a closed circular DNA system that replicates and transcribes within the organelle itself. As yet, few species of duikers had no intervening sequences, spacers, or repetitive DNA. Moreover, mitochondrial DNA