CHAPTER 4

COMBINED APPROACH, CONSERVATION GENETICS, AND GENERAL CONCLUSIONS

TOTAL EVIDENCE

Independent genetic markers can provide different levels of resolution which, in turn, allow for the accurate reconstruction of evolutionary events ranging from the most distant to the most recent. For example, mitochondrial DNA sequence data are frequently used to reconstruct recent evolutionary events (although the use of appropriate genes would allow resolution of deep nodes), whereas changes in diploid number and intrachromosomal rearrangements may be invariant among closely related taxa, as is the case for duiker antelope (see Chapter 3). To clarify evolutionary relationships between duiker antelope, data were gathered using three independent approaches: the sequencing of selected mitochondrial genes (cytochrome *b* and 12S rRNA), comparative cytogenetics, and fluorescence *in situ* hybridization of satellite DNA sequences.

There has been an increase in the number of studies designed to test hypotheses based on combining independent data sets (e.g. De Queiroz *et al.* 1995). Two approaches are generally followed. The first involves the separate analysis of each data set where the resultant topologies are examined for congruence among them. Secondly, a search for the single most parsimonious solution using all available data, the so-called "total evidence" approach (Kluge 1989), can be performed. The rationale is that different data sets may provide phylogenetic signal at different hierarchical levels so that in combination they may improve the overall resolution of a phylogeny (Hillis 1987). In the present investigation, a "total evidence" approach was adopted in which the comparative and molecular cytogenetic characters were combined with the molecular sequence data after a partition homogeneity test (Farris *et al.* 1995 in Paup 4.0b2a) showed no significant conflict among them (P=0.51).

Initially the cytochrome *b* and 12S rRNA sequences from all 19 recognized duiker taxa were combined with the FISH and comparative cytogenetic data which are unfortunately limited to only seven of the 19 species. The cytogenetic characters were coded as present or absent

and are presented in Table 9. Due to the large number of taxa for which there was missing data a largely unresolved topology resulted (Fig. 16A). A reanalysis of the data including only taxa for which both sequence and chromosomal information are available resulted in a single tree of 909 steps (Fig. 16B). The inclusion of the cytogenetic and FISH characters significantly improved the support for most of the nodes. These finding would suggest that the three independent data sets (molecular sequence data, conventional cytogenetics, and FISH) compliment each other in the combined analysis. In addition, a high degree of correspondence was found between the results of the combined analysis and those from markers analyzed independently.

GENETICS AND WILDLIFE CONSERVATION

The role of genetics in conservation biology centers mostly around the description of biodiversity and the patterns of genetic variation among populations and species. The data presented herein have implications beyond simply providing an assessment of the duiker phylogeny and provide wildlife managers with an evolutionary framework that may be useful in reassigning conservation priorities to some of the species, in particular the Ruwenzori red duiker and Weyne's duiker.

The Ruwenzori red duiker *(C. rubidus)* has commonly been regarded as a subspecies of the wide spread black-fronted duiker, *C. nigrifrons* (St. Leger 1936, Ansell 1971, Groves & Grubb 1981) and has therefore enjoyed little attention as a conservation priority in its own right. However, the results of this study suggest otherwise. The Ruwenzori duiker groups for most part within the west African red duiker clade and is quite distinct from the black-fronted duiker which clusters as the sister taxon to *C. rufilatus* in the east African red duiker lineage (Chapter 2). This association is maintained irrespective of the method of analysis or weighting scheme used. Moreover, the sequence divergence separating *C. rubidus* and *C. nigrifrons* approximates that delimiting all recognized species; should this hold (the inadequacies of sample size withstanding) it is reasonable to assume that the Ruwenzori red duiker may in fact represent a distinct species and, if valid, clearly raises the conservation profile of this species. This finding is all the more important given its restricted distribution (it occurs only on the Ruwenzori mountains at altitudes above 3 000 meters), and diminishing population numbers (Kingdon 1982, 1997).

Table 9 Five FISH and comparative cytogenetic characters used in the combined analyses. The characters are coded as presence/absence data. The cytogenetic data of Robinson *et al.* (1996) is included.

	A	B	C	D	E
Outgroup	0	0	0	0	0
C. monticola	1	0	1	0	0
C. maxwellii	1	0	1	0	0
C. natalensis	0	0	0	1	0
C. silvicultor	0	1	0	0	1
C. spadix	0	1	0	0	1
C. dorsalis	0	1	0	0	1
S. grimmia	0	0	0	1	0

A=Pericentromeric heterochromatic inversion of the X chromosome, B=Absence of a G-negative juxtacentromeric band on the X chromosome, C=FISH hybridization of satellite probes (*Eco*RI-Max, *Eco*RI-Blue, *Pst*I-Blue, *Pvu*II-Max) to both the X and Y chromosome, D=FISH hybridization of satellite probes (*Eco*RI-Max, *Eco*RI-Blue, *Pst*I-Blue, *Pvu*II-Max) to the X chromosome but not the Y chromosome, E=Absence of FISH hybridization of satellite probes (*Eco*RI-Max, *Eco*RI-Blue, *Pst*I-Blue, *Pvu*II-Max) to both the X and Y chromosomes.

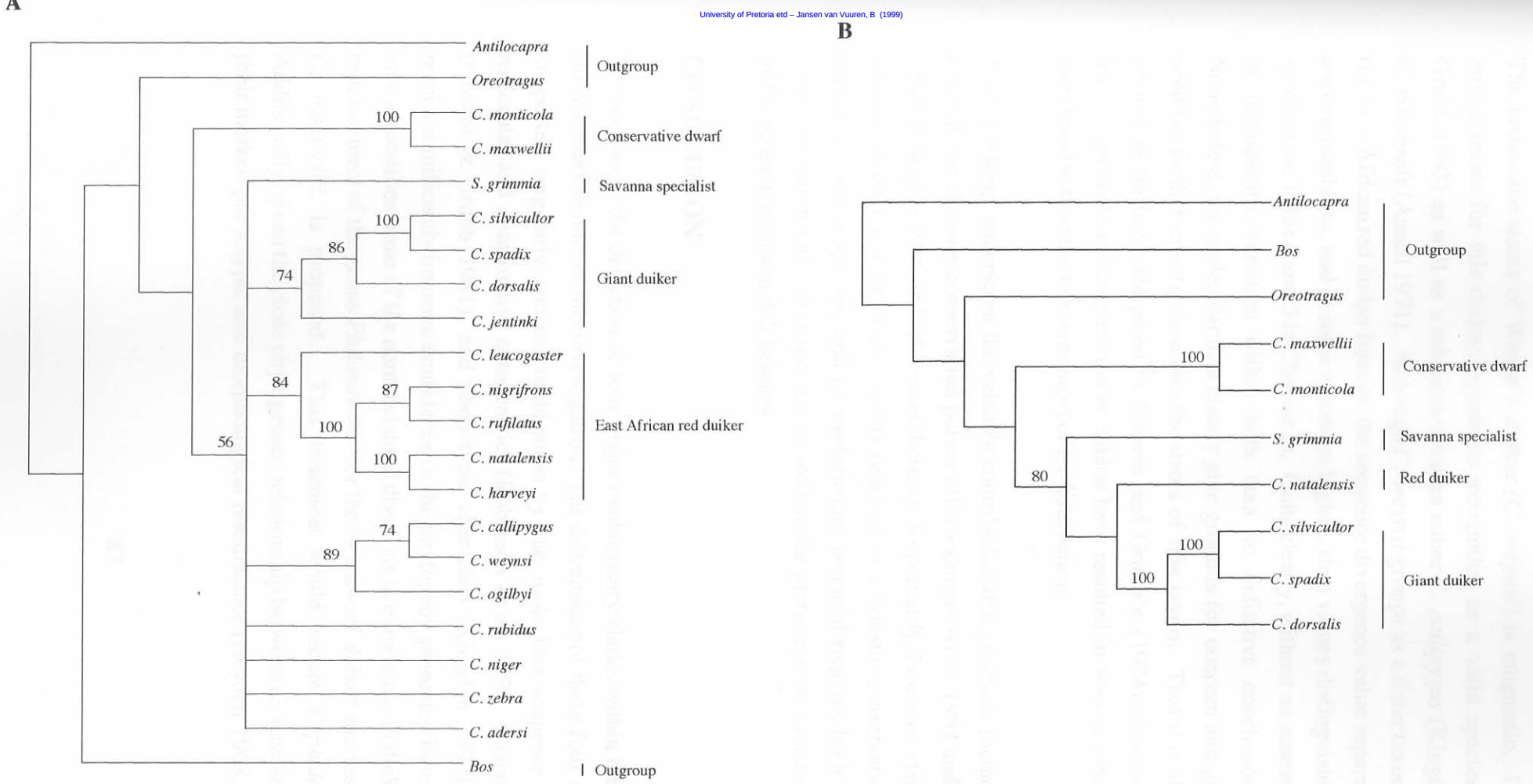

Figure 16 Results of the unordered parsimony analyses based on the combined molecular and cytogenetic data sets. (A) The bootstrap consensus tree (1 000 replicates) obtained when all duiker taxa are included. Nodes that received less than 50% support were collapsed. (B) The single most parsimonious tree obtained when only taxa for which cytogenetic data were available are included. The tree was produced with the heuristic search option in Paup 4.0b2a (Swofford 1999) with 100 random replacements. Bootstrap support obtained from 1 000 iterations is shown above the branches.

The taxonomic status of Weyne's duiker *(C. weynsi)* is enigmatic. Previous taxonomic arrangements for this duiker include its recognition as a valid species (St. Leger 1936, Grubb 1993) as well as a subspecies within either *C. callipygus* (Kingdon 1982, 1997) or *C. natalensis* (Ansell 1971). Although *C. weynsi* groups as a sister taxon to *C. callipygus* in the west African red duiker lineage, the sequence divergence value separating these two taxa are comparable to, and in some instances higher, than values distinguishing well recognized species (see Table 4 and 5 in Chapter 2). Quite clearly, without an assessment of the degree of intraspecific variation within both taxa no definitive conclusions can be drawn. Nonetheless, the molecular data clearly give grounds for concern and give emphasis to the need for further investigation into the status of this taxon. That this should enjoy a high priority is further highlighted by Groves and Grubb's (1974) observation that increased hunting pressure and degradation of habitat have resulted in Weyne's duiker being severely threatened with extinction over most of its distribution.

These findings underscore the valuable contribution that genetics, including phylogenetics, can make in shaping conservation policies (for example Avise 1994 and references therein, Smith & Wayne 1996 and references therein). Importantly, however, it must be realized that genetics is only one of many aspects required in a holistic conservation approach. The conservation of a species, and its evolutionary potential requires both the preservation of genetic variation within that species, as well as the protection of its habitat and the integrity of the ecosystem to which it belongs.

CONCLUSION

In conclusion, the detection of four major evolutionary clades within the Cephalophini was the hall mark feature of this investigation. The delimitation of these four adaptive lineages is supported singularly and in combination by the molecular sequence data (Chapter 2), molecular and comparative cytogenetics (Robinson *et al.* 1996, Chapter 3), morphology (Groves & Grubb 1981), and phenotypic characters (Kingdon 1997). The concordant results significantly improve confidence in the phylogeny presented herein. Moreover, this study questions some of the nomenclatural divisions in current use in duiker taxonomy. The reinstatement of the genus *Philantomba* for the two dwarf duiker species, *C. monticola* and *C. maxwellii,* is proposed. This treatment would secure *Cephalophus* monophyly. Additionally, given the close phylogenetic relationship between *C. natalensis* and *C. harveyi,* their marked phenotypic and morphological resemblance (Heyden 1968, Ansell 1971), and

evidence of hybridization between them (Kingdon 1982), it is not unreasonable to suggest that *C. harveyi* be relegated to a subspecies of the Natal red duiker, *C. natalensis* .