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DNA-led rediscovery of the giant sable antelope in Angola

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Abstract The giant sable antelope (*Hippotragus niger variani*), unique to Angola, was feared extinct after almost three decades of civil war. Comparisons of mitochondrial DNA sequences derived from dung samples recently collected in the field and from old museum specimens of certain provenance provide the first documented evidence to date that this enigmatic antelope has survived. Its DNA-led rediscovery in the former combat zone was subsequently confirmed by photographic evidence. The Angolan isolate constitutes a distinct monophyletic group that shows a dramatic population decline from historic levels. It represents a diagnosable conservation unit which is characterised by unique cranial morphological features (Blaine 1922 1922:317–339), a highly restricted range, and the presence of fixed genetic differences in all of its common relatives.

Keywords *Hippotragus niger variani* · Giant sable antelope · Dung mitochondrial DNA · Species identification

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Introduction

The giant sable, which is endemic to central Angola, was first reported in the early 1900s (Thomas 1916; Blaine 1922). Impressiveness, rarity, isolation and uncertain taxonomic position all contribute to making this animal the most enigmatic of Africa's antelope (Walker 2002). Except for three field studies (Frade and Sieiro 1960; Cabral 1970; Estes and Estes 1974), little is known of its habits, life history, population size and intraspecific relationship. The last accepted sightings of the giant sable occurred in Angola in 1982 (Estes 1983). The paucity of information is further exacerbated by 30 years of armed conflict in Angola, leading to its placement on the IUCN's Red List as "critically endangered" and, most recently, to fears that it may not have survived the civil unrest. In spite of anecdotal reports and ground and aerial surveys, no evidence existed to suggest that it may persist in the Luando Integral Nature Reserve and the Cangandala National Park, two areas in which giant the sable have previously been recorded (Estes and Estes 1974) (Fig. 1). The former reserve lies in the Malange province and is approximately 8,280 km^2 in size. For 240 km of its length, it is bounded by the Cuanza and Luando rivers. The Cangandala National Park, situated about 40 km north of the Luando Integral Nature Reserve, comprises about 600 km^2 and is similarly situated in the Malange province. Dung samples collected at localities within these two refuges, and provisionally identified in the field as being derived from giant sable, provided an opportunity for the independent verification of its persistence in these protected areas.

Using DNA-based species-identification analysis, we provide first documented evidence that this spectacular antelope survived in the former combat zone. Because species-oriented conservation programs currently attempt to analyse and maintain intraspecific variation (DeSalle and Amato 2004), the DNA sequence data are used in a cladistic analysis to identify the phylogenetic relationships of the giant sable and to evaluate its status as a minimal unit for conservation management.

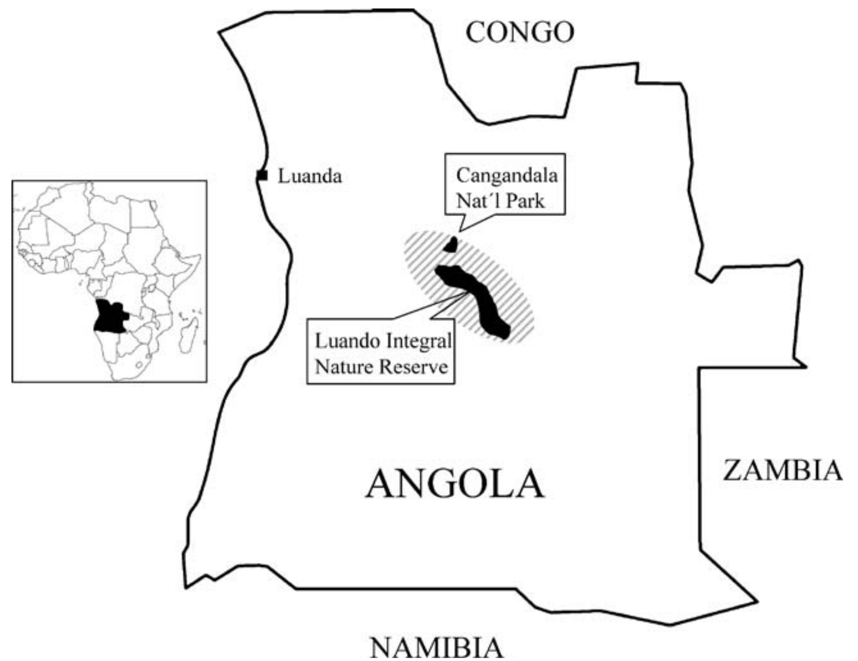

Fig. 1 A map of Angola showing the locations of regions where dung pellets were sampled as well as the historical range of *H. n. variani* (*shaded area*) where the museum specimens were collected

Materials and methods

Specimens

Nineteen dung samples, thought to be of giant sable, were collected in 2004 by personnel from the Catholic University of Luanda during excursions to the Luando Integral Nature Reserve (11°58′ S, 17°43′ E) and the Cangandala National Park (9°54′ S, 16°44′ E) in the Malange province in central Angola (Fig. 1). Most samples were collected around natural salt licks. Each dung pellet was placed separately into a zip-lock bag without contact of human skin and transported to the laboratory where they were stored at room temperature until DNA extraction.

To obtain reference sequences of the giant sable, we produced homologous sequences from four museum specimens of certain provenance (principally, the locality and date of collection). These were GSA, GSB and GSC all of which were collected in 1921 by Major Powell-Cotton and are deposited in the Powell-Cotton Museum (Kent). The fourth, GSD, was collected in 1930 and forms part of the collections of the Field Museum of Natural History, Chicago. All specimens were collected in the Cuanza river region within the historical range of the giant sable and which marginally extends the area covered by the newly established reserves described above (Fig. 1). The museum specimens were collected well before the introduction of animal translocations which characterise modern-day game-ranching practices. Our genetic findings (below) spurred further efforts in field research and were subsequently confirmed by photographical evidence (Fig. 2).

Laboratory protocols

Since species-specific DNA sequences obtained from shed rectal cells provide an excellent tool for species identification (Kohn and Wayne 1997), we extracted total genomic DNA for use in polymerase chain reaction (PCR)-based amplifications and subsequent sequencing of mitochondrial and nuclear DNA fragments (Table 1) using the primer pairs shown in Table 2. The outer layer of the dung pellet was removed with a wooden stick and processed for DNA extraction using the all-tissue DNA extraction kit (GEN-IAL, Troisdorf, Germany). All amplifications were performed in 50 μl of reaction medium containing 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 2.0 mM $MgCl_2$, 0.5× GC-rich solution, 2 μl Fast Start *Taq* DNA Polymerase (Roche), 0.2 mg/ml BSA, 200 μM each dNTP, 1 μM of each primer, and 100 ng DNA. In each case, three PCR products were designed to cover 724 and 1,074 bp of the cytochrome *b* gene and the D-loop region, respectively. The resulting PCR amplicons had a minimum of 375 bp and maximum of 418 bp overlap (without primers). The PCR was performed on a GeneAmp 2400 machine (Perkin Elmer) under the following conditions: one cycle of denaturation at 95°C for 6 min, followed by 35 cycles at 95°C for 30 s, annealing at 50°C for 30 s and extension at 72°C for 60 s; a final extension period at 72°C for 7 min completed the reaction.

To reduce the risk of contamination, the outer layers of the skin snips from museum specimens were removed prior to extraction. All extractions were carried out in a laminar flow cabinet in a DNA-free laboratory. Extractions were performed using the QIAamp DNA Micro Kit (Qiagen). To

Fig. 2 The picture shows a pregnant female giant sable antelope, *dark brown* in colour, with characteristic long horns and the distinctive facial markings. To her *left* is another pregnant cow suggesting that they form part of a breeding herd. The animals were photographed by a remote-sensing camera near a natural salt-lick in central Angola. (Photo: P. Vaz Pinto)

continuously test for carry-over contamination, negative controls were included throughout the various procedures (DNA extractions and amplifications). Standard PCR reactions contained 1× reaction buffer, 2.0 mM $MgCl_2$, one unit SuperTherm Polymerase, 2 mM of each dNTP, 1 μM of each primer and ~100 ng DNA. Amplifications were performed under the following temperature regime: an initial denaturation step at 96°C for 5 min followed by 40 cycles of 96°C for 30 s, 50°C for 30 s and 72°C for 1 min. A final extension step at 72°C for 5 min completed the reaction. Given that DNA extracted from museum specimens frequently comprises only short fragments, our primers (Table 2) were selected to target relatively short stretches within each region. All amplifications were performed in a GeneAmp 2700 (Applied Biosystems) PCR machine. Amplicons were cleaned through Qiaquick PCR purification kit columns (Qiagen). Both strands were sequenced using BigDye chemistry (Applied Biosystems) according to the manufacturer's instructions and analysed on an ABI-Prism 3100 capillary DNA sequencer. To ensure the authenticity of our museum and dung DNA sequences, all extractions, amplifications and sequencing reactions were performed more than once and were separated temporarily.

Data analysis

Initial sequence comparisons and measures of variability were performed using MEGA 2.0 (Kumar et al. 1993). The D-loop data set comprised 62 distinct sequences and 499 manually aligned positions. Maximum likelihood and bootstrap analyses with 100 iterations used PAUP* 4.0b10 (Swofford 2001) with likelihood settings from the best-fit model (HKY+G) selected by hLRT in Modeltest 3.06 (Posada and Crandall 1998): Base=(0.3740, 0.2491, 0.1308), Nst=2, TRatio=15.0934, rates=gamma, shape=0.4768, Pinvar=0. Neighbour-joining and maximum parsimony methods in-

Table 1 Dung and museum samples from *Hippotragus niger variani* DNA fragments, and corresponding GenBank accession numbers of sequences reported in this study

Sample ID	Source	Origin	Cytochrome *b*	D-loop	Zfy
L3	Dung	Luando	AY875640	AY875643	AY875641
L2	Dung	Luando	AY970693	AY970699	AY970700
B2	Dung	Cangandala	AY970688	AY970697	Absent
B3	Dung	Cangandala	AY970689	AY970698	Absent
B4	Dung	Cangandala	AY970690	AY970694	Absent
B6	Dung	Cangandala	AY970691	AY970695	Absent
B8	Dung	Cangandala	AY970692	AY970696	Absent
GSA	Museum	Cuanza River	AY971585	AY971589	n.d.
GSB	Museum	Cuanza River	AY971586	AY971590	n.d.
GSC	Museum	Cuanza River	AY971587	AY971591	n.d.
GSD	Museum	Cuanza River	AY971588	AY971592	n.d.

n.d. not determined

Table 2 Primers used for amplification and sequencing

Name[a]	Sequence (5′-3′)[b]	Source
Dung samples		
Cytochrome *b*:	5′-tga ctt gaa raa cca ycg ttg	Palumbi et al. 1991
GludgL	5′-ccc tca gaa tga tat ttg tcc tca	Kocher et al. 1989
CB2H		
CB1L	5′-cca tcc aac atc tca gca tga tga aa	Kocher et al. 1989
CB2H	5′- ccc tca gaa tga tat ttg tcc tca	Kocher et al. 1989
CB1L	5′-cca tcc aac atc tca gca tga tga aa	Kocher et al. 1989
CB3_770H	5′-gtg tag ttg tct ggg tct cc	Burger et al. 2001
D-loop:		
ProL	5′-cta cct cca rcw ccc aaa gc	Palumbi et al. 1991
HiCTR2H	5′-ata gct tga gtc caa gca tcc	This study
HiCTR2L	5′-agt aca tta cat gat tta ccc c	This study
HiCTR2H	5′-ata gct tga gtc caa gca tcc	This study
HiCTR3L	5′-cga tgg att aat gat taa tca gc	This study
HiPheH	5′-agc att ttc agt gcc ctt gc	This study
Zfy intron:		
LGL331	5′-caa atc atg caa gga tag ac	Aasen and Medrano 1990
LGL335Y	5′-gaa gca rgc aca ttc ata gag ga	Aasen and Medrano 1990
LGL331Y	5′-gaa acc caa tta aaa tat atg aag ca	Aasen and Medrano 1990
LGL335	5′-aca cct gat tcc aga cag tac ca	Aasen and Medrano 1990
Museum samples		
cytochrome *b*:		
L15162	5′-agc ttc tac cat gag gac aaa tat c	Pääbo and Wilson 1988
H15494	5′-tag ttg tca ggg tct cct ag	Irwin et al. 1991
D-loop:		
L15910	5′-gaa ttc ccc ggt ctt gta aac c	Hoelzel et al. 1991
H16498	5′-cct gaa gta gga acc aga tg	Shields and Kocher 1991
Internal H-primer	5′-ttg ctt ata tgc atg ggg c	Matthee and Robinson 1999

[a]The letters L and H refer to the light and heavy strands, respectively
[b]Degenerate sites are indicated by *y*=*c* or *t*; *r*=*a* or *g*; *w*=*a* or *t*

variably resulted in identical trees except for branch-swapping among haplotypes within evolutionary clades (data not shown). Units of conservation management were delimited using a cladistic approach (Vogler and DeSalle 1994) including population aggregation analysis (Davis and Nixon 1992).

Results and discussion

Authenticity of the *H. n. variani* sequences

Contaminations, nuclear insertions and errors in procedural design can influence authenticity of DNA data obtained from dung and museum specimens. Therefore, in each case, the authenticity of the sequences reported in this study (Table 1) was tested by various criteria (Hofreiter et al. 2001):

1. DNA extractions and amplifications on dung and museum skin snips were conducted in separate laboratories in Berlin (Germany) and Stellenbosch (South Africa).
2. Extraction and PCR negative controls were always included.
3. Sequences were reproduced various times from at least two independent extractions that were separated in time and which involved a total of at least two independent PCR amplifications.
4. Overlapping PCR amplicons always produced the same sequence.
5. The DNA sequences obtained from both dung and museum specimens were unique, making it extremely unlikely that the amplicons arose from DNA contamination.
6. The observations that the cytochrome *b* sequences can be translated into an identifiable protein without nonsense mutations, that the D-loop sequences contained the bovine Rs2 repeat motif and that the nucleotide diversity was five times higher in the non-coding region (0.109±0.009) than in the coding region (0.022±0.005) make it unlikely that nuclear copies of mtDNA genes were sequenced accidentally.
7. All sequences were compared with entries in the GenBank using the BLAST program (National Center

for Biotechnology Information, Bethesda, MD) to identify DNA fragments of high similarity.

Species identification

Mitochondrial DNA sequences were chosen in this investigation for testing provenance of dung samples. We based this on ease of recovery of multiple mtDNA copies from samples that are partially degraded. The hypervariable D-loop region was chosen over parallel cytochrome *b* sequences because it offers a higher range of resolution and is one of the most widely applied regions of the mitochondrial genome for intraspecific comparisons (Avise 2004). Fourteen of 19 dung pellets (74%) were successfully amplified and sequenced: seven were positively identified as giant sable, and the other seven were classified as the sympatric sister species, the roan antelope *Hippotragus equinus*, based on the sequence found to be the closest match (Table 3). Three haplotypes were identified among the four museum specimens analysed herein, each of which was distinct from the two haplotypes that were retrieved from the dung samples. As anticipated, the resolution provided by D-loop sequence analysis was approximately ten times higher than that by the cytochrome *b* sequences (Table 3), and we, therefore, base our phylogenetic interpretation on the former.

The male-specific Zfy sequences obtained from two dung samples (Table 1) were identical to those of *Hippotragus niger* (AY875642), when searched against 36 ruminant species in the GenBank and, therefore, of no use in giant sable identification.

Conservation phylogenetics

Our D-loop sequences were included in a previously published data matrix comprising 50 specimens (separate haplotypes) from 17 localities representative of seven countries throughout the majority of the sable antelope's range (Matthee and Robinson 1999; Pitra et al. 2002). Thus, the mtDNA tree shown in Fig. 3 reflects the most comprehensive assessment of genetic diversity within the species and gives an indication of geographic limits of the currently recognized subspecies. Ansell (1971) and others (Groves 1983; Walther 1990; Wilson and Reeder 1993) recognized four distinct subspecies of *H. niger*. These are *H. n. kirkii* Gray, 1872 in west Tanzania, *H. n. roosevelti* Heller, 1910 in southeastern Kenya and east Tanzania, *H. n. niger* Harris, 1838 in southern Africa, and *H. n. variani* Thomas, 1916 in a limited area of Angola. Additionally, Groves (1983) described a further subspecies, *H. n. anselli*, which is thought to range from eastern Zambia and Malawi to southeastern Tanzania. However, these subspecific classifications were based generally on restricted geographical sampling of few specimens and single morphological characters which undergo extensive individual variation (Groves 1983).

Our molecular phylogeny (Fig. 3) suggests that the nominate *H. niger* is comprised of at least three genetically well-differentiated, geographically structured mtDNA lineages and is partially consistent with the conventional assignment of subspecies. There was high bootstrap support for reciprocal monophyly of three haplogroups corresponding to the subspecies *H. n.roosevelti* (97%), *H. n. kirkii* (100%), and *H. n. niger* (93%). The validity of the subspecies *H. n. anselli* could not be evaluated due to lack of material. Surprisingly, the giant sable sequences from Angola are nested within the southern African clade (*H. n. niger*) questioning the legitimacy of its status as a separate subspecies, *H. n. variani* (sensu Thomas 1916). The most closely related haplotypes to *variani* were found in the southern region of central Tanzania (a distance of some 2,000 km). Given the patterns suggested by an earlier study (Pitra et al. 2002), it seems likely that the peripheral populations of *H. n. niger* in Angola and south Tanzania were founded in the recent past by episodic long-distance colonisations from a common source population. Similar phylogeographic patterns, probably shaped by Pleistocene fluctuations in climate, were also observed in other savannah antelope species as the hartebeest, topi and wildebeest (Arctander et al. 1999) and the roan antelope (Alpers et al. 2004). However, despite recent proposals (Sites and Marshall 2003; Herbert et al. 2003), mtDNA evidence should not be used as a primary criterion for taxon boundaries because of potential limitations including retention of ancestral polymorphism, male-biased gene flow and introgression after hybridization (Avise 2004). We suggest, therefore, that our molecular data might rather stimulate taxonomic re-evaluation of morphological and ecological characteristics and, if warranted, the taxonomic revision of *H. niger*.

The fact that giant sable is not yet extinct has important implications for conservation. There are several lines of

Table 3 Mean number of differences±SE among groups of hippotragid DNA sequences

Group[a]	1	2	3	4
1. Dung DNA (7/5)	–	4.3±1.0	42.1±4.0	94.0±7.6
2. *H. n. variani*, museum specimens (4/3)	0.5±0.3	–	41.6±3.8	94.0±7.5
3. *H. niger* sp., GenBank (11/50)	4.0±1.1	4.5±1.2	–	94.9±6.6
4. *H. equinus* sp., GenBank (9/9)	12.4±3.1	12.9±3.1	14.6±3.1	–

Observed sequence differences among aligned parts of cytochrome *b* sequences (208 bp) are given below the diagonal and D-loop sequences (499 bp) above the diagonal

[a]The numbers of cytochrome *b*/D-loop sequences per group are presented in parentheses

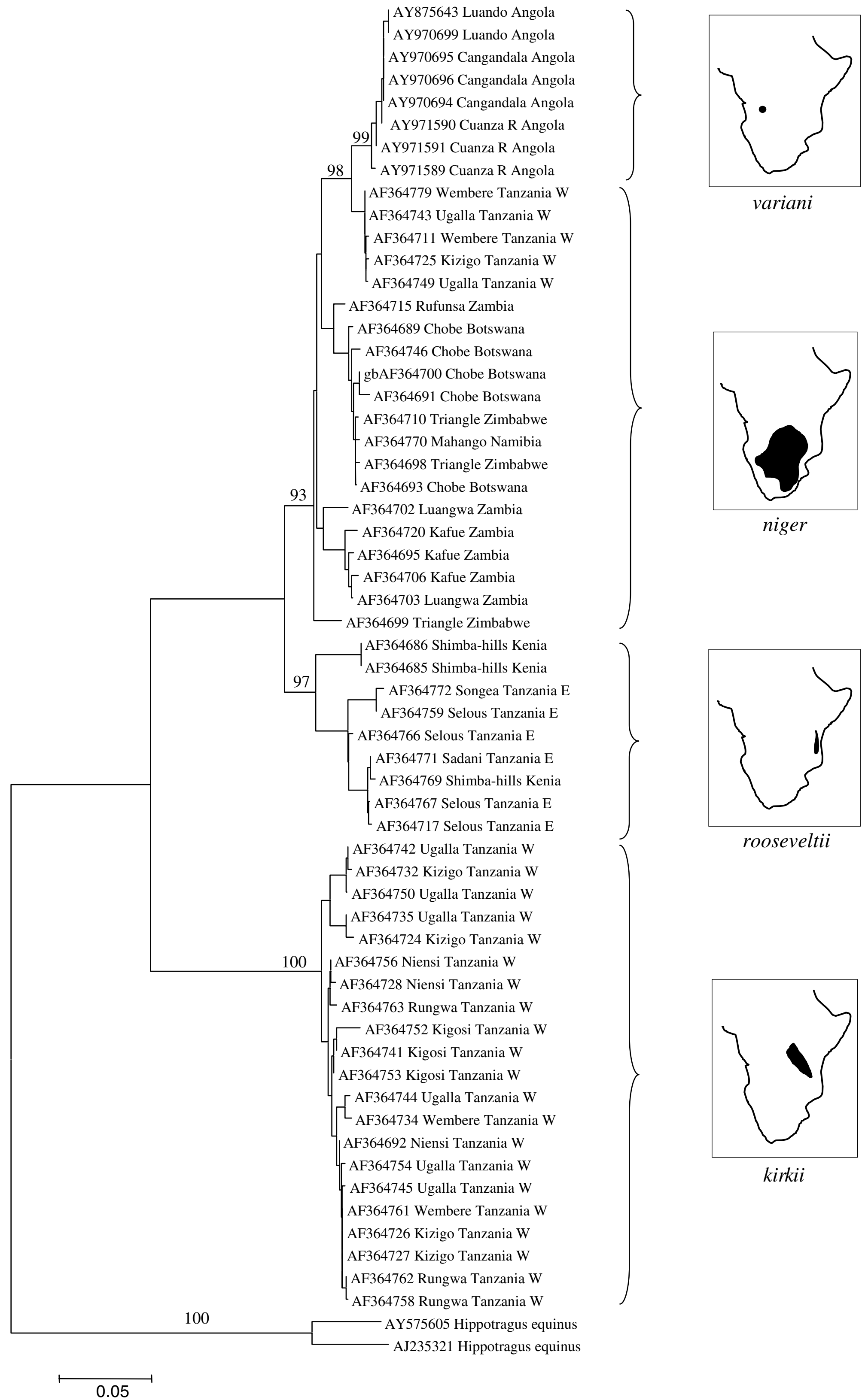

Fig. 3 Maximum likelihood tree ($\ln L = -1{,}958.8$) showing the degree of similarity of mitochondrial D-loop sequences within a phylogenetic framework of *Hippotragus niger* specimens (Pitra et al. 2002), with the subspecies names and boundaries presented. The GenBank accession numbers of the sequences and sampling localities of the specimens are shown. The sister-species *H. equinus* was used as an outgroup. Bootstrap values (%) for nodes supporting current subspecies designation are presented

evidence to suggest that the Angolan population should be treated as a separate unit of conservation (Vogler and DeSalle 1994). First, the haplotypes observed in the giant sable antelope form a monophyletic group within the *H. niger* mtDNA tree (99% bootstrap support, Fig. 3), and the population aggregation analysis identified four diagnosable nucleotide sites (three C to T transitions and one A to T transversion) in the D-loop that are present in all

individuals but not in other conspecific populations. Assuming an estimated substitution rate of 15.7% per million years (Mannen et al. 1998) for the bovid D-loop region, the Angolan and southern African lineages diverged 200,000 years ago. Second, there are distinct morphological differences underpinning its recognition in prior taxonomic studies. Although the giant sable is similar in size to the *H. n. niger*, it does not have the full white eye-to-eye nose line (Fig. 2) found in the nominate. Additionally, the two taxa differ markedly in horn length and presentation —in the case of the Angolan variant, the horns rise vertically to a height of 165 cm in adult males (Mochi and Carter 1971). Thirdly, there are important national considerations for its conservation. Generally, conservation in Angola has been severely compromised by widespread lawlessness during the lengthy civil war, and many large mammal populations are near local extinction (Walker 2002). The giant sable, the national symbol of the Angolan natural heritage, is undoubtedly a flagship species around which conservation initiatives can be built. Finally, the apparently geographically isolated population in Angolan has undergone marked population decline. The number of giant sable is thought to have been reduced from an estimated 1,000–2,000 (Frade and Sieiro 1960; Cabral 1970; Estes and Estes 1974; Estes 1983) four decades ago to an elusive relict population for which precise numbers have yet to be determined. Clearly, our results hold out hope that extensive faecal sampling combined with multilocus genotyping may be useful in providing robust estimates of population size (Palomares et al. 2002; Creel et al. 2003) and a more accurate delimitation of the giant sable distribution in Angola. Prudent management by the Angolan authorities involving local communities could include steps to prevent exploitation of this antelope by a potentially large international market and, in the broader sense, the ongoing homogenization of the species by ill-considered translocations that form part of current game-ranching and ecotourism practices in Southern Africa.

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References

Aasen E, Medrano JF (1990) Amplification of the *ZFY* and *ZFX* genes for sex identification in humans, cattle, sheep and goats. Biotechnology 8:1279–1281

Alpers DL, VanVuuren BJ, Arctander P, Robinson TJ (2004) Population genetics of the roan antelope (*Hippotragus equinus*) with suggestions for conservation. Mol Ecol 13:1771–1784

Ansell WFH (1971) Order Artiodactyla. In: Meester J, Setzer HW (eds), The mammals of Africa: an identification manual, part 15. Smithsonian Institution, Washington, District of Columbia, p 184

Avise JC (2004) Molecular markers, natural history, and evolution. 2nd ed., Sinauer Associates, Sunderland (Massachusetts)

Blaine G (1922) Notes on the zebras and some antelopes of Angola. Proc Zool Soc Lond 1922:317–339

Burger J, Pfeiffer I, Hummel S, Fuchs R, Brenig B, Herrmann B (2001) Mitochondrial and nuclear DNA from (pre)historic hide-derived material. Anc Biomol 3:227–238

Cabral JC (1970) Alguns Aspectos da Ecologia da Palanca Real (*Hippotragus niger variani* Thomas). Bull Instituto de Investigação Científica de Angola 7:5–38

Creel S, Spong G, Sands JL, Rotella J, Zeigle J, Joe L, Murphy KM, Smith D (2003) Population size estimation in Yellowstone wolves with error-prone noninvasive microsatellite genotypes. Mol Ecol 12:2003–2009

Davis JI, Nixon KC (1992) Populations, genetic variation, and the delimitation of phylogenetic species. Syst Biol 41:421–435

DeSalle R, Amato G (2004) The expansion of conservation genetics. Nat Rev Genet 5:702–712

Estes RD (1983) Sable by moonlight. Anim Kingd 86:10–16

Estes RD, Estes RK (1974) The biology and conservation of the giant sable antelope *Hippotragus niger variani* Thomas, 1916. Proc Acad Nat Sci Philadelphia 126:73–104

Frade F, Sieiro D (1960) Palanca preta gigante de Angola. Junta de Investigações do Ultramar, Lisboa, Garcia de Orta 8:21–38

Groves CP (1983) A new subspecies of sable antelope, *Hippotragus niger* (Harris 1938). Rev Zool Afr 97:821–828

Herbert PDN, Cywinska A, Ball SL, deWaard JR (2003) Biological identifications through DNA barcodes. Proc R Soc Lond B Biol Sci 270:313–321

Hoelzel AR, Hancock JM, Dover GA (1991) Evolution of the cetacean mitochondrial d-loop region. Mol Biol Evol 8:475–493

Hofreiter M, Serre D, Poinar HN, Kuch M, Paabo S (2001) Ancient DNA. Nat Rev Genet 2:353–359

Irwin DM, Kocher TD, Wilson AC (1991) Evolution of cytochrome *b* gene of mammals. J Mol Evol 32:128–144

Kocher TD, Thomas WK, Meyer A, Edwards SV, Paabo S, Villablanca FX, Wilson AC (1989) Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proc Natl Acad Sci U S A 86:6196–6200

Kohn MH, Wayne RK (1997) Facts from feces revisited. TREE 12:223–227

Kumar S, Tamura K, Nei M (1993) MEGA: molecular evolutionary genetics analysis, version 1.02. Pennsylvania State University, University Park

Mannen H, Tsuji S, Loftus RT, Bradley DG (1998) Mitochondrial DNA variation and evolution of Japanese black cattle (*Bos taurus*). Genetics 150:1169–1175

Matthee CA, Robinson TJ (1999) Mitochondrial DNA population structure of roan and sable antelope: implications for the translocation and conservation of the species. Mol Ecol 8:227–238

Mochi U, Carter TD (1971) Hoofed mammals of the world. Charles Scribner's Sons

Paabo S, Wilson AC (1988) Polymerase chain reaction reveals cloning artefacts. Nature 334:387–838

Palomares F, Godoy JA, Piriz A, O'Brien SJ, Johnson WE (2002) Faecal genetic analysis to determine the presence and distribution of elusive carnivores: design and feasibility for the Iberian lynx. Mol Ecol 11:2171–2182

Palumbi SR, Martin A, Romano S, McMillan WO, Stice L (1991) The simple fool's guide to PCR. Department of Zoology and Kewalo Marine Laboratory, University of Hawaii, Honolulu

Pitra C, Hansen AJ, Lieckfeldt D, Arctander P (2002) An exceptional case of historical outbreeding in African sable antelope populations. Mol Ecol 11:1197–1208

Posada D, Crandall KA (1998) Modeltest: testing the model of DNA substitution. Bioinformatics 14:817–818

Shields GF, Kocher TD (1991) Phylogenetic relationships of North American ursids based on analysis of mitochondrial DNA. Evolution 45:218–221

Sites JW, Marshall JC (2003) Delimiting species: a Renaissance issue in systematic biology. TREE 18:462–470

Swofford DL (2001) PAUP*: phylogenetic analysis using parsimony (*and Other Methods) Sinauer Associates, Sunderland, Massachusetts

Thomas O (1916) A new sable antelope from Angola. Proc Zool Soc Lond 1916:298–301

Vogler AP, DeSalle R (1994) Diagnosing units of conservation management. Conserv Biol 8:354–363

Walker JF (2002) A certain curve of horn: the hundred-year quest for the giant sable antelope of Angola. Atlantic Monthly

Walther FR (1990) Roan and Sable Antelopes. In: Parker SP (ed) Grzimek's Encyclopedia of mammals, vol. 5. McGraw-Hill, New York, pp 437–447

Wilson DE, Reeder DM (1993) Mammal species of the world (2nd edn). Smithsonian Institution, Washington