

Distribution and genetic diversity of the Endangered Abbott's duiker *Cephalophus spadix* in the Udzungwa Mountains, Tanzania

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ABSTRACT: Abbott's duiker *Cephalophus spadix* is a forest antelope endemic to a very few highland forests in Tanzania. Apparently extinct over much of its historical range, the species is listed as Endangered by the International Union for Conservation of Nature based on its rarity and its known current distribution in only 5 isolated upland areas: Kilimanjaro, Southern Highlands, West Usambara, Rubeho and Udzungwa Mountains. In contrast to the situation in the rest of its range, Abbott's duiker is relatively well documented and locally abundant in parts of the Udzungwa Mountains, which may now be the only stronghold for the species. We review the distribution of Abbott's duiker within the Udzungwa Mountains and present new information based on the non-invasive genetic identification of dung piles collected from the majority of forest blocks between 2006 and 2010 (73 confirmed dung samples). Our results include new records from outlying forest blocks where the presence of Abbott's duiker was previously unknown. Moreover, we present the first population-level analysis of genetic structure and diversity in this endangered species based on nuclear microsatellites and mitochondrial sequence data. While these genetic results are limited due to small sample sizes, they indicate differentiation from other Abbott's duiker populations, as well as low genetic diversity relative to sympatric antelope species. Finally, we discuss threats to Abbott's duiker and identify broad trends within the differently managed Udzungwa Mountain forests, and elsewhere, that suggest potentially successful conservation strategies for this neglected species.

KEY WORDS: Eastern Arc Mountains · Faecal DNA · Phylogeny · Camera-traps · Duikers

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INTRODUCTION

The highland forests of Tanzania are amongst the most important areas in the world for biodiversity conservation due to the exceptional density of threatened and endemic species found there (Burgess et al.

2007). These forests are also of great value to the people of Tanzania through their provision of ecosystem services such as watershed protection and carbon sequestration (Burgess et al. 2009). The endemic species of Tanzania's highlands are valuable indicators of the health of these important ecosystems.

One of the most threatened highland endemic mammals of Tanzania is Abbott's duiker *Cephalophus spadix*, a forest antelope found in only a few upland areas (Fig. 1). This duiker species is notable for its head crest of pink or red hair and its large body size (Kingdon 1997). Despite these characteristics it is very rarely seen due to its secretive behaviour (often crepuscular or nocturnal), densely vegetated habitats and naturally low population density. The species is threatened primarily by hunting, particularly with snares, and also by habitat loss due to agricultural encroachment and selective logging (Moyer et al. 2008).

Not much is known about the historical distribution of Abbott's duiker, but the species has long gone unrecorded in many sites where it was formerly known, including the Uluguru and East Usambara Mountains, the Gregory Rift forests, and the Njombe escarpment in southern Tanzania (Moyer 2003). This apparent decline resulted in the species' IUCN Red List status being changed from Vulnerable to Endangered in 2008 (Moyer et al. 2008). This assessment considered Abbott's

duiker to survive in just 4 isolated mountain ranges: Kilimanjaro, Udzungwa, West Usambara and the Southern Highlands (Mount Rungwe and Livingstone forest). A small isolated population had also been discovered in the southern Rubeho Mountains in 2006 (Rovero et al. 2008). No information on abundance was available from Kilimanjaro or West Usambara, and the species was considered very rare in the Southern Highlands, leaving the Udzungwa Mountains as the only known stronghold for the species.

The Udzungwa Mountains in south-central Tanzania are the largest block of the Eastern Arc Mountains (Fig. 2). Many of the area's forests are protected by the Udzungwa Mountains National Park, and the more recently gazetted Kilombero Nature Reserve, although other forests are less well protected and threatened by illegal activities; for further details see, e.g., Rovero et al. (2010). This variation in protected status was reflected in the Red List's assessment of Abbott's duiker within the Udzungwas, with the species listed as 'locally common' in the forest blocks known as Mwanihana, Luhomero and Ukami and 'rare' or 'scarce' in Matundu (including Lumemo, Ruipa and Matundu West), Nyumbanitu and Uzungwa Scarp (Moyer et al. 2008). The status of Abbott's duiker in several other forests was unknown.

Knowledge of the status of Abbott's duiker in the Udzungwas has increased greatly since the last Red List assessment, due to extensive survey work and the use of remotely triggered camera-traps (Rovero et al. 2005) and non-invasive genetics (Bowkett et al. 2009). These techniques not only provide more reliable survey methods than traditionally available, but also a wealth of further information on abundance (Rovero & Marshall 2009), habitat-use (Bowkett et al. 2008), and, in the case of genetic analysis, population structure and genetic health (Beja-Pereira et al. 2009). Here we present results from recent surveys of Abbott's duiker, including all major forest blocks in the Udzungwa Mountains, and an exploratory analysis of genetic information recovered from dung samples collected during this work.

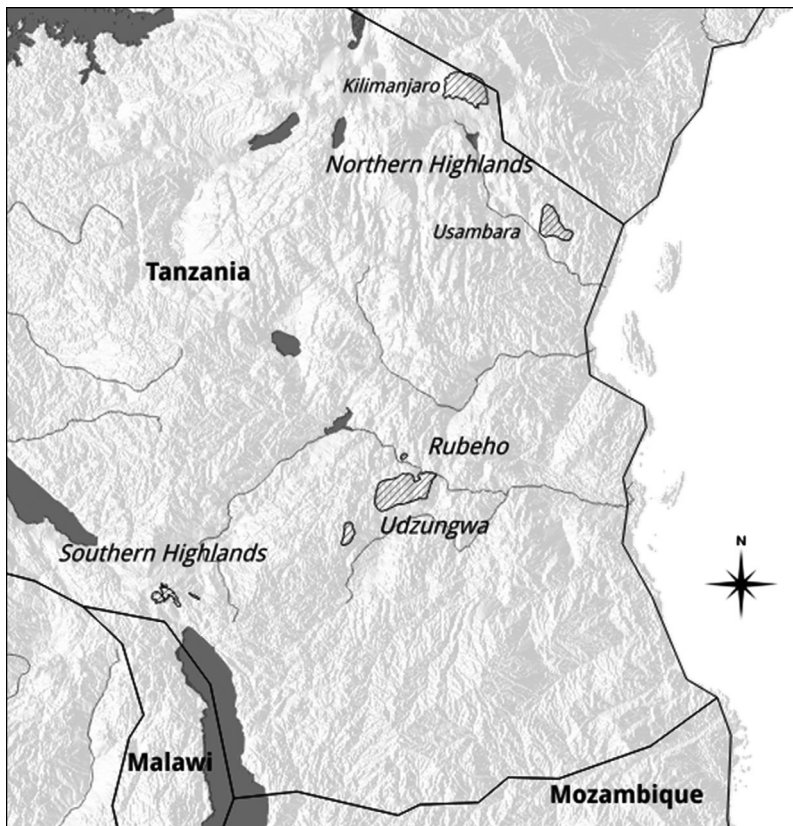


Fig. 1. *Cephalophus spadix*. Global distribution of the Endangered Abbott's duiker (hatching). Highland areas and mountain ranges referred to in the text are italicized. Modified from Moyer et al. (2008)

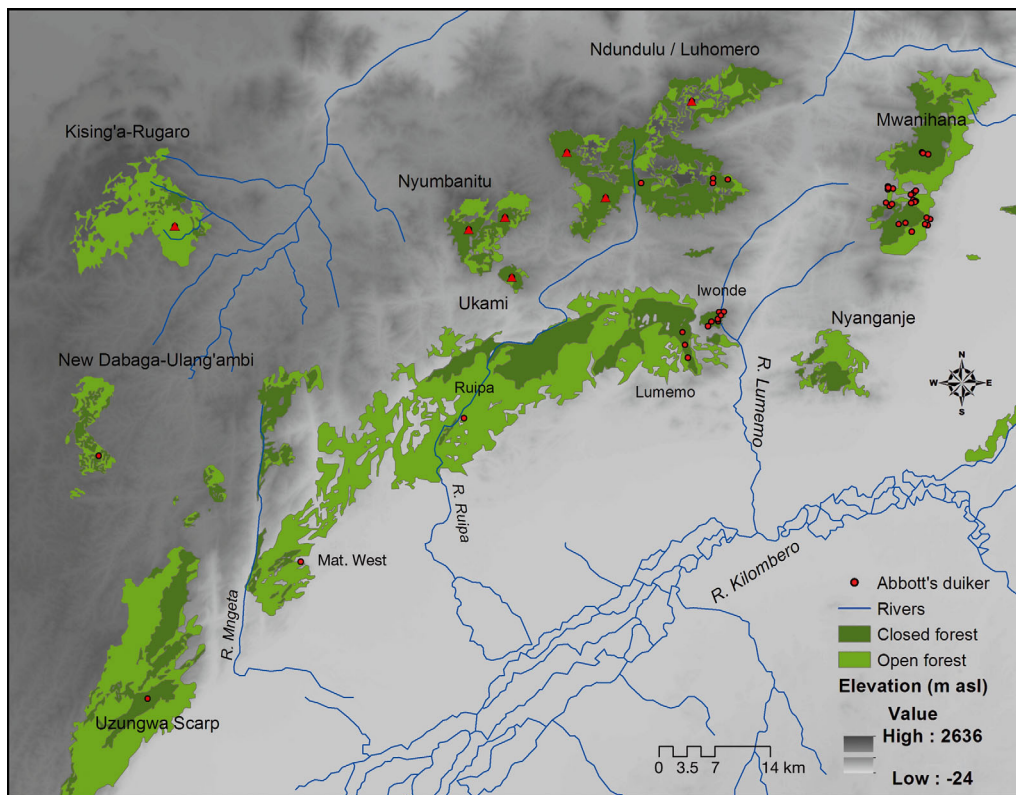


Fig. 2. *Cephalophus spadix*. Map of the Udzungwa Mountains, Tanzania, showing recorded presence of Abbott's duiker (red circles are geo-referenced, and red triangles approximate, dung sample locations), see Table 1 for more details. Outlined shapes represent closed- and open-canopy forest identified from satellite imagery by Marshall et al. (2010)

MATERIALS AND METHODS

We surveyed 24 sites within 10 forests throughout the Udzungwas between 2006 and 2010 (Fig. 2, Table 1). Within each forest we walked reconnais-

sance transects in a triangular configuration (typically 3 km d⁻¹) using hip-chains to record distance. Single linear transects were also sometimes undertaken to sample particular forest areas. Suspected Abbott's duiker *Cephalophus spadix* dung piles,

Table 1. *Cephalophus spadix*. Forest characteristics and Abbott's duiker survey results for the 10 forest blocks studied in the Udzungwa Mountains, Tanzania. CT: camera-trap; S: sightings; m a.s.l.: metres above sea level. Size and elevation data from Marshall et al. (2010). Blanks indicate no other records

Forest	Size (km ²)	Elevation (m a.s.l.)	Walked transects (km)	Faecal DNA records	Other records
Matundu (Lumemo, Ruipa and Matundu West)	526	279–1046	40	6	CT
Uzungwa Scarp	314	290–2144	25	1	S ^a
Luhombero-Ndundulu	231	1105–2520	76	17	CT, S
Mwanihana	151	351–2263	68	33	CT, S
Kising'a-Rugaro ^b	116	1627–2322	30	1	
Nyumbanitu	57	1074–2322	27	2	CT
Nyanganje ^b	42	350–1038	23	0	
New Dabaga-Ulang'ambi ^b	40	1764–2081	21	4	
Ukami	7	902–1651	10	0 ^c	CT
Iwonde	5	980–1472	9	9	CT

^aSighting by Arafat Mtui, 25 March 2005; ^bcamera-traps were not used in these forests; ^cdung samples were not collected from Ukami

encountered along transects or elsewhere, were recorded and collected for genetic analysis (unless desiccated). Tissue samples were recovered on an opportunistic basis from antelope found dead in the field or from remains found in poaching camps. Samples from the Southern Highlands, collected using similar methods, were donated by the Wildlife Conservation Society–Tanzania Programme. Sample collection and transfer were conducted in accordance with institutional, national and international guidelines. We also employed camera-traps in 7 of the forests, both specifically to detect Abbott's duiker and as part of other research programmes (e.g. Bowkett et al. 2008, Rovero & Marshall 2009, Jones 2014).

Dung pellets were processed, and species identity was established, as described elsewhere (Bowkett et al. 2009, Ntie et al. 2010a). In brief, to verify species identity and sample mitochondrial DNA (mtDNA) variation, we targeted a ~600 bp fragment of the left-hand domain of the mtDNA control region using a combination of various primers and PCR conditions, see Ntie et al. (2010a) for details. To sample nuclear DNA variation we used 7 microsatellite markers in 2 pre-PCR multiplexes: MPLX1 = INRA40 (Beja-Pereira et al. 2004), BM1225, BM2113 and BRRIBO (Bishop et al. 1994), and MPLX2 = BM143 (Bishop et al. 1994), INRA05 (Vaiman et al. 1994) and SR12 (Ntie et al. 2010b modified from Kogi et al. 1995). Primer sequences and PCR conditions are provided in the Supplement at www.int-res.com/articles/suppl/n024p105_supp.pdf. PCR products were processed on a Beckman Coulter capillary sequencer and scored using CEQ 8800 software (Beckman Coulter).

Sequence data were aligned using MUSCLE (Edgar 2004) and checked in SEAVIEW (Gouy et al. 2010). Species identity was established by visual inspection of aligned sequences and confirmed using the BLAST programme (NCBI, Bethesda, MD, USA). We undertook a Bayesian phylogenetic analysis of recovered haplotypes in MrBayes 3.2 (Huelsenbeck & Ronquist 2001). This analysis implemented 2 Metropolis-coupled Markov-Chain-Monte-Carlo (MCMC) searches, each consisting of 1 cold and 3 heated chains. Multiple chains are recommended to avoid false convergence on a local optimum. Model parameters were left at default settings, except that instead of pre-selecting a substitution model we sampled across the entire general time reversible (GTR) model space (Huelsenbeck et al. 2004) by averaging different models according to their posterior proba-

bility (lset nst = mixed rates = gamma) and checking for convergence across different runs with the *sump* command (Ronquist et al. 2011). Convergence of MCMC runs was achieved by allowing the analysis to continue until the standard deviation of split frequencies reached the recommended threshold of 0.01 (between 1 and 1.5 million iterations in our case). The default burn-in fraction of 25% was applied to the convergence diagnostic and the subsequent parameter and tree summaries. Effective sample sizes, log-likelihood values and stationarity plots were checked using the *sump* command.

The tree was rooted for display with 2 sequences for bay duiker *C. dorsalis*, a monophyletic sister group to Abbott's duiker, reflecting published duiker phylogenies (Jansen van Vuuren & Robinson 2001, Ntie et al. 2010a). The Bayesian analysis included all haplotypes recovered from dung and tissue samples in the Udzungwas (Table 1) and in the Southern Highlands, plus all published control region sequences for Abbott's duiker and its sister species, the yellow-backed duiker *C. sylvicultor* (Johnston & Anthony 2012). We conducted an AMOVA to test for partitioning of control region variation between the Udzungwas and Southern Highlands in Arlequin 3.5.1.2 (Excoffier et al. 2005).

For microsatellite loci, we scored each allele at least 4 times from separate PCRs to avoid the problems associated with reproducing consistent profiles from faecal DNA; this multiple-tube approach is generally regarded as standard when working with faecal DNA (Taberlet et al. 1999). We constructed a neighbour-joining tree using DAS shared allele distance (Chakraborty & Jin 1993) in the program POPULATIONS (Langella 1999), including all available genotypes from the Udzungwas and Southern Highlands. We were also able to genotype 5 Northern Highlands samples (Kilimanjaro SUN118 and Usambara SUN115, SUN120 to 122) from the tissue collection at the University of Stellenbosch, South Africa (Jansen van Vuuren & Robinson 2001). Microsatellite data variation was also explored using a principal coordinates analysis (PCA) in GenAlEx 6 (Peakall & Smouse 2006). Standard genetic diversity values for both data sets, and deviations from Hardy-Weinberg and linkage equilibria in the microsatellite data, were tested for using Arlequin and in GENEPOP 4.0.10 (Raymond & Rousset 1995) with False Discovery Rate correction (Verhoeven et al. 2005). Null alleles were tested for with the program MICROCHECKER (Van Oosterhout et al. 2004).

Comparative data for sympatric populations of Harvey's duiker *C. harveyi*, blue duiker *Philotomba monticola* and suni *Neotragus moschatus* were extracted from Bowkett (2012). To control for differences in sample size, we used rarefaction for microsatellite allelic richness in the program HP-Rare (Kalinowski 2005) and for control region haplotypic richness in CONTRIB (Petit et al. 1998).

RESULTS

A total of 73 antelope dung samples from 8 different forests were confirmed as Abbott's duiker through our alignment of mtDNA sequences (Table 1). Many samples identified as Abbott's duiker in the field were in fact Harvey's duiker or bushbuck *Tragelaphus scriptus*, and so were excluded from this study (Bowkett et al. 2009, 2013). In addition, we obtained camera-trap records from 6 forests in the Udzungwa Mountains (Fig. 3), including Ukami for which we were unable to collect dung samples.

For the majority of samples we were unable to replicate microsatellite genotypes according to our multiple-tubes criteria, probably due to poor quality or insufficient nuclear DNA. Therefore, only 19 microsatellite genotypes from Udzungwa samples were included in our analysis. Two samples had missing values at 1 locus. Identical genotypes were excluded to avoid including multiple samples from the same individual (1 case in the Udzungwas, 1 in the Southern Highlands). Data for locus BM2113 failed to meet Hardy-Weinberg expectations ($p = 0.016$), although this was not significant after correction for false discovery rate, and also showed evidence of potential null alleles ($p < 0.025$). There was no evidence of significant linkage disequilibrium within the data set. Loci BM1225 and SR12 were monomorphic within the Udzungwas, although not in other regions.

While we included 14 control region haplotypes in our phylogenetic analy-

sis, only 6 were recovered from the Udzungwas, and 57 of the 73 samples shared 1 particular haplotype (Table 2). All Udzungwa haplotypes were unique to the region (Table 2). As anticipated, there was strong bootstrap support for the monophyly of Abbott's duiker with respect to its sister species (Fig. 4). Both the microsatellite neighbour-joining tree and the



Fig. 3. *Cephalophus spadix*. Abbott's duiker photographed in Mwanihana, Udzungwa Mountains National Park, Tanzania

Table 2. *Cephalophus spadix*. Frequency of Abbott's duiker mtDNA control region haplotypes recovered from the Udzungwa Mountains, together with available data from other regions. MA: Matundu; LU: Luhomero-Ndundulu; MW: Mwanihana; UZ: Udzungwa Scarp; NY: Nyumbanitu; ND: New Dabaga-Ulang'ambi; I: Iwonde; KR: Kising'a-Rugaro

Haplotype	Udzungwa	Southern Highlands	Northern Highlands	Udzungwa forests
AM903084	0	0	2 ^a	
SA18	57	0	0	MA, LU, MW, NY, ND, I
AB004	7	0	0	MA, UZ, MW, ND
AB035	3	0	0	MA, MW
AM903086	0	6	0	
WCS07	0	2	0	
WCSB465	0	2	0	
AM903085	0	2	0	
TJ005	1	0	0	KR
TJ050	4	0	0	LU, MW, I
AM080	1	0	0	LU
AM903083	0	0	2	
FJ823349	0	0	2	
FJ823348	0	0	1	

^aAlso Ilole forest, Rubeho Mountains (1×)

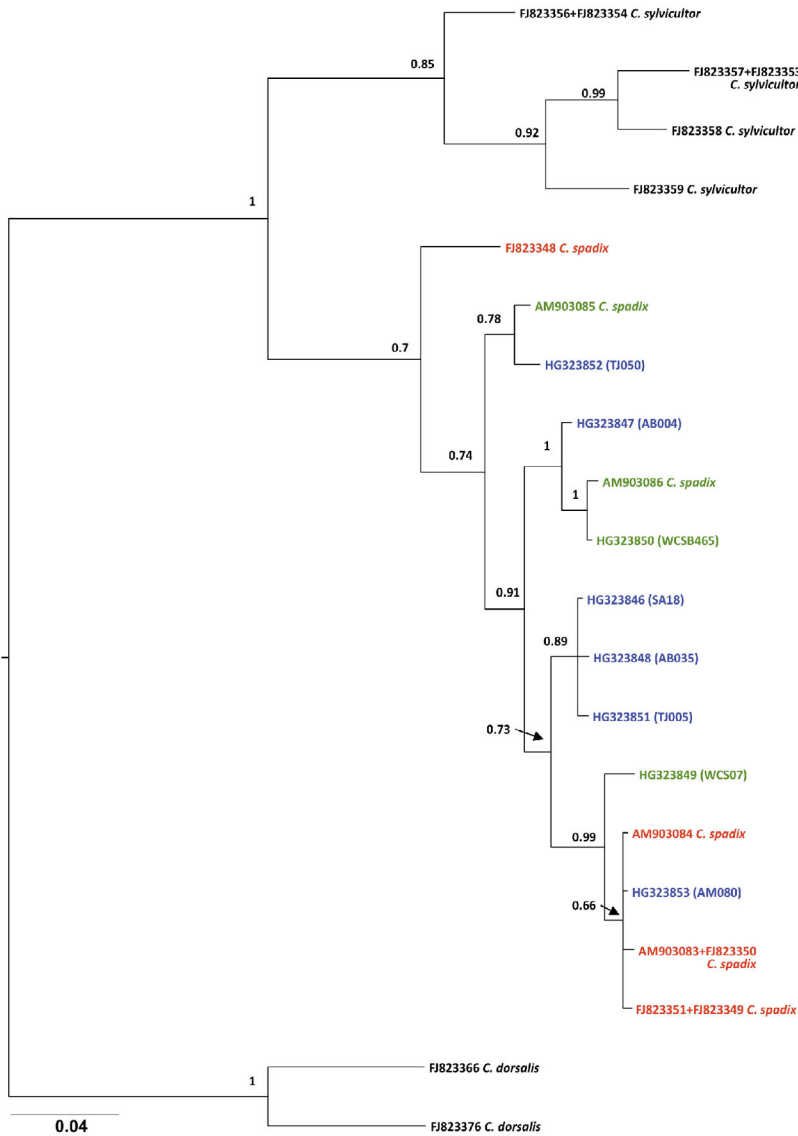


Fig. 4. *Cephalophus spadix*. MtDNA control region (~600 bp) Bayesian consensus phylogeny for Abbott's duiker from the Udzungwa Mountains. Node values are posterior probability (values <0.5 not shown, results shown as polytomy). Haplotypes from the Udzungwas are labelled in blue; Southern Highlands, in green; and Northern Highlands, in red. Sample codes for sequences derived from this study are given in parentheses

PCA plot suggested regional differentiation with a distinct Southern Highlands clade (Figs. 5 & 6). Control region variation was heavily partitioned between regions (AMOVA: among-region variation = 56.4%, within-region variation = 43.6%, global $F_{ST} = 0.56$, $p < 0.001$).

Overall genetic diversity values were low for Abbott's duiker, with all measures at least twice as high in the sympatric Harvey's duiker and other species (Tables 3 & 4).

DISCUSSION

As an endangered species found only in Tanzania, our results for Abbott's duiker in the Udzungwa Mountains have significant conservation importance. These surveys have confirmed the presence of Abbott's duiker in 9 forests in the Udzungwa Mountains, including 3 areas lacking molecular or camera-trap records prior to this study (Jones & Bowkett 2012). While the species had previously been reported from New Dabaga-Ulang'ambi (Dinesen et al. 2001), we provide the first records from Iwonde, a small forest patch within the National Park, and from Kising'a-Rugaro, a much larger outlying forest that has been heavily hunted and logged (Marshall et al. 2010, Jones 2014)

We found little evidence for geographic structuring of mitochondrial lineages within Abbott's duiker. Almost all the recovered control region haplotypes were unique to individual regions (Table 2), but several were too similar to be resolved by our phylogenetic analysis, and many clades with strong bootstrap support contained sequences from >1 region (Fig. 4). One possible explanation for this is incomplete lineage sorting, whereby haplotypes may have undergone small sequence changes, but there has not been sufficient time for groups of related haplotypes to become fixed in particular areas (Maddison & Knowles 2006).

In contrast, our microsatellite analysis appears to differentiate genotypes from the 3 sampled regions (Figs. 5 & 6). This result may reflect the more rapid evolution of microsatellite markers compared to mtDNA, but caution should be taken in interpreting this preliminary analysis, given the restricted sample sizes and marker limitations (see 'Results'). Furthermore, bootstrap support was generally low for the main clades (Fig. 5), although this may reflect the limited information available for inferring evolutionary relationships from a relatively small number of microsatellite loci.

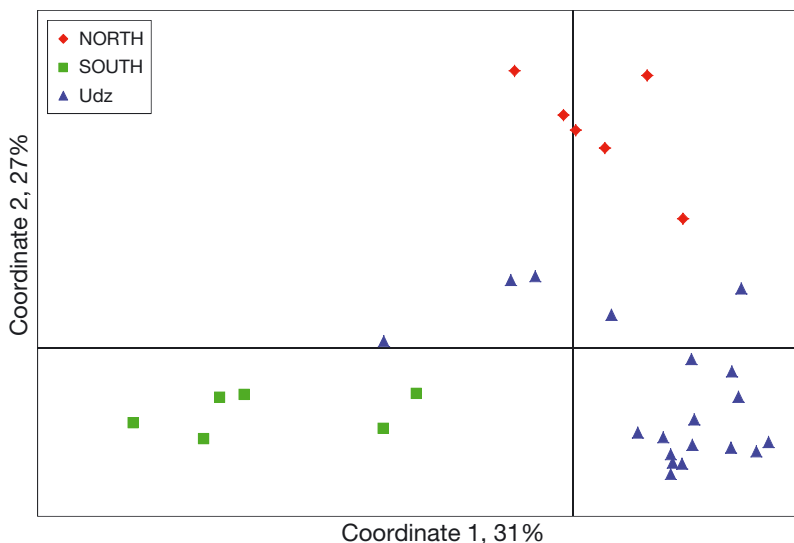
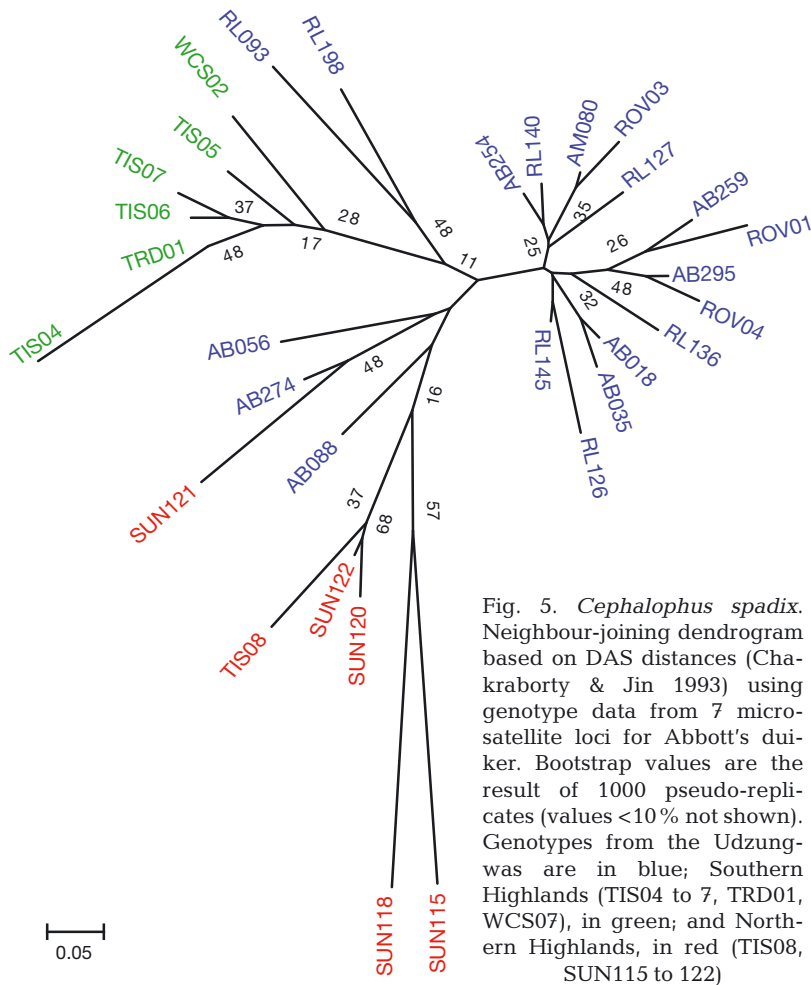


Fig. 6. *Cephalophus spadix*. Two-dimensional principal components plot of Abbott's duiker microsatellite genotypes labelled by geographical regions in Tanzania. NORTH: Northern Highlands (Usambara and Kilimanjaro); SOUTH: Southern Highlands; Udz: Udzungwa Mountains

Overall, genetic diversity values were very low for Abbott's duiker compared to Harvey's duiker in the Udzungwa Mountains (Tables 3 & 4) or published values for other mammal species (see Garner et al. 2005 and Appendix 1 of Gebremedhin et al. 2009). In addition, 2 of the microsatellite loci examined in this study appear to have undergone fixation within the Udzungwa Mountains, although they were polymorphic in other regions. This low diversity and allelic fixation is highly indicative of small population sizes and long-term isolation from populations in other regions (as indicated by AMOVA; $F_{ST} = 0.56$). In the past, lowland forests may have connected highland regions, allowing gene flow to occur, particularly during Pleistocene inter-glacial periods (deMenocal 2004). However, contemporary connectivity with other regions is presumably low or absent due to the arid habitats found between mountain ranges.

Low genetic diversity may also be the result of more recent population declines due to habitat loss and particularly illegal hunting. Antelope hunting occurs throughout the Udzungwas, including within the National Park, but is far more prevalent in the outlying forest reserves (Rovero et al. 2010, Jones 2014), including Kising'a-Rugaro and Uzungwa-Scarp where our surveys suggest Abbott's duiker is much less abundant (Table 1). Therefore, enforcement of laws prohibiting hunting in protected areas may help to prevent further loss of genetic diversity.

There is some evidence that Abbott's duiker populations may be able to recover in areas where conservation action has been implemented. Joint Forest Management (JFM), devolving management rights to elected Village Natural Resource Councils, has established patrols in several forest reserves in the Udzungwas. Nielsen & Treue (2012) report decreased hunting activity and a significant increase in relative antelope density 7 yr after implementation of JFM in New Dabaga-Ulang'ambi. These effects were associated with the apparent re-estab-

Table 3. *Cephalophus spadix*, *C. harveyi*. Number of alleles (N_a), observed (H_o) and unbiased expected (uH_E) heterozygosities for 7 microsatellite loci in Abbott's duiker ($n = 19$) and Harvey's duiker ($n = 149$) in the Udzungwa Mountains

Locus	N_a		H_o		uH_E	
	<i>C. spadix</i>	<i>C. harveyi</i>	<i>C. spadix</i>	<i>C. harveyi</i>	<i>C. spadix</i>	<i>C. harveyi</i>
BM2113	5	8	0.47	0.71	0.72	0.78
INRA40	4	12	0.37	0.83	0.47	0.86
BM1225	1	3	–	0.27	–	0.30
BRRIBO	4	13	0.58	0.75	0.63	0.87
BM143	3	13	0.32	0.69	0.28	0.78
INRA05	2	4	0.06	0.27	0.06	0.36
SR12	1	10	–	0.61	–	0.64
Mean	2.86	9	0.26	0.59	0.31	0.66
(rarefied ^a)	(2.81)	(6.79)				

^aMean number of alleles (allelic richness) rarefied to the smallest number of complete genotypes (17)

ishment of a population of Abbott's duiker that was not recorded in the baseline survey before implementation of JFM. Similarly, Abbott's duiker appears to have partly recovered from overhunting in the forests of Mount Rungwe in the Southern Highlands following a combination of education, community development initiatives and law enforcement (T. R. B. Davenport, unpubl. data).

On the other hand, results from Kilombero Nature Reserve in the Udzungwas, where a ranger station staffed by Tanzania National Parks was constructed in addition to the implementation of JFM, indicate that the increased patrolling effort displaced hunters from savanna woodland areas into Ndundulu and Nyumbanitu forests, thereby increasing the threat to Abbott's duiker (Nielsen 2011). These results indicate that enforcement of hunting bans can facilitate recovery of populations of Abbott's duiker and other species. However, it can also lead to unforeseen consequences, such as increased hunting in adjacent areas, if not associated with improvements in local livelihoods and other benefits as intended by policies

such as JFM (Nielsen & Treue 2012, Nielsen & Meilby 2013).

While our distribution results provide renewed hope for the survival of Abbott's duiker, it is clear that the species remains threatened and potentially vulnerable to the negative impacts of small population size. We strongly recommend further survey work and non-invasive genetic sampling of Abbott's duiker, not only in the Udzungwas, but also throughout the species' historical range. Specifically, surveys should target sites such as Kilimanjaro and Usambara, where the current distribution and abundance of Abbott's duiker remains unknown. Genetic samples from these

sites would provide sequence data for a more comprehensive assessment of evolutionary relationships between extant regional populations. Additional microsatellite genotypes from all regions would provide a more robust analysis of population structure, connectivity and genetic diversity. Such information is likely to prove invaluable for identifying and prioritizing conservation management units. In the meantime, prevention of further habitat loss and poaching is essential for the survival of the small isolated populations reported in this study and for the long-term viability of the species as a whole.

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Table 4. Mitochondrial control region diversity in 4 species of forest antelope in the Udzungwa Mountains, Tanzania

	Suni <i>Neotragus moschatus</i>	Blue duiker <i>Philantomba monticola</i>	Harvey's duiker <i>Cephalophus harveyi</i>	Abbott's duiker <i>Cephalophus spadix</i>
Sequences	157	48	334	73
Haplotypes	28	13	59	6
Haplotypic richness ^a	16.37	12	23.92	4.27
Polymorphic sites	81	61	131	27
Gene diversity	0.922	0.905	0.953	0.381
Nucleotide diversity	0.039	0.026	0.037	0.006

^aHaplotype number rarefied to the smallest sample size (48)

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