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# Molecular identification versus local people's information for accurate estimates of bushmeat utilization from the Serengeti ecosystem, Tanzania

Stella Bitanyi<sup>1,6\*</sup>, Gro Bjørnstad<sup>1,4</sup>, Marit Nesje<sup>2</sup>, Eblate M. Ernest<sup>1,5</sup>, Robinson H. Mdegela<sup>3</sup> and Knut H. Røed<sup>1</sup>

<sup>1</sup>Department of Basic Sciences and Aquatic Medicine, Norwegian School of Veterinary Science, P.O. Box 8146 Dep., N – 0033 Oslo, Norway.

<sup>2</sup>Department of Production Animal Clinical Science, Norwegian School of Veterinary Science, P.O. Box 8146 Dep., N – 0033 Oslo, Norway.

<sup>3</sup>Department of Veterinary Medicine and Public Health, Faculty of Veterinary Medicine, Sokoine University of Agriculture, P.O. Box 3015, Morogoro, Tanzania.

<sup>4</sup>Department of Archaeology, Conservation and History, University of Oslo, P.O. Box 1008 Blindern, N – 0315 Oslo, Norway.

<sup>5</sup>Tanzania Wildlife Research Institute, P.O. Box 661, Arusha, Tanzania.

<sup>6</sup>Ministry of Livestock Development and Fisheries - Veterinary Investigation Centre - Temeke, P.O. Box 9254, Dar-Es-Salaam, Tanzania.

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**Sustainable wildlife management assumes thorough knowledge of the factors of importance for species population dynamics. In this study, we examined the diversity of wildlife species that are illegally harvested in the Serengeti ecosystem, Tanzania. A total of 124 bushmeat samples were obtained from hunters, middlemen and consumers in 79 sub-villages adjacent to the protected areas in western Serengeti. The species identity was verified in 118 bushmeat samples through molecular sequencing of the cytochrome oxidase subunit I (COI) and phylogenetic assignments to established reference sequences of the respective species. The species diversity among the bushmeat samples was high with 15 identified species altogether. Wildebeest (*Connochaetes taurinus*) was clearly the most common species (n = 52), but also buffalo (*Syncerus caffer*, n = 15), eland (*Tragelaphus oryx*, n = 11), zebra (*Equus burchelli*, n = 10), topi (*Damaliscus lunatus*, n = 8) and impala (*Aepyceros melampus*, n = 7) were relatively frequently identified. The correctness of the species identification given by the bushmeat providers was relatively low (59%) with error rates higher in consumers than in hunters and middlemen. This high error rate suggests that care should be taken in relying on local peoples' information for accurate estimates of biodiversity of bushmeat utilization.**

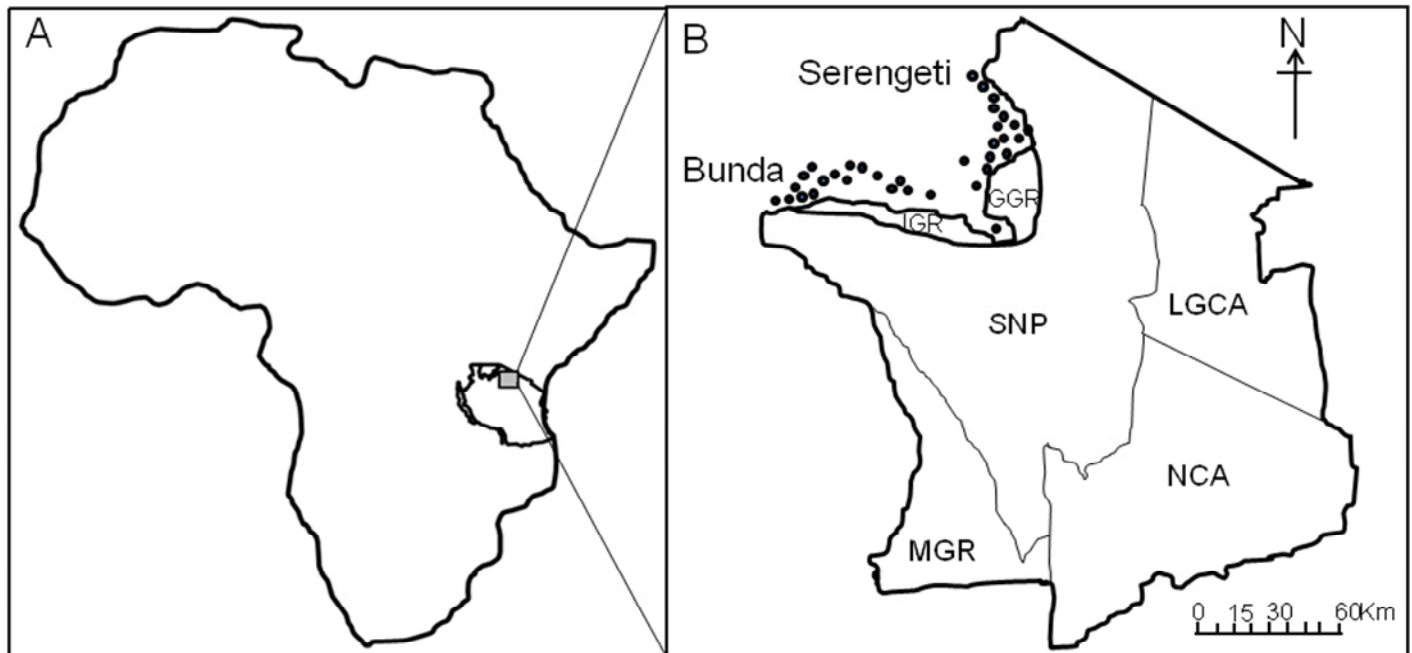
**Key words:** Bushmeat, illegal hunting, species identification, cytochrome oxidase subunit I (COI) sequencing, western Serengeti.

## INTRODUCTION

African wildlife has been increasingly restricted to protected areas that need additional conservation efforts

(Hilborn et al., 2006; Chantal et al., 2007). However, such efforts have been threatened by unsustainable exploitation of wildlife species (Campbell et al., 2001). Increased human populations, expansion of agricultural areas, illegal hunting and excessive trophy hunting have been identified as major threats to sustainable conservation (Bohne, 2008). As human populations

\*Corresponding author. E-mail: [nstelbi@yahoo.co.uk](mailto:nstelbi@yahoo.co.uk). Fax: +4722466072.



**Figure 1.** Map of Africa showing the location of Tanzania and Serengeti Ecosystem (A), with its protected areas Serengeti National Park (SNP), Ngorongoro Conservation Area (NCA), Grumeti Game Reserve (GGR), Ikorongo Game reserve (IGR), Maswa Game Reserve (MGR), Loliondo Game Controlled Area (LGCA). (B) Dots represent the locations of the villages in Bunda and Serengeti districts where bushmeat sampling was done.

continue to grow, pressures on wild species and natural ecosystems are becoming increasingly severe; with increasing rural establishment particularly around the protected areas (Madulu, 2001), resulting to increase in illegal hunting and use of bushmeat (Milner-Gulland et al., 2003). The increases in illegal harvests have resulted to biodiversity loss and it is becoming a major management challenge for conservation authorities in Africa (Robinson et al., 1999; Redmond et al., 2006). In formulating conservation strategies, it is important to know the composition of harvested species so that accurate management plans can be completed.

In Tanzania, wildlife is more restricted to protected areas. About a third of the country's total area is protected as National Parks, Game Reserves, Forest Reserves and Marine Parks (Thirgood et al., 2004). The Serengeti ecosystem (Figure 1), well known for its abundance and diversity of wild, large mammals (Sinclair et al., 2008), is located in North-western Tanzania and extends to South-western Kenya. The ecosystem covers an area of about 25,000 km<sup>2</sup> and includes Serengeti National Park (SNP), Ngorongoro Conservation Area (NCA), Grumeti Game Reserve (GGR), Ikorongo Game Reserve (IGR), Maswa Game Reserve (MGR) and Loliondo Game Controlled Area (LGCA) (Figure 1). SNP covers the largest area (14,763 km<sup>2</sup>) and is highly protected with human activities limited to photographic tourism. By comparison, trophy hunting is allowed in the game reserves adjoining the Serengeti National Park,

based on a quota system set out annually in hunting blocks (Baldus and Cauldwell, 2004). Local communities in Serengeti are not allowed to hunt and there is no open market for wild meat (Ndibalema and Songorwa, 2008). However, the close proximity of the communities to wildlife protected areas makes illegal hunting relatively easy and a common practice. The estimates of illegal harvests of wild animals in the ecosystem have been reported to range from 40,000 to 200,000 animals per year, with the vast majority being wildebeests (Hofer et al., 1996; Mduma et al., 1998). Studies by Campbell and Borner (1995) and Hofer et al. (1996) showed that illegal hunting practices in the area affected populations of buffalo (*Syncerus caffer*), giraffe (*Giraffa camelopardis*), impala (*Aepyceros melampus*) and topi (*Damaliscus lunatus*).

In the western corridor of the Serengeti National Park, illegal hunting has been highest around densely populated areas (Holmern et al., 2002; Loibooki et al., 2002). The human population in the area is estimated to be over two million (United Republic of Tanzania, 2002), composing more than 25 diverse tribes, dominated by Ikoma, Ikizu, Kuria, Natta and Sukuma (Evjen, 1998). Local communities surrounding western Serengeti have been relying on bushmeat hunting for food security and income generation (Loibooki et al., 2002; Kaltenborn et al., 2005). Trade of illegally acquired bushmeat is a major source of income for many traders. Barnett (2000) and Campbell et al. (2001) showed that 75% of hunters

arrested during anti-poaching patrols admitted that they were hunting for cash. The commercialization of bushmeat trade combined with market forces and internal pressures such as poverty makes the conservation and management efforts of these resources a great challenge.

Species identification of bushmeat in western Serengeti has traditionally been based on information given by local people. Relying on people's identification alone might not give the precise information needed as the bushmeat trade is sometimes complex and poorly understood (Bowen-Jones et al., 2002). The true species identity of bushmeat samples might be subjected to errors that arise in the chain of trade as the meat passes through many middle traders before reaching the consumers. The reliability of the information given by the purchaser may also be affected by the desire to meet the meat preference of the consumers (Nyahongo et al., 2007; Ndibalema and Songorwa, 2008).

The use of DNA diagnostic techniques has proven to be effective as a species identification tool, overcoming the problems of traditional morphology based identification methods (Wong and Hanner, 2008). Mitochondrial DNA (mtDNA) is frequently used for species identification and possesses advantages over nuclear DNA. The presence of enough point mutation in mtDNA allows discrimination of closely related species, and as mtDNA is maternally inherited, sequences ambiguities from heterozygous genotypes are avoided (Unsel'd et al., 1995; Gupta et al., 2005). Particularly, cytochrome c oxidase subunit I (COI) mitochondrial gene have been used as standard DNA barcode tool for species identification. Hebert et al. (2003) revealed a deep genetic divergence among 13,000 closely related species groups from different animal phyla using COI, thus enabling reliable species identification. COI has also been used to identify bushmeat samples to species level (Eaton et al., 2010). In a recent study, we reported the ability of using COI to identify antelope and bovid species from Tanzania using sequences down to 100 base pairs (bp) (Bitanyi et al., 2011). The study involved analyses of most potential species traded as bushmeat in the area.

In this study, the diversity of wildlife species that are illegally hunted and consumed in western Serengeti was examined through molecular sequencing of COI of bushmeat samples. To test for reliability of the species information given by the bushmeat providers, the molecular identification was compared with the information given by consumers, middlemen and hunters.

## MATERIALS AND METHODS

### Sample collection

A total of 124 bushmeat samples were purchased from villages adjacent to the Serengeti National Park, on the western side, in Serengeti and Bunda districts (Figure 1). The villages were also bordering Grumeti and Ikorongo Game Reserves, where hunting is

not allowed both inside and outside protected areas (in the villages), therefore any bushmeat found in these villages are from illegal hunting. In Tanzania, a village is a registered legal and political unit that can cover an area of 30 to 45 km across, with population size ranging from 1000 to 4000 individuals. Villages are composed of several administrative areas known as sub-villages. In this study, a total of 79 sub-villages within 34 villages were sampled. An average of 1 to 2 samples was obtained from each sub-village. To reduce the possibility of purchasing multiple samples from same specimen, samples were purchased from different sub-villages and no more than two samples of the same locally identified species were bought from each sub-village. There was no species preference spoken when purchasing the samples.

The sampling sessions were distributed over three years (2007 to 2009), from mid September to December. This is the period when the wildebeest migrations have moved far north outside western Serengeti to Maasai Mara Reserve (Rusch et al., 2005). The seasonal sampling was aimed to enable a fair inclusion of both resident and migratory species. The total number of samples collected and their origin are shown in Supplementary Table 1 in the Appendix. The samples consisted of fresh and processed (dried and smoked) samples that were provisionally identified by hunters, middlemen and consumers. In bushmeat trade, middlemen include both men and women who buy from the hunters and resell to the public (consumers) either as smoked or fresh meat (Akumsi, 2003). A species identity was given for all samples except for seven samples which were given as 'swala'; a common Swahili name for small to medium sized antelopes. Samples were stored in 95% ethanol at room temperature.

For species identification, COI sequences obtained from antelopes and bovids from the Serengeti ecosystem (Bitanyi et al., 2011) were used. However, these reference sequences did not cover all given species of the provided bushmeat samples. Therefore an additional blood sample was obtained from darting a free ranging zebra (*Equus burchelli*) by a qualified veterinarian involved in the project. The sample was collected in EDTA vacutainer tube and stored at -21°C. In addition, COI sequences of giraffe, elephant (*Loxodonta africana*), hippopotamus (*Hippopotamus amphibius*) and bushpig (*Potamochoerus spp*) were obtained from NCBI GenBank to serve as reference sequences for these species.

### DNA extraction, amplification and sequencing

DNA extraction and PCR amplification was performed as described by Bitanyi et al. (2011). In short, DNA was extracted using DNeasy® Blood and Tissue Kit (QIAGEN) and a 650 base-pair fragment was amplified using the primers COIbF (5' -TTTCAACCAACCACA AAGACATCGG - 3') and COIbR (5' -TATACTTCAGGGTGT CCAAAGAATCA - 3') (Bitanyi et al., 2011). PCR reactions were carried out in 25 µl final reaction volumes containing 10× reaction buffer with 2.5 mM of MgCl<sub>2</sub>, 0.2 mM each dNTP, 10 mM each primer, 1.5 units of Hot star Taq polymerase (QIAGEN) and about 1µl of template DNA. The PCR parameters consisted of 15 min at 95°C for initial polymerase activation, followed by 35 cycles of 30 s at 94°C, 30 s at 55°C and 1 min at 72°C and finished with 10 min at 72°C. PCR products were purified using ExoSap-IT® (GE Healthcare, USA) and sequenced on ABI 3100 automated sequencer (Applied Biosystems) using the Big Dye® terminator chemistry version V1.1, following the manufacturer's protocol.

Among the purchased samples, wildebeest was most numerous. To test for possible repeated sampling of the same specimen, we analysed 37 wildebeest samples obtained in 2009 for polymorphism in 16 microsatellite loci (CT 02, CT 03, CT 07, CT 08, CT 10, CT 12, CT 13, CT 14, CT 17, CT 18, CT 19, CT 21, CT 23, CT 25, CT 27, CT 30) as described by Røed et al. (2011).

**Table 1.** Number (N) and percentage (%) of identified species among bushmeat samples obtained from Serengeti and Bunda districts of western Serengeti including all obtained samples (n = 118) versus the first obtained sample from each sub-village (n = 74).

Species identified		Number and % of identified spp from all samples		Number and % of identified spp. using one sample from each sub village	
Common name	Scientific name	N	%	N	%
Wildebeest	<i>Connochaetes taurinus</i>	52	44.1	25	33.7
Buffalo	<i>Syncerus caffer</i>	15	12.7	14	18.9
Eland	<i>Tragelaphus oryx</i>	11	9.3	9	12.1
Zebra	<i>Equus burchelli</i>	10	8.4	7	9.4
Topi	<i>Damaliscus lunatus</i>	8	6.7	5	6.7
Impala	<i>Aepyceros melampus</i>	7	5.9	4	5.4
Warthog	<i>Phacochoerus aethiopicus</i>	4	3.3	2	2.7
Hartebeest	<i>Alcelaphus buselaphus</i>	3	2.5	1	1.4
Reedbuck	<i>Redunca redunca</i>	2	1.7	1	1.4
Giraffe	<i>Giraffa camelopardalis</i>	1	0.8	1	1.4
Hippopotamus	<i>Hippopotamus amphibius</i>	1	0.8	1	1.4
Bushpig	<i>Potamochoerus spp</i>	1	0.8	1	1.4
Elephant	<i>Loxodonta africana</i>	1	0.8	1	1.4
Thomson gazelle	<i>Eudorcas thomsonii</i>	1	0.8	1	1.4
Bushbuck	<i>Tragelaphus sylvaticus</i>	1	0.8	1	1.4

### Data analyses

Six samples did not amplify and therefore were not included in the analyses. Bidirectional contig assembly was carried out using SEQSCAPE (version 2.1.1; Applied Biosystems). Sequences were edited by eye using Proseq (version 2.91; Filatov, 2002) and all polymorphic bases were checked using the original chromatograms in MEGA (version 4.0.2, Tamura et al., 2007).

Phylogenetic methods were used to identify the species of the bushmeat samples. MEGA was used to calculate nucleotide sequence divergence and to construct neighbour-joining (NJ) phylogenetic trees incorporating the Kimura 2 parameter model (Kimura, 1980). Bootstrap values for the internal topology were estimated using 1000 replicates. The NJ tree enabled the identification of bushmeat samples based on their location among the reference sequences by Bitanyi et al. (2011), the obtained sequence of zebra and the downloaded NCBI GenBank sequences. Bootstrap support greater than or equal to 70% was used as a set up limit for species clusters identification.

Simpson's diversity index (Simpson, 1949) was used to describe the species diversity of the bushmeat samples. The diversity index values vary from 0 to 1, where the values near zero correspond to highly diverse or heterogeneous samples, while values near 1 correspond to a more homogenous data set. The identity test for repeated sampling of the same individual based on the microsatellite genotypes was performed in the computer program CERVUS (Kalinowski et al., 2007) with criteria for non-identity of at least two mismatched loci.

### RESULTS

Readable sequences were obtained from 118 bushmeat samples, with sequence length from 100 to 470 bp. The different sequence lengths showed strong bootstrap support with their respective species clusters comprised

of the reference sequences, and species identity was verified for all bushmeat samples. The seven samples provided as 'swala' were identified as two reedbuck (*Redunca redunca*), three wildebeest, one impala and one Thomson gazelle (*Eudorcas thomsonii*). Intra-species nucleotide divergence ranged from 0 to 1.1%, which gave additional support for correct species assignment since intraspecies COI nucleotide divergence rarely exceeds 2% (Avice, 2000).

The species diversity among the bushmeat samples are shown in Table 1. A total of 15 species were identified. Wildebeest constituted the highest number (44.1%) of the identified samples. In addition, buffalo (12.7%), eland (9.3%), zebra (8.4%), topi (6.7%) and impala (5.9%) were relatively frequently identified among the bushmeat samples. Giraffe, hippopotamus, bushpig, Thomson gazelle, bushbuck and elephant had only one verified sample each. Analyses involving one sample from each sub-village had similar species composition as when all samples were used (Table 1). The Simpson's species diversity index (I) was somewhat lower when one sample from each sub-village was used (I = 0.172) compared to the whole sample set analyses (I = 0.230). The panel of microsatellite markers gave high confidence in identity testing, revealing that none of the wildebeest samples obtained in 2009 were from the same specimens, illustrating that the degree of repeated sampling in this present study is overall low.

Comparing the species identity of bushmeat samples determined by molecular sequencing with that given by the sample providers revealed a 59.3% overall agreement

**Table 2.** Accuracy of species identification given by providers of bushmeat samples obtained from Serengeti and Bunda districts of western Serengeti.

Species	Number of samples	Number of samples with correct identity	% correct sample identity
Wildebeest	41	29	70.7
Topi	19	6	31.6
Eland	13	8	61.5
Zebra	13	9	69.2
Buffalo	12	8	66.7
Giraffe	3	1	33.3
Warthog	3	3	100
Hippopotamus	1	1	100
Bushpig	1	1	100
Hartebeest	1	1	100
Elephant	1	1	100
Impala	1	1	100
Bushbuck	1	1	100
Thomson gazelle	1	0	0

**Table 3.** Percentages of correct species identity of bushmeat samples provided by hunters, middlemen and villagers in Serengeti and Bunda districts of western Serengeti

Sample provider	Number of sample provided	Number of correctly identified sample
Hunters	34	25 (73.5%)
Middlemen	28	20 (71.4%)
Villagers	56	25 (44.6%)

(Supplementary Table 2; Appendix). A total of 46 samples had a different species identity than that provided when purchasing the samples. The accuracy in information for the different species is summarized in Table 2. Wilde-beest, buffalo, zebra and eland had relatively similar percentages of identity accuracy (60 to 70%), while topi had the lowest identification accuracy of 31.6%. Among the 19 samples purchased as topi, only six samples were verified by the molecular analyses.

The proportions of correct species information given by hunters, middlemen and consumers varied significantly ( $\chi^2 = 9.40$ ,  $df = 2$ ,  $p < 0.01$ ). As given in Table 3, middlemen and hunters had relatively similar and higher percentage of correct identity as compared to the identification given by consumers.

## DISCUSSION

The diversity of species found to be locally consumed in the western Serengeti ecosystem includes a wide range of antelope species, elephants, giraffes, zebras, bushpig and warthogs. As part of analysing the genetic structure of wildebeest in the Serengeti ecosystem, microsatellite analyses of the 37 wildebeest samples obtained in 2009

suggested overall low degree of repeated samples in the present material. The somewhat lower species diversity observed when using the whole dataset as compared to including only one sample in each sub-village might be due to some bias in repeated sampling of some specimens. The observed species biodiversity index of  $H' = 0.172$  when only one sample from each sub-village was used could thus be closer to a true value of the biodiversity of the bushmeat utilization in western Serengeti. Generally, the low diversity index values observed in both scenarios illustrates the high species diversity of the illegal hunt in the area. Furthermore, six bushmeat samples did not amplify in this study, possibly due to variation in the primer regions, and these samples could represent additional species. The use of an index for estimating the biodiversity of the illegally harvested wildlife could be of value in conducting comparative studies from different communities or ecosystems.

Hunting for meat has remained a major challenge to the existence of various herbivore species in the Serengeti ecosystem. The patterns of bushmeat consumption and preferences are usually based on availability of species, taste of meat and motives for hunting (Barnett, 2000; Kaltenborn et al., 2006; Ndibalema and Songorwa, 2008; Mfunda and Røskaft, 2010). In this

study, wildebeest was clearly the most common species identified. Campbell and Hofer (1995) have previously suggested this species to be the most common illegally hunted wildlife species in and around Serengeti. It has been observed that the deeply rooted culture of hunting in Kuria and Ikoma tribes of western Serengeti has been depending much on the annual migration of the wildebeest (Kaltenborn et al., 2005). The wildebeest is the most common large herbivore in the Serengeti ecosystem, with an estimated population size of 1,300,000 animals (Sinclair et al., 2008). The species is well known for its large herds migrating throughout the ecosystem in an annual and cyclic pattern (Sinclair and Arcese, 1995; Thirgood et al., 2004), although, the species is also found in most of Serengeti year around. It has been indicated that the more resident wildebeest, particularly those within western Serengeti, may represent a separate population (Thirgood et al., 2004). Most of the wildebeest obtained and identified within this study are probably from such resident animals since most sampling were done several weeks after the large wildebeest herds had left the western Serengeti area on their migration towards north. However, during the migration, wildebeest may roam through villages in the region and animals may easily be caught and slaughtered for consumption (Kaltenborn et al., 2005). It can therefore not be excluded that some of the processed samples were from the migratory herds.

The buffalo was the second most common species among the bushmeat samples. Recently, buffalo populations in SNP have been reported to be 32,000, which is the highest number observed in the past 14 years (TAWIRI, 2010). Despite this population increase, there is a need to continue instituting strictly conservation measures as the trend and estimates of illegal harvests observed in this study might result to the future declining of buffalo populations. Based on the estimate of 40,000 to 200,000 animals illegally harvested per year in the Serengeti ecosystem (Hofer et al., 1996; Mduma et al., 1998), our finding that almost 20% of the bushmeat samples were buffalo, gives an annual estimate of some 8,000 to 40,000 buffaloes illegally harvested in the Serengeti ecosystem. Such high estimated number from illegal hunting when coupled with harvests through the regulated trophy hunting may well be of a threat to the population. Illegal hunting has previously been pointed out to be the principal factor explaining the significant reduction of the buffalo population in the Serengeti ecosystem (Dublin et al., 1990). However, other factors such as anthropogenic, climatic driven habitat loss and diseases (Metzger et al., 2010; Kideghesho, 2010) may act as well as threats to the buffalo population. The interaction between the illegal hunting and the accelerating human settlements in the areas surrounding the national parks (Madulu, 2001) may be a particular challenge for future sustainable conservation of the species.

This study has revealed relatively low accuracy in the species information given by the local people providing the bushmeat samples. The low identification capacity reflects probably the general difficulties in organoleptic identification of bushmeat samples. Most of the present bushmeat was processed as dried meat which made the species identification extremely difficult. The difficulties in identification of processed meat has been reported in a study by Nyahongo et al. (2007) where most people failed to identify the correct bushmeat species based on testing of pre-cooked meat. The consumer's preferences for meat from certain wildlife species might influence hunters and traders to provide false species identity in order to fulfil market requirements (Adeyoju, 2010). This may partly explain the higher species identification accuracy among hunters and middlemen as compared to the consumers. The high error rate among the 19 samples provided as topi (70%) may also be influenced by the consumer's preference and demand for this species. Topi meat has been known to be preferred among the tribes in the area due to its taste (Ndibalema and Songorwa, 2008). High demand among the consumers for topi may increase the erroneous species specifications given by the providers.

This study has shown that relying on people's knowledge in identification of bushmeat harvested from protected areas might not give the accurate picture of levels of species exploitation. If such information is to be obtained, more reliable information is provided by hunters and middlemen than by consumers. Furthermore, wildlife managers must ensure that accurate population data of the most harvested species is obtained to detect changes in population that occur over time. This would help in identifying species that appear to experience sharp declines and thus taking necessary measures for their conservation.

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## APPENDIX

**Supplementary Table 1.** Origin and number of bushmeat samples used in this study.

District/ Location	Village of origin	Number of sub-villages sampled	Number of samples
Serengeti district	Bonchugu	6	24
	Nyamburi	5	11
	Robanda	3	5
	Mbilikiri	3	4
	Bwitengi	2	4
	Issenye	2	4
	Bisarara	3	4
	Iharara	2	3
	Mbalibali	2	3
	Singisi	3	3
	Natta	3	3
	Kisangura	2	2
	Nyichoka	2	2
	Merenga	1	1
	Nyiberekera	1	1
	Machochwe	1	1
	Nyamakendo	1	1
	Kibeyo	1	1
Koreri	1	1	
Park Nyigoti	1	1	
Rwamchanga	1	1	
Bunda district	Mihale	5	10
	Mariwanda	4	6
	Hunyari	3	6
	Nyamatoke	4	5
	Kyandege	3	3
	Kihumbu	3	3
	Mugeta	2	2
	Sarawe	2	2
	Sanzate	2	2
	Bukore	2	2
	Nyangere B	1	1
Kunzugu	1	1	
Changuge	1	1	

**Supplementary Table 2.** Species identification of bushmeat samples after COI analyses compared to field identification by local people.

<b>Correctly identified samples</b>		
<b>Local sample identification</b>	<b>COI sample identification</b>	<b>Total number of samples</b>
Wildebeest	Wildebeest	29
Buffalo	Buffalo	8
Eland	Eland	8
Topi	Topi	6
Zebra	Zebra	9
Giraffe	Giraffe	1
Warthog	Warthog	3
Hippopotamus	Hippopotamus	1
Bushpig	Bushpig	1
Hartebeest	Hartebeest	1
Elephant	Elephant	1
Impala	Impala	1
Bushbuck	Bushbuck	1
<b>Incorrectly identified sample</b>		
<b>Local sample identification</b>	<b>COI sample identification</b>	<b>Total number of samples</b>
Wildebeest	Topi	2
Wildebeest	Buffalo	5
Wildebeest	Warthog	1
Wildebeest	Impala	2
Wildebeest	Eland	2
Topi	Buffalo	2
Topi	Impala	1
Topi	Wildebeest	9
Topi	Hartebeest	1
Buffalo	Eland	1
Buffalo	Wildebeest	3
Eland	Impala	1
Eland	Zebra	1
Eland	Wildebeest	3
Zebra	Hartebeest	1
Zebra	Wildebeest	3
Giraffe	Wildebeest	2
Thomson gazelle	Impala	1
	Reedbuck	2
Antelopes species ('swala')	Impala	1
	Thomson gazelle	1
	Wildebeest	3