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Wildlife Forensics

Assessing the utility of DNA barcoding in wildlife forensic cases involving South African antelope

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ABSTRACT

Poaching of South African wildlife is considered a threat to biodiversity. In the absence of diagnostic morphometric traits, DNA barcoding is considered as a method of choice for species identification. Here, we report on forensic case work involving the illegal hunting of antelope species. Three forensic cases which included confiscated material were submitted between 2018 and 2019 and for species identification. Laboratory procedures including DNA extraction and sequencing of cytochrome c oxidase 1 (COI) and cytochrome b (cytb) were conducted following forensic procedures to determine species identification. Generated sequences matched to reference sequences on the National Centre for Biotechnology Information (NCBI) and the Barcode of Life Data Systems (BOLD) to impala (*Aepyceros melampus*, 99.4–99.7 % homology), eland (*Tragelaphus oryx*, 99.8–100 % homology) and kudu (*T. strepsiceros*, 99.6–99.7 % homology). Phylogenetic analysis and intra- and interspecies distance further confirmed species identification with high bootstrap support (96–100 %). Average intraspecies sequence divergence was 0–1.15% and pairwise comparisons between taxa satisfied the 10-fold genetic distance. Thus both COI and cytb barcoding genes are suitable methodologies for forensic identification of species in the cases presented here. However, analysis of the reference samples identified species where barcoding may potentially fail. These include taxa that have undergone recent, rapid radiations resulting in high intraspecies distance or species that can hybridize. We thus recommend in these cases a reference database that includes geographically widespread samples is required and analysis with additional mitochondrial and/or nuclear markers.

Introduction

A significant decline in biodiversity can be largely attributed to illegal hunting, poaching and trade of wildlife [1]. It has been estimated that more than 300 mammalian species are threatened with extinction due to illegal activities [2]. In South Africa, commonly poached and traded species in the illegal wildlife trade include rhino and elephant for their horns and ivory that are used for decorative purposes and in traditional medicine markets [3]. Large African cats such as cheetah, lion and leopard are poached for the International pet trade with body parts being used in traditional medicine markets and skins being sold for traditional clothing [4]. Pangolin are commonly poached for their perceived mystical powers in traditional Asian and African medicine markets [5]. However, the extent of illegal wildlife trade and poaching of South African antelope on private and public reserves have not been previously reported on. It is currently unknown which species are commonly involved in wildlife forensic cases. It has been estimated that there are more than 18 million individual game on privately owned wildlife ranches with many more occurring on National and provincial reserves. Common species found on South African game ranches and reserves include: Cape buffalo (*Syncerus caffer*), blue wildebeest (*Connochaetes taurinus taurinus*), blesbok (*Damaliscus pygargus phillipsi*), impala (*Aepyceros melampus*), sable antelope (*Hippotragus niger*), roan antelope (*H. equinus*), gemsbok (*Oryx gazella*), kudu (*Tragelaphus strepsiceros*) and eland (*Tragelaphus oryx*). In general, antelope in South Africa are hunted for local consumption. Species that are commonly legally hunted in South Africa include springbok (*Antidorcas marsupialis*), impala, blesbok and kudu [6].

DNA barcoding has been identified as a rapid and practical molecular tool that can be used to identify species due to species-specific variation in short mitochondrial DNA (mtDNA) sequences from one or a few genomic regions [7,8]. DNA barcoding has been applied to wildlife forensic cases [9–12]. The standard region to identify a species is cytochrome c oxidase 1 (COI) [13] or cytochrome b (cytb) with changes to the sequence

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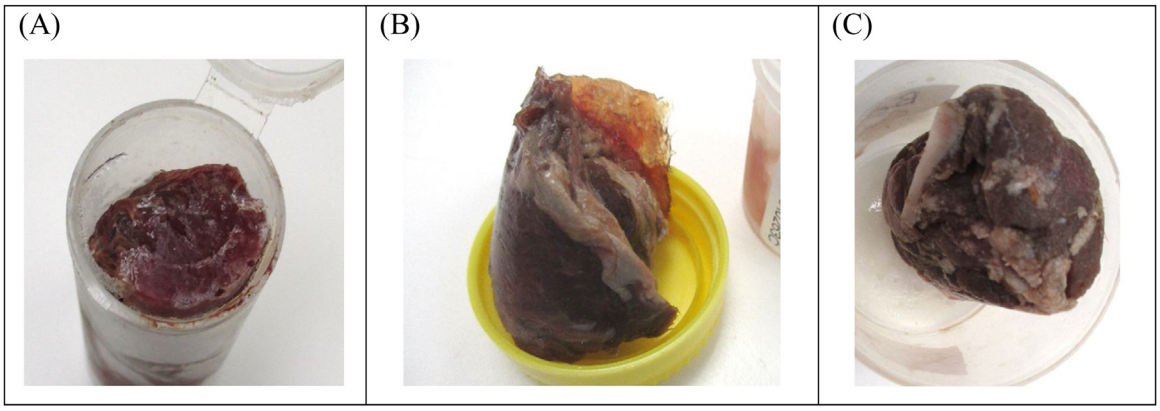

Fig. 1. Samples received for species identification. (A) tissue sample from Case 1, (B) tissue sample from Case 2 and (C) tissue sample from Case 3.

occurring more slowly in COI compared to cytb [14]. A 100 % match between the reference sequence and the unknown samples, will result in assignment of species. However, this is under the assumption that the reference sample has been correctly classified and that there is an absence of intra-species variation. Inter-specific and intra-specific variation can be determined by an averaged Kimura-2 parameter (K2P) distance. Variation is reported to vary widely between and within species and between molecular markers used [13]. An alternative analysis method for

Table 1
List of reference species for cytochrome c oxidase 1 (COI) and cytochrome b (cytb) obtained from National Centre for Biotechnology Information (NCBI) and Barcode of Life Data Systems (BOLD).

Scientific name	Common name	Genbank/BOLD Accession number (COI)	Genbank/BOLD Accession number (Cytb)
Aepyceros melampus	Impala	HQ603134.1	AF022056.1
Aepyceros melampus	Impala	HQ603135.1	AF034966.1
Aepyceros melampus	Impala	HQ603143.1	AF036289.1
Aepyceros melampus	Impala	JN632592.1	JN632592.1
Aepyceros melampus	Impala	–	JN632592
Alcelaphus buselaphus	Hartebeest	JN632593.1	AF016640
Alcelaphus buselaphus	Hartebeest	JN632594.1	AJ222681
Alcelaphus buselaphus	Hartebeest	HQ603087.1	JN632593
Alcelaphus buselaphus	Hartebeest	HQ603088.1	NC020676
Alcelaphus buselaphus	Hartebeest	HQ603089.1	–
Alcelaphus buselaphus	Hartebeest	HQ603090.1	–
Alcelaphus buselaphus	Hartebeest	HQ603091.1	–
Antidorcas marsupialis	Springbok	JX436990.1	JN632593.1
Antidorcas marsupialis	Springbok	JN632596.1	JF728761
Antidorcas marsupialis	Springbok	JX436991.1	NC020678
Antidorcas marsupialis	Springbok	JX436992.1	AF022054.1
Antidorcas marsupialis	Springbok	–	AF036281.1
Philantomba monticola	Blue duiker	GQ144530.1	AF153891.1
Philantomba monticola	Blue duiker	HQ644101.1	AF153892.1
Philantomba monticola	Blue duiker	GQ144533.1	AF153893.1
Philantomba monticola	Blue duiker	HQ644102.1	JN632686.1
Philantomba monticola	Blue duiker	JN632687.1	JN632687.1
Cephalophus natalensis	Red duiker	HQ644103.1	AF153890.1
Cephalophus natalensis	Red duiker	HQ644104.1	JN632618.1
Cephalophus natalensis	Red duiker	JN632618.1	–
Connachaetus gnou	Black wildebeest	JX436976.1	AF016637.1
Connachaetus gnou	Black wildebeest	JX436977.1	JF728762
Connachaetus gnou	Black wildebeest	JN632626.1	NC020698
Connachaetus gnou	Black wildebeest	–	JN632626
Connachaetus taurinus	Blue wildebeest	JN632627.1	NC020699
Connachaetus taurinus	Blue wildebeest	JQ690393.1	JN632627.1
Connachaetus taurinus	Blue wildebeest	HQ603109.1	JN632628.1
Connachaetus taurinus	Blue wildebeest	HQ603118.1	AF016638.1
Connachaetus taurinus	Blue wildebeest	HQ603119.1	AF034969.1
Damaliscus pygargus	Blesbok/Bontebok	JX436981.1	NC020627
Damaliscus pygargus	Blesbok/Bontebok	JX436982.1	AF016639.1
Damaliscus pygargus	Blesbok/Bontebok	JX436983.1	AF036287.1
Damaliscus pygargus	Blesbok/Bontebok	JX436984.1	FJ207530.1
Damaliscus pygargus	Blesbok/Bontebok	JX436985.1	–
Damaliscus pygargus	Blesbok/Bontebok	JX436986.1	–
Damaliscus pygargus	Blesbok/Bontebok	JX436987.1	–
Damaliscus pygargus	Blesbok/Bontebok	JX436988.1	–
Damaliscus pygargus	Blesbok/Bontebok	JX436989.1	–
Hippotragus equinus	Roan	JN632647.1	NC020712
Hippotragus equinus	Roan	KY650663	AF022060
Hippotragus equinus	Roan	HQ603145	JN632647

Table 1 ***(continued)***

Scientific name	Common name	Genbank/BOLD Accession number (COI)	Genbank/BOLD Accession number (Cytb)
Hippotragus equinus	Roan	–	JF728769
Hippotragus niger	Sable	JN632648.1	AF022061
Hippotragus niger	Sable	KY628411	JF728770
Hippotragus niger	Sable	KY628406	JN632648
Hippotragus niger	Sable	KM245339	NC020713
Kobus ellipsiprymnus	Waterbuck	HQ603158.1	JF728771
Kobus ellipsiprymnus	Waterbuck	HQ603159.1	AF022059.1
Kobus ellipsiprymnus	Waterbuck	JQ690391.1	AF096624.1
Kobus ellipsiprymnus	Waterbuck	JN632651.1	JN632651.1
Kobus ellipsiprymnus	Waterbuck	HQ603160.1	–
Neotragus moschatus	Suni	JN632669.1	JN632669.1
Neotragus moschatus	Suni	JN645581	AF022051
Neotragus moschatus	Suni	–	AJ222683
Neotragus moschatus	Suni	–	FJ959386
Oreotragus oreotragus	Klipspringer	JN632675	AF022052
Oreotragus oreotragus	Klipspringer	JN645583	HQ641311
Oreotragus oreotragus	Klipspringer	HQ603154	JN632675
Oreotragus oreotragus	Klipspringer	–	NC020731
Ourebia ourebi	Oribi	JN632680.1	AF320574
Ourebia ourebi	Oribi	–	NC020731
Ourebia ourebi	Oribi	–	JN632680
Oryx gazella	Gemsbok	JX436995.1	AF249973.1
Oryx gazella	Gemsbok	JF444372.1	JN632678.1
Oryx gazella	Gemsbok	NC016422.1	NC016422
Oryx gazella	Gemsbok	JN869312.1	JN869312
Oryx gazella	Gemsbok	–	KC282640
Ovis aries	Sheep	NC016422.1	HM236175.1
Raphicerus campestris	Steenbok	JN632693	AF022068
Raphicerus campestris	Steenbok	KX012655	NC020741
Raphicerus campestris	Steenbok	–	JN632693
Redunca arundinum	Southern reedbuck	JN632694	NC020794
Redunca arundinum	Southern reedbuck	–	AF096628.1
Redunca arundinum	Southern reedbuck	–	JN632694.1
Sylvicapra grimmia	Common duiker	HQ644118.1	AF153904.1
Sylvicapra grimmia	Common duiker	HQ644119.1	AF153905.1
Sylvicapra grimmia	Common duiker	JN632701.1	JN632701.1
Tragelaphus angasii	Nyala	JN632702.1	JF728783
Tragelaphus angasii	Nyala	JX436993.1	AF091633.1
Tragelaphus angasii	Nyala	–	AF022066.1
Tragelaphus angasii	Nyala	–	JN632702
Tragelaphus angasii	Nyala	–	JN632702.1
Tragelaphus oryx	Eland	HQ603173.1	AF022057.1
Tragelaphus oryx	Eland	HQ603174.1	JF728786
Tragelaphus oryx	Eland	JQ690384.1	AF036278.1
Tragelaphus oryx	Eland	JX436994.1	JN632704.1
Tragelaphus oryx	Eland	JN632704.1	–
Tragelaphus scriptus	Bushbuck	HQ603157.1	JF728787
Tragelaphus scriptus	Bushbuck	JN632705.1	AF022067.1
Tragelaphus scriptus	Bushbuck	JN632706.1	AF036277.1
Tragelaphus scriptus	Bushbuck	JN632707.1	JN632705
Tragelaphus scriptus	Bushbuck	–	MH792162
Tragelaphus strepsiceros	Kudu	HQ603161.1	AF022063.1
Tragelaphus strepsiceros	Kudu	HQ603162.1	AF022063
Tragelaphus strepsiceros	Kudu	JN632708.1	AF036280.1
Tragelaphus strepsiceros	Kudu	–	HQ641312.1
Tragelaphus strepsiceros	Kudu	–	HQ641313.1
Tragelaphus strepsiceros	Kudu	–	JF728789
Tragelaphus strepsiceros	Kudu	–	JN632708.1

species identification is the construction of a phylogenetic tree to determine the evolutionary relationships between the unknown sample and known reference sequences [15–17]. Difficulties associated with DNA barcoding include: lack of taxonomic coverage of certain species in accessible databases [18], heteroplasmy [19], co-amplification of numts [20], existence of past hybridization events between certain species [21] and use of a single gene to assign species [22]. Wilson-Wilde et al. [23] suggested that for law enforcement purposes when investigating wildlife crime, both COI and cytb should be used. The authors further recommended a suite of mitochondrial and nuclear markers should be used to resolve more distantly related populations. The aims of the present study were to (1) determine the species of samples submitted as forensic cases using COI and cytb sequencing and (2) estimate genetic distance and phylogeny of reference and unknown samples in order to assess the efficiency of the two mitochondrial gene regions to identify the specific species.

Case history

Three (3) forensic cases were analyzed in 2018 and 2019 for molecular identification of species. In Case 1, a suspect was arrested for possession of game meat and a sample was collected. The officer further visited the scene of the crime and took a second sample from a carcass that was found. In Case 2, a suspect was found in possession of two different carcasses and

it was unknown whether they were from game or domestic animals. Here, two samples were collected from each carcass. Lastly, in Case 3, a carcass was found in a suspect's procession and the examination request was to determine if the sample was kudu or bovine (Fig. 1).

Materials and methods

Genomic DNA was isolated from the samples using the Applied Biosystems™ PrepFiler™ Forensic DNA extraction kit following the manufacturer's protocol. Regions of the mitochondrial genes COI and cytb were amplified and sequenced using primer sets as described in Kocher et al. [24], Hebert et al. [25], Bitanyi et al. [26] and using the Applied Biosystems 2720 Thermal Cycler. Polymerase chain reaction (PCR) amplification was carried out in a total volume of 25 micro litres (μL). PCR was conducted with Ampliqon *Taq* DNA Polymerase Master Mix RED (Ampliqon, Odense, Denmark). The final reaction conditions were as follows: 1 X PCR Mastermix, 10 pico mol (pmol) of each of the forward and reverse primer and 50−100 nano gram (ng) genomic DNA template. The conditions for PCR amplification were as follows; 5 min (min) at 95 °C denaturation, 35 cycles for 30 s (sec) at 95 °C, 30 s at 50−53 °C and 1 min at 72 °C followed by extension at 72 °C for 10 min. PCR products were separated by electrophoresis in a 2% agarose gel for 30 min at 100 V in 1 x Tris-Borate-EDTA Buffer (TBE) followed by clean-up of PCR products using the ExoSAP protocol. The purified PCR products were cycle sequenced using BigDye Terminator v3.1. Cycle Sequencing Kit (Life Technologies). The cycle sequencing products were purified with the BigDye XTerminator Purification Kit (Life Technologies) before sequencing on an ABI 3500 genetic analyser (Life Technologies) in forward and reverse.

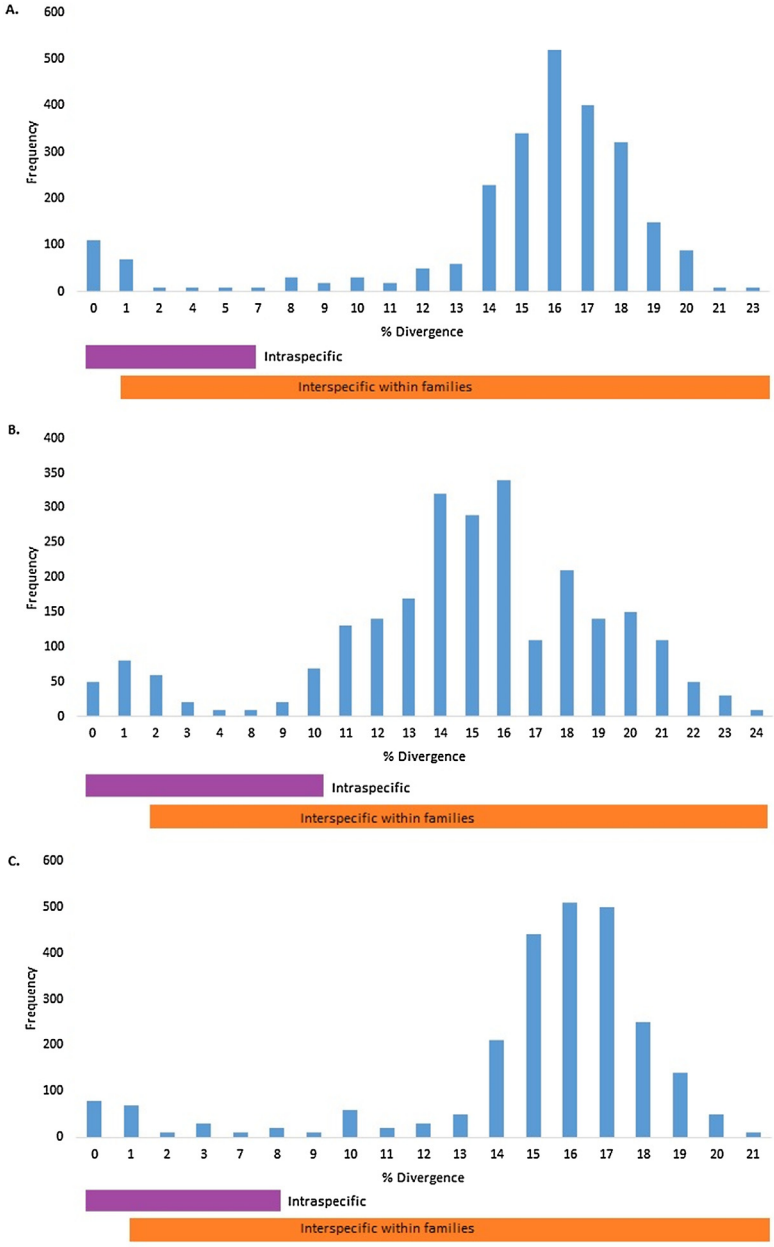

Fig. 2. (A-C) Histogram of Kimura 2-parameter for intraspecific (purple) and interspecific (gray bars) divergence values for cytochrome c oxidase 1 (A), cytochrome b (B) and concatenated cytochrome c oxidase 1 and cytochrome b (C). (D-F) Boxplot summary of pairwise divergence values per species. Red crosses indicate maximum interspecific divergence and pink crosses indicate intraspecific divergence for cytochrome c oxidase 1 (D), cytochrome b (E) and concatenated cytochrome c oxidase 1 and cytochrome b (F).

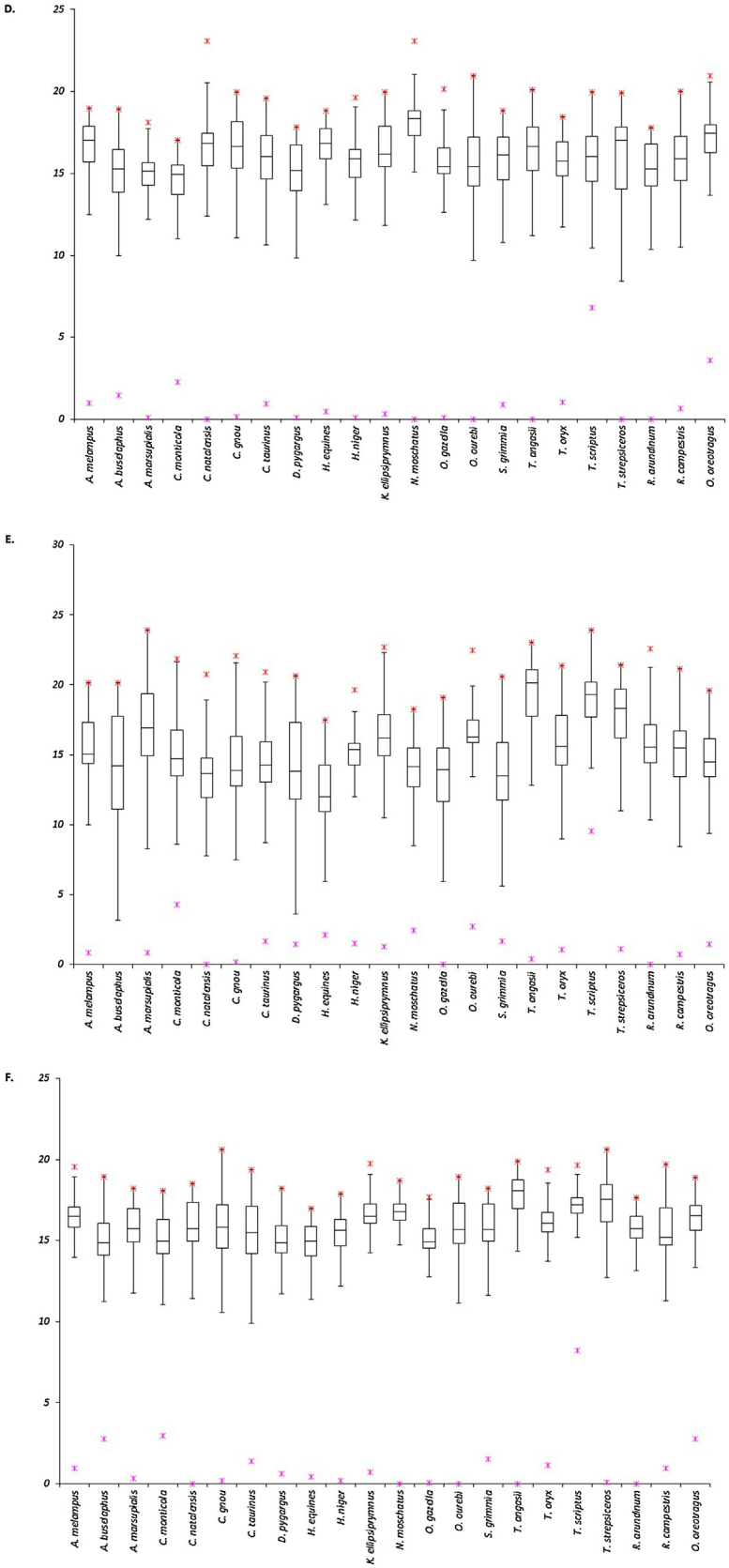

Fig. 2. (*continued*).

Data analysis

The raw sequence data for each mtDNA gene were checked, edited and aligned manually in GENEIOUS v10.2.3. The sequences obtained for the unknown samples were compared to the sequence database of National Centre for Biotechnology Information (NCBI) using the Basic Local Alignment Search Tool (BLAST) program [27] and the database of the Barcode of Life Data Systems (BOLD) [28]. A dataset of 83 COI and 95 cytb reference sequences were downloaded from NCBI and BOLD (Table 1) based on similarity to the unknown sequences. Multiple sequence alignment of the reference and unknown sequences were performed in GENEIOUS v10.2.3 using ClustalW alignment. Final sequence assembly was obtained using a uniform sequence length of 465 bp for COI and 307 bp for cytb. Neighbour-joining (NJ) phylogenetic trees were constructed in MEGA 7 with the K2P model using 1000 bootstrap replications. Intra- and interspecies distances for both genes were determined using the K2P model in MEGA.

Results and discussion

Sequence length of the samples from the three cases varied from 463 to 631 bp for COI and was 307 bp in length for cytb. Intra- and interspecies distances for various South African antelope species were determined for COI (Fig. 2A, D), cytb (Fig. 2B, E) and concatenated COI and cytb (Fig. 2C, F) using the K2P method. Intraspecies distance varied from 0 to 9.6 % for COI, varied from 0 to 6.8 % for cytb and varied from 0 to 8.21 % for the concatenated sequences (Supplementary 1). Intraspecific divergence in different taxa has been reported to be below 2% in fish [29], reptiles [30] and mammalian species [26,31,32]. However, here we identified intraspecific divergence above 2% for several species. Higher within-species distance for COI was observed in klipspringer (*Oreotragus oreotragus*, 3.6 %), bushbuck (*Tragelaphus scriptus*, 6.82 %) and blue duiker (*Cephalophus monticola*, 2.27 %). With regards to cytb, higher intra-species distance was observed in bushbuck (*T. scriptus*, 9.56 %), oribi (*Ourebia ourebi*, 2.7 %), suni (*Neotragus moschatus*, 2.44 %), hartebeest (*Alcelaphus buselaphus*, 3.18 %), blue duiker (*C. monticola*, 4.3 %) and roan (*Hippotragus equines*, 2.09 %). When concatenated sequences were considered, the following species displayed intraspecies distances higher than 2%: bushbuck (*T. scriptus*, 8.21 %), blue duiker (*C. monticola*, 2.98 %), klipspringer (*O. oreotragus*, 2.77 %) and hartebeest (*A. buselaphus*, 2.76 %). Higher intraspecies genetic variation may be attributed to lack of monophyly due to recent divergence from a common ancestor. Paraphyletic lineages have been previously reported in *T. scriptus* and *C. monticola* and phylogenies of these species have not been resolved adequately using cytb [32]. Both species are reported to be geographically widespread and have undergone rapid and recent divergence. Thus, they do not do not satisfy the 10-fold genetic distance rule described by Hebert et al. [25]. A study conducted to determine the reliability of commercial labeling of game meat in South Africa using cyb and COI barcoding has reported high intraspecies distances in African antelope [6]. Whereas, a species identification study on Tanzanian antelope identified low divergence (below 2%) [26]. All studies support the use of reference samples from broad geographic ranges and more than one marker in order to examine within species variability, which may be especially pertinent in monophyletic groups.

Interspecies distance varied from 0.97 to 23.1 % for COI, 2.32–23.04% for cytb and 1.45–20.6% for the concatenated sequences (Supplementary 1). The lowest interspecies variation (0.97–2.32 %) was identified between blue and black wildebeest (*Connachaetus taurinus* and *C. gnou*) and the highest was observed between suni (*N. moschatus*) and duiker (*C. natalensis*) for COI (23.1 %), between bushbuck (*T. scriptus*) and springbok (*Antidorcas marsupialis*) for cytb (23.04 %) and between kudu (*T. strepsiceros*) and black wildebeest (*C. gnou*) for concatenated sequences (20.6 %). Pairwise comparisons of K2P distance between taxa satisfied the 10-fold genetic distance [25] except between bushbuck (*T. scriptus*) and eland (*T. oryx*) as well as kudu (*T. strepsiceros*) and eland (*T. oryx*) for COI. For cytb, the interspecific distance (7.57 %) between eland (*T. oryx*) and kudu (*T. strepsiceros*) was not 10 fold higher. This can be attributed to high intraspecies variability in combination with recent divergence of species from one another. Interspecific hybridization can additionally create taxonomic uncertainty and pose a problem for DNA barcoding. Hybridization is the crossing of genetically distinguishable groups or taxa leading to the production of viable hybrids [33]. Hybridization and introgression are influenced globally by human interaction with the environment (habitat modification, translocation). Although hybridization plays an important role in the evolution of many species, it has also contributed to the extinction of species [34]. There are several species in South Africa that have been known

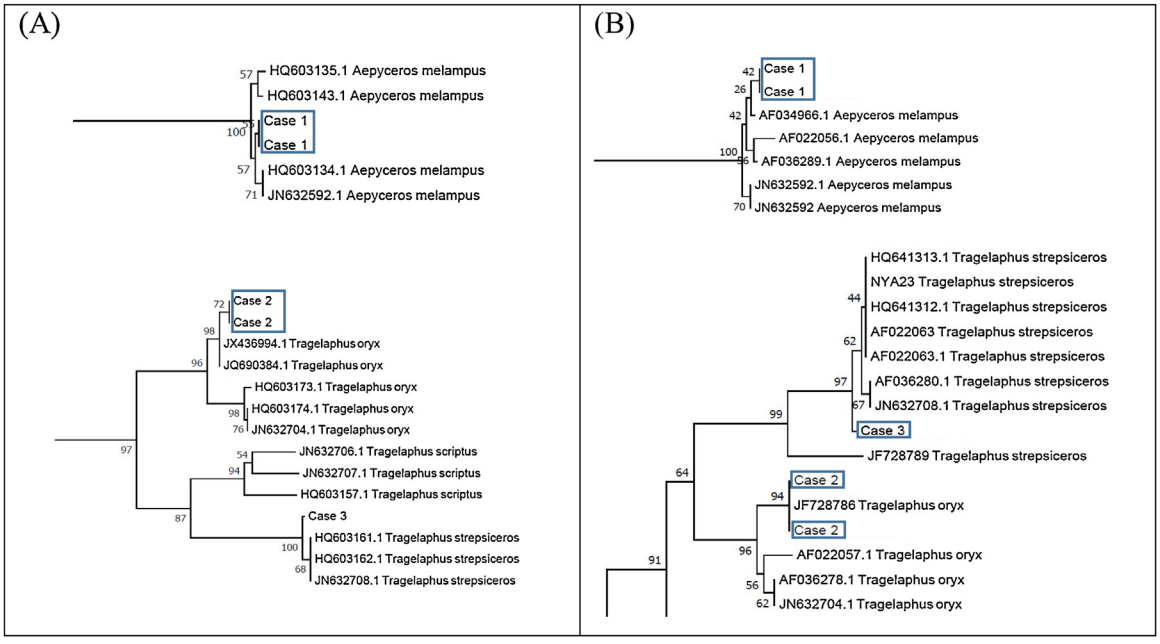

Fig. 3. Excerpt of neighbour-joining (NJ) phylogenetic trees using the Kimura 2-parameter (K2P) model with 1000 bootstrap replicates. (A) Cytochrome c oxidase 1 (COI) and (B) cytochrome b (cytb). Case samples indicated in blue blocks.

to hybridize and have fertile offspring. These include amongst others the bontebok and blesbok as well as the blue and black wildebeest [35,36]. An evolutionary divergence time of approximately 1 million years has been calculated for the two wildebeest species [37], indicating a close phylogenetic relationship for these species. Low levels of differentiation based on barcode genes for recently diverged species has been previously reported in white-headed gulls [38] and Mallard/Black ducks [39]. Thus, for identification of hybrids and in poaching cases barcoding will fail to correctly identify a species and/or sub-species that have the potential to hybridise. In South Africa, this would include: black faced impala x common impala [40]; red hartebeest and blesbok [41] and gemsbok and scimitar-horned oryx [42]. The inability to differentiate between two species has been reported in the *Gallus* genus. Lack of variation in the COI gene of grey jungle fowl (*G. sonneratii*) and chicken (*G. gallus*), two species known to hybridise has been reported by Dawnay et al. [43]. Therefore, analysis of fast evolving nuclear genes such as microsatellites will be required in species with the potential to hybridize in order to correctly identify the species and/or sub-species in forensic cases [35,36].

Here, homology analysis of COI using BLAST identified that the two samples from Case 1 matched impala (*Aepyceros melampus*) with 99.4 % and 99.5 %, respectively, In addition, homology analysis of cytb matched impala (*A. melampus*) with 99.7 %. Samples from Case 2 matched eland (*T. oryx*) with 99.8 % (COI) and 100 % (cytb). Lastly, in Case 3, the sample matched kudu (*T. strepsiceros*) COI with 99.6 % and cytb with 99.7 %. Phylogenetic analysis for COI (Fig. 3A) and cytb (Fig. 3B) corroborated the findings of the BLAST analysis with samples grouping with the above identified species with high bootstrap support (96–100 %). Intraspecies distance for these three species varied from 0 to 1.05 % for COI, 0.86–1.12% for cytb and 0.09–1.15% for concatenated sequences (Fig. 2).

Conclusions

Here, all unknown samples were classified to species level due to high similarity to reference specimens at two different gene regions and additionally these samples clustered with high bootstrap support to multiple database entries in phylogenetic trees. Thus, in these three forensic cases, consistent results were obtained in order to reliably identify species. However, the study highlighted some weaknesses of the DNA barcoding method for species identification in forensic cases of African antelope. High within species variation was observed consistently for bushbuck (*T. scriptus*) and blue duiker (*C. monticola*). Thus, COI and cytb should not be used to evaluate taxonomic relationships within the genus *Cephalophus* and *Tragelaphus*. It is recommended that for species where recent speciation processes have contributed to high within and low between species differentiation, representative sampling from various geographic areas is required. When concatenated sequences of COI and cytb were considered, the accuracy of the results did not improve. Thus additional data is needed to further examine their systematic relationships, and the use of other markers such as D-loop, 16S, 12S or nuclear markers should be considered. However, availability of sequences from reference samples for these gene regions may be limited on publically accessible databases and need to be further expanded. In addition, very low interspecies variations were observed between blue and black wildebeest (*Connachaetus taurinus* and *C. gnou*), two species that are known to hybridize. In cases where accurate species identification is required for wildebeest, nuclear markers such as microsatellites or Single Nucleotide Polymorphisms (SNPs) are recommended.

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Declaration of Competing Interest

None.

CRediT authorship contribution statement

Desiré Lee Dalton: Conceptualization, Formal analysis, Writing - original draft. **Marli de Bruyn:** Data curation, Validation, Formal analysis, Writing - review & editing. **Tia Thompson:** Writing - review & editing, Methodology. **Antoinette Kotzé:** Project administration, Writing - review & editing.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.fsir.2020.100071.

References

[1] D.W.S. Challender, S.R. Harrop, D.C. MacMillan, Towards informed and multi-faceted wildlife trade interventions, Glob. Ecol. Conserv. 3 (2015) 129–148, doi:http://dx.doi.org/10.1016/j.gecco.2014.11.010.
[2] W.J. Ripple, K. Abernethy, M.G. Betts, G. Chapron, R. Dirzo, M. Galetti, T. Levi, P.A. Lindsey, D.W. Macdonald, B. Machovina, T.M. Newsome, Bushmeat hunting and extinction risk to the world's mammals, R. Soc. Open Sci. 3 (10) (2016) p.160498.
[3] J.S. Adams, T.O. McShane, The Myth of Wild Africa: Conservation Without Illusion, Berkeley, (1992) .
[4] G.L. Warchol, L.L. Zupan, W. Clack, Transnational criminality: an analysis of the illegal wildlife market in Southern Africa, Int. Crim. Justice Rev. 13 (1) (2003) 1–27.
[5] M. Mwale, D.L. Dalton, R. Jansen, M. De Bruyn, D. Pietersen, P.S. Mokgokong, A. Kotzé, Forensic application of DNA barcoding for identification of illegally traded African pangolin scales, Genome 60 (3) (2016) 272–284.
[6] M.E. D'Amato, E. Alechine, K.W. Cloete, S. Davison, D. Corach, Where is the game? Wild meat products authentication in South Africa: a case study, Investig. Genet. 4 (1) (2013) 6.
[7] D.M. Spooner, DNA barcoding will frequently fail in complicated groups: an example in wild potatoes, Am. J. Bot. 96 (6) (2009) 1177–1189.
[8] Y.-Y. Shen, X. Chen, R.W. Murphy, Assessing DNA barcoding as a tool for species identification and data quality control, PLoS One 8 (2) (2013) e57125, doi:http://dx.doi.org/10.1371/journal.pone.0057125.
[9] S.K. Verma, G.K. Goswami, DNA evidence: current perspective and future challenges in India, Forensic Sci. Int. 241 (2014) 183–189.
[10] G.D. Khedkar, S.B. Abhayankar, D. Nalage, S.N. Ahmed, C.D. Khedkar, DNA barcode based wildlife forensics for resolving the origin of claw samples using a novel primer cocktail, Mitochondrial DNA Part A 27 (6) (2016) 3932–3935.
[11] P.F. Gonçalves, A.R. Oliveira-Marques, T.E. Matsumoto, C.Y. Miyaki, DNA barcoding identifies illegal parrot trade, J. Hered. 106 (S1) (2015) 560–564.
[12] F.M. Khan, K. William, S. Aruge, S. Janjua, S.A. Shah, Illegal product manufacturing and exportation from Pakistan: revealing the factuality of highly processed wildlife skin samples via DNA mini-barcoding, Nucleosides Nucleotides Nucleic Acids 37 (3) (2018) 179–185.
[13] P.D.N. Hebert, A. Cywinska, S.L. Ball, J.R. deWaard, Biological identifications through DNA barcodes, Proc. Biol. Sci. 270 (1512) (2003) 313–321, doi:http://dx.doi.org/10.1098/rspb.2002.2218.PMID:12614582.
[14] M. Lynch, P.E. Jarrell, A method for calibrating molecular clocks and its application to animal mitochondrial DNA, Genetics. 135 (1993) 1197–1208.
[15] J.C. Avise, W.S. Nelson, C.G. Sibley, Why one-kilobase sequences from mitochondrial DNA fail to solve the Hoatzin phylogenetic enigma, Mol. Phylogenet. Evol. 3 (2) (1994) 175–184.
[16] J. Roman, B.W. Bowen, The mock turtle syndrome: genetic identification of turtle meat purchased in the south-eastern United States of America, Anim. Conserv. Forum 3 (1) (2000) 61–65 February, Cambridge University Press.
[17] S.K. Verma, L. Singh, Novel universal primers establish identity of an enormous number of animal species for forensic application, Mol. Ecol. Notes 3 (1) (2003) 28–31.
[18] L.M. Boykin, K.F. Armstrong, L. Kubatko, P. De Barro, Species delimitation and global biosecurity, Evol. Bioinform. 8 (2012) pp.EBO-S8532.
[19] K.N. Magnacca, M.J. Brown, Mitochondrial heteroplasmy and DNA barcoding in Hawaiian Hylaeus (nesoprosopis) bees (Hymenoptera: colletidae), BMC Evol. Biol. 10 (1) (2010) 174.
[20] J.V. Lopez, S. Cevario, S.J. O'Brien, Complete nucleotide sequences of the domestic cat (Felis catus) mitochondrial genome and a transposed mtDNA tandem repeat (Numt) in the nuclear genome, Genomics 33 (2) (1996) 229–246.
[21] F. Di Rocco, D.M. Posik, M.V. Ripoli, S. Díaz, M.L. Maté, G. Giovambattista, et al., South American camelid illegal traffic detection by means of molecular markers, Leg. Med. 13 (2011) 289–292.
[22] R. De Salle, M.G. Egan, M. Siddall, The unholy trinity: taxonomy, species delimitation and DNA barcoding, Philos. Trans. R. Soc. B 360 (2005) 1905–1916.
[23] L. Wilson-Wilde, J. Norman, J. Robertson, S. Sarre, A. Georges, Current issues in species identification for forensic science and the validity of using the cytochrome oxidase I (COI) gene, Forensic Sci. Med. Pathol. 6 (3) (2010) 233–241.

[24] T.D. Kocher, W.K. Thomas, A. Meyer, S.V. Edwards, S. Pääbo, F.X. Villablanca, A.C. Wilson, Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers, Proc. Natl. Acad. Sci. 86 (16) (1989) 6196–6200.
[25] P.D.N. Hebert, M.Y. Stoeckle, T.S. Zemlak, C.M. Francis, Identification of birds through DNA barcodes, PLoS Biol. 2 (2004) 1657–1663.
[26] S. Bitanyi, G.R.O. Bjørnstad, E.M. Ernest, M. Nesje, L.J. Kusiluka, J.D. Keyyu, R.H. Mdegela, K.H. Røed, Species identification of Tanzanian antelopes using DNA barcoding, Mol. Ecol. Resour. 11 (3) (2011) 442–449.
[27] S.F. Altschul, W. Gish, W. Miller, E.W. Myers, D.J. Lipman, Basic local alignment search tool, J. Mol. Biol. 215 (3) (1990) 403–410.
[28] S. Ratnasingham, P.D. Hebert, BOLD: the Barcode of Life Data System (http://www.Barcodinglife. org), Mol. Ecol. Notes 7 (3) (2007) 355–364.
[29] R.D. Ward, T.S. Zemlak, B.H. Innes, P.R. Last, P.D. Hebert, DNA barcoding Australia's fish species, Philos. Trans. R. Soc. B: Biol. Sci. 360 (1462) (2005) 1847–1857.
[30] M.J. Eaton, G.L. Meyers, S.O. Kolokotronis, M.S. Leslie, A.P. Martin, G. Amato, Barcoding bushmeat: molecular identification of Central African and South American harvested vertebrates, Conserv. Genet. 11 (4) (2010) 1389–1404.
[31] E.L. Clare, B.K. Lim, M.D. Engstrom, J.L. Eger, P.D. Hebert, DNA barcoding of Neotropical bats: species identification and discovery within Guyana, Mol. Ecol. Notes 7 (2) (2007) 184–190.
[32] S. Ntie, A.R. Johnston, P. Mickala, A.E. Bowkett, B. Jansen van Vuuren, M. Colyn, P. Telfer, F. Maisels, O. Hymas, R.L. Rouyer, R.A. Wallace, A molecular diagnostic for identifying central African forest artiodactyls from faecal pellets, Anim. Conserv. 13 (1) (2010) 80–93.
[33] J. Mallet, Hybridization as an invasion of the genome, Trends Ecol. Evol. 20 (5) (2005) 229–237.
[34] F.W. Allendorf, R.F. Leary, P. Spruell, J.K. Wenburg, The problems with hybrids: setting conservation guidelines, Trends Ecol. Evol. 16 (11) (2001) 613–622.
[35] A.M. van Wyk, A. Kotzé, E. Randi, D.L. Dalton, A hybrid dilemma: a molecular investigation of South African bontebok (Damaliscus pygargus pygargus) and blesbok (Damaliscus pygargus phillipsi), Conserv. Genet. 14 (3) (2013) 589–599.
[36] P. Grobler, A.M. van Wyk, D.L. Dalton, B.J. van Vuuren, A. Kotzé, Assessing introgressive hybridization between blue wildebeest (Connochaetes taurinus) and black wildebeest (Connochaetes gnou) from South Africa, Conserv. Genet. 19 (4) (2018) 981–993.
[37] S.W. Corbet, T.J. Robinson, Genetic divergence in south african wildebeest: comparative cytogenetics and analysis of mitochodrial DNA, J. Hered. 82 (6) (1991) 447–452.
[38] P.A. Crochet, J.Z. Chen, J.M. Pons, J.D. Lebreton, P.D. Hebert, F. Bonhomme, Genetic differentiation at nuclear and mitochondrial loci among large white-headed gulls: sex-biased interspecific gene flow? Evolution 57 (12) (2003) 2865–2878.
[39] C.D. Ankney, D.G. Dennis, L.N. Wishard, J.E. Seeb, Low genic variation between black ducks and mallards, Auk 103 (4) (1986) 701–709.
[40] W.C. Green, A. Rothstein, Translocation, hybridization, and the endangered black-faced impala, Conserv. Biol. (1998) 475–480.
[41] T.J. Robinson, D.J. Morris, N. Fairall, Interspecific hybridization in the Bovidae: sterility of Alcelaphus buselaphus × Damaliscus dorcas F1 progeny, Biol. Conserv. 58 (3) (1991) 345–356.
[42] R.K. Brooke, P.H. Lloyd, A.L. De Villiers, Alien and Translocated Terrestrial Vertebrates in South Africa, (1986) .
[43] N. Dawnay, R. Ogden, R. McEwing, G.R. Carvalho, R.S. Thorpe, Validation of the barcoding gene COI for use in forensic genetic species identification, Forensic Sci. Int. 173 (1) (2007) 1–6.