

A septennium review of wildlife forensic DNA analysis in South Africa

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ABSTRACT

The application of scientific research tools and technologies in wildlife forensic analysis is fundamental to support law enforcement in the regulation and enforcement of illegal criminal activities. Validated genetic technologies and techniques have proven to be critical in securing successful prosecutions specifically through the examination of DNA from physical exhibit material. In South Africa, DNA techniques and tools have been implemented to identify and characterise biological evidence of wildlife, in answering questions that arise during crime investigation and prosecution. Here, we describe, and review wildlife forensic cases analysed in South Africa (by South African National Biodiversity Institute (SANBI) and the Veterinary Genetic Laboratory (VGL)) over a seven-year period (August 2017 to July 2024). In total, 3 763 wildlife forensic cases were analysed. The taxonomic representation was skewed towards mammals encompassing 94.1 % of all cases due to large amount of wildlife cases involving black and white rhinoceros, African elephant, lion and antelope. These cases were predominantly from the north-eastern parts of the country including Limpopo, Mpumalanga and KwaZulu-Natal provinces which have previously been classified as a 'hotspot' for poaching. The type of analysis requested varied between the different taxonomic groups with 90 % of mammal cases submitted for DNA comparison, while bird, reptile, fish and invertebrate cases were mainly submitted for species identification (>87 %). This paper further reviews the successes and challenges encountered from a South African perspective and provides future recommendations.

1. Introduction

Ranked as one of the world's 17 megadiverse nations, South Africa is known for its rich biodiversity, high species richness and vast variety of ecosystems. Species richness is attributed to South Africa being home to approximately 5 % of the world's mammals, 7 % of birds and 4 % of reptiles of which a large proportion is endemic (endemism ranging from 5 % to 67 % for different taxonomic groups) [1]. The 2018 National Biodiversity Assessment (NBA) assessed 23,312 indigenous taxa and estimated that approximately 14 % are listed as threatened [1]. The mega diversity, high levels of endemism and high number of rare and unique wildlife species all contribute to South Africa being disproportionately targeted for illegal wildlife trade. The unsustainable illegal wildlife trade threaten not only species with extinction but can result in loss of biodiversity, disruption of ecosystems and the dispersal of invasive species [2–4].

South African species targeted for illegal trade purposes include amongst others black rhinoceros (*Diceros bicornis*), white rhinoceros

(*Ceratotherium simum*), Temminck's ground pangolin (*Smutsia temminckii*) and African elephant (*Loxodonta africana*) which have been listed as some of the most commonly poached or trafficked species worldwide [5–8]. These species, as well as many others, are targeted for purposes such as traditional medicine, culinary delicacies, bushmeat, pet trade, decorations and as status symbols [9,10].

Black and white rhinoceros are specifically targeted for their horns that are commonly used in traditional medicine as the horn is believed to cure various ailments including fever, gout, hallucinations and food poisoning [11]. A dramatic increase in the number of African rhino's killed for their horns per year was observed and ranged from 36 individuals in 2006 to a maximum of 1349 in 2015. The majority of these rhinos were poached in the Kruger National Park (KNP), South Africa, with a 59 % decline in the number of rhinos within the park since 2013. The dynamics of rhino poaching continue to evolve, with patterns of illegal activity shifting in response to conservation strategies, enforcement pressure, and market demands. For example, since 2015, and as a result of conservation and law enforcement efforts in the park there has

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been a steady decline in numbers of rhino poached in the KNP with only 499 recorded in 2023. However, this subsequently led to increased poaching in the KwaZulu-Natal, Limpopo and Eastern Cape provinces of South Africa [12].

Worldwide, all eight (8) pangolin species are targeted for their meat and scales [13]. Pangolin meat is consumed as luxury food or as bushmeat while the scales are considered highly valuable in traditional medicine [14,15]. Within the southern African region, the number of confiscated Temminck's ground pangolin has shown a significant increase from 2 individuals in 2000 to > 30 in 2013, suggesting a growth in illegal trade of this species [16].

African elephant population declines are mainly associated with the ivory trade due to its use as ornaments, jewellery and in traditional medicine. A total population decline of 60–70 % between 1979 and 2002 [17] was reported due to poaching and it is suspected that the population is continuing to decline [18]. In South Africa, predominantly in KNP, the total number of elephants killed increased from 22 individuals in 2015–67 in 2017 [19]. An increase in poaching of several other indigenous South African species such as sungazers [20], tortoises [21] and abalone [22], has also been observed.

Combatting illegal wildlife trade and poaching requires a multidisciplinary approach including amongst others wildlife DNA forensics and the provision of genetic evidence where illegal wildlife trade or poaching is suspected. This paper aims to review the case work analysed in South Africa over a seven-year period ranging from August 2017 to July 2024 and the efforts made to support law enforcement in combatting illegal wildlife trade.

2. Legislative protection of species

International trade in wild animals and plants is governed by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) which aims to ensure that trade does not threaten the survival of wild populations [23]. Countries voluntarily become part of CITES and are required to adhere to the laws of the Convention. South Africa, as a signatory to CITES, has developed domestic legislation to align with its international obligations. The Threatened or Protected Species (ToPS) Regulations, promulgated under the National Environmental Management: Biodiversity Act 10 of 2004 (NEM:BA Act 10 of 2004), serves to complement CITES by regulating not only international but also domestic trade in listed species including 32 mammals, 21 birds, 20 reptiles, 7 fishes and 24 invertebrates [24]. While CITES is primarily concerned with controlling cross-border trade in endangered species, ToPS extends its scope to broader conservation objectives, including habitat protection, species recovery planning, and the regulation of restricted activities such as hunting, possession, and breeding. Further, South Africa's legal framework for biodiversity conservation is multi-tiered. At the national level, NEMBA establishes overarching standards and principles for the management and sustainable use of biodiversity, including the creation of the South African National Biodiversity Institute (SANBI) and the Scientific Authority to guide decision-making on species trade. At the provincial level, each of the nine provinces maintains its own conservation agency empowered to enact and enforce regulations within the national framework, which can result in jurisdictional complexity and variation in implementation [24].

3. Wildlife forensic science and molecular tools used

Wildlife forensic science is the application of scientific methods to assist or solve legal issues related to crimes against wildlife [25]. The application is typically guided by the investigative question and various molecular markers, such as mitochondrial DNA sequencing (or DNA barcoding), Short Tandem Repeats (STRs) and Single Nucleotide Polymorphisms (SNPs) are applied to answer these questions [26] and provide valuable information on species, populations, geographical origin, individuals and parentage [27].

3.1. Species identification

Species identification is one of the most common applications in wildlife forensic analysis and molecular techniques such as DNA barcoding is recommended and have been successfully applied to identify species. DNA barcoding is the amplification of an approximately 650 base pair (bp) region of the cytochrome oxidase I (COI) region and comparing it to known reference material commonly published on international repositories such as NCBI GenBank (www.ncbi.nih.gov) or the Barcode of Life Database system (BOLD; www.boldsystems.org). The COI region is considered the standard for animal species identification [28,29] and has been validated as a diagnostic marker for forensic identification applications [30,31]. Other mitochondrial regions such as cytochrome b (cytb) [32–34], 12S ribosomal ribonucleic acid (rRNA) [35,36] and 16 s rRNA [37,38] have also been employed. DNA barcoding has been successfully applied in numerous studies across a wide range of taxa and species such as mammals [39], carnivores [40], birds [41,42] and marine species [43]. In 2020, Dalton *et al.* assessed the utility of DNA barcoding to solve antelope forensic cases in South Africa using the cytochrome oxidase I and cytochrome b regions. Consistent results were obtained to identify forensic evidence as impala (*Aepyceros melampus*), eland (*Tragelaphus oryx*) and kudu (*Tragelaphus strepsiceros*). However, the authors identified weaknesses in DNA barcoding related to high intraspecies variation, low interspecies variation and hybridisation [44].

Species identification forms a crucial part in South African wildlife forensic analysis to enforce CITES and national legislation, but is dependent on the availability of accurate, well curated wildlife forensic reference databases. Through the Barcode of Wildlife Project (<https://barcodeofwildlife.org>) a COI and cytb reference database was constructed for South African species commonly encountered in wildlife forensic investigations including several look-alike species including mammals, birds, reptiles and marine species. The database was constructed using samples collected under full chain-of-custody and taxonomically verified by experts to ensure admissibility in court. Baxter *et al.* 2024 [45] reported on the establishment of the mammal reference database under this project which included 37 species for COI and 23 species for cytb.

3.2. Individual identification

In wildlife forensic investigations it is often necessary to link crime scene samples to samples recovered from suspects such as clothing, tools or processed animal parts [28]. This is accomplished through the generation of an individual DNA profile using highly polymorphic, informative molecular markers such as STRs or SNPs. Profiling systems are often developed for conservation research purposes and lack the validation required for use in forensic casework [46]. Currently, there are no commercial STR-based DNA profiling test kits available for wildlife species found in South Africa of which many are endemic to the country or continent of Africa. Laboratories involved with wildlife forensics, therefore, have to develop, optimize and validate their own STR-based DNA profiling tests for each species encountered in wildlife cases. In South Africa, STR-based DNA profiling tests have been developed amongst others for white and black rhinoceros [47], Temminck's ground pangolin [48], cranes [49], cheetah [50] and lion [51]. These are frequently applied to support legal trade regulation or for use in wildlife forensic case work and has supported successful convictions and sentencing in wildlife cases. However, there is rarely feedback regarding the outcomes of cases and the full impact cannot be assessed yet.

To calculate the statistical probability of evidentiary items originating from the same individual, reference allele frequency data representing the species and populations is needed [52]. This is achieved through the establishment of comprehensive reference databases, which is often difficult to establish for rare and endangered species. In South Africa, the RhODIS (Rhinoceros DNA Index System) database was developed using 23 STR markers for individual identification of

rhinoceros species representing data from across their distribution [53]. The DNA profiling system and reference database includes over 80,000 DNA profiles and continues to be successfully applied in support of rhinoceros crime prosecutions [54]. SANBI manages genetic databases for several threatened species for conservation and forensic applications. For Temminck's ground pangolin, the database comprises 30 STR markers and genetic profiles from 134 individuals. A comprehensive crane database, which includes blue (*Anthropoides paradiseus*), wattled (*Bugeranus carunculatus*), and grey-crowned (*Balearica regulorum*) cranes, has been validated using 251 samples and includes 16 STR markers along with two primers for avian sex determination. The cheetah database features 218 SNPs, validated across 16 family groups to ensure reliability. Additionally, a lion database has been developed, incorporating 21 STR markers and genetic data from 227 individuals.

3.3. Geographical origin

To assess the legal status of wildlife within countries and whether a crime has been committed, it has become critical to prove geographic origin especially where large consignments are investigated. This provides valuable insight into trade routes, patterns and hotspot areas for illegal wildlife trade. Geographic assignment of evidentiary items is based on the level of genetic variation between species and populations and requires hypervariable loci that can distinguish between populations of a single species [55].

In a case study done by Kotze et al. [56], the illegal trade of cheetah (*Acinonyx jubatus*) over the Namibian and Botswana borders were investigated. The source of the confiscated cheetah was established through microsatellite marker comparisons with the relevant reference population allele frequency databases linking the cheetahs back to South Africa. Similar techniques, using both mitochondrial and nuclear DNA have been applied in case studies involving elephant (*Loxodonta spp.*, [57], www.loxodontalocalizer.org), pangolin (*Manis tricuspis*, [58]), tortoises (*Testudo hermanni*, [59]) and nyala (*Tragelaphus angasii* [60]). Ogden et al. [46] reported that geographic identification is still in its infancy and further suggested that in a forensic context, the most likely genetic geographic origin based on the hypotheses given by the defence and the prosecution should be determined by using assignment testing. However, the effectiveness of assignments is dependent on availability of population allele frequency databases [52] which are generally unavailable and need to be developed for several South African wildlife species.

4. Wildlife forensic case work in South Africa

In South Africa, laboratories responsible for wildlife forensic DNA analysis are contracted by local enforcement authorities (South African Police Service (SAPS) or Department of Forestry, Fisheries and the Environment (DFFE)) to provide this service based on their expertise and experience. Two laboratories within South Africa have mainly been responsible for the provision of wildlife forensic genetic analysis, namely the South African National Biodiversity Institute (SANBI) Genetic Services Unit (GSU) and the Veterinary Genetics Laboratory (VGL) of the University of Pretoria. The Forensic Science Laboratory (FSL) of the South African Police Service (SAPS) has been responsible for managing this contract service since 2017. The VGL has been contracted to conduct the genetic analysis of rhinoceros species (white rhinoceros and black rhinoceros), while SANBI GSU has conducted analysis of species such as Temminck's ground pangolin (*Smutsia temminckii*), and abalone (*Haliotis midae*). Some wildlife species have been analysed by both laboratories and include amongst others African elephant and lion (*Panthera leo*). This paper aims to review the case work analysed by SANBI GSU and VGL over a seven-year period ranging from August 2017 to July 2024.

On average, 537 cases were analysed in South Africa (SANBI GSU and VGL) per annum amounting to a cumulative 3 763 wildlife forensic

cases (8279 samples) over the seven-year period. The number of cases analysed per year is indicated in Fig. 1. Year 3 represented a significantly lower number of cases per year (12) because no SAPS contract was in place with any laboratory between September 2019 to October 2020 and does not necessarily represent a decline in wildlife crime. The Covid 19 epidemic that led to movement restrictions in the country during this period also contributed to delays in the contracting processes.

Geographical representation of the total cases analysed (see Fig. 2) indicated that most cases analysed originated from regions across the north-eastern parts of the country including the Limpopo (17%), Mpumalanga (40%) and KwaZulu-Natal (KZN, 25%) provinces. This is most likely due to the high number of illegal trade seizures and poaching cases that occur in and around KNP which is known as a hotspot area for both rhinoceros and African elephant poaching. Many perpetrators are apprehended within the park itself, often in possession of weapons and tracking gear. In other cases, suspects are intercepted while transporting illicit wildlife products, such as rhino horn, beyond park borders, highlighting the park's role as both a target and a transit point in the illegal trade [61,62]. A growing concern is the increase of rhinoceros poaching in the Hluhluwe-iMfolozi Park, KZN accounting for 62% of rhinoceros poached in 2023 [63]. The low representation across the remaining six provinces (Gauteng (5%), Northwest (6%), Eastern Cape (3%), Northern Cape (2%), Western Cape (1%) and Free State (2%)) could suggest that there is less poaching in these areas, due to fewer of these species being present in these areas or it could point to under-resourced monitoring, limited ranger presence, or gaps in intelligence-led enforcement. Strengthening detection mechanisms and inter-agency coordination in these underrepresented provinces could help uncover hidden trends and improve national anti-poaching strategies.

4.1. Taxonomic and species representation

Mammals represent the most prevalent target taxonomic group, accounting for 94% of all cases analysed (taxonomic representation indicated in Fig. 3). This is followed by invertebrates (3.3%) and fish (1.9%) with the lowest representation across reptiles (0.4%) and birds (0.3%). The high predominance of mammals is mainly because of the large numbers of rhinoceros species (white rhinoceros and black rhinoceros) analysed which accounts for more than 72% of all species encountered over the seven-year period. The high percentage of mammals is further attributed to large numbers of African elephant (6.5%), lion (5.3%) and greater kudu (*Tragelaphus strepsiceros*, 2.7%) species analysed. Together these five mammal species account for 87% of all species analysed over the seven-year period. Species representation is indicated in Fig. 4.

Birds and reptiles have not been observed frequently over the seven-year period. Birds were represented by small numbers of white-backed vultures (*Gyps africanus*, 17%) and ostrich, *Struthio camelus* (30%). The remainder were exotic species encountered as part of live confiscations. Reptiles commonly encountered include Nile crocodile (*Crocodylus niloticus*) (21%) and pythons (*Python spp.*) (18%) predominantly submitted as dried skins. In addition to these two species, small numbers of lizards (10%), gecko's (7%) and tortoises (14%) were observed.

Fishes were more prevalent (1.9%), mainly due to the large number of sharks analysed. These were mainly shark fins confiscated from points of export and included mostly CITES Appendix II listed species. In a report published by TRAFFIC [64] South Africa is a primary source country for shark fins and recorded a total mass of 930 tonnes shark fins imported worldwide from South Africa between 2012 and 2020. This remains an ongoing trend.

Invertebrates were predominantly represented by abalone. According to TRAFFIC [65] approximately 96 million individual abalone have been poached over a study period of 10 years (2006 – 2016) with an average mass of 2 174 tonnes illegally poached per year. The abalone illegal fishery has since grown into a highly organised system driven by

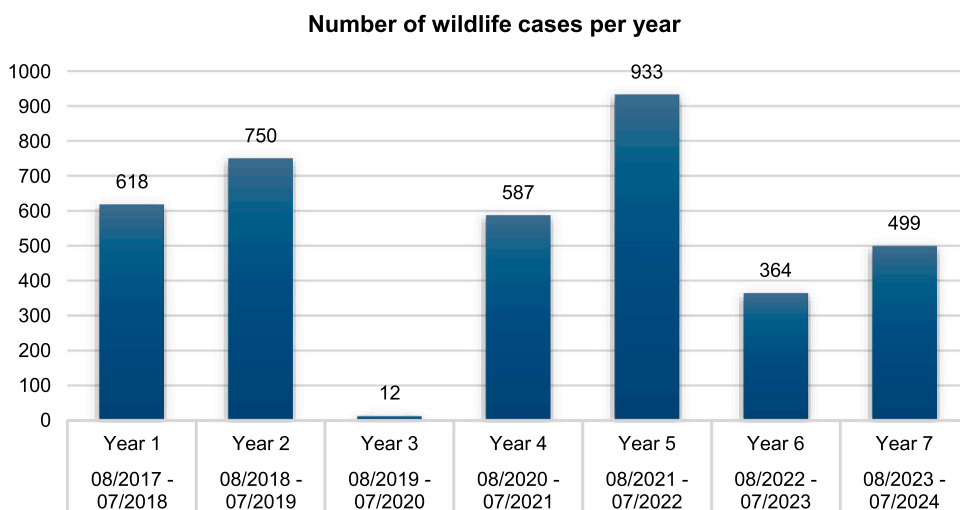


Fig. 1. Number of cases analysed per year over the seven-year period.

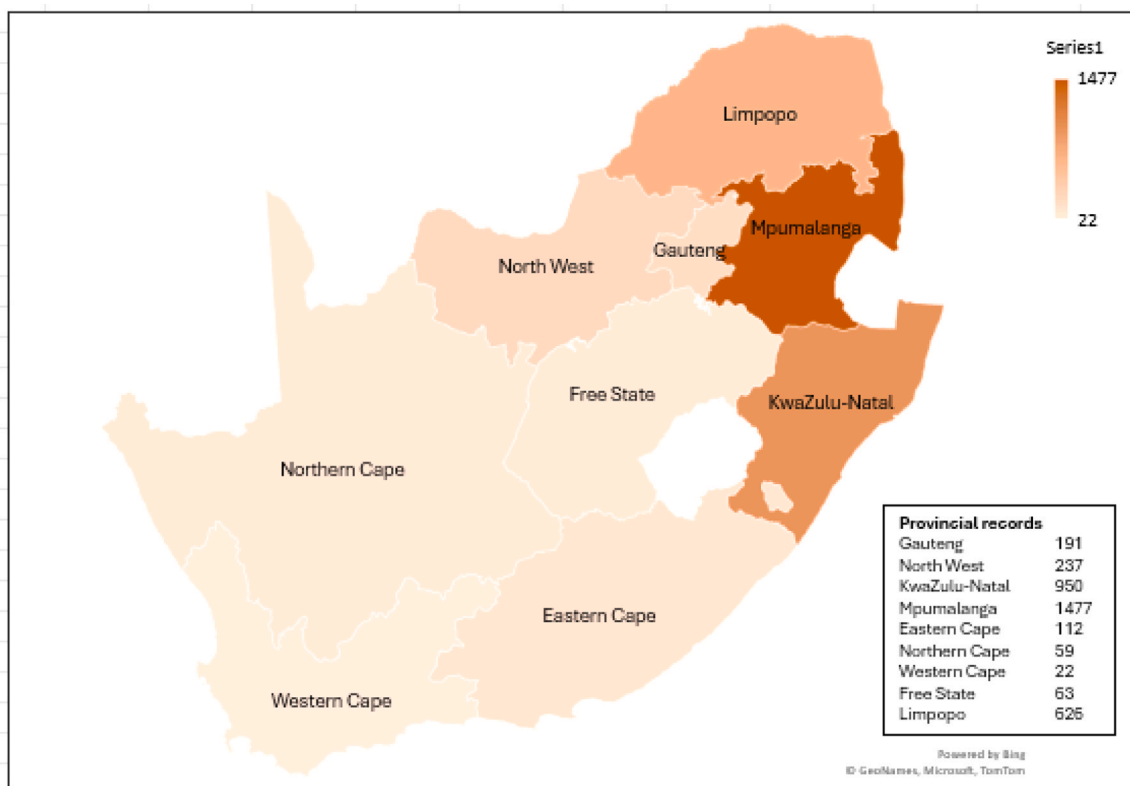


Fig. 2. Geographical representation of cases analysed across the nine provinces in South Africa.

international syndicates facilitated by the high values on black markets, low risk of detection and poor conviction rates. Poaching is driven by a combination of factors, including low risk of detection due to limited surveillance capacity along South Africa’s extensive coastline, and weak deterrence due to low fines and penalties as well as poor conviction rates. Efforts to curb the illegal trade have included the listing of abalone under CITES Appendix III, the implementation of territorial user rights systems, and targeted enforcement operations. However, these measures have had limited success in dismantling the entrenched networks that control the trade [66–68].

4.2. Analysis requests

Within South Africa, requests are generally grouped under 1) DNA analysis for species identification and 2) DNA analysis for comparison. Comparison analysis typically includes individual identification for matching of samples or comparison against the existing DNA database. In some instances, requests are for addition to the database for future comparison. An example of this is when an elephant carcass is discovered with tusks removed, samples are collected for profiling, and the DNA profile of the individual is added to an elephant database kept by the respective laboratories. Once tusks are recovered from national law enforcement operations the DNA profile from the tusk can be compared

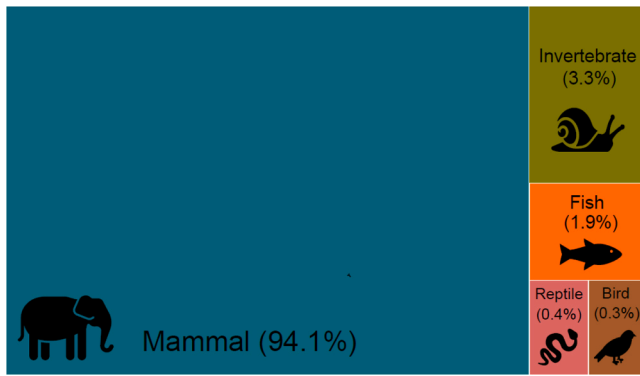


Fig. 3. Taxonomic representation of cases analysed in South Africa over a seven-year period (August 2017 to July 2024).

to the database in order to establish a match/link. Occasionally, both species identification and comparison analysis are requested. In these cases, species identification usually serves as an initial screening test to ensure that a suitable STR panel is selected and used. Samples of rhinoceros are recovered from all poached animals as part of the crime scene investigation and submitted to the VGL to be added to the RhODIS

database for comparison whenever horns are recovered within South Africa or internationally. Matching requests to the database also include blood that is found on clothing or equipment used in the commissioning of the crime such as axes and knives. Approximately 10 % of cases submitted to RhODIS are horns, equipment or clothing where the request is a match to DNA profiles from carcasses recorded on the database. Fig. 5 represents the distribution of the type of analysis across the different taxonomic groups.

Other mammal species encountered for comparison analysis purposes include African elephant, lion and various antelope species including greater kudu, springbok (*Antidorcas marsupialis*), gemsbok (*Oryx gazella*), impala (*Aepyceros melampus*) and nyala. The majority of requests for the other taxonomic groups is for species identification purposes (birds (87 %), reptiles (100 %), fish (100 %) and invertebrates (100 %)) while only a small proportion (>10 %) of mammals are analysed for identification of species, largely due to the nature of how mammalian specimens are typically seized. In many cases involving mammal whole carcasses are recovered during enforcement operations. This allows for species identification to be conducted through morphological assessments, which are often sufficient when diagnostic features are intact. Regardless, species commonly identified included Temminck's ground pangolin, hippopotamus (*Hippopotamus amphibius*), white-backed vulture, Nile crocodile, abalone and sea cucumber

Mammal	
Rhinoceros, <i>Ceratotherium simum</i> / <i>Diceros bicornis</i>	5642
Aardvark, <i>Orycteropus afer</i>	16
Aardwolf, <i>Proteles cristata</i>	1
African elephant, <i>Loxodonta africana</i>	505
Black-bellied pangolin, <i>Phataginus tetradactyla</i>	11
Blesbok, <i>Damaliscus pygargus phillipsi</i>	10
Blue wildebeest, <i>Connochaetes taurinus</i>	10
Brown hyena, <i>Parahyaena brunnea</i>	1
Buffalo, <i>Syncerus caffer</i>	25
Bushbuck, <i>Tragelaphus scriptus</i>	22
Chacma baboon, <i>Papio ursinus</i>	6
Cheetah, <i>Acinonyx jubatus</i>	2
Common duiker, <i>Sylvicapra grimmia</i>	21
Cow, <i>Bos taurus</i>	26
Donkey, <i>Equus asinus</i>	7
Eland, <i>Tragelaphus oryx</i>	2
Fallow deer, <i>Dama dama</i>	2
Gemsbok, <i>Oryx gazella</i>	24
Giraffe, <i>Giraffa camelopardalis</i>	1
Goat, <i>Capra hircus</i>	2
Greater kudu, <i>Tragelaphus strepsiceros</i>	207
Hare, <i>Lepus saxatilis</i>	5
Hartebees, <i>Alcelaphus buselaphus</i>	4
Hippopotamus, <i>Hippopotamus amphibius</i>	16
Horse, <i>Equus caballus</i>	2
Impala, <i>Aepyceros melampus</i>	37
Leopard, <i>Panthera pardus</i>	25
Lion, <i>Panthera leo</i>	410
Mongoose, <i>Herpestes javanicus</i>	1
Nyala, <i>Tragelaphus angasii</i>	25
Pig, <i>Sus scrofa</i>	8
Porcupine, <i>Hystrix cristata</i>	3
Reedbuck, <i>Redunca spp.</i>	6
Sable, <i>Hippotragus niger</i>	16
Sheep, <i>Ovis aries</i>	7
Southern reedbuck, <i>Redunca arundinum</i>	6
Spotted hyena, <i>Crocuta crocuta</i>	5
Springbok, <i>Antidorcas marsupialis</i>	12
Steenbok, <i>Raphicerus campestris</i>	9
Temminck's ground pangolin, <i>Smutsia temminckii</i>	54
Tiger, <i>Panthera tigris</i>	8
Tsessebe, <i>Damaliscus lunatus</i>	4
Warthog, <i>Phacochoerus africanus</i>	52
Waterbuck, <i>Kobus ellipsiprymnus</i>	10
White rhinoceros, <i>Ceratotherium simum</i>	11
White-bellied pangolin, <i>Phataginus tricuspis</i>	20
Wildebeest, <i>Connochaetes spp.</i>	1
Zebra, <i>Equus burchelli</i>	11

Bird	
Amazon parrots, <i>Amazona spp.</i>	5
Blue-and-yellow macaw, <i>Ara ararauna</i>	2
Ostrich, <i>Struthio camelus</i>	7
Red-fan parrot, <i>Deroparyus accipitrinus</i>	1
Sulphur-crested cockatoo, <i>Cacatua galerita</i>	1
White cockatoo, <i>Cacatua alba</i>	3
White-backed vulture, <i>Gyps africanus</i>	4

Reptile	
Armadillo girdled lizard, <i>Ouroboros cataphractus</i>	2
Common giant ground gecko, <i>Chondrodactylus angulifer</i>	2
Geometric tortoise, <i>Psammobates geometricus</i>	1
Hinge-back tortoise, <i>Kinixys spp.</i>	1
Karoo girdled lizard, <i>Karusasaurus polyzonus</i>	1
Nile crocodile, <i>Crocodylus niloticus</i>	6
Puff adder, <i>Bitis arietans</i>	1
Python, <i>Python spp.</i>	5
Red-eared slider, <i>Trachemys scripta elegans</i>	2
Southern African rock python, <i>Python natalensis</i>	2
Speke's hinge-back tortoise, <i>Kinixys spekii</i>	2
Water monitor, <i>Varanus salvator</i>	1

Fish	
Blacktip shark, <i>Carcharhinus limbatus</i>	1
Blue shark, <i>Prionace glauca</i>	67
Bowmouth guitarfish, <i>Rhina ancylostoma</i>	1
Common pike conger, <i>Muraenesox bagio</i>	8
Copper shark, <i>Carcharhinus brachyurus</i>	4
Giant guitarfish, <i>Rhynchobatus djiddensis</i>	1
Giraffe seahorse, <i>Hippocampus camelopardalis</i>	21
Great hammerhead, <i>Sphyrna mokarran</i>	3
Pigeye shark, <i>Carcharhinus amboinensis</i>	3
Scalloped hammerhead, <i>Sphyrna lewini</i>	4
Sea pony, <i>Hippocampus fuscus</i>	1
Sharks, <i>Carcharhinus spp.</i>	1
Shortfin mako shark, <i>Isurus oxyrinchus</i>	29
Smooth hammerhead, <i>Sphyrna zygaena</i>	1
Smoothnose wedgefish, <i>Rhynchobatus laevis</i>	1
Spinner shark, <i>Carcharhinus brevipinna</i>	2
Tiger shark, <i>Galeocerdo cuvier</i>	2
White-spotted guitarfish, <i>Rhynchobatus australiae</i>	3

Invertebrate	
Abalone, <i>Haliotis midae</i>	218
Sea cucumber, <i>Stichopus naso</i>	24
Sea cucumber, <i>Actinopyga echinites</i>	5
Sea cucumber, <i>Holothuria scabra</i>	3
Sea cucumber, <i>Sticophus chloronotus</i>	6
Sea cucumber, <i>Holothuria nobilis</i>	1

Fig. 4. Species representation of cases analysed in South Africa over a seven-year period (August 2017 to July 2024).

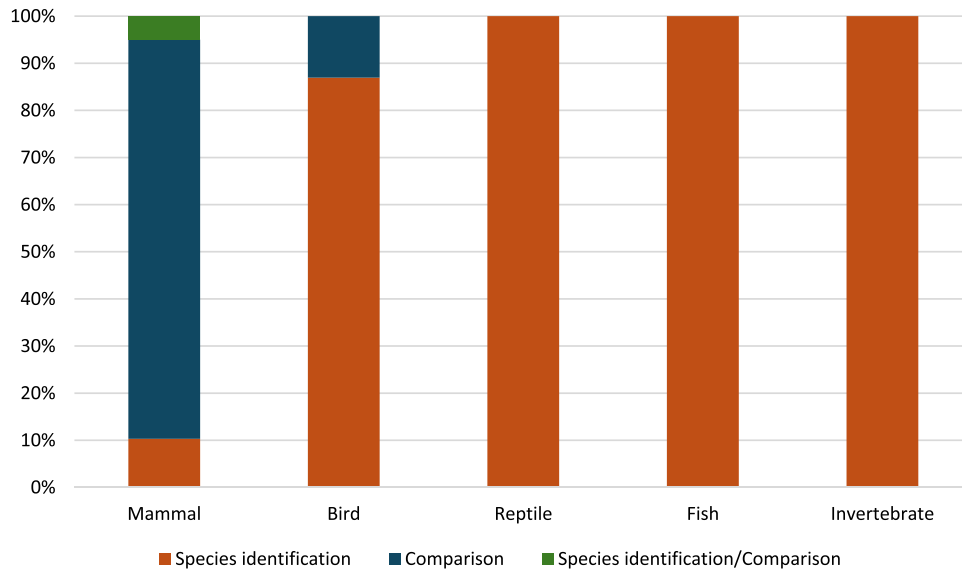


Fig. 5. Representation of type of analysis across different taxonomic groups for cases analysed in South Africa in a seven-year period (August 2017 to July 2024).

(*Stichopus naso*). The identification of species remains critical in supporting successful prosecutions where illegal trade or possession of CITES or ToPS listed species is suspected.

Biological evidence submitted include blood (1.00 %), tissue/skin (39.00 %), hair (0.62 %), horns (18.20 %), ivory (4.54 %), scales/claws/nails (6.95 %), bones (11.30 %), swabs (4.04 %), clothing (0.74 %), tools or gear (9.15 %) and whole carcasses (1.40 %) (Fig. 6). Blood samples are often collected from crime scenes where an animal has been killed or poached and can include dried blood from soil, rocks and leaves. Occasionally, blood samples are submitted that were collected from live confiscations of for example pangolins or parrots. Tissue samples are submitted most commonly and are usually collected from carcasses of animals such as rhinos killed for their horns, lions poisoned with their heads and paws removed or shark fins confiscated. Tissue samples are also often collected from suspects in possession of meat. Rhinoceros horn as well as ivory samples are commonly submitted

for confirmation of species and to link to carcasses. Carcass samples from many rhinoceros poaching cases also include bone samples due to the presence of predators and scavengers that remove most of the meat from the carcass rapidly, particularly in the larger game parks. Tools used for poaching of for example rhinoceros are also commonly submitted to link suspects to a crime scene. While in abalone cases the lifters and diving gear are submitted for detection of abalone DNA. Whole carcasses are frequently seen in cases of sea cucumbers or seahorses for identification of species. Other types of samples include amongst other powders, bile/gallbladders, fluids, faeces and slime.

5. Challenges encountered in non-human forensic analysis and future recommendations

The growing demand for non-human forensic DNA testing in South Africa has underscored the urgent need for robust, locally validated

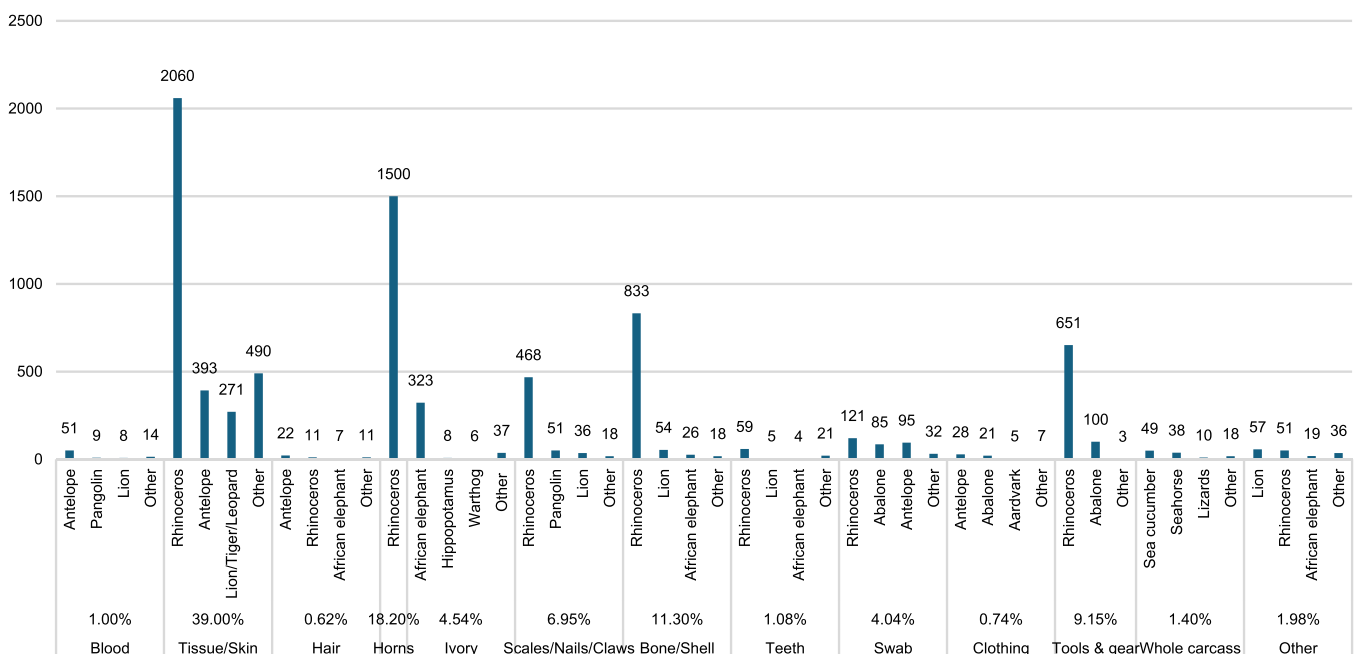


Fig. 6. Representation of biological evidence of cases analysed in South Africa in a seven-year period (August 2017 to July 2024).

molecular techniques that are responsive to the country's extraordinary biodiversity and complex legal framework. South Africa is home to a vast array of endemic and threatened species, many of which are targeted by transnational wildlife trafficking networks. As a result, forensic genetics has become an increasingly vital tool in the investigation and prosecution of wildlife crime. However, despite notable scientific progress, the implementation of wildlife forensic genetics in South Africa remains constrained by a constellation of technical, institutional, and logistical barriers.

Technical challenges add a layer of complexity to wildlife forensic genetics. One of the most prominent differences between human and non-human forensics is the number of species encountered in wildlife forensic investigations compared to human forensic investigations. Often limited species and population information are available for species encountered in wildlife forensic casework. Reliable species identification depends on comprehensive and validated reference databases. However, many of South Africa's endemic and threatened species are underrepresented in global databases, and local efforts to build these repositories are hampered by difficulties in obtaining voucher specimens, especially for rare or protected species. Obtaining voucher specimens for development of reference database for forensic purposes can be logistically challenging due to the rarity of species that is often targeted for illegal trade purposes. Collecting wildlife samples and obtaining the necessary permits, from various geographic origins is expensive and time consuming with various risks, including animals being distressed or dying during invasive sampling. For some species, such as the giant ground pangolin (*Smutsia gigantea*), not many individuals are left in the wild and due to the transboundary nature of wildlife crime, species encountered might not be indigenous to the country.

In some instances, taxonomy is not clearly defined, or changes occur in taxonomy. One specific example is the classification of African savanna elephant (*Loxodonta africana*) and African forest elephant (*Loxodonta cyclotis*) as two separate species rather than subspecies. The subsequent challenge is that only African savanna elephant is listed in the ToPS list and protected within South Africa from a legislative perspective. However, genetic technologies are not yet developed or appropriately validated to distinguish between these two species for forensic purposes. A similar challenge arises with giraffes. For decades, giraffes were classified as a single species (*Giraffa camelopardalis*) with up to nine subspecies. However, genetic research by Fennessy *et al.* (2016) [69] and Coimbra *et al.* (2021) [70] has challenged this view and have identified four genetically distinct species: the northern giraffe (*G. camelopardalis*), reticulated giraffe (*G. reticulata*), Masai giraffe (*G. tippelskirchi*), and southern giraffe (*G. giraffa*), while the IUCN currently lists giraffes as a single species classified as "Vulnerable".

This is further complicated because of hybridisation between species. Hybridisation is when individuals from two different species reproduce offspring with a combination of both species DNA. This poses a serious challenge in wildlife forensic analysis and can result in misidentification of species due to the maternal inheritance of mitochondrial DNA used for the identification of species. In South Africa, several species are known or suspected to hybridise. These events are often driven by wildlife ranching practices, where species are moved between farms. Such translocations increase the likelihood of contact between previously isolated taxa, making hybridisation more common across private and commercial game farms. Among mammals, for example, hybridisation between bontebok (*Damaliscus pygargus pygargus*) and blesbok (*D. p. phillipsi*) has been widely documented, threatening the genetic integrity of the bontebok population. Similarly, anthropogenic translocations have led to hybridisation between black-faced impala (*Aepyceros melampus petersi*) and common impala (*A. m. melampus*) [71,72]. In these instances, it is necessary to supplement mtDNA with nuclear DNA technologies to correctly identify the species. Here, it is of utmost importance to obtain prior information on the species and populations.

The co-amplification of sections of the mitochondrial and nuclear

DNA (numts) can also pose a challenge in the identification of species and is particularly common in the *Panthera* genus [73]. This resulted in several erroneous sequences on public reference databases which can subsequently lead to misidentification of species in wildlife forensic casework. Here, it is important to develop specific DNA technologies to avoid the amplification of numts but also to obtain appropriate reference specimens and reference data for use in casework.

The development of technologies for forensic application relies largely on conservation genetics research. However, these technologies and methods need to adhere to international standards and be validated to ensure that results derived from it are admissible in a court of law. In order to ensure standardization of methods applied in wildlife forensic analysis the International Society of Forensic Genetics (ISFG) published a set of 13 recommendations [52] followed by several review papers on the standards and guidelines for non-human forensics [74,75]. These recommendations focus on reference samples, species identification and individual identification in non-human analysis. However, there are several challenges in the implementation of these standards. For example, many species targeted by poachers in South Africa, such as pangolins, leopards, and rhinos, exhibit low genetic diversity due to population bottlenecks and inbreeding. This reduces the discriminatory power of genetic markers and complicates individual identification.

In addition, wildlife forensic research in South Africa is chronically underfunded which can limit the development and validation of new markers and technologies tailored to local conservation needs. While human forensic services already face severe backlogs, wildlife cases often receive lower priority, further delaying justice and undermining deterrence. Infrastructure limitations are compounded by a shortage of trained personnel with expertise in non-human DNA analysis. This skills gap hampers the development and validation of species-specific markers and reduces the capacity to interpret complex genetic data, such as that arising from hybridisation or low-diversity populations. Further, ensuring the integrity of forensic evidence from field to courtroom is a persistent challenge. Many wildlife officers and veterinarians lack formal training in crime scene management, increasing the risk of contamination or procedural errors that could render evidence inadmissible.

In addition, understanding the impact of wildlife forensic genetic analysis may be helpful to obtain funding for further research. Thus, tracking of successes and challenges when wildlife forensic case reports are submitted to courts in South Africa would be meaningful to provide insight into the effectiveness of genetic evidence in increasing convictions and sentences.

It is thus recommended that investment in validated marker panels, curated reference databases, and personnel training should be prioritized. Doing so will enhance the reliability and impact of wildlife forensic investigations in South Africa, ensuring that scientific evidence can effectively support biodiversity conservation and the prosecution of wildlife crime.

CRediT authorship contribution statement

Mamadi Theresa Sethusa: Writing – review & editing, Conceptualization. **Cindy K Harper:** Writing – review & editing, Writing – original draft. **Desiré Lee Dalton:** Writing – review & editing, Writing – original draft, Conceptualization. **Marli de Bruyn:** Writing – original draft, Formal analysis, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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