SANBI
Biodiversity for Life
South African National Biodiversity Institute

National Biodiversity Assessment 2018

TECHNICAL REPORT

Volume 7:

Genetic Diversity

The NBA 2018 was undertaken primarily during 2015 to early 2019 and therefore the names and acronyms of government departments in existence during that period are used throughout the NBA reports. Please refer to www.gov.za to see that changes in government departments that occurred in mid-2019.

This report forms part of a set of reports, datasets and supplementary materials that make up the South African National Biodiversity Assessment 2018. Please see the website [http://nba.sanbi.org.za/] for full accessibility to all materials.

SYNTHESIS REPORT
For reference in scientific publications
Skowno, A.L., Poole, C.J., Raimondo, D.C., Sink, K.J., Van Deventer, H., Van Niekerk, L., Harris, L.R., Smith-Adao, L.B., Tolley, K.A., Zengeya, T.A., Foden, W.B., Midgley, G.F. & Driver, A. 2019. National Biodiversity Assessment 2018: The status of South Africa's ecosystems and biodiversity. Synthesis Report. South African National Biodiversity Institute, an entity of the Department of Environment, Forestry and Fisheries, Pretoria. http://hdl.handle.net/20.500.12143/6362

For reference in non-scientific publications
South African National Biodiversity Institute (SANBI). 2019. National Biodiversity Assessment 2018: The status of South Africa's ecosystems and biodiversity. Synthesis Report. South African National Biodiversity Institute, an entity of the Department of Environment, Forestry and Fisheries, Pretoria.

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2: Inland Aquatic (Freshwater)
Van Deventer, H., Smith-Adao, L., Collins, N.B., Grenfell, M., Grundling, A., Grundling, P-L., Impson, D., Job, N., Lötter, M., Ollis, D., Petersen, C., Scherman, P., Sieben, E., Snaddon, K., Tererai, F. & Van der Colff, D. 2019. *South African National Biodiversity Assessment 2018: Technical Report. Volume 2: Inland Aquatic (Freshwater) Realm*. CSIR report number CSIR/NRE/ECOS/IR/2019/0004/A. South African National Biodiversity Institute, Pretoria. http://hdl.handle.net/20.500.12143/6230

3: Estuarine
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4: Marine
Sink, K.J., Van der Bank, M.G., Majiedt, P.A., Harris, L., Atkinson, L., Kirkman, S. & Karenyi, N. (eds). 2019. *South African National Biodiversity Assessment 2018 Technical Report Volume 4: Marine Realm*. South African National Biodiversity Institute, Pretoria. South Africa. http://hdl.handle.net/20.500.12143/6372

5: Coast
Harris, L.R., Sink, K.J., Skowno, A.L. & Van Niekerk, L. (eds). 2019. *South African National Biodiversity Assessment 2018: Technical Report. Volume 5: Coast*. South African National Biodiversity Institute, Pretoria. http://hdl.handle.net/20.500.12143/6374

6: Sub-Antarctic Territory
Whitehead, T.O., Von der Meden, C., Skowno, A.L., Sink, K.J., Van der Merwe, S., Adams, R. & Holness, S. (eds). 2019. *South African National Biodiversity Assessment 2018 Technical Report Volume 6: Sub-Antarctic Territory*. South African National Biodiversity Institute, Pretoria. http://hdl.handle.net/20.500.12143/6375

7: Genetics
Tolley, K.A., da Silva, J.M. & Jansen van Vuuren, B. 2019. South African National Biodiversity Assessment 2018 Technical Report Volume 7: Genetic Diversity. South African National Biodiversity Institute, Pretoria. http://hdl.handle.net/20.500.12143/6376

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GLOSSARY OF TERMS

Adaptation – An evolutionary process whereby specific alleles or gene mutations result in phenotypes that may be better suited to specific environmental conditions, and provide an advantage to such organisms.

Allele – A variant of a gene. Within the nuclear genome, each gene will have two alleles, which were inherited from each parent. Different alleles can lead to different expression of a trait.

Allelic diversity (N_A) – The presence and number of different alleles at a gene locus.

Bioinformatics – An interdisciplinary field science that combines biology, computer science, mathematics and statistics to analyse and interpret biological data.

Biomonitoring – The act of observing and assessing the state and ongoing changes in ecosystems, components of biodiversity and landscape, including the types of natural habitats, populations and species.

Deleterious mutation – Mutations that are selected against, or removed from, populations because of an unfavoured phenotypic expression.

Effective population size (N_e) – The size of an ideal population that would have the same rate of genetic change as the population under consideration. Ne influences the rate of loss of genetic variation, the efficiency of natural selection and the accumulation of mutations. As a rough guideline, Ne approximates the number of breeding individuals producing offspring that live to reproductive age.

Environmental DNA (eDNA) - Trace DNA in samples such as water, soil, or faeces. eDNA is a mixture of potentially degraded DNA from many different organisms. It is important to note that this definition remains controversial due to the sampling of whole microorganisms that might appear in an environmental sample. Although metagenomic microbial studies might use environmental sampling, they cannot always be defined as true eDNA studies because some methods first isolate microorganisms from the environment before extracting DNA.

Evolutionary distinctiveness (ED) – the distinctiveness of a species as measured by the amount of its unique evolutionary history in a phylogeny.

Fixation index (F_{ST}) – A standardized index of the distribution of genetic variation between populations on a scale between 0 (identical allele frequencies among populations) and 1 (populations fixed for different alleles).

GenBank – A repository of genetic information hosted and managed by the National Institute of Health in the USA.

Gene – The fundamental unit of heredity, made up of DNA. Genes are organised into chromosomes in the cell nucleus, or found in the mitochondria or chloroplasts (plants only).

Genetic diversity – The number of different alleles or haplotypes for a population or species. High genetic diversity means the sample of individuals from a population or species have many different versions (alleles or haplotypes) of the gene that was quantified.

Gene flow – The transfer of genetic variation from one population to another.

Genetic diversity – The number of different alleles or haplotypes for a population or species. High genetic diversity means the sample of individuals from a population or species have many different versions (alleles or haplotypes) of the gene that was quantified.

Genetically Modified Organism (GMO) – An organism who's DNA has been altered or modified in some way through genetic engineering. Genetic engineering can either incorporate novel genes in their entirety or manipulate existing gene sequences.

Haplotype – A DNA marker, or combination of markers, that are passed on from one generation to the next as a single unit. Individuals share a haplotype if their inherited DNA for a specific section or marker-set is identical.

Haplotype – In a haploid genome (e.g. mitochondrion or chloroplast), a variant of a gene.

Haplotype diversity – The probability that two randomly sampled alleles are different.

Heterozygosity – The condition when an individual has two different alleles for one gene.

Heterozygous – An organism is heterozygous for a given locus if they have two different alleles.

Homozygosity – The condition of an individual that has the same allele for one gene.

Homozygous – An organism is homozygous for a given locus if they have two identical alleles.

Introgression – Refers to the transfer of genetic material or gene flow from one population to the next. Can have negative consequences if gene flow is between genetically distinct populations or species.

Metabarcoding – Taxonomic identification of multiple species extracted from a mixed sample (community DNA or eDNA) which have been PCR-amplified and sequenced on a high-throughput platform (e.g. Illumina, Ion Torrent).

Microsatellite – Short tandem repeat sequence, usually comprising variable numbers of repeats of 2–5 nucleotides (e.g. CA). Different numbers of repeats result in different lengths of alleles.

Mitochondrial DNA (mtDNA) – The genome found within the mitochondria of eukaryotic cells. In sexually reproducing organisms, the mitochondrial genome is maternally inherited.

Next-generation sequencing (NGS) – NGS, or high-throughput sequencing, allows the sequencing of DNA and RNA much more rapidly and cheaply than the prior technology of Sanger sequencing, thus 'revolutionising' genomics and molecular biology. It is a catch-all term to describe a number of different sequencing methodologies including Solexa (Illumina), Roche 454, Proton/PGM, PacBio, GridION/ MinION and SOLiD sequencing.

Nucleotide diversity – The average proportion of nucleotide differences between all possible pairs of sequences in the sample.

Outbreeding depression – Can result from the breeding of individuals that derive from very different environments and/or with markedly different adaptations in their DNA. Typically lead to the disruption of co-adapted gene complexes, with the offspring suffering from reduced fitness.

Phylogenetic – The evolutionary relationships among biological entities, often species.

Phylogenetic Diversity – A biodiversity richness metric that incorporates phylogenetic differences between taxa.

Phylogenetic Endemism – A biodiversity richness metric that incorporates phylogenetic differences between taxa, weighted by range size.

Phylogeographic – The historical processes that may be responsible for the contemporary geographic distributions of populations, as examined by their genetics.

Polymerase Chain Reaction (PCR) – A widely used technique used in molecular biology to exponentially amplify a single copy or a few copies of a specific segment of DNA to generate thousands to millions of copies of a particular DNA sequence.

Population – A set of interbreeding individuals of a species, where gene flow is not reduced, resulting in a homogenous gene pool.

Population – A group of interbreeding individuals.

Recombination – Occurs when individuals from different populations, or with different genetic make-up, produce offspring which have traits that are very different to either parent. Can also refer to the exchange of genetic material during meiosis.

Single nucleotide polymorphism (SNP) – A nucleotide site in a DNA sequence where more than one nucleotide (G, A, T or C) is present in the population.

EXECUTIVE SUMMARY

Life on earth relates directly to the diversity of genes in space and time. The genomes of organisms encode the basic biological structures that define them, and allows individuals to survive and persist through time in changing environments. To this end, DNA can best be described as the foundation of all life on earth, it is recognised as an important component of biodiversity (together with species diversity and ecosystem diversity) and the importance of maintaining genetic diversity has been highlighted by the Convention on Biological Diversity.

Genetic diversity can be defined as the amount of variation observed in the DNA of distinct individuals, populations or species. The maintenance of this diversity is of the utmost importance as genetic diversity allows species or populations to adapt to an ever-changing environment. Risks to genetic diversity include genetic erosion through e.g. habitat fragmentation and habitat loss, hybridisation and inbreeding, unsustainable use of species, disease via translocations of individuals, and species extinctions. Genetically modified organisms also present a risk through the escape of undesirable genes into native populations.

To recognise and minimise genetic erosion, genetic diversity should be monitored over time for a given species or population. The value of long-term monitoring is well recognised; however, globally, there is a lack of temporal genetic datasets, as well as a lack of genetic diversity indicators and thresholds, with which data can be compared (such indicators have been developed, but lack specific genetic input). To date within South Africa, few short-term monitoring studies have been carried out that explicitly monitor temporal shifts in the genetic diversity of South African taxa. These studies serve as a baseline and provide valuable insight into ongoing and potential future monitoring programmes.

The indicators to establish the status and to track trends for genetic diversity are not yet established. Using a case study to test indicators, trends at the national level were tracked by interrogating several high level metrics as indicators of genetic erosion. The case study analyses showed that the greatest historical impacts to phylogenetic richness for reptiles are in the northeast, southwest and the coastal margin of KwaZulu-Natal Province. For the case study, there are several hotspots of elevated genetic erosion in the last few decades, in particular northern KwaZulu-Natal Province, south-eastern Mpumalanga Province, northern Gauteng Province and southern and northern Limpopo Province. The case study highlights the types of indicators that could be used, but additional indicators and other case studies should be examined in the future.

To promote future genetic monitoring programmes and studies, a national genetic monitoring framework is required that outlines how to prioritise species for monitoring, what genetic markers and metrics to use, how often populations should be monitored. Moreover, such a framework would not only outline how genetic diversity can be monitored at a population or species level, but be extended to include monitoring for genetic erosion at the national level.

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1. SOUTH AFRICA'S BIODIVERSITY AND THE NATIONAL BIODIVERSITY ASSESSMENT

The National Biodiversity Assessment (NBA) is a collaborative effort to synthesise and present the best available science on South Africa's biodiversity. It aims to inform policy, planning and decision making in a range of sectors for the conservation and sustainable use of biodiversity.

The NBA is a platform for reporting on the current state of biodiversity within South Africa. It describes the key pressures on biodiversity and, where possible, identifies important trends. It covers the terrestrial, inland aquatic[1], estuarine and marine realms, as well as the coast and South Africa's sub-Antarctic territory as cross-realm zones. The NBA is used to illustrate the benefits that biodiversity and intact ecosystems provide to the economy, society and human wellbeing. Finally, the systematic approach of the NBA allows us to identify important national knowledge gaps and research priorities linked to biodiversity.

> **Biodiversity** is defined as the 'variability among living organisms from all sources including, *inter alia*, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and across ecosystems' (Convention on Biological Diversity).
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> Biodiversity incorporates diversity at the genetic, species and ecosystem level – which together form the foundation of ecosystem services and are integrally linked to human wellbeing.

1.1. South Africa's biodiversity profile

Identified as one the world's 17 megadiverse nations, South Africa ranks as one globally for plant species richness and third for marine species endemism. With million km[2] and surrounding seas of 1.1 million km[2], South Africa[2] is among the smaller of the world's megadiverse countries – which together contain more than two thirds of the world's biodiversity. South Africa also holds three of the world's 35 biodiversity hotspots (a measure of biological diversity combined with vulnerability to threats): the Cape Floristic Region, Succulent Karoo biome, and Maputaland-Pondoland-Albany centre of endemism.

Current statistics have the number of South African animal species estimated at 67 000, while 20 401 plant species have been described. Approximately 7% of the world's vascular plants; 5% of mammals; 7% of birds; 4% of reptiles; 2% of amphibians; 1% of freshwater fishes are found in South Africa. There is limited information on invertebrate groups, but South Africa has almost a quarter of global cephalopods (octopus, squid, and cuttlefish). Some terrestrial invertebrate groups have high richness relative to the global fauna.

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[1] Inland aquatic realm refers to rivers and inland wetlands – also referred to as the 'freshwater realm' – and may include saline inland water systems.

[2] South Africa's sub-Antarctic territories of Prince Edward Island, Marion Island and their surrounding seas cover an additional 0.5 million km[2.]

For example, 13% of the world's sunspiders (Solifugae), ticks (Ixiodidae) and silverfish/fishmoth (Zygentoma), and nearly 5% of butterflies occur in South Africa.

Around half of the mammals, reptiles, amphibians, butterflies and freshwater fishes found in South Africa are endemic. Plants have even higher levels of endemism, with two thirds of species considered endemic to South Africa – mostly linked to the unique Cape Floristic Region. High marine species endemism has consistently been reported for the Agulhas ecoregion on the south coast, which lies entirely within South Africa's territory and is geographically isolated from the globe's other warm temperate regions. Approximately 40% of South Africa's estimated 10 000 marine animal species are endemic, the vast majority of which are invertebrates.

Table 1.1. Summary of species richness and endemism for selected South African taxonomic groups, including global estimates.

Taxonomic group	Species	Endemics	Endemics as % of total SA species	Global estimates of number of species	Species in SA as % of global total
Amphibians	125	62	50%	7 934	1.6%
Birds	732	38	5%	11 122	6.6%
Butterflies	799	418	52%	17 500	4.5%
Dragonflies	162	28	17%	5 680	2.9%
Fishes (freshwater)	118	58	49%	14 953	0.8%
Fishes (marine)	~ 2 000	261	13%	~20 000	10.0%
Mammals	336	57	17%	6 399	5.3%
Octopus, squids	195	unknown	unknown	800	24.3%
Plants (vascular)	20 401	13 763	67%	304 419	6.7%
Reptiles	404	200	50%	10 793	3.7%
Seaweeds	563	~17	3%	~17 000	3.3%
Spiders	2 088	unknown	unknown	40 700	5.1%

South Africa's wide range of bioclimatic, oceanographic, geological and topographical settings have resulted not only in high species diversity and endemism, but also high ecosystem diversity and endemism across all realms. There is a wide variety of terrestrial biomes and marine ecoregions in South Africa, its surrounding seas and sub-Antarctic territory; ranging from the unique Fynbos biome to the extensive savannas and grasslands of the eastern interior, and from the subtropical Indian Ocean through the warm temperate Agulhas shelf to the cold upwelling influenced shelf of the southern Benguela (Figure 1.1.1). Situated 1 700 km south of the country, the Prince Edward Islands and their surrounding seas add a cold, sub-Antarctic set of ecoregions and biomes to South Africa's territory (Figure 1.1.1).
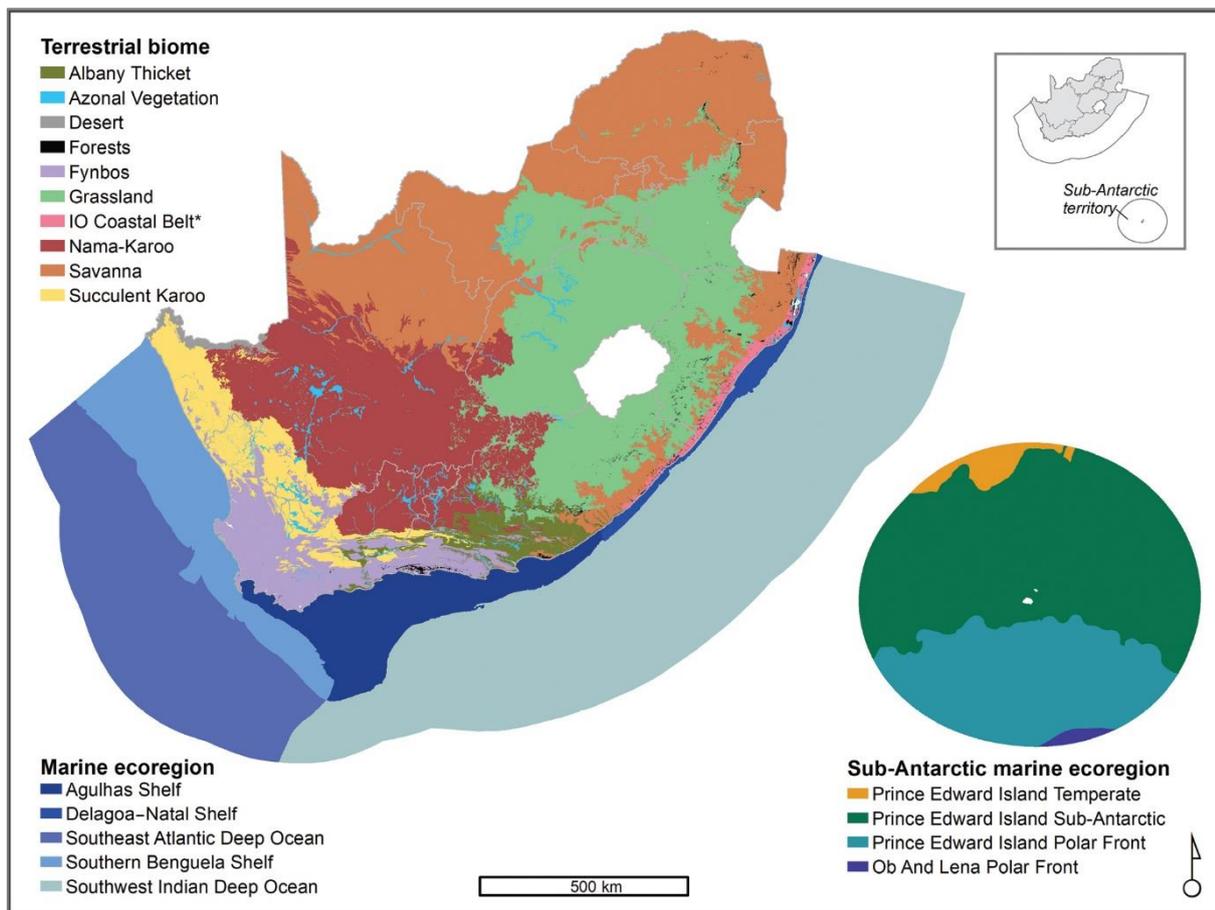
Figure 1.1. Terrestrial biomes and marine ecoregions of South Africa and the marine ecoregions surrounding the Prince Edward Islands group (South Africa's sub-Antarctic territory) lying 1 700 km southeast of the mainland. The vegetation of the sub-Antarctic islands is classified as either Sub-Antarctic Tundra or Polar Desert biome. *IO Coastal Belt refers to Indian Ocean Coastal Belt.

South Africa's **terrestrial** realm can be categorised into nine biomes and 458 ecosystem types, approximately 80% of which are endemic. The moist, winter-rainfall region in the southwest of the country is home to the unique Fynbos biome. Adjacent to this lies the Succulent Karoo biome, an arid winter-rainfall biome with the highest diversity of succulent plants in the world. The Nama-Karoo biome covers the arid, summer-rainfall, western interior. The Savanna biome (the largest biome in southern Africa) dominates the northern and eastern summer rainfall regions of South Africa. The Grassland biome occurs mostly on the cooler high lying central plateau and has high levels of endemism. The Albany Thicket biome occurs in the eastern and southern cape and contains a unique combination of plant forms with an Eocene origin and unique evolutionary history. The Forest biome (with warm temperate and subtropical types) is the smallest biome and is characterised by patches distributed across the winter and summer rainfall areas of the country. The Indian Ocean Coastal Belt biome represents the southernmost extent of the wet tropical seaboard of East Africa. The Desert biome occupies a small portion of the extreme northwest of the country, forming the southernmost extent of the Namib Desert.

South Africa's **marine** realm includes the Atlantic, Indian and Southern Oceans with the contrasting cold Benguela upwelling systems and the warm, fast-flowing Agulhas current. This diverse oceanographic setting combined with complex geology and topography drives exceptional marine biodiversity and a wide array of ecoregions and ecosystem types. Three shelf ecoregions are recognised; the cool temperate Southern Benguela, the warm temperate Agulhas and the subtropical Natal-Delagoa. The deep ocean beyond the

shelf edge includes two further ecoregions in the form of the Southeast Atlantic and the Southwest Indian. The Southern Benguela includes two sub-regions, the Namaqua and Cape regions, which separate at Donkin Bay (north of St Helena Bay) on the west coast. In addition, the Natal-Delagoa ecoregion includes the Delagoa, KwaZulu-Natal Bight and KwaZulu-Natal-Pondoland regions which have distinct biodiversity patterns. These ecoregions and sub-regions include 150 marine ecosystem types that include several functional ecosystem groups: Sandy Shores, Rocky and Mixed Shores, Islands, Bays, Kelp Forests, Soft Shallow Shelf, Shallow Reef and Rocky Shelf, Deep Soft Shelf, Deep Rocky Shelf, Slope, Plateau and Abyss.

South Africa is among the most water scarce countries per capita in the world, and has a high temporal and spatial variability of rainfall. This results in highly variable runoff and river flow regimes, and a relative scarcity but surprisingly rich variety of inland wetlands. The diversity of **river and inland wetland** ecosystem types (together comprising the inland aquatic realm) is underpinned by the strongly contrasting bioclimatic zones – the arid western interior (summer rainfall), the mesic eastern grassy biomes (summer rainfall), the arid western coastal regions (winter rainfall) and the mesic winter rainfall south Western Cape. The latest mapping data indicates that inland wetlands cover 2.2% of South Africa's surface area, though this is likely to be an underestimate. These wetlands are classified into 135 distinct ecosystem types on the basis of vegetation bioregions and hydrogeomorphic units. The diversity of river ecosystem types is driven by ecoregions, bioclimatic variation and geomorphological factors, resulting in 222 distinct types.

South Africa has 290 **estuaries** and 42 micro-estuaries which have been classified into 22 estuarine ecosystem types and three micro-system types. This represents a high diversity of estuary types stemming from diverse climatic, oceanographic and geological drivers. The comparatively small, wave-dominated South African estuaries generally have restricted inlets, with more than 75% closing for varying periods when a sandbar forms across the mouth. Four bioregions apply to South African estuaries: the Cool Temperate (Orange to Ratel), the Warm Temperate (Heuningnes to Mendwana), the Subtropical (Mbashe to St Lucia) and the Tropical (uMgobezeleni to Kosi).

For the NBA 2018, an ecologically determined **coast** (cross-realm) was defined and used, which spans the terrestrial, estuarine and marine realms. The South African coast is microtidal (<2 m range) and mostly high energy, with generally exposed to very exposed conditions from the subtropical northeast coast to cold temperate west coast. It comprises of dunes, cliffs, beaches, rocky and mixed shores, estuaries, mangroves, kelp and reefs, bays, river-influenced shelf regions and a wide range of coastal vegetation types (from forests to arid shrublands). With this heterogeneity comes exceptionally high coastal biodiversity and high levels of endemism, especially among dune plants, beach fauna and other invertebrate taxa. There are 186 ecosystem types that are considered coastal: 25 estuarine, 79 terrestrial and 85 marine, all of which are fundamentally influenced by both the land and sea.

South Africa's **sub-Antarctic territory** (cross-realm) consists of Prince Edward Island, Marion Island and surrounding seas (collectively known as the Prince Edward Islands, PEI), and is situated 1 700 km southeast of the mainland. These tiny islands and surrounding seas have a very different biodiversity profile from that of the mainland and its oceans. The islands are volcanic in origin and experience a cold temperate or polar climate with a strong oceanic influence; with five terrestrial ecosystem types described. There are 29 marine ecosystem types covering the shore, the territorial waters and exclusive economic zone, and these range from subtropical ecoregions in the north to polar ecoregions in the south.

1.2.   The importance of biodiversity

South Africa's biodiversity provides a wide array of benefits to the economy, society and human wellbeing. These benefits that nature can provide are dependent on intact ecosystems, healthy species populations and genetic diversity. Human activities present a range of direct and indirect pressures on biodiversity that need to be carefully considered with the need to maintain and protect biodiversity, and the benefits that are derived from biodiversity.

Biodiversity-related jobs number approximately 418 000 and the biodiversity-based tourism industry is worth over R30 billion per year. Intact ecosystems and high species diversity are essential for agricultural production, providing healthy populations of crop pollinators and natural predators of agricultural pests. Healthy rangelands support both livestock and wildlife ranching (the latter worth R14 billion per year). Intact catchments, wetlands and riparian systems help clean water supplies, attenuate floods and store water for times of drought – in so doing, they protect people from floods and droughts and help with adaptation to a changing climate. The harvesting of edible plants, edible insects, medicinal plants and building or weaving materials from the wild is widely practiced in South Africa and is an important part of the rural economy. The natural ecosystems, plants and animals have also influenced cultural and spiritual development, and are woven into languages, place names, religion and folklore. This web of associations with biodiversity forms part of South Africans' national identity and heritage.

Nelson Mandela said, '*Our people are bound up with the future of the land. Our national renewal depends upon the way we treat our land, our water, our sources of energy, and the air we breathe. …Let us restore our country in a way that satisfies our descendants as well as ourselves.*' This recognition of peoples' reliance on the natural environment and biodiversity was later further enshrined in the Constitution of o the Republic of South Africa (1996), which states that everyone has the right to an environment that is not harmful to their health or wellbeing; and to have that environment protected for the benefit of present and future generations through reasonable measures.

While biodiversity is a national asset and a powerful contributor to inclusive growth and job creation, its protection is at times cast as a hurdle to socio-economic development. This is unfortunate considering the extent to which biodiversity and use of biodiversity can contribute to the objectives in the National Development Plan 2030. The primary goals of reducing poverty and inequality in South Africa through stimulating the economy, improving employment figures, building an inclusive rural economy and providing affordable health care; all rely to some extent on biodiversity, healthy ecosystems, resilient ecological infrastructure and environmental sustainability.

> **Ecological infrastructure** refers to naturally functioning ecosystems that generate or deliver valuable services to people.

Every decision taken, whether by governments or individuals, affects the future of biodiversity. By investing in the restoration, protection and management of our biodiversity assets and ecological infrastructure, we enhance social and economic development and contribute to human wellbeing.

1.3. Purpose and structure of the NBA

The NBA is the primary tool for monitoring and reporting on the state of biodiversity in South Africa. It is prepared as part of the South African National Biodiversity Institute's (SANBI) mandate[3] to monitor and report regularly on the status of South Africa's biodiversity, and is a collaborative effort from many institutions and individuals. The NBA focusses primarily on assessing biodiversity at the ecosystem and species level, with efforts being made to include genetic level assessments. Two headline indicators that are applied to both ecosystems and species are used in the NBA: threat status and protection level. The products of the NBA include seven technical reports, a technical synthesis report and several popular outputs.

The primary **purpose** of the NBA is to provide a high-level summary of the state of South Africa's biodiversity at regular points in time, with a strong focus on spatial information. Each NBA builds on decades of research and innovation by South African scientists, and makes that science available in a useful form to users both inside and outside of the biodiversity sector. As a body of work the NBA is not prescriptive; it presents important information that can be adopted by government and civil society in various decision-making processes to support socio-economic imperatives, human wellbeing, and the best management and conservation of South Africa's biodiversity.

Like the previous assessments in 2004 and 2011, this third iteration of the NBA will feed into a range of processes within the environmental sector and beyond. Key applications include:
- Informing policies and strategies in the biodiversity sector (e.g. National Biodiversity Framework, National Protected Area Expansion Strategy), and other key sectors responsible for natural resources utilisation and/or protection, such as the water, agriculture, fisheries, and mining sectors (*e.g*. Mining and Biodiversity Guidelines).
- Providing information to help prioritise the often limited resources for managing and conserving biodiversity; including datasets that feed into site and regional level planning and assessment (e.g. Strategic Environmental Assessments and Environmental Impact Assessments) and provincial and municipal Bioregional Plans and Marine Spatial Plans (*i.e*. systematic biodiversity planning).
- Creating a key reference and educational work for use by scientists, students, consultants, decision makers and funders.
- Serving as an effective national level platform for encouraging and facilitating collaboration, information sharing and, importantly, capacity building in the biodiversity sector in South Africa.
- Providing information for a range of national and international level monitoring, reporting and assessment processes such as state of environment reporting and reporting on commitments to international conventions (e.g. linked to the United Nations Convention on Biological Diversity (CBD), the Sustainable Development Goals (SDGs) and the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES).

The NBA has a varied audience, of which each have different needs, hence the NBA is presented in various forms. The NBA website is the primary portal through which you can access all information and products [http://nba.sanbi.org.za/]. The NBA website also provides factsheets and presentations summarising the NBA for non-technical audiences, using graphics and easily interpretable language.

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[3] **SANBI's mandate is outlined in the National Environmental Management: Biodiversity Act (10 of 2004), hereafter referred to as the 'Biodiversity Act'.**

The NBA 2018 has *seven technical reports*: one for each realm (terrestrial, inland aquatic, estuarine and marine); two cross-realm technical reports (the coast and South Africa's sub-Antarctic territory); and a technical report on genetic diversity. The technical reports are comprehensive volumes covering all input data used for the assessments, detailed explanations of methods and approach, full results and discussion, key messages for decision makers, limitations and knowledge gaps, and priorities for the future. These reports are for a scientific and technical audience, and are fully referenced and peer- reviewed. The technical reports refer to the various supplementary technical documents, maps and datasets; all of which are available through the NBA website with accompanying metadata.

The *synthesis report* focuses on the main findings and key messages from each technical report. As the technical reports give full details of the methods and input data used for the NBA, the synthesis report only briefly discusses the building blocks and approach used on a broad level. The synthesis report is divided into four parts:
- Part One introduces the NBA, its contextual framework and relevance in the biodiversity sector, and provides a biodiversity profile for South Africa.
- Part Two contains the integrated national findings across all realms and presents the key messages from the NBA 2018.
- Part Three presents the main findings for each realm (terrestrial, inland aquatic, estuarine and marine), and two cross realm systems: the coast and the sub-Antarctic territory.
- Part Four addresses some of the interventions from the biodiversity sector that are aimed at addressing key pressures on biodiversity and outlines priority actions for enhancing these interventions. It reflects on the limitations of the current assessment and identifies research and monitoring required to strengthen future assessments.

1.4. Assessing genetic diversity for the National Biodiversity Assessment

The Convention on Biological Diversity highlights the importance of genetic diversity as a fundamental component of biodiversity. Therefore, an assessment of genetic diversity has been included in the National Biodiversity Assessment (NBA) for the first time. This assessment should be considered preliminary, and is aimed at highlighting issues regarding risks to the maintenance of genetic diversity, proposing methods for assessment and monitoring of genetic diversity, and testing of potential indicators. The current assessment is not a complete treatment of South Africa's genetic diversity, nor does it cover all potential methods that might be used. Furthermore, given that the NBA is aimed at assessing the *status and trends* of South Africa's biodiversity, the genetic component of the NBA is not a review of literature relating to studies that have utilised genetic techniques to quantify genetic diversity for South African taxa. Such studies are numerous and there are several literature reviews available (Linder *et al*. 2010; Lexer *et al*. 2013; Tolley *et al*. 2014; Verboom *et al*. 2014). Furthermore, individual studies of genetic population structure (or higher level diversity), while a valuable element of our baseline knowledge, are not meant to address *status and trends* of genetic diversity for South Africa. The majority of existing literature relate to uncovering population or species level differentiation and cover a single (or short) temporal point, providing a snapshot in time. Therefore, it is not possible to amass the literature to assess trends of genetic diversity over time. Neither can these studies provide an overall view of the *status* of genetic diversity for South Africa because they are not within a unifying framework. Rather, they report genetic patterns of various taxa within different landscapes and at different time points. Thus, this first addition of a genetic component to the NBA was set up to highlight these issues, to motivate for a comprehensive framework, and to test the waters regarding possible indicators.

The genetic component provides a motivation as to why including genes as a fundamental component of biodiversity is important, and highlights the factors that could pose a risk to maintaining genetic diversity. The need for a genetic monitoring framework to guide research in South Africa is underscored, and speaks to the goal of understanding the *status and trends* of priority taxa on a national scale. Finally, some novel approaches for potentially tracking the erosion of genetic diversity on the landscape at a phylogenetic level are investigated using a case study.

There are many aspects of genetic diversity that are not covered by the current genetic component of the NBA. There are additional taxa, other methods, and other objectives that could be developed in the future. The benefits of and risks to genetic diversity have been treated fairly comprehensively, but additional factors could be uncovered in the future. Although the establishment of a national genetic monitoring framework is advocated, the framework itself still requires development through by a multi-stakeholder engagement, collaboration with global bodies such as the GEOBON Genetics Working Group, and with careful consideration of all the possible indicators and approaches.

The assessment of genetic diversity as a component of biodiversity is in its infancy. To develop this further, a larger-scale, and more comprehensive strategy is needed that includes engagement of experts and stakeholders, as is an implementation team that can move the strategy forward from planning to analyses to actions.

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2. RISKS AND IMPACTS TO GENETIC DIVERSITY, PRESSURE AND BENEFITS

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Chapter Overview

This chapter covers the risks and impacts to biodiversity, as well as the genetic diversity relating to wild relatives of domestic species, game species and translocations/ hybrids/captive bred.

2.1. Benefits of genetic diversity

Life on earth relates directly to the diversity of genes in space and time. The genomes of organisms encode the basic physiological, phenological, behavioural, and biological structures that define them, and allows individuals to adapt and survive through time in changing environments. To this end, DNA can best be described as the foundation of all life on earth. DNA variation in a variety of species has secondary benefits to humans through contributions to horticulture, forestry, fisheries, game breeding and hunting, and agriculture.

2.1.1. Genetic diversity

Genetic diversity is recognized as an important component of biodiversity (together with species diversity and ecosystem diversity). It can be defined as the amount of variation observed in the Deoxyribonucleic acid (DNA) of distinct individuals. The maintenance of diversity is of the utmost importance as genetic diversity equates to evolutionary potential, and thus allow species or populations to adapt to an ever-changing environment. Importantly from a conservation perspective, the levels of genetic diversity seem to vary greatly in natural populations and species.

Although the field of genetic diversity has taken enormous strides forward (for example in understanding the effect of one migrant per generation exchanges between populations, the effects of habitat fragmentation, the strong relationship of plant traits and measures of genetic differentiation, and the 50/500 rule), the exact drivers of variation, and particularly the influence that species' biology (e.g., dispersal capability), mating system, social system, ecology (e.g., habitat preference), and population history has on this, remains to be fully understood. In addition, genetic diversity is often linked to mutations and adaptation across space and time. Genetic diversity is therefore a complex, multi-faceted concept which is influenced by a multitude of interrelated factors (organismal, climatic, and geological). To effectively understand the drivers behind genetic diversity, and to conserve these processes and manage potential risks, is a multi-disciplinary field which draws together several scientific disciplines.

Genetic information can be gathered across multiple spatial scales, from the individual and/or population level (population genetics, or landscape genetics sensu (Manel *et al.* 2003), at intermediate spatial scales (phylogeography sensu (Avise *et al.* 1987), or across large spatial scales, often involving a range of taxa or higher taxonomic groups (biogeography sensu (MacArthur & Edward 2001). Scale-dependant questions are typically asked, ranging from relatedness and genetic diversity within a population, how landscape influences local movement and gene flow within and between neighbouring populations, whether genetic lineages with independent evolutionary trajectories are present, and whether congruent genetic patterns characterize higher taxonomic groups. Species distributions are often climatic and/or habitat-dependent,

and the landscape (in conjunction with the ecology and biology of species) is therefore crucial in determining genetic structures in space and time. A plethora of studies report the phylogeographic structure for a range of South African taxa (see e.g. (Tolley *et al.* 2014) for studies done in the Fynbos biome) while landscape genetic studies are largely lacking for most South African groups. Although landscape genetics is a more recent discipline, the lack of genetic studies at local spatial scales presents a gap in our current knowledgebase. Landscape changes may facilitate or impede the movement of individuals, affecting relatedness within populations (at local scales) and gene-flow between populations, thereby aiding the maintenance of genetic variation in meta-populations or driving fragmentation and divergence in isolates (Storfer *et al.* 2007).

2.1.2. Genetic diversity linked to conservation management

Management of populations and species were historically based on crude assessments of threats, habitat degradation, genetic diversity (notably allozyme and restriction fragments length variation) and other ecological factors. With the advent of more sensitive molecular tools, notably DNA sequence data and microsatellite information based on polymerase chain reaction amplifications, genetic information started to play an increasingly important role in decision making. These studies started to unravel the spatial distribution of variation as well as variation within populations or species, and were mostly based on non-adaptive (i.e., neutral) DNA sequence data or low numbers of microsatellite markers. The next and current management revolution comes from utilizing full or partial genomes (the genomics era), and a better understanding of the different genomic regions (both role and function).

With the availability of full genomes for a variety of species (see e.g. (*Genome 10K Project* 2018), and various other similar initiatives for a range of taxonomic groups), our approaches to species management have seen improvements. Genomic studies analysing intraspecific and interspecific differences have become an effective and significant conservation tool. In addition, investigations based on full (or large) genome spreads are increasingly based on genetic information which is adaptive in nature. Complete genome studies allows new understanding of the biology of species. Specifically, comparisons of genomes leads to a better understanding of genome architecture, including the identification of loci under selection (resulting in adaptations).

Genetic diversity is directly involved in the persistence of individuals, populations, and species in two notable ways. First, phenotypic plasticity allows short-term responses (acclimation, acclimatization, learned behaviour) in a single individual. Although non-heritable (compared to adaptations), these responses allow individuals to persist, thrive and reproduce in changing environments through immediate changes or responses (typically over hours or weeks). In contrast, adaptation refers to changes in a heritable trait (physiological, behavioural, and structural) occurring in individuals within populations and/or species. Adaptations are linked to evolutionary potential, which can be defined as the potential of an organism's genome to evolve novel functions, and therefore adapt to alternative or changing environments across generations. Evolutionary potential therefore relates to natural selection. Novel environments will favour novel or variant genes and gene complexes.

Both phenotypic plasticity and evolutionary potential relies on genetic diversity. As such, these determine whether an organism is adaptively matched to its environment, allowing it to reproduce. Conversely, a lack of genetic diversity may impair this adaptability to match a phenotype to a specific environment, which may result in the extinction of populations or even species. This loss of biodiversity has important implications for community assemblages and ecosystem function. Ecosystem function can therefore be linked, directly or indirectly, to genetic diversity. Perturbation of community assemblages may result in altered ecosystem function (especially in functions which are beneficial to humans) if species integral to

this processes or interactions are lost (see e.g. (Maestre *et al.* 2012). Even local changes in biodiversity can change community assemblage and ecosystem function and impede ecosystem resistance to changing environments and natural disasters. Understanding genetic diversity, linked to population and species' persistence, ecological communities and ultimately ecosystem services, is key to conserving biodiversity and mitigating biodiversity loss.

In summary, the application of genetic and genomic information is far-reaching in the fields of biodiversity management (population evaluation and monitoring), improved management, phylogeography (the spatial distribution of genetic variation), demographic management of small populations, and epidemiology (Ryder 2005). Genome (genetic) variation provides the basis for understanding the geographical distribution of variation (including in a landscape genetic framework and broader), infer demographic events (bottlenecks or population expansion), identify loci under selection (the basis for adaptations), and provide assessment of population structure and behavioural ecology. Importantly, one may include a temporal scale, inferring whether relevant processes occurred in the recent or more distant past.

2.2.   Extrinsic Benefits

2.2.1. Genetic rescue (including *ex situ* or *in situ* breeding)

For decades conservation biologists have focused primarily on the demographic contributions of immigrant individuals in the management of small, isolated populations. More recently, the genetic effects of a small population size on population persistence have received substantial attention, and a growing body of research supports an important, and previously undervalued, genetic role for immigrant individuals (Hedrick *et al.* 2011; Karsten *et al.* 2011; Frankham 2015).

Small isolated populations of once outbreeding species are at substantial risk of losing genetic variation via genetic drift. Played out over many generations, loss of genetic variation increases the chance of matings between genetically similar individuals, and in so doing, increases occurrences of the negative phenotypic effects of inbreeding. The resulting impacts on survival and fitness i.e. inbreeding depression, act to reduce population size, thereby increasing the probability of further inbreeding depression and ultimately leads to increased risk of local extinction (Allendorf & Luikart 2009). This pathway to extinction via inbreeding is captured in famous models of the extinction vortex, giving conservation biologists a powerful mathematical tool with which to understand the dynamics of population and species extinctions in the context of their causes (Gilpin 1986). Within this framework, a number of natural and experimental studies have demonstrated that immigrants can bring about both demographic and genetic rescue effects in small inbred at-risk populations. By alleviating inbreeding depression and boosting fitness, ongoing population decline can often be turned around by the (re)introduction of genetic variation alone i.e. the genetic rescue effect (reviewed in (Frankham 2015).

Despite substantial evidence of its positive effects in small populations, genetic rescue has not been as widely applied to the management of many threatened populations and species as one would expect (Whiteley *et al.* 2015). Like demographic rescue, genetic rescue is highly interventional, requires ongoing monitoring of receiving populations, and thus carries considerable costs. Nonetheless, it is an important management tool that can stem biodiversity loss and increase population resilience in an age of substantial environmental change (Whiteley *et al.* 2015).

Example: *Hyperolius pickersgilli (*Pickersgill's Reed Frog) is a small, narrow-range endemic frog species restricted to fewer than 20 wetland sites along the KwaZulu-Natal coastline. The species is listed as Endangered (IUCN 2018) due to its extremely small area of occupancy where it is threatened by the use of pesticides for mosquito control, and draining of wetland habitats for urban and agricultural development.

At the Amphibian Species Prioritisation Workshop (Johannesburg 2008) Pickersgill's Reed Frog was identified as a species requiring *ex-situ* rescue and supplementation, and the species' Biodiversity Management Plan was ratified in 2017. In 2012 an *ex-situ* breeding program was initiated at the Johannesburg Zoo. This programme is supported by two further breeding programs at the South African Association for Marine Biological Research in eThekwini, and at the National Zoological Gardens in Pretoria. Offspring have been successfully reared by the populations housed in eThekwini and Pretoria, and additional founder animals will be added to these to ensure sustainable insurance populations with high conservation genetic value (these principles hold true for all *ex situ* breeding programmes). The Endangered Wildlife Trust's (EWT's) Threatened Amphibian Programme is working together with these captive breeding programs to ensure that reintroductions take place into well-managed habitat.

2.2.2. Biobanking

Well-established and well-managed collections of biomaterials (including frozen tissue, seed banks, botanical and zoological gardens) are becoming increasingly valuable due to declining populations of wildlife in nature, habitat loss and degradation, illegal wildlife trafficking and trade, fragmented populations, hybridisation, and loss of genetic variability and diversity, amongst others. These biodiversity repositories, or Biobanks, can be used for supporting genetic population management and conservation research, and provide platforms for rapidly increasing demand for research in forensic sciences, toxicology, pathology reproduction technologies, and wildlife disease epidemiology. Advances in molecular biology allow low cost options for storing the genetic diversity of wildlife species, thereby maximising future options for restoring species if necessary. Mismanaged or poorly curated collections will be of no advantage to genuine conservation efforts, therefore established repositories need to ensure that their processes, protocols, and operations all adhere to international guidelines and best practice for these collections to be useful and accessible well into the future, as well as ensuring sustainable utilisation of our biodiversity heritage.

Biobanking includes the systematic collection, processing and storing of various biomaterials at optimal storage temperatures. These temperatures range from +23° C to -196 °C, and store hair, feathers, blood and blood derivatives, extracted DNA, herbaria collections, formalin fixed paraffin embedded wax blocks, biopsies, semen, pathology samples, and fibroblasts. In addition, data associated with the sampled individual, including information pertaining to the collecting event, taxonomy, dates, morphology, photographs, and biomaterials, are all stored on dedicated collection databases. In addition, these are often associated with metadata with a digital object identifier (DOI) number, provided by public repositories such the Global Biodiversity Information Facility (GBIF), or the South African Biodiversity Information Facility (SABIF) node.

Biobanks are ordinarily situated in dedicated facilities comprising different laboratories for processing of biomaterials, and banks for the long-term storage and curation of these samples. However, these need to be well-planned, thought-out facilities with the following considerations:

- Long-term funding;
- What, why, where, and how?
    - What are you storing?
    - Why are you storing it (disciplines)?
    - Where are you storing it?
    - How long are you planning on storing it?

- Staffing complement;

- Well maintained and supported database; and

- Institutional vision with regards to high quality ethics, access and utilisation of these samples.

Biomaterials accessioned into any collection need to follow identified specimen pathways. The access and utilisation of these tissue types need to meet specified criteria evaluated by established committees, and the various agreements (data or specimen transfer, access to material, etc.) need to be firmly in place.

The importance of biobanks as national repositories and facilities is gaining traction. To this end, the Department of Science and Technology (DST) has selected Biodiversity Biobanks as one of the research infrastructure projects being implemented as part of the South African Research Infrastructure Roadmap. SANBI is currently managing the development of a full proposal and will be driving at least the initial implementation of the project. A National Biodiversity Biobank is envisaged, which will hold and supply biobank materials for South African species, available to national and global researchers. The source of the materials still needs to be finalised, however, it is anticipated that samples will be of high quality and verified, and provided by researchers who have completed projects or through samples collected specifically for biobanking using standard protocols. Issues relating to ethics, permitting, material transfer agreements, and access and benefit sharing will all need to be dealt with. A central facility to deal with these will assist researchers and ensure that national and international requirements are met. The South African initiative is moulded on international best practises, with several international biodiversity biobanks and networks that provide useful models for finalising the form of a national biobank. A National Biodiversity Biobank will increase the efficiency and cost effectiveness of research, reduce the workload of researchers, and contribute to conservation and potentially economic development as well (personal communication with Michelle Hamer 2018).

2.2.3. Forensic genetics (including barcoding)

Forensic genetics is the application of scientific research and technologies in molecular biology to support law enforcement in the regulation of illegal criminal activities. The ability to identify biological samples is critical in the investigation of prohibited trade in protected wildlife (flora and fauna) and their derivative products. Species protection is necessary as a large number of named species are in danger of regional or global extinction due to illegal wildlife trade, which has continued to escalate. Illegal trade activities also disrupt the ecological processes necessary for the provision of ecosystem services that provide valuable financial security and benefits to society. Effective crime investigation aimed at protecting national biodiversity assets including iconic wildlife species (such as the rhinoceros) and valuable ecological infrastructure is thus vital for conservation management and sustainable use of biodiversity.

The value of forensic genetics

Genetic technologies play a major role in forensic analyses, and have proved to be critical in securing successful prosecutions specifically through the examination of DNA (genotyping) from physical exhibit material that may include all types of biological traces containing nucleated cells. DNA genotyping techniques help to identify and characterise biological evidence by answering questions that arise during crime investigation and prosecution in the criminal justice system. With regards to protected species, these may include information about:

- The species involved (DNA barcoding);

- The geographic origin of the specimen;

- The individual identification of the specimen;

- The source of the specimen (wild or captive) based on parentage;

- The sex of the specimen; and

- The age of a specimen.

Questions arising during crime investigations require a specific or specialised genetic approach when analysing forensic evidence. Additionally, as all processes involved in the forensic analysis of evidence may be subject to legal scrutiny, a very high standard of operational systems is necessary. As such, data and laboratory procedures have to be maintained. This has led to the development of tools specifically designed for this purpose that have continued to develop and improve, due to the on-going advances in next generation or high throughput sequencing technologies.

DNA barcoding

DNA barcoding has been described as a taxonomic method that uses one or more standardized short genetic markers in an organism's DNA to identify it as belonging to a particular species based on comparison to a reference library (Hebert *et al.* 2003).

The use of DNA barcoding reference records generated via Sanger sequencing for species identification is now a routinely accepted accurate and convenient tool for forensic testing in South Africa. The DNA barcoding initiative uses a unified methodological approach, with the targeted gene region for vertebrates being the 5' region of the gene encoding mitochondrial cytochrome oxidase subunit 1 (COI) (Hebert *et al.* 2003), while between two and three markers (*rbcL*, *matK*, and ITS) are typically recommended for plants (Hollingsworth *et al.* 2009). Single gene chloroplast mutation rates are typically too slow for species-level identification in plants, such that a combination of plastid and nuclear regions are required. For animals, the main advantage of using mitochondrial DNA is that there is a higher increased probability of survival in samples that contain a low amount of DNA (e.g. hair shafts) and old or degraded biological samples as there are a large number of mitochondria copies per cell compared to nuclear DNA. Furthermore, most taxa have lower geographical variation in cytoplasmic DNA (mitochondrial or chloroplast) compared with data derived from faster evolving nuclear markers, such that a few records are sufficient for effective species-level resolution.

DNA barcoding (*sensu lato*; as an approach which used DNA data as an identification tool) can also be used to address questions of geographic origin, individual identification, to assess whether individuals are wild or captive-bred, and to assess the age and gender of specimens (see Box 2.1 for an application of this technique).

Geographic origin: Molecular analysis of geographic origin includes the use of mitochondrial and chloroplast DNA variation, microsatellite markers or single nucleotide polymorphisms (SNPs) to assign individuals to a particular population. The methods rely on genetic differentiation between populations due to isolation that results in fixed differences. In the cases of microsatellite and SNP markers, differences in allele frequencies can be used to characterise genetic structure. To investigate geographic origin, the development of a large genetic database is required where the allele frequencies of each population are investigated, and individuals are then assigned to a particular population or genetic lineage.

Individual identification: Multiple markers such as microsatellites or SNPs can be used to generate a unique profile for an individual. Individual identification may be used when a DNA match is being investigated, for example when evidence needs to be matched between a crime scene and a suspect or confiscated evidence. Individual DNA profiles may also be required to regulate legal trade of species that are subject to quotas or can be used to determine captive breeding of an animal (where databases exist for captive stock

such as for rhinoceroses in South Africa). The probability of a match (Random Match Probability or RMP) is determined with the use for suitable allele frequency databases (Iyengar 2014). Calculations of RMP should also include estimates of genetic structure (for example, FST). In addition, incorporation of the inbreeding coefficient (FIS) within RMP calculations should be conducted when assessing wildlife populations where inbreeding may be high. The presence of inbreeding in a population may increase the likelihood of observing homozygous genotypes. Another individual identification estimator is the probability of identity, or the probability that two individuals drawn at random from a population will have the same genotype at multiple loci (Waits *et al.* 2001).

Box 2.1. The South African Barcode of Wildlife Project (BWP)

The South African Barcode of Wildlife Project (BWP) was established to develop a comprehensive and validated DNA barcode reference database of endangered and protected (priority) species that are illegally trafficked in large numbers as well as species that are considered as look-alikes (closely related substitutes). The main approach of the project was to use expertly identified reference voucher specimens collected under a chain of custody sampling protocol in line with the approved International Society for Forensic Genetics (ISFG) recommendations for forensic reference databases. The BWP process was implemented through field and laboratory information management systems (FIMS and LIMS) to automate workflows and effectively manage DNA sequencing and all associated laboratory data for application in genetic services and forensics.
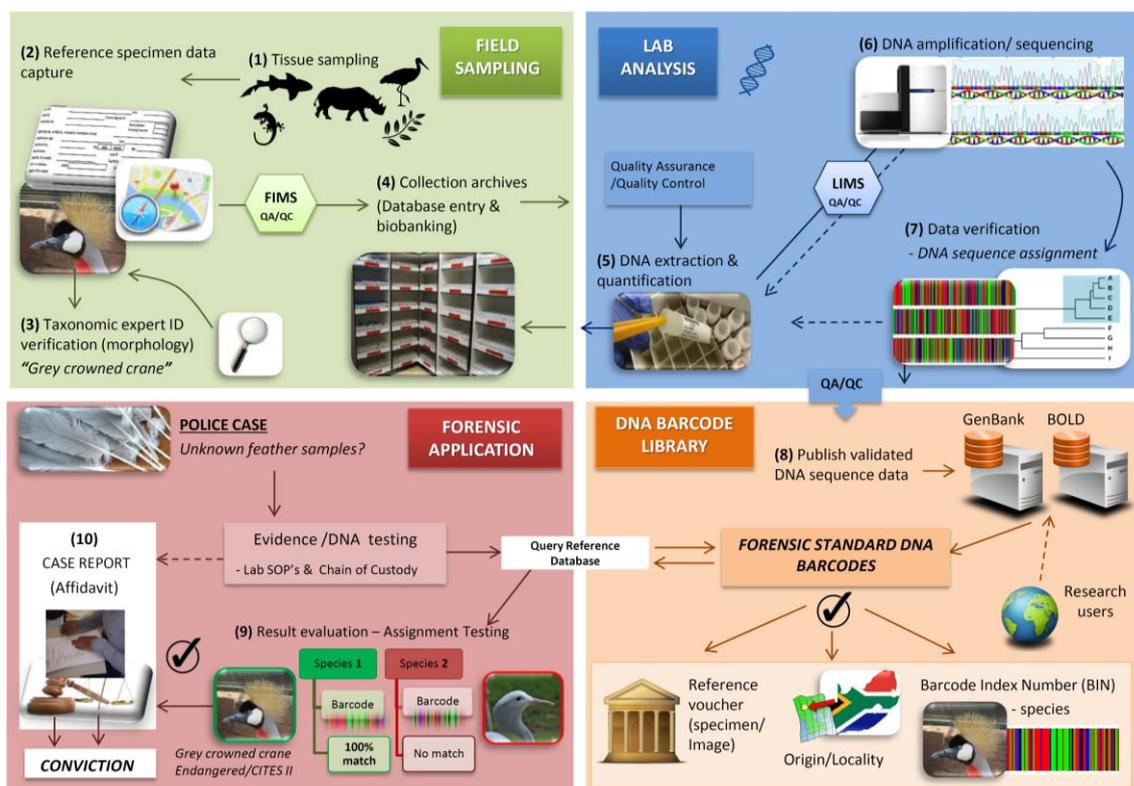
The South African Barcode of Wildlife Project workflow for forensic application

Wild or captive source of the specimen based on parentage

Establishing levels of relatedness in forensic investigations is generally employed to differentiate between captive bred and wild caught animals. The patterns of inheritance from parent to offspring allow DNA profiles generated from either SNPs or microsatellites to be used to verify family relationships, or relatedness. Parentage is confirmed if alleles present in putative parents are also present in offspring. However, analysis may be complicated and several factors need to be considered when selecting a microsatellite panel for parentage or relatedness investigations such as (1) mutation rate at each marker (which may not be possible for all wildlife species), and (2) the quality of the data obtained should be assessed for genotyping errors such as null alleles which can lead to false parentage assignments (Dakin & Avise 2004).

Sex determination

Sex determination is an invaluable tool for monitoring harvests and investigating forensic cases. Non-molecular methods based on sexual dimorphism or size difference may be unreliable and complicated. Direct observation of the genital region may be possible depending on species and if the full carcass is available. Additional methods, such as testosterone levels and pelvic-girdle morphology, have been used for some species, however, these are dependent on age class and morphological or physiological variation and can often not be obtained from free-ranging animals. Molecular sex-determination can be done when only parts of the animal are available or when sex-specific characters are either absent or difficult to observe. Standardized DNA tests are available to sex birds (Griffiths *et al.* 1998) and mammals (Shaw, Wilson & White 2003); however, such tests are not readily available for many other groups because of intra- and interspecific variation in sex-determination (e.g. temperature dependent) or in sex chromosomes.

Age

In some wildlife crime investigations it may be necessary to determine the age of a sample. For example, if a rhino horn was collected prior to 1947, then it pre-dates laws prohibiting trade in rhino horn. Various methods can be used to achieve this including a form of stable isotope analysis known as radio carbon dating. During the early part of the 1950s, atmospheric nuclear weapons testing became common which resulted in an artificial increase in the amounts of different carbon isotopes, particularly carbon 14, which had doubled in abundance by the mid-1960s (Nydal & Lovseth 1983). As such, rhino horn that pre-dates this period will be expected to have a lower ratio of carbon 14 than more modern specimens. Morphological features of internal structures, such as growth rings in fish otoliths (Campana 2001), and tooth cementum annulation (Wittwer-Backofen, Gampe & Vaupel 2004) in mammals can provide accurate age estimation of dead animals. Determining the age of live animals largely relies upon external features which change predictably over time in a discriminate fashion. Research on the genetic aspects relating to the aging process has made significant advancements due to the availability of more advanced technology to study and quantitate molecules putatively involved in aging. It is now known that many aspects of the natural aging process are under genetic control and therefore a programmed process (Horvath 2013) or results from unrepaired environmental genetic damage (Burgstaller *et al.* 2018). Such age-related changes are apparent in fluctuating abundance of transcripts, the sequence itself, or epigenetic modifications to specific DNA, RNA and protein regions. These methods therefore present a novel and promising method of age estimation in animal populations, which would have to be validated using samples of known age obtained from captive and wild individuals.

Forensic genetic applications in South Africa

In South Africa, wildlife forensic technologies and technical applications have benefitted greatly from Sanger sequencing reference records generated for species identification and traceability, largely driven by South Africa's commitment to contribute records to the growing reference database BOLD (Barcode of Life Data System). This approach has been widely used and applied across a range of taxa, for example African pangolin (*Pholidota*). Scales, derived from a 3.3 ton illegal trade confiscation of the CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) Management Authority in Hong Kong, were though to belong to pangolins of African descent. DNA barcoding was used to accurately identify several African species namely Giant Ground Pangolin (*Smutsia gigantea*), Temminck's Ground Pangolin (*S. temminckii*), Black-Bellied Pangolin (*Phataginus tetradactyla*), and White-Bellied Pangolin (*P. tricuspis*) and one Asian species, the Sunda Pangolin (*Manis javanica*) (see Mwale *et al.* 2016). Similarly, barcoding has been used to provide baseline data for the identification of small antelope species used in illegal bush-meat trade (Ntie *et al.* 2010), to trace the colonization routes and origins of invasive species (Jansen Van Vuuren & Chown 2007; Kaleme *et al.* 2011; Karsten *et al.* 2015), to confirm the continued existence of species previously believed to be extinct (Pitra *et al.* 2006) and to assist with the management of economically important game species in South Africa (Alpers *et al.* 2004; Jansen Van Vuuren *et al.* 2010; Jansen Van Vuuren *et al.* 2017).

Recently, single-nucleotide polymorphisms (SNPs) have also been be successfully used in wildlife forensic analyses. The main advantage of SNP tests is that short DNA sequence fragments (~50 bp) from severely degraded and old samples can be used in discrimination tests compared to microsatellite markers or DNA barcoding. In South Africa, a rapid allelic discrimination real-time polymerase chain reaction (qPCR) assay has been developed for the confirmation of Lion (*Panthera leo*) or Tiger *(P. tigris*) DNA (Dalton *et al. unpublished)*. The proposed real-time PCR assay can be applied for the accurate confirmation of either lion or tiger DNA that could be used by law enforcement agencies around the world as a tool to monitor illegal trade of tiger bones.

Other forensic applications have been associated with the development of individual and species-specific DNA profiling systems for priority species such as cheetah, elephant, lion and rhino. For example, SANBI maintains a national cheetah database which includes unique genotype profiles obtained from cheetah samples collected following a chain of custody sampling procedures. The DNA profile database is used to distinguish different individuals as well as kinship analysis. Once kinship has been verified, a passport is issued that verifies captive breeding of cheetah offspring. Similarly, all rhinoceros individuals in South Africa are genotypes by the Veterinary Genetics Laboratory at the University of Pretoria. A specific database has been developed (RhODIS 2018) which his invaluable in the prosecution of poaching cases involving rhinoceroses. To date, more than 15 000 samples have been analysed and documented.

The application of DNA barcoding for seafood traceability in South Africa was explored by (Von Der Heyden *et al.* 2010; Cawthorn *et al.* 2012). Both studies reported high levels of mislabelling of fish products, ranging from 9% to 50%, across retailers and wholesale outlets. Some species, such as Kob (*Argyrosomus* spp.) had only about ~15% correctly labelled samples, with substitutes of four other fish species, some not found within the Exclusive Maritime Zone of South Africa (Von Der Heyden *et al.* 2010). Currently, there are no organised processes to check the species identification and provenance of seafood species traded in South Africa, leaving fish stocks, including sharks, for which mislabelling is rife elsewhere (Bornatowski *et al.* 2013) at an increased risk from mismanagement and overexploitation.

2.2.4. Bioprospecting including medicinal bioprospecting, ethnobotany, and indigenous knowledge

Bioprospecting, also known as biodiversity prospecting, refers to the exploration of biological material for properties of commercial value, be it of a genetic or biochemical nature. South Africa has a wealth of indigenous knowledge, driven by a strong belief in traditional medicines. Bioprospecting this wealth is gaining traction. Scientific studies covers a range of environments and habitats – marine: Davies-Coleman & Sunassee, 2012; Bolton *et al.* 2013, aquatic: Gumbi *et al.* 2017, terrestrial: Avrelija & Walter 2010, plants: Street & Prinsloo 2013; Chinsembu 2015, insects: Srivastava *et al.* 2009, microbes and endophytes: Abdalla & McGaw 2018, with mining for a diverse range of properties (e.g. Eckelmann *et al.* 2016). Attention has also been given to the legal implications and ownership OR benefit sharing (Myburgh 2011; Morris 2016; Wynberg 2017).

While the exact mechanism of how the benefits from the biological material is derived may not be known, there are several advantages to having this information at ones disposal. First, it would allow for the exploration of similar properties from other biological sources, while it will also facilitate the process of ensuring the safety of the product or derivative. The role of indigenous knowledge is therefore also vital to expediting the value that can be realised from the development of sustainable benefits through bioprospecting. This is as prior knowledge of the plant/animal/microbial derived product properties and applications provides one with a good starting point from which to identify and characterise the source of these properties in the biochemical and genomic sense. Being able to pinpoint these features in turn greatly facilitates the often tedious process of ensuring the safety of the product.

There are a number of applications of Next Generation Sequencing (NGS), which are applied in various forms, which makes access to vast amounts of genomic information available to be screened for their bioprospecting potential. Some of these are highlighted below.

Whole Genome Sequencing (WGS): WGS refers to the technique whereby the entire genomic complement of an organism is sequenced using one of the available NGS platforms. The typical procedure involves the fragmentation of the genome into millions of small pieces (in the range of 100 – 400 bp, depending on the platform), followed by the determination of the nucleotide order for each of these fragments, where after these fragments are aligned to each other (or to an available reference) in order to generate the assembled genome. The technique, because it generates the full genome of the organism, therefore allows for the availability of the full search space for the discovery of the genetic component(s) which contributes to the bioprospecting value of the organism.

Transcriptome Sequencing: This derivative of the WGS targets only those regions of the genome that is actively expressed and that yield protein products, which is in most cases responsible for the trait that is sought for bioprospecting. The reduced representation of the genomic information from this technique, in comparison to WGS, means that only features that are of consequence to the production of certain proteins will be detectable. An additional feature of transcriptome sequencing is that the representation of the genomic regions that express the proteins is highly quantifiable. This feature therefore assists researchers in identifying the trait responsible for the bioprospecting benefit, given that the trait would generally be overexpressed and therefore detectable through a comparison of levels of expression with other genes.

RAD sequencing/genotyping by sequencing: In another derivative of WGS, one is able to choose specific landmarks within the genome of an organism which are selectively captured through molecular techniques and for which the nucleotide order for each of the captured fragments is then determined using NGS.

Instead of generating information for the entire genomic complement of an organism, this technique only generates information relative to the position of the landmarks in the genome. These landmarks are typically regions in the genome which can be fragmented and therefore allows for a point of reference from which to generate the sequence information. The application of the technique is primarily for the comparison of the genetic relationship between organisms at the genomic level, rather than to explore the genomic space for the identification of genetic features of value to bioprospecting. The potential for understanding whether a species is related to another species, known to be used for bioprospecting, could potentially harbour the same characteristics, can be explored with this technique.

Metagenome sequencing: In another application of NGS, metagenomics offers an opportunity to explore the genomic information of the collection of organisms from a specific environment/plant/sample. Rather than focusing on any one specific organism, this technique looks at the sum of the genetic information in a specific environment. The bioprospecting benefits that can be derived can therefore be assessed in one step for multiple organisms as opposed to just for a singular organism. The application of, and bioinformatics support for, this technique is still in its relative infancy, making it difficult to reproduce all the genomes for the collection of organisms assessed in one go. This strategy is, however, particularly useful for doing an exploratory analysis of the potential for discovering new genomic elements out of a pool of organisms, often associated to a common environment or theme.

2.2.5. Take pride in diversity including tourism and biodiversity hotspots

Preserve the range of the wild population variability (for monetary and non-monetary values)

There is little doubt globally as to the value of biodiversity to both local and regional economies (OECD 2004). Within this well-developed framework, biodiversity value is measured in the many benefits that are derived from it, both tangible and intangible. Many of these have been demonstrated to be already significant in areas where they are measured in market activities. For example, the Department of Environmental Affairs (DEA) programme on the Biodiversity Economy of the country has, as its central focus, the *Wildlife Economy* comprising the collective economic biodiversity values of game ranching, hunting, and wildlife bio-prospecting. On the other hand, several studies exploring the contingent valuation of biodiversity exclude reference to its economic benefits, and highlights the importance that familiarity and biophilia (Wilson 1984) have on attitudes towards biodiversity conservation (Martín-López *et al.* 2007). This combination of non-monetary together with monetary value can be utilised in developing a sense of national pride embedded in a biodiversity conservation framework that benefits citizens by serving the economy (UNESCO 2010) The government's National Strategy for Sustainable Development and the green economy recognises the importance of this approach and supports a range of campaigns to instil national pride in our natural heritage (NSSD 1 2011-2014).

South Africa is exceptionally rich in natural heritage. South Africa's biodiversity credentials include nine major terrestrial biomes (Thuiller *et al.* 2006) and several of the eight UNESCO World Heritage Sites in South Africa are natural sites; the Cape Floristic Region, the iSimangaliso Wetland Park, the Richtersveld Cultural and Botanical Landscape, and the uKhahlamba Drakensberg Park. A number of South Africa's terrestrial biomes are prominent due to their species richness and levels of endemism, and contribute to the occurrence of three of the world's 36 identified Biodiversity Hotspots (Myers 1990; Myers *et al.* 2000); the Cape Floristic Region, the Succulent Karoo, and the Maputaland-Pondoland-Albany region. A number of recent meta-analyses indicate that the hotspot conservation designation is relatively effective at capturing both species and phylogenetic diversity of a region (Forest *et al.* 2007; Brum *et al.* 2017). Furthermore, in most systems studied to date species diversity is generally positively correlated with genetic diversity (reviewed in Kahilainen *et al.* 2014), suggesting that in the absence of species- and system-specific funding

for the conservation of genetic variation, prioritising the conservation of biodiversity hotspots may, in certain systems, be an effective tool for conserving inter- and intra-specific contemporary genetic variation.

Biodiversity tourism

Biodiversity-centred ecotourism is one of South Africa's key economic growth sectors. The tourism sector contributed 2.9% to South Africa's GDP in 2016 (Stats SA, Annual Tourism Satellite Account 2017) and sustainable ecotourism has been identified by the DEA as an important growth sector (DEA Biodiversity and Sustainable Tourism Initiative 2017). Also worth noting is Brand South Africa's significant focus is on the economic value of biodiversity to both local and regional tourism revenue (Brand South Africa 2018). For example, in the 2016/17 financial year, six million people visited the 19 national parks that are managed by South African National Parks (SANParks) and recent estimates of the standing value of South Africa's biodiversity only serve to highlight its importance for future economic growth. Approximately 15 years ago, (Turpie *et al.* 2003) estimated the total economic value of the Cape Floristic Region (both as Biodiversity Hotspot and a World Heritage Site) to be at least R10 billion per year, equivalent to over 10% of the regional Gross Geographic Product for that time. More recently, researchers have also estimated the standing economic value of high conservation profile species to regional GDP. For example, the African penguin (*Spheniscus demersus*) is currently listed as Endangered (BirdLife International 2018) and continues to decline with fewer than 26,000 breeding pairs remaining (Crawford *et al.* 2011). The mainland breeding colony at Boulders Beach in Cape Town is managed by SANParks as part of the Table Mountain National Park. Penguin-based tourism to this colony is a substantial contributor to the Western Cape tourism sector and accounted for R14.5 million in gate revenues alone in 2009/2010 (Lewis *et al.* 2012). When analysed together with tourist transport and associated expenditures of visiting the colony, penguins bring in R160 million per annum, the majority of which (78%) comes from international tourism (Van Zyl 2014).

It's clear that the more value a country derives economic growth from its biodiversity, the greater the conservation importance that is attached to the areas that are rich in biodiversity (Chevallier & Milburn 2015). Given that genetic variation is the building blocks for all measures of biodiversity i.e. structural, compositional, functional, and phylogenetic diversity (Noss 1990; King 2009), a strong and compelling case can be made for developing indicators of its importance to biodiversity and thereby its economic value. Substantial evidence already demonstrates the future economic benefit or option value of genetic diversity within natural populations (Jump *et al.* 2009). W while it is difficult to predict the novel selection pressures to which populations will be exposed to as we move into a future of significant environmental change, it would be judicious to ensure that sufficient standing genetic diversity remains so as to ensure the persistence of South Africa's biodiversity.

2.2.6. Marine Protected Areas as reservoirs for genetic diversity

The ecosystems of the South African coastline and associated offshore areas are shaped by one of the most dynamic and variable oceanographic regimes in the world, providing South Africa with a distinct geographic advantage in terms of understanding the historical and contemporary processes that shape marine biodiversity, including anthropogenic impacts on genetic and genomic variation. The region has established a strong baseline understanding of the spatial genetic patterns in various species along the coastline, particularly using mitochondrial DNA and to a lesser extent nuclear data such as microsatellite markers (von der Heyden 2009; Teske *et al.* 2011; Wright *et al.* 2015), as well as offshore stocks of commercially exploited species such as hake (von der Heyden, Lipinski & Matthee 2007; Von Der Heyden *et al.* 2010) and kingklip (Henriques *et al.* 2017). More recently, research has focussed on epigenetics (Baldanzi *et al.* 2017), as well as genome wide scanning of various coastal species (ongoing work on corals, seagrasses, molluscs, urchins, crabs, sardines, hakes, kingklip, and various coastal fish species) to identify intra-specific, genome-

wide variability and structure. In particular, given the heterogeneous marine environment of South Africa that spans ecological gradients of temperature, salinity and primary productivity (amongst others), is promising to provide crucial insights into population connectivity and signals of local adaptation. These may be important for population and species persistence into the future. Identifying potential variation, even in high gene flow environments, should be factored into conservation decision making (von der Heyden 2017) because locally adapted variants or populations with high number of outlier loci may be more resilient to future change, although this remains to be tested for many natural populations. In South Africa, only one published study exists on genomic variation of two marine species (Nielsen *et al.* 2018). This study indicates that despite large effective population sizes and high levels of gene flow, the northernmost populations of the South African west coast were characterised by unique SNP variants.

From a conservation and biodiversity planning perspective, genetic and genomic data are not currently incorporated into marine spatial planning, but it is highly likely that their inclusion will help capture areas of evolutionary importance, persistence and resilience. It is well understood that the South African coastline has several regions across which gene flow are limited. These include Cape Point, a transition zone along the south-east coast, as well as a break south of the border with Mozambique, although several other regions of genetic discontinuity have been identified (Teske *et al.* 2011). Breaks in gene flow generally tend to overlap between taxonomically diverse species and strongly suggest shared environmental drivers that can in some instances be traced back at least 70,000 years (Toms *et al.* 2014).

Importantly, (Wright *et al.* 2015) show that the South African marine protected area network is poorly connected from a genetic perspective, which may lower the ability of species and their populations to respond to change. This is due to (1) the spatial arrangement of coastal marine protected areas is geographically unlinked, leaving unique populations of some species vulnerable, and (2) the network currently excludes some sites that have been identified as being of evolutionary importance as is seen for many populations and localities along the South African West Coast. Despite potentially high gene flow facilitated by the Benguela Current, this region is characterised by high levels of population structure across several species, for both mitochondrial (Mertens *et al*. in press; personal communication with Mbongwa) and SNP data (Nielsen *et al.* 2018). In particular, the northernmost populations (Kleinzee and further northwards) tend to be genetically differentiated hinting at unique evolutionary significant units. Worryingly, these localities are highly threatened yet enjoy no formal protection. Including these, and other populations, in marine spatial planning and assessing the state of marine conservation genetics in South Africa in general, is urgently required to ensure that unique evolutionary patterns are protected thus ensuring better resilience and the long-term persistence of South Africa's marine biodiversity (Box 2.2).

Box 2.2. Anthropogenic impacts contribute towards the loss of genomic diversity in an estuarine keystone species

Zostera capensis is a seagrass found in South African estuaries and sheltered lagoons, were it provides crucial ecosystem services that benefit both biodiversity (habitat for seagrass associated species such as juvenile fishes and invertebrates, as well as benthic biodiversity) and human societies (support of coastal and offshore fisheries, sediment binding, and maintenance of water quality). Like all seagrasses globally, *Z. capensis* is threatened by human activities such as pollution, coastal development, eutrophication and habitat destruction and it is currently listed as Vulnerable by the IUCN. In South Africa, some populations have shown severe declines in cover and biomass, with at least one in local extinction in the St. Lucia estuary, with recovery uncertain (Adams, 2016).

Genomic variability is seen as a key component contributing towards the resilience and persistence of natural populations under changing conditions, with several studies providing evidence for genetic and genomic variability positively correlated with phenotypic traits supporting resistance and resilience to change in local populations (Ehlers *et al.* 2008; Massa *et al.* 2013; Jahnke *et al.* 2015). Within this context, the genomic variation of *Z. capensis*, spanning its entire distribution, was mapped for ten population in South Africa using ~ 1200 SNPs and measures of diversity (nucleotide diversity, heterozygosity and allelic richness) calculated for each of the populations. Diversity measures were tested for associations with four environmental stressors (change in flow, habitat loss, sand mining and fishing effort), through generalised linear models. Notably, each of the measures of diversity was negatively associated with at least one environmental stressor, with nucleotide diversity negatively associated with increased habitat loss, sand mining and fish effort (N. Phair, pers. comm.). This suggests that anthropogenic activities are already impacting the genomic signatures of Z. capensis, which may contribute towards lowering the resilience of this important ecosystem engineer, thereby increasing the potential for local population declines and potentially the loss of entire populations.

Populations of the seagrass *Zostera capensis*, such as the one pictured here in the Breede River, support both biodiversity and human society, but anthropogenic impacts threaten their genetic integrity and resilience to future change.

2.3. Risks

2.3.1. Loss of diversity

Habitat fragmentation and reduced population connectivity

At the global scale, population fragmentation via habitat loss is one of the most serious threats to biodiversity. While local populations do naturally differ in size, and local populations of some species may go through local extinction events and then be recolonized by migrants from other populations, the effects of population size and connectivity on population genetic variation and structure require reconsideration within the framework of ongoing anthropogenic loss of habitat and fragmentation (Templeton *et al.* 1990). Population fragmentation is a significant problem for species that have evolved with continuous distributions across broad geographic areas, and now find themselves restricted to increasingly smaller and more isolated patches of habitat. This is because the genetic impacts of population fragmentation depend critically on gene flow among fragments. Reduced gene flow leads to substantial changes in the local effective population size (N_e), increasing the probability of inbreeding and thus further enhancing loss of genetic diversity within fragments. Reduced population size and connectivity also promotes fluctuations in temporal and spatial genetic differentiation via enhanced genetic drift (or the random fluctuations in allele frequencies from one generation to the next), and leads to greater risks of extinction, in the long term, than for a single population of the same total size. It is also important to note, that the relative impact of fragmentation on genetic variation may be buffered, to some degree, by life history traits, particularly when species occur as a series of naturally fragmented metapopulations (Gilpin & Hanski 1997). Nevertheless, maintenance of variation in fragmented population in order to avoid inbreeding depression and to ensure adaptive potential is a major conservation goal worldwide.

Within South Africa, only a handful of studies have explicitly assessed the impacts of anthropogenic habitat fragmentation on population genetic parameters in animal and plant species. In animals, examples include an assessment of genetic variation and structure in severely fragmented populations of the Geometric Tortoise (*Psammobates geometricus*), the most endangered tortoise on mainland Africa due to loss of its renosterveld habitat to agriculture (Cunningham *et al.* 2002). And more recently a study of highly fragmented, fenced populations of Lion (*Panthera leo*) within South African national parks and small, privately owned game reserves (Miller *et al.* 2015). In plants, examples include studies that focused on genetic variation of species in the highly fragmented renosterveld vegetation of the Cape lowlands e.g. the Bearded Nemesia (*Nemesia barbata*) (Heelemann *et al.* 2014) and a comparative study of the annual *Hemimeris racemosa* and the shrubby perennial *Eriocephalus africanus* (Heelemann *et al.* 2015), all of which are impacted by significant intensification in agricultural land use.

Urbanisation and genetic fragmentation – an important area for future research

Among the many human activities that cause biodiversity decline, *urbanisation is one of the most important, resulting* in both habitat loss and habitat fragmentation concurrently. In the period 2010-2050, the proportion of the world's population living in urban areas is predicted to increase from ~52% to ~67% around the world, increasing the current extent of urban development three fold (Liu *et al.* 2016). While human alteration of natural habitats continues at an unprecedented rate, relatively little research globally has focused on the response of species to the novel selective pressures of urbanization and the consequences for biodiversity are generally poorly understood.

As areas of urban-wildland interface expand, wildlife populations are increasingly confronted by novel and potentially stressful anthropogenic changes in their environments. Roads, vehicular traffic, and the physical urban matrix fragment habitat, act as barriers to dispersal and gene flow (Keyghobadi 2007), increase

mortality (Riley *et al.* 2003), and alter behavioural patterns (Baker & Harris 2007). Additionally, human introductions of non-native species increase disease exposure for native wildlife, further placing populations at risk (Riley *et al.* 2004). In increasingly urbanized landscapes, understanding the factors that affect population persistence and genetic variation is clearly vital to the maintenance of biodiversity. Globally, relatively few studies have assessed aspects of genetic connectivity, neutral and adaptive genetic variation, disease and survival in urban systems, and those that have are predominantly focused in the United States and Europe. Within South Africa, there have not been any published studies to date, however, a number of ongoing research programs are assessing genetic variation and connectivity in both Baboons (*Papio ursinus*) and Caracal (*Caracal caracal*) in and around the urban extent of Cape Town and surrounding natural and agricultural landscapes, as well as the connectivity between green spaces in Johannesburg and eThekweni using small mammals and arthropods as models.

Hybridization and Inbreeding

Inbreeding involves the successful mating (i.e., production of offspring) of genetically closely related individuals. This process usually results in homozygous individuals, which may increase the frequency of (typically recessive) deleterious alleles, thereby leading to a reduction in adaptive fitness (also called inbreeding depression). This decreases the viability of individuals to survive and reproduce, which may ultimately decrease population fitness (also known as an extinction vortex).

Conversely, genetic hybridization involves the successful mating of two individuals which are genetically well-differentiated (genetically distinct). Admixture and introgression are major threats to species conservation. The ability to accurately identify introgression is critical to the management of species (Gompert 2012), and may even provide unprecedented insights into evolutionary processes. Because of the movement of animals (either naturally or human-facilitated), admixture and the effects thereof become increasingly more important to understand and manage. A well-documented case is the hybridization between domestic cattle and bison in North America (Halbert *et al.* 2005). Using a suite of linked microsatellite markers, researchers showed low levels (< 2%) of introgression of cattle DNA into wild bison populations. If it were not for this genomic approach, such low levels of hybridization would not have been detected.

Anthropogenic hybridization is recognised to be a threat to native species due to the increased frequency and/or mixing of species, subspecies or evolutionary significant units (ESUs) that would never have naturally encountered each other. (Allendorf *et al.* 2001) categorised anthropogenic hybridization into three types namely (1) hybridization without introgression (sterile offspring produced), (2) widespread introgression, or (3) complete admixture (fertile offspring produced). In cases were fertile hybrid offspring are produced, this can result in wide spread introgression where both pure and hybrid individuals exist, or it can lead to complete admixture if hybridization is not detected and conservation measures are not enforced (Allendorf *et al.* 2001). Hybridization without introgression is the mating between different species where sterile offspring is produced. Sterility can occur due to genetic incompatibility that can include either (or both) genic and chromosomal differences. Anthropogenic hybridization may occur due to fostered changes in the abundance and distribution of the species on private properties, with such consequence as reduced fertility in the rare taxon, genetic swamping or assimilation (Levin, Francisco-Ortega & Jansen 1996; Wolf *et al.* 2001) reported that both reduced fertility and genetic swamping or assimilation may result in extinction. Additionally, foreign alleles are introduced through hybridization that may ultimately segregate independently leading to maladaptive phenotypic changes, which result in the loss of local environmental adaptations (outbreeding depression).

Fitzpatrick *et al.* (2015) identified three primary threats including (1) hybrid subpopulations may have a greater probability of extinction, (2) hybrids may be culturally undesirable (for example, ecological inauthentic), and (3) they may have negative impacts on native species or ecosystems.

Intentional introductions are a method by which inbreeding and inbreeding depression may be mitigated in wild populations or those in captivity. Through the introduction of conspecific individuals which carry different alleles and/or are more genetically diverse than the recipient population, this may allow such populations to regain genetic diversity through outcrossing and therefore increase their adaptive potential through hybrid vigour. It is possible to establish a fully outbred or heterozygous population from two inbred populations, provided they are homozygous for different alleles. This approach is often useful in small, managed populations. Similarly, many of South Africa's agricultural strains, such as maize, is developed in this way, through selection for particular traits in parent lines, which are then crossed to produce viable commercial lines.

When considering intentional introductions, conspecific populations for the recipient population frequently do not exist (either due to population reductions and/or extinctions). In these instances, and when required, it is possible to introduce extra-limital, albeit closely related individuals, to increase genetic diversity in impaired populations. Extra-limital populations are populations which are geographically and genetically distinct from the particular recipient species (because of geographic separation, and independent evolutionary trajectories). Introduction of extra-limital populations runs the risk of leading to outbreeding depression (following hybridisation) should the introduced individuals be too genetically different or adapted to different environment conditions. Care should therefore be taken to introduce individuals which are genetically diverse, but not exceedingly so. Additionally, introductions should preferably proceed from populations occupying similar environments with regards to climate, precipitation, temperature, vegetation etc. As a rule of thumb, introductions should first be considered from geographically close populations, as was recently suggested for Oribi Antelope (*Ourebia ourebi*; Jansen Van Vuuren *et al.* 2017).

Wildlife economy: Game breeding

The commercial wildlife breeding industries in South Africa has experienced an almost exponential growth since the mid-2000s. This industry largely focusses on the breeding of rare or economically important game species (e.g. Cape buffalo [*Syncerus caffer]*; Sable Antelope [*Hippotragus niger]*, Roan Antelope [*Hippotragus equinus]*, and Cape Eland [*Taurotragus oryx]*) for the purposes of commercial sale, and also intended for the trophy hunting industry. In addition, exotic species, or colour variants of more common species, are also bred.

Breeding practices frequently involve the keeping of animals in relatively small, but always fenced, enclosures and with resultant small effective population sizes. Furthermore, the possibilities of assortative mating are negated through selection of breeders for specific herd compositions and the mating of specific individuals to accentuate certain phenotypic traits (e.g. longer horn length or phenotypic markings) which are desirable during commercial sales. As a result, these small populations are at risk of inbreeding and inbreeding depression. Conversely, outbreeding depression or hybridisation are also possible as animals from disparate regions across species' ranges are commonly included in breeding programs. A recent study by Visser and Jansen van Vuuren (unpublished data) investigated the effects of commercial breeding on genetic diversity, including strong selection for phenotypes or breeding lines, in two rare antelope species (sable and roan antelope). When considering sable antelope from all the different game farms as a single population, genetic diversity was higher than in natural populations. However, when considering different game farms separately, diversity was low. For roan antelope, diversity was lower in farmed animals

compared with natural populations across the range of roan, possibly because of the small founding population for roan on commercial farms. This maintenance of genetic diversity in these commercially bred species likely results from the continuous movement of animals among different breeding farms, due to breeders favouring novel diversity during trait selection. As such, a large effective population size is maintained, in contrast to what would be found in the natural situation where natural breeding herds are geographically discrete.

The financial incentives created by the high commercial value of rare animals in South Africa apparently aids in the exchange of such animals across large spatial scales, thereby creating a large metapopulation. Even though genetic diversity seems to be maintained overall, two potential problems present themselves. First, as certain animals are selected for intensive breeding purposes due to phenotypic traits favoured in this wildlife industry, certain genotypes seems to be propagated extensively while the genotypes of less favoured animals remain comparatively rare. This is in contrast to the natural situation, as anthropogenic selection trumps natural selection, thereby effectively creating an artificial population of certain selected genotypes. Animals which are extensively propagated due to their phenotype, also propagate their genotype, which may not necessarily be well-adapted to situations in the wild, or may carry deleterious alleles. As animals are frequently held in relatively small enclosures subject to relaxed natural conditions, outbreeding depression may be difficult to detect and therefore go unmanaged. As such, potential hybrid animals may have reduced fitness to conditions in the wild, but are sustained and propagated under relaxed conditions (e.g. feeding, antibiotics etc.) which may increase the chances of domestication.

Another potential problem is that the propagation of commercially bred species is market-dependent. Only certain favoured species are propagated, while most remain neglected. This anthropogenic selection of species for (indirect) conservation is not uncommon, but does create the possibility that initiatives may be short-lived or even futile. This is exacerbated by country laws, as rare species such as rhinoceros remain neglected during commercial breeding as they are illegal to hunt or to harvest products from (such as horn). Because of this, there is no incentive to propagate or breed rhinoceros in South Africa and therefore population numbers remain low, and will likely suffer losses of genetic diversity.

Taken together, the commercial breeding of rare species seems to be double-edged sword. On the one hand, it may ensure the increase in numbers of certain favoured species with the maintenance of genetic diversity under a metapopulation approach. Conversely, this practice is market and law-dependent, and may lead to anthropogenic selection and propagation of certain (possibly naturally unfit) genotypes, and outbreeding depression or domestication due to relaxed natural circumstances.

In South Africa, extensive translocation of species outside of their natural range has led to hybridization in several cases. However, this threat can be managed through biodiversity management plans via genetic testing. Introgressive hybridization can be detected using molecular methods which include microsatellites (Evans *et al.* 2001; Nijman *et al.* 2003; Gaubert *et al.* 2005; Gay *et al.* 2008; Gompert *et al.* 2010a), mitochondrial DNA analysis (Evans *et al.* 2001; Nijman *et al.* 2003; Gaubert *et al.* 2005; Gay *et al.* 2008), Y-chromosome markers (Kikkawa *et al.* 2003) and single nucleotide polymorphisms (Gompert *et al.* 2010b). Hybridisation due to natural overlapping species range can be considered as the most severe threat as this cannot be managed through translocation and metapopulation policies, for example the African Wildcat (*Felis silvestris*), which hybridises with domestic cats. A list of species reported to hybridize in South Africa is provided in Table 2.1.

Table 2.1. List of species reported to hybridize in South Africa.

Species		Reference
Blesbok *Damaliscus pygargus phillipsi*	Tsessebe *Damaliscus lunatus.*	(Bothma *et al.* 1990)
Blesbok *Damaliscus pygargus phillipsi*	Bontebok *Damaliscus pygargus pygargus*	(van Wyk *et al.* 2013)
Red hartebeest *Alcelaphus buselaphus*	Blesbok *Damaliscus pygargus phillipsi*	(Robinson *et al.* 1991)
Red hartebeest *Alcelaphus buselaphus*	Tsessebe *Damaliscus lunatus*	(Robinson *et al.* 2015)
Red hartebeest *Alcelaphus buselaphus*	Lichtenstein hartebeest *Alcelaphus lichtensteinii*	(Flagstad *et al.* 2001)
Blue Wildebeest *Connochaetes taurinus taurinus*	Black wildebeest *Connochaetus gnou*	(Grobler *et al.* 2018)
Buffalo (Cape) *Syncerus caffer caffer*	Domestic cattle *Bos indicus*	(Owiny *et al.* 2009)
Eland (Cape) *Tragelaphus oryx oryx*	Kudu *Tragelaphus strepsiceros.*	(Van Gelder 1977)
Plains Zebra *Equus quagga*	Cape mountain zebra *Equus zebra zebra*	(Dalton *et al.* 2017)
Hartmann Mountain Zebra *Equus zebra hartmannae*	Cape mountain zebra *Equus zebra zebra*	(Novellie *et al.* 2002)
Plains Zebra *Equus quagga*	Grevy's zebra *Equus grevyi*	(Cordingley *et al.* 2009)
Waterbuck (Southern) *Kobus ellipsiprymnus ellipsiprymnus*	Lechwe *Kobus leche*	(Birungi & Arctander 2001)
Roan Antelope (Southern) *Hippotragus equinus*	Sable Antelope (Southern) *Hippotragus niger niger.*	(Robinson & Harley 1995)
Roan Antelope (Southern) *Hippotragus equinus*	Roan Antelope (Western) *Hippotragus equinus*	Van Wyk *et al*. unpublished
African Wildcat *Felis lybica*	Domestic cat *Felis catus.*	(Wiseman *et al.* 2000)
Kudu (Southern Greater) *Tragelaphus strepsiceros.*	Nyala *Tragelaphus angasii.*	(Dalton *et al.* 2014)

Small population management

Effective management of small or disconnected populations has been identified as a core problem in conservation biology since the inception of the discipline. To set effective measures in wildlife conservation, it is essential to have comprehensive knowledge about the species. Population genetics as a discipline aims to provide information about populations and their demographic development, which is unattainable with other methods. Genetic data are particularly useful in providing information about population structure, level of inbreeding, their distribution and the connectivity of habitats (Schwartz *et al.* 2007). *In-situ* and *ex-situ* conservation management of endangered and vulnerable wild animals is increasingly based on genetic studies. Genetic variation including local adaption is a premise for future adaptive changes, the avoidance of fitness decline because of inbreeding depression and thus for the long-term survival of a species. Without genetic variation, it is not possible for a population to evolve in response to a changing environment (e.g. a new disease), and the risk of extinction increases (Hedrick 2013). Therefore, the identification and conservation of genetically distinct local populations are important to reduce the risk of extinction (Luck *et al.* 2003). Because the identification of conservation units is critical in conservation management, several terms have been defined. The Evolutionary Significant Unit (ESU) (Moritz 1994; Crandall *et al.* 2000; Ryder 2005) describes a population or a group of populations with high distinctiveness because of adaptive variation.

Rhino extinction, genetic erosion and conservation options: The Black Rhinoceros *(Diceros bicornis)* is again on the verge of extinction due to unsustainable poaching in its native range. A study conducted by (Moodley *et al.* 2017) examined the range-wide genetic structure of historic and modern populations using the largest and most geographically representative sample of black rhinoceros material ever assembled. Using both mitochondrial and nuclear datasets, these authors quantified range-wide genetic erosion and

structure in this species for the first time, describing a staggering loss of 69% of the species mitochondrial genetic variation, including the most ancestral lineages that are now absent from modern populations. Populations in countries such as Zambia, Angola, Uganda, Somalia, Ethiopia and Chad no longer exist. Among several highly structured, but hitherto cryptic mitochondrial haplogroups/nuclear populations, they found that the historic range of the West African subspecies (*D. b. longipes*), declared extinct in 2011, extends into southern Kenya, where a handful of individuals survive in the Masai Mara. Conservation units were identified that will help maintain evolutionary potential. The results suggest a complete re-evaluation of current conservation management paradigms for this species.

Diversity in the Toll-like receptor genes of the African penguin (*Spheniscus demersus*): The African Penguin (*Spheniscus demersus*), is listed as Endangered by the IUCN Red List of Threatened Species due to the drastic reduction in population numbers over the last 20 years. To date, the only studies on immunogenetic variation in penguins have been conducted on the major histocompatibility complex (MHC) genes. It was shown in humans that up to half of the genetic variability in immune responses to pathogens are located in non-MHC genes. Toll-like receptors (TLRs) are now increasingly being studied in a variety of taxa as a broader approach to determine functional genetic diversity. In a study conducted by (Dalton *et al.* 2016), the authors confirm low genetic diversity in the innate immune region of African Penguins similar to that observed in New Zealand Robin that has undergone several severe population bottlenecks. Single nucleotide polymorphism diversity across TLRs varied between *ex situ* and *in situ* penguins with the number of non-synonymous alterations in 14 *ex situ* populations being reduced in comparison to 16 *in situ* populations. Maintaining adaptive diversity is of vital importance in the assurance populations as these animals may potentially be used in the future for re-introductions. Therefore, this study provides essential data on immune gene diversity in penguins and will assist in providing an additional monitoring tool for African Penguin in the wild, as well as to monitor diversity in *ex situ* populations and to ensure that diversity found in the *in situ* populations are captured in the assurance populations.

Saving the world's rarest flufftail: The White-Winged Flufftail (*Sarothrura ayresi*) is known to occur in the highland marshes of Ethiopia, as well as almost 4 000 km in South Africa. The White-Winged Flufftail is listed as Critically Endangered with fewer than 250 adults remaining in suitable wetland habitats in the wild. The South African population is estimated to be less than 50 birds. The species was uplisted from Endangered to Critically Endangered in 2013 due to the limited number of suitable breeding sites and the severe threat of habitat degradation at those sites, both in Ethiopia and South Africa. Whether or not the birds migrate between Ethiopia and South Africa has long been an enigma. The AEWA White-Winged Flufftail International Single Species Action Plan (ISSAP), produced in 2008, emphasizes the limited knowledge on the movements of the birds (whether these are intra-African migrants or altitudinal migrants), which can be an indirect threat to species survival. It is known that the birds occur in Ethiopia between July and September (boreal summer), and in South Africa from November to March (austral summer). White-winged flufftails have rather specific habitat needs with the presence/absence of the birds being linked to water level: they are absent when it is too deep or too dry. In Ethiopia they favour seasonal flooded wetlands, stretches of grass and sedge standing about knee high in ankle-deep water in summer. In South Africa, by contrast, the wetlands used by these flufftails are permanently or semi-permanently wet. The availability of habitat is likely to be associated with high rainfall during this time of year and an increase in the abundance of aquatic food sources. The species' movements during the remaining months (September, October, April, May and June) are unclear. Dates of occurrence in both countries largely do not overlap and studies undertaken on morphological comparisons show no distinguishable differences between birds collected from locations in Ethiopia or South Africa. The peak breeding season is during July/August in Ethiopia in a single wetland, and this South African bird was most likely an early arrival. The

dates of observations of a few enigmatic low-altitude records of birds in Kwa-Zulu Natal, Eastern Cape and Mpumalanga provinces in South Africa roughly correspond with times of the year when birds should be expected to disperse to or return from high-altitude wetlands. The role of low-altitude or coastal wetlands is poorly understood but it could be argued that it provides temporary suitable habitat before departure or after arrival from elsewhere.

Various methods can be used to study the movements of birds, including ringing, telemetry, stable light isotopes and genetic analyses. In the case of the White-Winged Flufftail, very few records are known and sightings are unreliable. Ringing is not a reliable option as it is unlikely that a bird will be successfully recaptured. In contrast, telemetry could provide a suitable solution, but the current available tracking devices (e.g. geo-locators) are still not suitable to be comfortably placed on birds in this size category. Thus far, no genetic studies have been conducted on *Sarothrura ayresi* to confirm genetic connectivity between the South African and Ethiopian populations. In a study conducted by (Dalton *et al.* 2018), analysis of mitochondrial and nuclear markers was conducted for White-Winged Flufftail samples from South African and Ethiopian birds, as well as Red-Chested Flufftail (*Sarothrura rufa*) for species comparison. Analyses of the DNA regions identified three variations between the two populations, supporting the hypothesis that these two populations are not different species or sub-species but are rather one migrating population with different seasonal occupied ranges. However, these results do not exclude the possibility of additional breeding and non-breeding sites. Low genetic diversity in the populations of White-Winged Flufftails was observed, which needs to be further elucidated with fast evolving co-dominant markers such as microsatellites, as this low diversity may ultimately contribute to the extinction of the species.

Co-adapted gene complexes linked to selection including colour variants in economically important game species

Colour variation (including albinism and melanism) has been recorded for a large number of species worldwide. Colour variants are, however, extremely rare in natural populations, largely due to natural selection against such colour morphs. Variants which deviate extensively from the natural colour are frequently subjected to higher visibility by predators, increasing the chances of predation and removal from the gene-pool. Additionally, colour variation often involves aberrant genes or gene complexes, or the homozygosity of rare and/or recessive genes.

South Africa has the one of the largest commercial wildlife breeding industries worldwide. This industry largely focusses on rare game species, but in recent years has also shifted to breeding colour variants of common or abundant species. Colour variants are therefore intensively commercially bred in the Blue Wildebeest (*Connochaetes taurinus*), Springbok (*Antidorcas marsupialis*), Blesbok (*Damaliscus pygargus phillipsi*), Impala (*Aepyceros melampus*), Gemsbok (*Oryx gazella*) and Lion (*Panthera leo*). These colour variants largely include albinistic (white), melanistic (black) or golden morphs, with several derivatives thereof.

Colour variant animals selected for the commercial industry are frequently subjected to intensive captive breeding in small enclosures, so as to anthropogenically select and strengthen these character traits. While this poses risks of domestication and mal-adaptation to the natural environment (due to relaxed pressures of natural selection, through decreased predation, provision of food and antibiotics etc.), the genetic effects of these intensive and deliberate actions remain largely unclear. The scarcity of colour-morph individuals in natural populations strongly suggests that colour morphs derive from the expression of recessive alleles (homozygous), although it is clear that these are not single locus traits given the complexity and range of the colour variants.

A possible genetic result of these practices includes the mixing of co-adapted gene complexes linked to selection. Certain gene complexes (frequently tightly linked loci) coordinate and encode physiological traits which are directly related to an individual's fitness (physiological performance and reproductive fitness) within its natural environment. As a result of natural selection (directional selection) and local adaptation to a specific environment, these gene complexes may be differentiated between isolated and geographically disparate populations within a species (Wright 1969; Mayr 1970; Wallace 1981). Within each population, these gene complexes are therefore co-adapted. As a result of this local adaptation, the interbreeding of such disparate/differentiated/genetically unique populations may result in inter-population hybrids which have reduced fitness (due to genetic incompatibility) in either parental environment (also known as outbreeding depression or hybrid breakdown; see (Endler 1977; Burton 1986; Burton *et al*. 1999). In natural situations, outbreeding depression is frequently mitigated through positive assortative mating. Mating preferences therefore (genotypically and phenotypically) favour individuals who are co-adapted to increase fitness (see Bleay & Sinervo 2007 for an empirical example). Alternatively, the lower fitness of hybrid individuals in the wild results in the removal of these mal-adapted genes through natural selection.

Under the artificial situation created by the commercial game breeding industry, colour variants from disparate regions of the species' range are joined under intensive breeding programmes in relatively restricted enclosures. This situation negates all chances of mate choice (positive *assortative* mating), and is likely to result in the admixture of co-adapted gene-complexes. These hybrid individuals therefore have reduced fitness to the natural environment, which is further propagated through relaxed natural selective pressures under these circumstances. In other words, the survival of these hybrids (which potentially suffer from outbreeding depression) is insured, which is counter-productive to the conservation of genetic diversity and adaptability within the species.

The commercial value of colour variant species has seen a sharp decrease since 2016, largely as the demand for such animals in the hunting industry is far less than the current supply. Not only do these steep prices deter prospective hunters, but the ethical aspect of hunting captive bred animals has also been a deterrent.

Potential risks of the practise of intensive and selective breeding

Over the last three decades the South African wildlife industry has been largely compatible with conserving biodiversity and as such has made a significant contribution thereto (Child *et al*. 2012). In recent years, selective breeding and the intensive management of game has emerged as a new and growing sector within the broader private wildlife industry (Cloete *et al*. 2015; Taylor *et al*. 2015). Concerns have been raised about the long-term and potential consequences of the practice on other sub-sectors of the wildlife sector, as well as the country's biodiversity and biodiversity economy (Cousins *et al*. 2010; Dalerum & Miranda 2016; Pienaar *et al*. 2017). Following concerns raised within the Scientific Authority of South Africa in 2009 and the subsequent request from the Minister of the Department of Environmental Affairs an expert task team, consisting of scientists with a diverse range of skills and expertise was established in February 2013 by the Scientific Authority. The purpose of the task team was to both identify and assess the full range of potential risks to biodiversity and the biodiversity economy, and to compile a report for submission to the Scientific Authority. The Scientific Authority, in accordance with Section 61 of NEMBA, would in turn advise the Minister on appropriate, policy and regulatory responses if required.

The assessment used the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services' (IPBES 2014) conceptual framework to identify seven potential biodiversity risks or issues using the best available published scientific literature, information obtained from members of the wildlife sector,

experts and the national dialogue process. From these seven potential issues, 17 potential Impacts (harms or stressors) were described with specific concerns highlighted under each Impact Statement. Each impact was then assessed and scored on the quality of scientific evidence available, the probability of occurrence within the industry, and the likely impact on an ecosystem and species level respectively. The quality of the evidence was evaluated for scientific rigour using the 'uncertainty approach' as used by the UK National Ecosystem Assessment and complemented by a 'likelihood of manifestation' scale. A hierarchical ranking method was used to rank the impacts on a gradient from highest (1) to the lowest impact (5) at an ecosystem and threatened species level respectively. To determine the impacts with the highest potential risk at an ecosystems level, impacts with a score of 1 (Virtually certain) or 2 (Likely) were used. This was followed by ranking the selected impacts according to quality of evidence, only selecting those impacts with a score of either 1 or 2, and lastly on the probability of occurrence within the industry. A similar process was followed for assessing the risk to Threatened or Near Threatened species.

 The assessment concluded that the intensive management and selective breeding of game poses a number of significant risks to biodiversity at the ecosystem and species levels, as well as to other sectors of the biodiversity economy of South Africa, and may compromise the current and future contribution of the wildlife industry to biodiversity conservation. It identified several important direct risks and impacts on biodiversity at different scales, as well as indirect collateral negative impacts on conservation and the broader wildlife economy. Risks associated with:

i)   the significant increase in the extent of impermeable fences;
ii)   the intensification of management practices, and subsequent control of species that are likely to have a negative impact on breeding practices i.e. predators;
iii)   the incorrect use of pesticides, and unlawful use of hazardous substances, that lead to mortalities in indigenous species, had the biggest effect at an ecosystem level. At a species level the intentional breeding for selected traits and the removal of wild specimens of naturally rare species, or species with a small population size, had the highest risk.

Potential impacts related to the intentional breeding for selective traits such as colour, or increased horn or body size considered in the report were 1) the expression of deleterious genes, 2) the loss of genetic and allelic diversity, 3) outbreeding depression, 4) physiological stress, and 5) domestication. The objective of commercial breeding programmes is to maximize the rate of genetic change for economically important traits. Where these traits are only expressed in recessive phenotypes inbreeding or line breeding is often used to maximize the genetic progress towards these traits. The benefits of inbreeding are increased uniformity, increased prepotency (ability to pass on traits to offspring) and fixing of desired traits and breed type. It is thus virtually certain that breeding practices, such as inbreeding, line breeding and artificial selection for specific phenotypic traits are taking place within sectors of the wildlife industry (Dry 2016). Even though there has been little work undertaken on the genetic basis for colour transmission in African game species, it is well established in the peer-reviewed scientific literature with a high level of agreement that the selection of specific traits through a process of inbreeding or otherwise is highly likely to lead to physical, behavioural and lethal outcomes (Laikre 1999; Cieslak *et al*. 2011; Hofreiter & Schöneberg 2010; Jensen & Andersson 2005). In addition, these practices are likely to lead to a loss of genetic and allelic diversity that in turn is highly likely to result in decreased fitness and in the long term reduce the evolutionary potential of populations to adapt to environmental change (Hedrick & Kalinowski 2000; Reed & Frankham 2003; Frankham 2005). When specific traits, such as coat colour, are selected using artificial selection, the adaptive value of the trait is seldom considered. This may have unforeseen consequences and is likely to counter natural selection pressures that adapt an animal to its environment. It has been established in the scientific literature that colour variation is likely to influence an animal's

thermoregulation (Hetem *et al*. 2009) and that this (altered thermoregulation behaviour) may influence camouflage and social interactions such as mate selection. However, further research is required to better understand the impact of coat colour selection on an animal's ability to adapt to its environment.

With the expansion of the wildlife industry over the past three decades, there has been concomitant increase in human-mediated movement (translocation) of animals, within and outside their natural distribution ranges (Castley *et al.* 2001, Spear & Chown 2009, Taylor *et al.* 2015). The consequences of mixing of genes from naturally separated gene pools are poorly understood and both positive and negative consequences have been documented depending on the environmental conditions (Laikre *et al.* 2010). It has been well-established in the scientific literature that domestication results in diverse phenotypic and behavioural changes to wild animals, including decreased flight responses, increased sociality, earlier reproduction, and modification of endocrine and metabolic systems (Waples 1999; Trut *et al*. 2009; Teletchea 2017). The probability that the process of domestication will take place within intensive breeding facilities is virtually certain and the impacts or effects of domestication are dependent on the number of generations in a controlled environment and the degree of animal husbandry applied.

The extent and severity of all the impacts described for this issue will depend on the potential of the affected individual to reproduce and the proportion of animals of a particular species that are exposed to these breeding activities versus the wild. The risk is thus especially high for species with low population numbers or where the largest number of the species are kept under intensive conditions i.e. Roan Antelope (*Hippotragus equinus*), but much lower for common or Least Concern species. The highest level of impact will be on the individual exposed to these practices.

A mix of regulatory, awareness raising and incentive-based systems need to be implemented in order to mitigate the risks posed by this sub-sector of the wildlife industry. Given the challenges and costs of a regulatory approach, wherever possible, incentive-based approaches should be used as well as taking advantage of market forces to reward practices that are more compatible with biodiversity conservation and that are less risky to the biodiversity economy. However, the necessary enabling legislative framework for this needs to be created. Lastly, government and all role players in the wildlife economy, should take cognisance of potential far reaching implications of developing new ventures and sub-sectors within the wildlife sector. Principles of business and environmental sustainability as entrenched in NEMA on governance that considers social, environmental and economic aspects within the current and future landscape of the country would be critical to ensure sustainable growth of the biodiversity economy to the benefit of all.

2.3.2. Disease Epidemiology

The Risks of Infectious Disease to Biodiversity Conservation in South Africa

South Africa occupies 2% of the Earth's total land surface but has almost 10% of global bird, fish and plant species and 6% of the mammal and reptile species. This rich biodiversity is of immense economic, political and cultural value. As such, numerous institutions, policies and programs exist at both private and government level to protect this biodiversity. Furthermore, South Africa has relatively strong legislative frameworks for managing and protecting its rich biodiversity, including identification of possible threats such as climate change, habitat loss, etc. However, there is a scarcity of information and research regarding the threat which infectious diseases pose to South Africa's biodiversity, particularly wildlife. A brief summary of selected key issues relating to this problem is outlined below.

Lack of tools for detection and identification of infectious diseases in wildlife

There are numerous bacterial, viral, protozoal and fungal pathogens and parasites which infect and cause significant morbidity and mortality in wildlife. When animals, particularly wildlife, become ill, it is important to accurately and rapidly identify the cause of the illness. Accurate identification of the etiological agent of disease is critical for the treatment of disease, prevention of disease spread, surveillance and monitoring of disease outbreaks, and initiation of effective preventative policies. However, in many clinical cases, rapid identification of the disease agent is not always possible thus veterinarians often struggle to accurately diagnose these infections, and treatment has to rely on a tentative diagnosis, which may not be correct (Wobeser 2007). This may result in death of several animals before the causative agent is reliably identified. This is even more important when considering conservation of endangered species, where invasive sampling is not always advisable or possible (Thompson *et al.* 2009).

Accurate identification of these pathogens often requires expensive laboratory equipment, highly trained personnel and may be time consuming (e.g. culture based identification and characterisation). A major challenge is that there is a distinct lack of diagnostics tests that have been developed, optimised and validated for use on wildlife. This makes identification and investigation of infectious diseases in wildlife very challenging. To address this, the Molecular Disease Epidemiology (MDE) sub-program at SANBI conducts research for development of molecular detection assays to identify and investigate infectious diseases in wildlife. Several molecular assays have been developed for detection and identification of *Toxoplasma gondii*, fungal dermatitis, *Cryptococcus gattii*, avian haemoparasites and viruses (pox, parvo, and papilloma) in wildlife. Research is on-going to develop molecular detection assays for other important pathogens and parasites (e.g. *Mycoplasma*, *Haemonchus*, *Nematodirus*, *Trichostrongylus*, and herpes virus) which cause disease in wildlife.

Lack of surveillance programs to monitor infectious diseases in wildlife

In South Africa, there is an ongoing shift in land use practises from traditional livestock farming to wildlife based activities such as eco-tourism, breeding, hunting among others (Bekker *et al*. 2012). As a result there is increasing interaction between wildlife, domestic or agricultural animals and humans. These interactions increase the risk for infectious diseases to spread from wildlife to domestic animals and humans and vice versa. There have been numerous outbreaks of infectious disease that have severely affected wildlife populations in South Africa. Many of these outbreaks are caused by zoonotic pathogens and thus also pose a threat to human health. For example, there have been cases of anthrax, botulism, brucellosis, rabies, toxoplasmosis, and tuberculosis in several species of wildlife in South Africa over the last two decades (Bekker *et al*. 2012). Information relating to the interaction of pathogens and parasites with wildlife is severely lacking, as is our understanding of the effects these pathogens have on wildlife health. This is particularly problematic with wildlife where accurate estimates can be difficult to achieve for many reasons such as limited sampling availability, inability to conduct invasive sampling in many species, animal migration etc. Furthermore, there are currently no formalised programs or legislation that are specifically targeted towards monitoring and surveillance of these and other infectious diseases in wildlife in South Africa. In contrast there is legislation governing animal diseases and trade in meat and meat products but these are aimed primarily at livestock and poultry and not wildlife. This legislation has resulted in significant effort to monitor and control the spread of several pathogens (e.g. foot and mouth disease) that infect and cause disease in agricultural and domestic animals and are thus of economic importance. The lack of on-going, formalised programs and legislation for monitoring, surveillance and epidemiology of infectious diseases in wildlife is a significant threat to *in-situ* and *ex-situ* conservation programmes in the country. Furthermore, the increased interaction between wildlife, humans and domestic animals poses a significant

threat to human and animal health. Thus, there needs to be legislation and formalised programs in South Africa for surveillance and epidemiology of known infectious diseases in wildlife.

Economic impact of disease on wildlife in South Africa

The global economy loses billions of dollars every year due to infectious diseases of agricultural crops and livestock (Brownlie 2012). There have been several outbreaks of infectious diseases in South African crops, livestock and also in humans that have had major negative effects on the economy. Epidemics of infectious diseases can have devastating economic impacts. Most recently in South Africa, the outbreak of avian influenza and listeriosis resulted in major losses to the poultry and food industries respectively as well as causing severe health crises. A 2011 outbreak of a Highly Pathogenic Avian Influenza (HPAI) in South Africa resulted in the preventative slaughtering of 50 000 birds and suspension of all poultry related exports resulted in export losses of 140 million dollars (Lebea *et al*. 2014). The Severe Acute Respiratory Syndrome (SARS) outbreak in 2003 resulted in a 2% fall in gross domestic product in Asia, despite causing less than a 1000 human deaths (Brownlie 2012).

 In South Africa, the wildlife industry has grown significantly over the past few decades. However, there are many pathogens and emerging infectious diseases which pose a threat to wildlife and the associated ecotourism industry. Furthermore, these diseases impede export and trade of wildlife which has negative economic effects on the wildlife industry (Lebea *et al*. 2014). For example, there have been several outbreaks of foot-and-mouth disease, which infects cloven-hoofed animals (domestic and wildlife) in Southern Africa between 2007 and 2010. Foot-and-mouth disease (FMD) is the most important viral disease of domesticated livestock (Lebea *et al*. 2014). Furthermore, FMD infects a wide variety of wildlife and its epidemiology is greatly influenced by the African buffalo which is a reservoir host for this disease and also plays a role in transmission of FMD to susceptible domestic animals (Lebea *et al*. 2014). Aside from FMD and avian influenza, there have been very few studies to investigate the economic impact of infectious diseases in wildlife in South Africa. There is significant potential for outbreaks to occur in wildlife which will result in significant mortalities and negatively affect wildlife conservation and associated industries (e.g. ecotourism).

2.3.3. Impacts on marine resources

Implications of marine aquaculture on the genetic integrity of South African marine species

South Africa has a growing mariculture sector that is highlighted in Operation Phakisa due to its high growth potential. However, currently the contribution of the mariculture is low to the seafood supply chain. Mariculture species in South Africa are dominated by invertebrates, particularly Abalone (*Haliotis midae*), Mussels (*Mytilus galloprovincialis*) and Pacific Oysters (*Crassostrea gigas*), although some finfish farming, including Kob, (*Argyrosomus* spp) and Yellowtail *(Seriola lalandii)* is occurring at small, exploratory scales. In addition, some seaweeds species including sea lettuce (*Ulva* spp.) and red algae (*Gracilaria* spp.) are also commercially grown. From a genetic perspective, the danger of mariculture lies primarily in the mixing of genetic lineages between commercially farmed and natural populations. However, both *M. galloprovincialis* and *C. gigas* are already invasive and naturalised in South Africa, with extensive populations along the coastline, but currently there is no evidence of hybridisation with native species.

For abalone*,* genetically structured populations were recovered for both mitochondrial (Evans *et al.* 2004) and nuclear markers (microsatellites, SNPs, see (Bester-van der Merwe *et al.* 2011), highlighting that the provenance of mariculture populations should be carefully considered, and should be based on a several lines of evidence. Further, (Rhode *et al*. 2012) showed similar levels of genetic diversity for natural and cultured populations of *H. midae*, although cultured populations were genetically distinct from wild

abalone, potentially as a result of selective pressures during the selection process of individuals, with strains selected to the environment of each mariculture facility. Both (Bester-van der Merwe *et al.* 2011; Rhode *et al.* 2012) highlight the need for maintaining genetically diverse natural populations to support the mariculture industry, which also includes making provisions that commercially grown abalone are not released, accidentally or otherwise, into natural systems, as the latter poses a serious risk to the genetic integrity of an already vulnerable stock. With the current plans for expanding mariculture activities around South Africa, any new venture needs to ascertain the levels of genetic structure and diversity of their species of interest, in order to minimise genetic pollution of naturally occurring populations.

Impacts on genetic integrity driven by over-exploitation: marine environment

Globally, there is increasing evidence for the impact of overfishing on the genetic integrity of exploited marine species, including a loss of genetic diversity on natural fish stocks (Pinsky & Palumbi 2014) and marine reptiles (Rodríguez-Zárate *et al*. 2013). This is not only confined to commercially or recreationally targeted species, but has also been shown in fishes collected for the aquarium trade (Madduppa *et al*. 2018). Depleting genetic variation is of high concern, because ultimately it can erode the potential for species to adapt to future change, thereby increasing extinction risk. Even though many marine species have large population sizes, including large effective population sizes, overexploitation as a driver of changes in measures of genetic diversity, population structure and adaptability remains troubling.

In South Africa, disentangling the effects of over-exploitation on marine species has not yet received much attention, mainly because there is a lack of pre-exploitation material that can be used to characterise genetic parameters prior to exploitation to which contemporary samples can be compared to. Furthermore, some species also show patterns of genetic chaotic patchiness that point to complex spatio-temporal patterns influenced by seasonal changes in the environment (von der Heyden 2009; Teske *et al.* 2011; Henriques *et al.* 2016). However, some attempts have been made to model genetic population dynamics through time using molecular markers for the Cape Hake and Kingklip, species that greatly contribute towards the demersal fishing industry of South Africa. For the Cape hakes, *Merluccius capensis* and *M. paradoxus*, (Henriques *et al.* 2016) provide evidence for a loss of genetic diversity over 15-50 generations, coinciding with increased fishing pressures in the 1970s and 1980s. This may have contributed towards lower levels of contemporary genetic diversity for both species. For Kingklip (*Genypterys capensis*), fishing pressure has also been invoked as an explanation for lower levels of current genetic diversity than historical measures (Henriques *et al.* 2017). These three species share several life history characterises including being relatively slow-growing and long-lived, that may make them more susceptible to loss of genetic diversity than faster growing and maturing species in the region. Genetic studies were also used to estimate the population size of the Great White Shark (*Carcharodon carcharias*) in South Africa. Although listed on CITES, the IUCN Red List and being a protected species in South Africa since 1991, genetic estimates showed a decline in population size of over 50% in recent years (Andreotti *et al.* 2016), placing the future of one of South Africa's iconic marine species in doubt. However, more emphasis should be placed not only on understanding the impacts of overexploitation, but also habitat loss and other anthropogenically driven processes, as well as climate change on offshore and coastal marine species in South Africa.

2.3.4. Genetically modified organisms

The Cartagena Protocol on Biosafety (Biosafety Protocol) under the Convention on Biological Diversity (CBD) describes Living Modified Organisms (LMOs) and Genetically Modified Organisms (GMOs) under one definition that refers to any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology (Falkner 2000). In some instances, these organisms are

also termed Genetically Engineered Organisms (GEOs). The Biosafety Protocol's main objective is to contribute towards ensuring an adequate level of protection in the field of the safe transfer, handling and use of living modified organisms resulting from modern biotechnology that may have adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health. For this purpose, Parties (countries) to the protocol need to develop comprehensive, transparent and scientific methods for adequate pre and post releases testing, management and monitoring, of GMOs released into the environment to ensure their environmental safety and sustainable use. Generally, GMOs are perceived to offer new options for meeting various needs (i.e. agricultural) in both developed and developing countries but can also pose several risks to the environment (Hilbeck *et al.* 2011).

The release of a GMO into the environment of South Africa is a regulated activity under the GMO Act, 1997 (Act 15 of 1997), which requires that an assessment of potential adverse effects to the environment be undertaken prior to conducting any activity with a GMO. Furthermore, the protection of the environment, in the context of GMOs, is informed by the National Environmental Management Act (NEMA), 1998 (Act No. 107 of 1998) and the National Environmental Management Biodiversity Act (NEM:BA), 2004 (No. 10 of 2004). The Environmental Risk Assessment Framework Guidance Document for GMOs (2008, under review) was also developed as an additional tool for environmental management consideration. For the purpose of risk assessment, an Environmental Risk Assessment (ERA) process is conducted to evaluate the likelihood that harm may occur as a result of exposure to a GMO. There are multiple scientific studies that sets a baseline on how an ERA can (should) be conducted, although strongly based on Genetically Modified Plants, also known as GM /Biotech crops (Suter II 2000; Garcia-Alonso *et al.* 2006; EFSA 2011; Devos *et al.* 2015). In conducting the assessment, information on potential adverse effects of a GMO on biotic and abiotic components of the environment, including non-target organisms, ecosystems and biodiversity must be evaluated. Ideally, assessment endpoints and protection goals set out by relevant legislations serve as a guide or form representative aspects of the environment that need to be protected from harm for ERA of GMOs intended for release into the environment.

The discussions around environmental risks and benefits of GMOs are common knowledge. These have been debated for decades on different scientific and social platforms. Nonetheless, there remain huge disparities in terms of evidence present to defend either argument. The 2017 Global Status of Commercialized Biotech/GM Crops, which covers 22 years of GM crop cultivation globally, reported that a total of 24 countries to be planting GM crops. According to the report, this highlights the recognition in benefits of GM crops across countries. Of the 24, 19 were developing countries and five developed (industrialized) countries. The land area of GM crop cultivation increased by 3% (4.7 million hectares) from 2016 to 2017. In fact, in 2016 GM crops constituted 185.1 million hectares compared to 189.8 million hectares in 2017. South Africa, which approved its first GM crop in 1997, is in the top ten globally in terms of GM crops planting, and continues to lead on the African continent. Since 1998, approximately 70 events have been approved for food, feed, and planting. These include five Argentine canola events, 10 cotton, 42 maize, 1 rice, and 12 soybean events. Other approvals (nonfood/feed/cultivated crops) include that of TB and poultry vaccines. In 2017, 2.73 million hectares were planted under GM crops compared to the 2.66 million hectares in 2016 (ISAAA 2017). GM Maize accounts for the most number of hectares planted (1.96 million), soybeans (736,535) and cotton (37,406). About 85% (down from 90% in 2016) of maize in SA is GM, whereas soybeans and cotton accounts for 95% and 100% respectively (ISAAA 2017).

GM crops and genetic flow concerns including genetic contamination

The three GM crops (cotton, maize and soybean) introduced and under cultivation in South Africa are part of the top four biotech crops globally, with the exception of canola (ISAAA 2017). All these crops are well

studied and documented in relation to perceived environmental concerns (Conner *et al.* 2003). For example, comprehensive studies on cotton (Hilbeck *et al.* 2006), maize (Dale *et al.* 2002) and soybeans (Turkec *et al*. 2016) are published. In most instances, the context of the studies cover the subject area on Non Target Organisms (NTOs), including generalist arthropod predators, pollinators and parasitoids; development of resistance, its management and monitoring (case of *Bacillus thuringiensis* (Bt) events); gene flow and pollen transfer resulting in hybridization between species, and in some cases GM crops contribution to biodiversity loss, increased invasive of weed species and the promotion in use of pesticides.

In reality, the release of any GMO into the environment possesses the general difficulty in predicting the occurrence and extent of long-term environmental effects even when a thorough ERA process has been followed. This is where protection goals and assessment end points are critical to clearly define the harm and make provisions to detailing the various pathways, and under which context each would be addressed. To unpack this in context of GM crops and gene flow, which is in most cases labelled as genetic contamination, it is first crucial to understand the applicability and context of each.

In most countries where GM crops have been introduced, they have no wild relatives. This also applies to South Africa. Studies that explore potential scenario in this regard, base their investigation on GM and non-GM crops in co-existence (Friesen *et al*. 2003; Pierce *et al.* 2005; Groenewald & Groenewald 2009). There are also examples of genetic contamination in maize for countries like Mexico, where no GM maize has been approved for commercial cultivation, but only for trial purposes (Quist & Chapela 2001). Whenever cases of gene flow are envisaged or arise between GM and non-GM populations, it becomes not only a complex situation in accounting for the environmental protection, but also for the ethical, financial (commercial), health (includes foodstuffs and labelling) and religious complexities.

For the environment, in the context of non-GM/wild relative populations, a few considerations, scenarios and questions need to be addressed for either risk mitigation, management, general surveillance or case by case monitoring. In no particular order of importance, the following need to be accounted for:

- There needs to be a clear definition on the context of harm perceived or resulting from the presence of GM material in non-GM populations (crop or non-crop/wild relative). This has to be guided by the ERA process taking into account the identification of harm, its characterization and problem formulation to determine the likelihood of harm;

- The harm identified and the likely pathways to harm should be investigated and subsequently have mitigation and management measures as well as a clearly defined long term monitoring plan guided by protection goals and assessments end points;

- The mitigation measures have to take into account that the presence of GM material in non-GM populations can take place at different levels, namely cross-pollination/ fertilization, during harvesting, transporting and storage - on and off farms; and due to seed exchange among farmers. The latter facilitates for greater and unpredictable distances for GM material introduction in foreign areas. In some instances, these might be areas not approved for GM material for cultivation or other related uses; and

- There is a need to determine specific separation distances for GM and non-GM populations in areas of coexistences. This requires scientifically sound measures guided by the ERA process and possible harm identified. It should be noted that the separation distances are likely to differ among crops and populations given different modes of pollination or their presence in the landscape.

Forecast on new crops and considerations for environmental research on gene flow

In the past few years, we have seen an accelerated emergence of research and trials on new GM crop traits, primarily driven by investments, economic, societal challenges and concerns. According to ISAAA (2017), there are about 13 crops currently undergoing research and trial on the African continent and some of these crops are likely to have general releases (approvals for commercialization cultivation) concerns due to the presence of wild relatives in their respective countries and environments. For South Africa in particular, the report indicates that new traits of cotton, maize and soybean are being investigated. There are other crops such as sorghum, sugarcane, cassava and grapevines currently being experimented on in South Africa for different desired traits. At the same time, the National Strategic Action Plan for the Conservation and Sustainable Use of Crop Wild Relatives in South Africa (2017 unpublished, http://www.cropwildrelatives.org) identified and provided a detailed checklist of crop wild relatives, which are wild species of plants that are closely related to crops used in subsistence or commercial agriculture. According to the action plan, these crop wild relatives are a vital component of agricultural biodiversity and important for food and nutritional security. Among the identified genera, there are a few with future potential concerns on the subject of gene flow due to their experimentation with GM traits as they have existing crop wild relatives. These include *Vigna* (cowpea), *Gossypium* (cotton), *Solanum* (potato/eggplant), *Sorghum* (sorghum), *Imperata* (sugarcane) and *Ipomoea* (sweetpotato).

The South African government acknowledges and encourages the use and adoption of Biotechnology and other New Breeding Techniques and this is document by various legislative documents (e.g. The Bio-Economy Strategy 2013). This is aimed at supporting and unlocking various economic opportunities and addressing health, food, societal and environmental challenges. Therefore, more experiments and research on crops either than those currently outlined here would be upon us in the near future. In tackling the research and data information gaps on gene flow as discussed above, there is a strong need for dedicated research as a proactive measure for South Africa both from an agricultural and conservation perspective.

2.3.5. De-extinction

The Earth has been through several mass extinction events, the worst being the Permian-Triassic event (ca. 251 million years ago) where 96% of species on the planet became extinct. Mass extinctions are thought to occur due to stochastic global events such as meteor strikes or extreme volcanism that causes severe changes to the atmosphere bringing about rapid shifts in environmental conditions to which life cannot quickly respond. Extinctions also occur at a steadier rate (background extinction rate; (De Vos *et al.* 2015) where species typically have a life-span and eventually die out most likely due to long term environmental changes to which they do not adapt (May 1995). For example, after glacial maxima, cold adapted species may die out due to the warmer conditions (e.g. woolly mammoth). Recently, the natural extinction rate has become elevated (up to 10,000 times higher than normal background rates) due to anthropogenic impacts (Pimm *et al.* 2006; De Vos *et al.* 2015), and as of 2017, the International Union for Conservation of Nature (IUCN) lists ca. 700 recent species extinctions (*Summary Statistics* 2018). Some of the more notable examples of these anthropogenic extinctions were the result of targeted hunting, such as the South African Bluebuck Antelope (*Hippotragus leucophaeus*), the South African Cape Lion sub-species (*Panthera leo melanochaita*), the Tasmanian Tiger (*Thylacinus cynocephalus*), the Great Auk (*Pinguinus impennis*), and the Passenger Pigeon (*Ectopistes migratorius*). Other extinctions are indirectly caused, due to the destruction of a species habitat and the species along with it. For example, South Africa has two extinct reptiles (*Tetradactylus eastwoodae* and *Scelotes guntheri*) who met their demise through the destruction of their habitat (Bates *et al.* 2014).

Recent technological advances in genetics have shaped the idea that extinctions do not have to be forever. That is, extinct species could be brought back (de-extinction) through either back-breeding, cloning, or genetic engineering (Sherkow & Greely 2013).

Back-breeding: An extinct species with close living relatives can be re-created through selective breeding guided by screening of the genome for desired traits. The phenotype of the extinct species is selectively bred into a few individuals of the living relative species. The population of individuals is then meant to be re-introduced into the original habitat. This approach already exists in South Africa, with an attempt to re-create the extinct subspecies of the plains zebra, the Quagga (*Equus quagga*; https://quaggaproject.org/). With other initiatives to back-breed extant species to produce the phenotype of extinct species (e.g. European auroch: (Sinding & Gilbert 2016), with the suggestion that such programs can meet this endpoint.

Cloning: The cloning technique has existed for a number of decades, although it is not widely applied. With cloning, an embryo containing the nucleus of the extinct species is implanted in a surrogate maternal host. However, this technique can only work if live cells of the extinct species were preserved. The method was used to revive the Spanish ibex (*Capra pyrenaica*), which went extinct in 2000 (Folch *et al.* 2009).

Genetic Engineering: This method is being promoted as a tool for reviving long extinct species where either back-breeding or cloning could not be used. The genome of extinct species are sequenced and scanned for specific mutations that characterise the extinct species. Using the CRISPR/9cas technology, the relevant mutations are inserted into the genome of an embryo from an extant relative.

South Africa has a number of species that are at risk of extinction, as well as several extinct species. Therefore de-extinction as a conservation tool is a tangible possibility. However, these methods do not actually restore the extinct species, because the original genome of the extinct species has not been recreated (Campbell & Whittle 2017; Shapiro 2017). With back-breeding, the restored population is simply a modified phenotype of the extant related species that resembles the extinct species. With genetic engineering, the restored population is a closely related species that is given certain mutations that were present in the extinct species. Cloning does generate a new individual of the extinct species, but it is a twin of the donor individual, so many individual and different clones would need to be generated to create a viable population of the extinct species e.g. (Bennett *et al.* 2017). These clones would all need to be derived from living cells from the extinct species, prior to its extinction.

De-extinction has been proposed as a conservation tool to either return recently extinct species to our planet, or to plan for species that are at severe risk of becoming extinct. Certainly, to maintain a healthy functioning ecosystem, species extinctions are in no way desired. So the benefit to de-extinction is that in cases where we lose species, we could potentially correct the situation. There are a vast number of epidemiological, ethical, legal and political issues related to this approach (e.g. (Cohen 2014; Campagna *et al*. 2017; Lacona *et al.* 2017) not to mention the potential implications for ecosystem functioning (e.g. (McCauley *et al.* 2017; Selbach, Seddon & Poulin 2018). Each of these issues has invoked heavy debate with no clear agreement or way forward at present.

Curiously, the risk to natural genetic diversity of species seems to be less prominent in the debate. Essentially, the released population of the 'reimagined' extinct species could potentially interbreed with extant species, resulting in genetic pollution of the extant species. The genetic pollution could bring about an unintended change in the genotype or phenotype of the extant species. Such changes could have consequences for the extant species survival through introduction of undesirable or even deleterious mutations in the extant species. Furthermore, the reimagined extinct species, which would be created through huge expense and effort, could also become genetically polluted through interbreeding with the

extant species. In both cases, there is a tangible risk to maintaining the genetic diversity of the species involved.

2.4. Conclusions

Life on earth relates directly to the diversity of genes in space and time, and DNA can best be described as the foundation of all life on earth. Genetic diversity is recognized as an important component of biodiversity (together with species diversity and ecosystem diversity). It can be defined as the amount of variation observed in the DNA of distinct individuals. The maintenance of diversity is of the utmost importance as genetic diversity equates to evolutionary potential (and thus allow species or populations to adapt to an ever-changing environment).

Importantly from a conservation perspective, the levels of genetic diversity seem to vary greatly in natural populations and species, however, the drivers of this variation, and particularly the influence that species' biology (e.g., dispersal capability), mating system, social system, ecology (e.g., habitat preference), and population history has on this, remains moot for the large part. In addition, genetic diversity is often linked to mutations and adaptation across space and time. Management of populations and species were historically based on crude assessments of threats, habitat degradation, genetic diversity (notably allozyme and restriction fragments length variation) and other ecological factors. With the advent of more sensitive molecular tools, notably DNA sequence data and microsatellite information based on polymerase chain reaction amplifications, genetic information started to play an increasingly important role in decision making. Extrinsic benefits to protecting genetic diversity include genetic rescue, biobanking, applications in forensic sciences, barcoding of species (known and unknown), bioprospecting, ethnobotany and indigenous knowledge, and tourism and biodiversity hotspots. Risk factors include habitat fragmentation which reduces genetic diversity, decreased connectivity between populations, urbanization, hybridization between distinct species, inbreeding, intensive breeding of economically important species (which may reduce genetic diversity through strong selection for economically important phenotypes), and disease epidemiology. Genetically modified organisms remain a contentious and often misunderstood area. New fields are emerging that hold promise as well as potential risks; de-extinction and gene modifications are included here.

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3. MONITORING TRENDS IN GENETIC DIVERSITY FOR PRIORITY TAXA IN SOUTH AFRICA

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Overview

This chapter discusses the status of genetic monitoring in South Africa, highlighting key considerations for future genetic monitoring programmes, such as which species to prioritize, how often should they be monitored, what molecular markers to use, and the type of indicators to be evaluated.

3.1. Introduction

The importance of genetic variation for maintaining biological diversity and evolutionary processes has been recognized by researchers for decades (e.g. Frankel 1974; Lande & Shannon 1996; Frankham 2005; Hughes *et al.* 2008; Bijlsma & Loeschcke 2012). Intraspecific genetic diversity, in particular, represents a species' evolutionary potential to evolve and adapt within a changing environment. In this way, genetic diversity drives the process of speciation and plays a pivotal role in ecosystem structure and function (Hughes *et al.* 2008; Whitham *et al.* 2008).

Many species are equipped with sufficient evolutionary resilience, or genetic diversity, to overcome rapid environmental change (Hughes *et al.* 2008); however, others are not, as is evident by the rapid loss of biodiversity reported globally (Millenium Ecosystem Assessment 2005). Most of these instances of decline have been documented in cultivated species or species that are heavily exploited. For wild species, however, there are few data on the actual changes in the magnitude and distribution of genetic diversity (Millenium Ecosystem Assessment 2005). This realization prompted the conservation of genetic diversity being listed as an explicit goal of various national and international agreements. In particular, the Convention on Biological Diversity (CBD) and its member nations explicitly agreed to 'promote the conservation of genetic diversity' (Goal 3: UNEP 2003) and sought 'to achieve, by 2010, a significant reduction in the current rate of biodiversity loss at the global, regional and national levels' (UNEP 2003). Despite this commitment, implementation of the conservation and monitoring of genetic diversity has lagged behind implementation for other levels of biodiversity (Laikre 2010; Laikre *et al*, 2016). This has generally been attributed to the lack of genetic diversity indicators and thresholds (Walpole *et al.* 2009; Laikre 2010; Tittensor *et al.* 2014; Laikre *et al*, 2016). Although there are examples of conservation genetic studies that have utilised genetic parameters, such as allelic richness and heterozygosity, to monitor changes in the genetic composition of species over time, there has been no consistent and comparable approach among them with respect to the number of sampling periods, sampling intervals, the type and number of genetic markers used, and the number of individuals sampled. Consequently, it has been difficult to identify simple and direct indicators sensitive enough to detect genetic erosion, resulting in genetic biodiversity indicators being largely overlooked until recently (Hoban *et al.* 2014).

With the release of the Aichi Biodiversity Targets in 2010, there is a renewed emphasis on the conservation of genetic diversity. In particular, Target 13 states: 'By 2020, the genetic diversity of cultivated plants and farmed and domesticated animals and of wild relatives, including other socio–economically as well as

culturally valuable species, is maintained, and strategies have been developed and implemented for minimizing genetic erosion and safeguarding their genetic diversity'. The specific mention of minimizing genetic erosion implies that genetic diversity be monitored over time in a given population. This temporal dimension is key as some studies claim to monitor genetic diversity when in fact they are actually 'assessing' genetic diversity at a single point in time (Schwartz *et al*. 2007). For example, using genetic data from a single year or pooled data from multiple years to provide a single-point estimate of a population's diversity and structure (e.g. Baker *et al.* 2000; Hua Yue *et al.* 2004; Cannas *et al.* 2016).

The value of long-term monitoring, in general, is well recognized. By repeating measurements through time, we understand what and how things are changing temporally. Monitoring has to be ongoing to detect change and a track record of data is required in order to be able to guide conservation planning and management so that we can respond effectively to change and its causes. Monitoring is equally important when little to no change is detected as it enables us to better understand stability and how it can be achieved (da Silva & Tolley 2018). However, most known long-term monitoring programs (i.e. those running over several decades) are strictly ecology-based and typically focus on population counts (e.g. Rijnsdorp *et al.* 1996; Lundie-Jenkins, Hoolihan & Maag 1999; Silvertown *et al.* 2006; Masubelele *et al.* 2013; Sauer *et al.* 2017). Due to the general lack of temporal genetic datasets globally, it was not surprising that a mid-term analysis of the Aichi Targets was unable to assess progress toward reaching Target 13 (i.e. maintaining and monitoring genetic diversity).

As a member nation, South Africa is committed and obliged to achieving the main objectives of the CBD, as well as the Aichi Biodiversity Targets. However, as of 2013, the country's main contributions to achieving Target 13 were guidance and framework documents relating to risk analysis of genetically modified organisms (DEA 2014). Since then, only a couple of studies have been published that explicitly monitor temporal shifts in the genetic diversity of South African taxa. One study looked at quantifying changes in genetic diversity within a narrowly endemic amphibian believed to have undergone an enigmatic decline (da Silva & Tolley 2018; Box 3.1); while the other investigated changes in genetic diversity within two generations of captive African penguin (Labuschagne *et al.* 2016; Box 3.2). Both of these studies are considered fairly short-term (i.e., under 20 years and/or within a few generations), and as such, may be considered uninformative and potentially overlooked given that there is less chance of detecting strong genetic change. However, even studies based on two time points can potentially provide invaluable information on how a population has changed, what underlying conditions precipitated the change, and how this can inform the future management of the population (Leonard 2008; Potvin *et al.* 2017). Even if no change is detected, such studies are a critical first step in establishing the appropriate genetic monitoring framework for a population or species (e.g. by helping to decipher the appropriate monitoring intervals, how many samples and markers to use). Without them, long-term monitoring programs may never be established.

Having explained why genetic monitoring is important, the rest of this chapter will focus on the what, when, and how of genetic monitoring to illustrate its usefulness in biodiversity conservation across all realms and how it can be incorporated into future research and management programmes within South Africa.

Box 3.1. A case study for genetic monitoring: Rose's Mountain Toadlet (*Capensibufo rosei*).

Capensibufo rosei (Cape Mountain Toad or Rose's Mountain Toadlet) is a small (c. 2–3 cm in length), range-restricted threatened amphibian endemic to the Cape Peninsula of South Africa. This toadlet is found only in the naturally fire-prone fynbos habitat (heathland vegetation type endemic to the Cape of South Africa: Mucina & Rutherford 2006). Unlike most anurans, it has no calling or auditory apparatus, making it the only known voiceless amphibian in southern Africa (Grandison 1980). These cryptic toadlets breed in small, shallow (10–30 mm depth, ca. 10–50 cm diameter) ephemeral pools formed by winter rainfall, with masses of males aggregating at these breeding pools during their relatively short breeding season (approximately 2 weeks in July or August) (Minter *et al.* 2004; Edwards *et al.* 2017). The pools are scattered within an area not usually more than a few hundred metres across. Individual females enter the pools briefly only to lay eggs (clutch size < 100 eggs: Grandison 1980), which are externally fertilized by males. Females leave as soon as spawning is complete, while males remain at the pool throughout the breeding season (Becker *et al.* 2018; Edwards *et a*l. 2017; K. A. Tolley, personal observation).

This toadlet is believed to have experienced an enigmatic decline, with the loss of several of its historical breeding populations despite the presence of apparently suitable habitat with natural vegetation and pools of water (Cressey *et al.* 2015). It is speculated that the suppression of natural fires and the loss of grazing wild animals from the Cape Peninsula may be the primary cause of this decline (Becker 2014; Cressey *et al.* 2015). Without these disturbances, the fynbos becomes overgrown and can even revert to thicket or forest (Bond 1997), and although some pools might still form, the pool characteristics (depth, size, shading, water retention) are probably affected. The loss of historical breeding sites has resulted in a considerable reduction in this species' distribution, with its's current Area of Occupancy at less than 10 km^2, resulting in it being listed as Critically Endangered on the IUCN Red List (SA-FroG 2017).
Currently, it is known from two populations, both with Table Mountain National Park – one historical site in Silvermine Nature Reserve and a new breeding site discovered in 2010 in the Cape of Good Hope (CGH) Nature Reserve (REF _Ref536524819 \h Figure 3). The Silvermine and CGH populations are approximately 20 km apart and population genetic analyses have shown that there is strong population structure with presumably no gene flow between sites, likely owing to the loss of the other historical breeding sites disconnecting dispersal pathways (Cressey *et al.* 2015; da Silva *et al.* 2016).
Moreover, within a seven-year time period (2008–2014), the survival and recruitment rates of *C. rosei* fluctuated considerably in response to rainfall, with high survival and low recruitment during low rainfall years, and the inverse during high rainfall years (Becker *et al.* 2018). Their isolation and the sharp fluctuations in their survival could potentially translate into population size fluctuations (e.g. Altwegg *et al.* 2003; McCaffery & Maxell 2010; Newell *et al.* 2013), which could result in changes in genetic diversity within a relatively short time period. To test this, a genetic monitoring study was conducted comparing the genetic diversity of toadlets from two time periods – 2011 and 2015 (da Silva & Tolley 2018). These dates were chosen because:(1) the five-year time interval fits within the temporal sensitivity requirement of genetic monitoring studies (i.e. at least one generation time of a species: Pereira *et al.* 2013; for *C. rosei*, this time period encompasses between two to five generations), (2) 2011 represents the earliest year in which samples from both populations were available, and (3) 2015 the most recent batch of samples at the time.

Box 3.1. Continued.

Map depicting the approximate locations of the two breeding sites (white circles) of Capensibufo rosei (inset) on the Cape Peninsula (taken from da Silva & Tolley 2018).

The study found that both populations were genetically stable over the five-year time interval in terms of retention of total diversity, and that the levels of genetic diversity increased slightly between sampling periods for both populations. Although this increase was not statistically significant, it does appear to correspond to increases in population size estimates detected by Becker and colleagues (2018) following a fire in 2015, likely suggesting that habitat quality is an important factor influencing the viability of these toads. Ongoing genetic monitoring at approximately five year intervals will build upon this initial assessment and help SANParks in the adaptive management of this species.

Box 3.2. A case study for genetic monitoring: African Penguin (*Spheniscus demersus*).

Spheniscus demersus (African penguin) is endemic to southern Africa, breeding mainly on islands off the coasts of South Africa and Namibia. The species is currently listed as 'Endangered' (BirdLife International 2018) and continues to decline, with fewer than 26 000 breeding pairs remaining (Crawford *et al.* 2011). Due to intensive exploitation of guano resources and egg collection in the early 20th century, followed by more recent population declines attributed to food prey shortages, environmental fluctuations, oil spills, and competition with commercial fisheries, the species has declined by >95% of its historic population size.

In South Africa, just eight colonies support 87% of the country's population and a number of established *ex situ* breeding programs are intended to provide population supplementation and possible restoration into the future. Given that *ex situ* populations of the African penguin are derived from a small number of founders, management informed by the risks of negative genetic changes (e.g. founder effects, inbreeding depression and genetic adaptations to captivity) is vital. Managing *ex situ* penguins in the in the absence of a genetic strategy will undoubtedly reduce their conservation value significantly. A recent study established important baseline estimates of neutral genetic variation in *ex situ* African penguins. This study provides an initial framework for future monitoring of *ex situ* population resources that is required to ensure their continued value to regional conservation efforts.

Labuschagne *et al.* (2016) genotyped 1 119 penguins from South African zoo and aquarium facilities at 12 microsatellite loci. While estimates of allelic diversity (mean N_A = 5) and heterozygosity (mean H_O = 0.58) were both moderate, the authors conclude that based on differences between first- and second-generation captive birds the *ex situ* population of African penguins is at risk of losing neutral genetic variation in the future. To increase the *ex situ* effective population size, and thereby reduce the rate of genetic drift, management will need to include exchange of birds between captive facilities in the management plans. More importantly, comparison of these findings with levels of *in situ* population variation is central to the design of an effective captive breeding program for the species.

3.2.   What to monitor?

Although it would be ideal if there were the necessary resources to monitor all of South Africa's diversity, this is unrealistic. A more realistic approach would be to establish a standard set of criteria with which to 'grade' or score potential candidates for genetic monitoring, and in doing so, create a priority list of species to monitor within South Africa. Until such time as such a scoresheet is developed, national and international biodiversity targets and threats should be examined to gain insight into what would benefit most from genetic monitoring.

South Africa's National Environmental Management: Biodiversity Act of 2004 (NEMBA) requires the monitoring and protection of 'species that are threatened or in need of protection to ensure their survival in the wild'. Moreover, Aichi Biodiversity Target 13 highlights the need to maintain genetic diversity of wild relatives of cultivated plants and domesticated animals. As such, as a starting point, we recommend that genetic monitoring be implemented on the following categories:

- *In situ* (wild) populations of threatened taxa
- *Ex situ* populations of threatened taxa
- Wild relatives of cultivated/domesticated species

3.2.1. *In situ* (wild) populations of threatened taxa

Threatened taxa, as designated by the three IUCN Red List categories (Vulnerable, Endangered, Critically Endangered), refers to species that are in immediate risk of extinction. The management of these taxa is fundamentally dependent on monitoring to follow trends in species recovery or decline and, in doing so, evaluate and inform conservation strategies. The inclusion of genetic data at the assessment and monitoring stages is vital as species with low genetic diversity are at higher risk of extinction due to increased inbreeding and genetic drift, which causes a decrease in effective population size (Crow & Kimura 1970; Frankel and Soulé 1981; Shaffer 1990; Frankham 1996; Spielman *et al*. 2004; Allendorf & Luikart 2007; Palstra & Ruzzante 2008). An evaluation of existing IUCN criteria for assigning threat status found that the criteria typically fail to identify species with low genetic diversity (Willoughby *et al.* 2015). This can be detrimental as there may be a critical threshold of genetic diversity below which species cannot recover. Even if the critical threshold has not been reached, a species with low genetic diversity may risk extinction long after its population size has recovered because such diversity can only be restored slowly, through the accumulation of mutations over many generations. Consequently, failure to monitor a species' genetic diversity could result in premature downgrading in its threat status. Although this is important for all threatened taxa, human-exploited threatened taxa in South Africa, such as many fisheries species (e.g. Seventy-four Seabream (*Polysteganus undulosus),* Dusky Kob (*Argyrosomus japonicas*), West Coast Rock Lobster (*Jasus lalandii*), South African Abalone (*Haliotos midae*) and medical plants such as *Warburgia salutaris* are in urgent need of genetic monitoring; yet, to date, many have not even been genetically assessed*.* For more species see Williams *et al*. (2013).

With regard to South African fisheries, the incorporation of genetic monitoring into existing monitoring programmes could easily be accomplished. Tissue samples from specimens, which are subsampled as part of the catch monitoring program, could be genetically analysed to estimate population demography and diversity. Combined with standard assessments, this genetic characterisation could provide further insight into population genetic thresholds that have bearing on long-term sustainable exploitation.

3.2.2. *Ex situ* populations of threatened taxa

Captive populations as the ultimate insurance against species extinction

To ensure the maintenance and recovery of viable populations, the core objectives of biodiversity conservation programs and supporting policy and legislation in South Africa are primarily achieved via zoological and botanical gardens, arboreta, aquaria, biobanks, and captive breeding programs, where they represent important sources for future population supplementation programs and the reintroduction of species. As insurance policies against extinction in the wild, it is therefore essential to (i) minimise genetic threats to *ex situ* resources, while (ii) maximising their representation in wild populations. Assessments of the genetic value of *ex situ* biodiversity resources are increasingly common. Coordinated monitoring plans, informed by knowledge of both captive pedigrees and standing levels of variation in wild populations, are required to ensure these resources remain relevant into the future.

While captive breeding programs of plants and animal species are an increasingly important tool in the conservation of threatened wild populations (Ebenhard 1995; Ballou *et al.* 2010; see Snyder *et al.* 1996), their effectiveness relies on maintaining detailed pedigrees across institutions, who then coordinate their respective breeding programs to meet demographic and genetic goals that can assist recovery of wild populations. The primary goal of most captive programs is the release of individuals into the wild. To ensure even a modicum of chance of surviving the challenges of release, captive individuals need to retain wild characteristics e.g. behavioural phenotypes, physiology and adaptive genetic variation (Woodworth *et*

al. 2002; Willoughby *et al.* 2017). For these programs to therefore be both effective and sustainable, monitoring of (i) genetic variation within captive populations and (ii) the genetic consequences of release on natural populations is required.

For captive populations to be used successfully in the restoration and supplementation of wild populations, they need to genetically match their wild founders as closely as possible (Ballou & Lacy 1995). Captive breeding programs therefore need to prevent two important processes: (1) loss of genetic variation, and (2) genetic adaptation to captivity. In other words, there is a need to temporarily stop the process of evolution. In both captive and wild populations, loss of genetic variation occurs via two processes, close-relative inbreeding and genetic drift. In captive populations, genetic drift is of particular concern, causing loss of both heterozygosity and allelic diversity that in the absence of unrelated 'immigrant' individuals is essentially irreversible. This reduced variation can have several consequences, in both the short- and long-term.

In the short-term, reduced variation increases the probability of inbreeding depression. While some degree of inbreeding is unavoidable in small captive populations, inbreeding depression is likely to limit population growth and reduce the probability of survival and persistence of introduced individuals. With detailed knowledge of the genetic composition of the population, pedigree inbreeding can be limited thereby increasing the short-term probability of releasing fit individuals into the wild. Limiting genetic drift is, however, more challenging and in the longer-term reduced variation will limit the ability of introduced populations to evolve in their new or changing environments. Equalizing reproductive success and thereby maximizing the effective population size is the primary method available to captive breeding programs. This requires knowledge of baseline levels of genetic variation and subsequent monitoring of both allelic and genotypic frequencies over time. Candidate loci for monitoring populations together with the relative importance of monitoring neutral versus adaptive genetic variation are discussed elsewhere in this chapter. To date, while there have been no studies in South Africa monitoring genetic variation in captive breeding populations, two recent studies report current levels of genetic variation in captive populations. Sasidharan-Priyadersini (2013) compared levels of genetic variation at neutral microsatellite loci in a captive South African Cheetah (*Acinonyx jubatus*) population to free-ranging Namibian and South African conspecifics. Despite sampling a larger number of captive individuals, a general trend of reduced variation in captive individuals relative to free-ranging populations is reported.

Monitoring genetic recruitment in wild populations

Informed and responsible management of *ex situ* population resources also requires monitoring of the genetic consequences of captive release on natural populations (Schwartz *et al.* 2007). Post-release genetic monitoring of populations receiving captive-bred individuals is vital for determining whether captive individuals successfully recruit into wild populations, and is the most reliable method to determine their relative reproductive contribution to subsequent generations (Schwartz, Luikart & Waples 2007). Globally, genetic monitoring of newly established and supplemented populations is rarely carried out and yet without such data it is impossible to determine the full conservation, ecological and evolutionary costs and benefits of this process (La Haye *et al.* 2017). While the challenge of generation time clearly limits the genetic monitoring of long-lived species, there have been many examples where monitoring would have been possible. To date there have been no studies in South Africa monitoring neutral or adaptive genetic variation in newly established populations or those supplemented with captive-bred individuals.

Monitoring to mitigate genetic adaptation to captivity

While many captive breeding programs try to avoid artificial selection of mates, some selection for favoured phenotypes is almost inevitable. This is particularly so when breeding pairs are selected on-site

rather than through thorough analysis of the global captive pedigree for a species. The result is inadvertent selection for captive-adapted traits that, in animals, may rapidly alter not only the genetic composition of captive populations, but also the 'biobehavioural' profile of individuals – the behaviour, physiology and morphology of individuals (McDougall *et al.* 2005; O'Regan & Kitchener 2005; Kaiser *et al*. 2015; Grueber *et al.* 2017). Breeding programs that ignore these possibilities risk leading ecologically and evolutionarily important captive populations toward domestication, thereby reducing their conservation genetic value (Williams & Hoffman 2009). For example, the genetic effects of domestication can substantially reduce fitness in subsequent generations, negatively impacting the sustainability of captive breeding stock of threatened species, and reducing their chances of successful re-introduction and supplementation of wild populations (Witzenberger & Hochkirch 2011; Table 3.1). Research shows that even a few generations of 'domestication via captive management' can negatively affect natural reproduction after re-introduction, leading to a cumulative fitness decline in wild populations (Araki *et al*. 2007). Through unintentional artificial selection, retention of alleles that may appear beneficial in captivity but are in fact maladaptive in the wild, can rapidly increase in frequency and are likely to be important contributors to fitness in wild populations. An alarmingly high proportion of wildlife reintroductions (animal and plant, alike) have failed to establish viable populations (Seddon *et al*. 2007; Godefroid *et al.* 2011), and while many factors interact to influence successful reintroductions, genetic adaptation to captivity is an important contributor (Williams & Hoffman 2009).

Using existing knowledge of priority species to inform genetic monitoring targets in captive populations

Despite extensive efforts to develop successful captive breeding programs in South Africa, it is highly likely that over the long-term substantial variation will exist between programs in their ability to maintain original levels of genetic diversity and fitness of the founder populations. Furthermore, given that release into the wild is an overarching aim of captive breeding programs, achieving and monitoring genetic targets in captive breeding programs has to be informed by knowledge of current standing variation in wild populations; this includes both neutral and adaptive genetic variation. While only a handful of studies have characterised genetic variation in South African captive wildlife populations, there are a large number of studies charactering neutral genetic variation in free-ranging, wild populations. Many of these studies are on species included in the Department of Environmental Affairs (DEA) Threatened or Protected Species (TOPS) list and can be used to inform a general monitoring framework for captive breeding programs that includes (i) establishing baseline targets for genetic variation in captive populations of priority species, (ii) monitoring captive genetic variation in priority species, (iii) managing captive populations to limit loss of genetic variation via inbreeding and genetic drift, and (iv) monitoring genetic variation in re-introduced and supplemented populations.

Table 3.1. Costs to fitness: domestication effects in captive breeding populations.

Effect on phenotype	Species	Reference
Behaviour		
- reduced antipredator and exploratory behaviours	Red Jungle Fowl (*Gallus gallus*)	Håkansson & Jensen (2008)
- reduced aggression and reproductive behaviour	Rainbow Trout (*Oncorhynchus mykiss*)	Marchetti & Nevitt (2003)
- loss of spatial memory skills	Golden Lion Tamarin (*Leontopithecus rosalia*)	Menzel & Beck (2000)
Morphology		
- limb paralysis	Cheetah (*Acinonyx jubatus*)	Palmer *et al.* (2001)
- reduced skull size	Indian Rhinoceros (*Rhinoceros unicornis*)	Groves (1982)
- changes in cranial morphology	Hyrax (*Procavia capensis*)	Lieberman *et al.* (2004)
- dental abnormalities	Cheetah (*Acinonyx jubatus*)	Fitch & Fagan (1982)
Physiological		
- earlier female sexual maturity	Yellow Baboon (*Papio cynocephalus*)	Altmann *et al.* (1981)
- declining fertility	Tasmanian Devil (*Sarcophilus harrisii*)	Farquharson *et al.* (2017)
- interruption to torpor	Feathertail Glider (*Acrobates pygmaeus*)	Geiser & Ferguson (2001)

3.2.3. Wild relatives of cultivated/domesticated species

In general, cultivated and domesticated species are easy to define. They are mainly found in agri-ecosystems and pertain to key commercial crops and livestock. While some of these taxa are threatened, some are not, but are still of interest given their economic value. Monitoring their genetic diversity is considered essential when wild relatives are present in an attempt to prevent genetic erosion. Within South Africa, Crop Wild Relatives (CWR) and wildlife ranching are the main groups or areas that would benefit from genetic monitoring.

Crop wild relatives

Crop wild relatives are wild species of plants that are closely related to crops. They are recognized as a vital component of agricultural biodiversity (DAFF 2016; Bruford *et al.* 2017; Magos *et al.* 2017). In contrast to their cultivated relatives, CWR have not passed through the genetic bottleneck of domestication (Tanksley and McCouch, 1997). As such, they tend to have higher levels of genetic diversity, are locally adapted and contain a range of traits for adapting crops to changing environmental conditions (such as drought or pest resistance), which could be used to develop improved varieties of domesticated crops (DAFF 2016).

Despite the recognition of their potential value in crop development, CWR are highly threatened by factors which impact all wild plant species, such as the effects of habitat destruction, nutrient enrichment, climate change, overgrazing, and overall poor veld management (Kell *et al*. 2012; DAFF 2016; Magos *et al.* 2017). As such, they are at risk of genetic erosion. However, to date, this has not been quantified very rigorously as data is often lacking or is anecdotal (Ford-Lloyd *et al*. 2006; Thormann & Engels 2015).

In South Africa, no CWR has been rigorously assessed for genetic erosion. However, nine CWR taxa are categorized as Critically Endangered and three are endangered – all 12 of which are not adequately represented in protected areas. *Secale strictum* subsp. *africanum* is an example of a threatened and

unprotected CWR (DAFF 2016). It is a range-restricted endemic species that is a close wild relative of rye. It is isolated to the Roggeveld Escarpment in the Northern Cape where it was once common. Yet currently, there are fewer than 50 mature individuals, none of which are protected. Because this CWR is also cultivated, attempts are being made to reintroduce it to other areas on the Roggeveld. However, before this is done, there needs to be a coordinated, systematic and integrated approach to CWR conservation that involves both *in situ* and *ex situ* strategies. This is likely to fall within the purview of the National Plant Genetic Resources Centre (NPGRC), which currently acts as a coordinator for all activities related to the *in situ* and *ex situ* conservation and sustainable use of plant genetic resources for food and agriculture in South Africa.

Wildlife ranching

Wildlife ranching in South Africa refers to the management of wildlife on private land for commercial purposes. The wildlife ranching sector comprises four sub-sectors (following Van der Merwe, Saayman and Krugell, 2004): 1) Ecotourism; 2) Live sales and breeding of high value species and colour variants; 3) Trophy and biltong hunting; and 4) Processed game products, which together contributes approximately R20 billion to the country's gross domestic product every year (Cloete *et al*. 2015). As of 2000, in excess of 9 000 wildlife ranches existed in South Africa, covering an area >200 000 km^2 and containing between 16 and 20 million wild animals (Taylor *et al*. 2015).

There is debate whether this sector involves the domestication of animals. However, if one considers the definition by Clutton-Brock (1989), which states 'A domestic animal is one that has been bred in captivity for purposes of economic profit to a human community that maintains complete mastery over its breeding, organization of territory and food supply' it may. The key component of domestication is selective breeding. Much of the recent growth in the wildlife ranching sector is due to deliberate breeding of wildlife under intensive or semi-extensive conditions (Taylor *et al*. 2015). This has generally involved "high-value" species, such as the African Buffalo and market-desired phenotypes of economic importance (e.g. large horns: African buffalo, Roan antelope; colour variants/morphs: Golden wildebeest, Black impala). These animals are held in small- to medium-sized fenced camps or enclosures, where they are protected from predators and provided with food, water and veterinary requirements. Consequently, these populations are not self-sustaining (Taylor 2016).

Despite the typical genetic implications associated with small captive populations (e.g. inbreeding, genetic drift: Allendorf *et al.* 2008; Russo *et al.* 2018) and artificially manipulating a population, to date there are no reports or studies that have shown that wildlife ranching has compromised the genetic integrity of their wild relatives (although see Van Wyk *et al*. 2013). However, potential risks do exist, namely through changes in the genetic composition, evolutionary trajectory and adaptive potential of wild populations through the introgression with captive populations. As has been widely documented in the aquaculture industry (Hindar *et al*. 1991), escapees from ranches have the potential to breed with natural populations and contaminate the wild gene pool. Moreover, animals are commonly relocated within and outside their natural distributions (Castley *et al*. 2001; Spear & Chown, 2009; Taylor *et al*. 2015). The consequences of this are poorly understood and both positive and negative consequences have been documented depending on the environmental conditions (Laikre *et al.* 2010).

3.2.4. Complement to traditional monitoring: detection of species and community assemblages through environmental DNA

Increasingly, molecular approaches are being employed to complement or replace traditional ecological monitoring practices, especially for rare, endangered and indicator species which typically occur at low densities. Environmental DNA (eDNA) has been instrumental in this.

As species interact with their environment, their DNA via faeces, saliva, urine, feathers and skin cells, for example, is continuously being shed into their surroundings (Baird & Hajibabaei 2012; Mächler *et al.* 2014; Rees *et al.* 2014; Thomsen & Willerslev 2015; Deiner *et al.* 2016). This DNA is known as eDNA. Depending on the environmental conditions at the time, this eDNA may become preserved in various media, such as ice cores or terrestrial and aquatic sediments, or it may begin to degrade rapidly as is found with surface soil and water. As such, eDNA can provide information on species composition from ancient and contemporary ecosystems, respectively (Baird & Hajibabaei 2012; Mächler *et al.* 2014; Rees *et al.* 2014; Thomsen & Willerslev 2015; Deiner *et al.* 2016). Moreover, because many species utilise the same environment, this method not only allows for the detection and monitoring of specific species (in terms of presence/absence), but also entire community assemblages with the help of metabarcoding and bioinformatics (Cristescu 2014; Kelly *et al.* 2014; Rees *et al.* 2014; Thomsen & Willerslev 2015; Evans *et al.* 2016).

The discovery and utility of eDNA has been a major technological breakthrough within the past decade, largely because it can overcome many of the limitations of traditional monitoring techniques, which typically rely on the physical identification of species by visual (count) or acoustic surveys (e.g. amphibians, birds, marine mammals), which are based on distinct morphological characters or calls, respectively. Some of the main limitations of these approaches include:

(a) Plasticity in morphological characters and calls;

(b) Morphologically cryptic taxa or difficult to detect taxa due to small size, low population densities, or being voiceless;

(c) Taxa possessing different phenotypes between sexes and life-stages or closely related species with very similar juvenile stages/phenotypes making morphological keys ineffective; and

(d) The high level of expertise needed to use the taxonomic keys required for species-level identifications.

Environmental DNA can overcome these challenges because it allows for the detection of organisms based only on their DNA. As such, this technique eliminates the need for on-site specialists trained in species identification, or the need for the preservation of samples for post-sampling identification when specialists are not immediately available on site. The process of preservation, in itself, has the potential to introduce unintended morphological changes which can result in erroneous species identifications later on. Studies have also found that eDNA can provide comparable or improved detection efficacy regardless of sampling medium (see Table 3.2), which can save researchers considerable time and expense, especially for monitoring extremely, small, elusive or rare species (e.g. Goldberg *et al.* 2011; Jerde *et al.* 2011; Thomsen *et al.* 2012; Mahon *et al.* 2013; Pilliod *et al.* 2013; Santas *et al.* 2013), as well as community assemblages (e.g. Guardiola *et al.* 2016; Sinniger *et al.* 2016; see also Table 3.2).

The application of Next-Generation Sequencing (NGS) technology to eDNA sampling makes it possible to monitor entire biological communities using bioinformatic processing of DNA metabarcoding. As such, fish, amphibians, and macroinvertebrates, along with reptiles, mammals, birds and plants, for example, can be surveyed from a single water sample. This broad untargeted approach has been termed 'passive'

surveillance in management applications (Deiner *et al.* 2017). While whole community assessments using eDNA metabarcoding are still not common, they have been shown to be a potentially powerful tool, which might greatly improve speed and accuracy of biodiversity assessments (for example, see Deiner *et al.* 2016; Hänfling *et al.* 2016; Bista *et al.* 2017).

Even though there are growing examples from the international conservation community where eDNA has been applied, by in large, this molecular approach has proven most successful in aquatic environments, especially freshwater, and hence has proven extremely useful in the field of freshwater conservation. One of the first seminal studies that retrieved eDNA from freshwater focused on the detection of the invasive American bullfrog in France (Ficetola *et al.* 2008). This study demonstrated that eDNA provided improved detection of an invasive species over traditional methods. Many subsequent studies continued to focus on eDNA to detect invasives (e.g. Dejean *et al.* 2012; Goldberg *et al.* 2013; Jerde *et al.* 2013; Piaggio *et al.* 2014). While eDNA has the potential to become a valuable monitoring tool for biological invasions, such as in the early detection of invasive populations, or surveillance of invasion pathways (e.g., ballast water of ships: Egan *et al.* 2015; Zaiko *et al.* 2015; the live bait trade: Nathan *et al.* 2015), to date, it is not routinely used for biosecurity regulation or enforcement. While the majority of freshwater eDNA studies have focused on fish or amphibians, macroinvertebrates make up a far greater proportion of the local diversity in aquatic systems, and their inclusion in biomonitoring is essential, yet to date very few studies have assessed or monitored them using an eDNA approach (Mächler *et al.* 2014).

The medium which has achieved the least success using eDNA has been the terrestrial environment. There are examples of contained experiments on the persistence of eDNA in terrestrial environments which have produced promising results (Walker *et al.* 2017). However, the application of these experiments in the natural environment has remained limited. In most of the successful work on the detection and genetic monitoring of terrestrial organisms the method for acquiring eDNA was obtained indirectly through the use of an aquatic source frequented and used by the organism. Saliva, either left behind on prey of a target species or on natural saltlicks, has also proven successful as a source of eDNA in a few studies on terrestrial organisms (Blejwas *et al.* 2006; Harms *et al.* 2015; Wheat *et al.* 2016; Ishige *et al.* 2017).

To date, the majority of eDNA studies have focused on spatial detection (e.g., early detection of invasive species, presence of rare or endangered species). Temporal estimates, which are essential for genetic monitoring, have been relatively neglected (but see Biggs *et al.* 2015; Bista *et al.* 2017).

Environmental DNA monitoring within South Africa is still in its infancy, with only a few studies currently underway that incorporate eDNA to survey biodiversity (Box 3.3; Ramond *et al.* 2015; Wilcox & Cowan 2016; Segobola *et al.* 2018). Considering South Africa's exceptional biodiversity and the urgent need to protect it, while also understanding the limited financial resources available to do so, it is almost certain that eDNA will become an important tool in species and ecosystem conservation within the country. However, in depth studies are required that compare eDNA metabarcoding approaches to conventional ecological surveys (Deiner *et al.* 2016), especially within a South African context. One such study is currently being initiated within the Table Mountain National Park. The aim is to test the utility of eDNA in correctly (1) detecting the presence of the Table Mountain Ghost Frog (*Heleophryne rosei*) – a Critically Endangered and elusive frog found in five streams on Table Mountain – and (2) identifying/surveying aquatic macroinvertebrates to quantify stream ecosystem health at an equal or greater resolution than the traditional SASS (South African Scoring System) and miniSASS methods. Given that many macroinvertebrates are believed to occupy a highly restricted range (e.g. Scodanibbio 2002; McGeoch *et al.* 2011; Scott *et al.* 2012), identification to species level would be valuable to guide conservation and management decisions, especially for taxa that are known to be highly sensitive to ecosystem disturbance

and health, such as members of the Ephemeroptera (mayfly) Order (Scodanibbio 2002; Bellingan *et al.* 2015).

Table 3.2. Representative studies comparing richness estimates with traditional sampling or historical data for a geographic location to that of eDNA metabarcoding (modified from Deiner et al. 2017).

Habitat	Macro-organism taxonomic focus	eDNA sample type	Traditional sampling method	eDNA efficacy	References
Freshwater	Fish	Flowing water	Depletion-based electrofishing.	Higher diversity	Olds *et al.* (2016)
	Invertebrates	Flowing water	Kicknet in stream and historical data.	Higher diversity	Deiner *et al.* (2016)
	Fish	Stagnant water	Gillnet, trapping, hydroacoustics, analysis of recreational anglers' catches	Complementary	Hänfling *et al.*(2016)
	Reptiles, amphibians	Stagnant water	Species distribution model based on historical data (i.e., distribution range and habitat type).	Increase species distribution knowledge	Lacoursière-Roussel *et al.* (2016)
	Amphibians, fish	Stagnant water; flowing water	Amphibians: visual encounter survey, mesh hand-net; fish: electrofishing, and/or netting protocols (fyke, seine, gill).	Greater detection probability	Valentini *et al.* (2016)
	Amphibians, fish, mammals, invertebrates	Stagnant water; flowing water	Active dip-netting, fresh tracks or scat, electrofishing with active dip-netting.	Complementary	Thomsen *et al.* (2012)
	Fish	Stagnant water; flowing water; surface sediment	Fyke net	Higher diversity	Shaw *et al.* (2016)
	Invertebrates	Water column; surface sediment	Sediment collected using a Van Veen grab.	Higher diversity	Gardham *et al.(2014)*
	Fish/Diptera	Surface and bottom water column	Long-term data, electrofishing (fish) and emerging traps (Diptera) at the time of eDNA sampling.	Higher diversity compared to sampling but lower diversity compared to long-term data	Lim *et al. (2016)*
	Invertebrates	Stagnant water		Complementary	Bista *et al.*(2017)
Marine	Fish	Surface and bottom water column	Long-term observation	Complementary	Yamamoto *et al.* (2017)
	Fish	Bottom water column	Trawl catch data	Similar family richness	Thomsen *et al.* (2016)
	Fish	Water column	Scuba diving	Higher diversity	Port *et al.* (2016)
Terrestrial	Mammals, plants	Midden pellets	Historical surveys	Higher diversity	Murray *et al.* (2012)
	Mammals	Saliva	Local knowledge (i.e., physical evidence) and camera data.	Complementary	Hopken *et al.* (2016)

Birds, invertebrates, plants	Top soil	Invertebrates: leaf litter samples and pitfall traps; reptiles: pitfall traps and under artificial ground covers; birds: distance sampling method; plants: above-ground surveys.	Complementary for plants and invertebrates	Drummond *et al.* (2015)
Earthworms	Top soil	Irrigated quadrats with 10 L of allyl isothiocyanate solution and hand-collected emerging worms.	Complementary	Pansu *et al.* (2015)
Vertebrates	Top soil	Local knowledge from safari parks, zoological gardens and farms; visual observations; historical surveys.	Complementary	Andersen *et al.* (2012)

Box 3.3. Towards biomonitoring of marine environments using eDNA approaches

South Africa has one of the most dynamic marine systems globally, with two contrasting current systems that drive strong environmental gradients including temperature and primary productivity. This in turn has shaped and continues to maintain a regional biodiversity that numbers more than 12 000 recognised species, with a high level of endemics (Griffiths *et al.* 2010), yet most of this biodiversity remains unexplored, particularly for smaller and cryptic taxonomic groups.

Current species description efforts are unlikely to significantly contribute towards plugging the biodiversity knowledge gap, which is problematic in the light of anthropogenic pressures that are have large impacts on coastal and offshore marine populations. In order to monitor and better understand changes, as well as quantify anthropogenic impacts on the distribution of marine species, eDNA approaches are being developed to provide better species distribution data that can support spatial planning and biodiversity planning in South Africa.

The main projects at present are focussing on mapping the distribution of coastal fishes, as well as community genetics (which includes all eukaryotic organisms) across 48 sites (see Figure (a) below) along the 3 650 km of South African coastline. Through repeat sampling that incorporates diverse coastal marine ecosystems including rocky and sandy shores, mangrove, seagrass and coral systems (see Figure (b) below) the aim is to identify patterns of biodiversity, as well as to construct networks that provide insights into the links between different marine ecosystems in South Africa. This data can be used to, for example, identify taxa that are able to utilise multiple ecosystems, as well as taxa restricted to specific areas or systems. Importantly, this provides a baseline for more targeted monitoring of coastal biodiversity and opens the possibility of tracking species and community shifts and invasive species, as well as linking anthropogenic impacts to changes in local diversity. These data can also be mapped and included for marine spatial planning and identifying areas for protection or sustainable utilisation. Finally, the use of eDNA holds great promise for supporting marine biomonitoring in South Africa and should urgently be extended to support ongoing efforts in mapping offshore pelagic and benthic habitats.

Box 3.3. Continued.

(a)

Map of 48 sampling localities for coastal eDNA along the South African coastline (Figure supplied by M. Czachur).

(b)

Examples of potential ecological links between the different marine habitats targeted in the study.
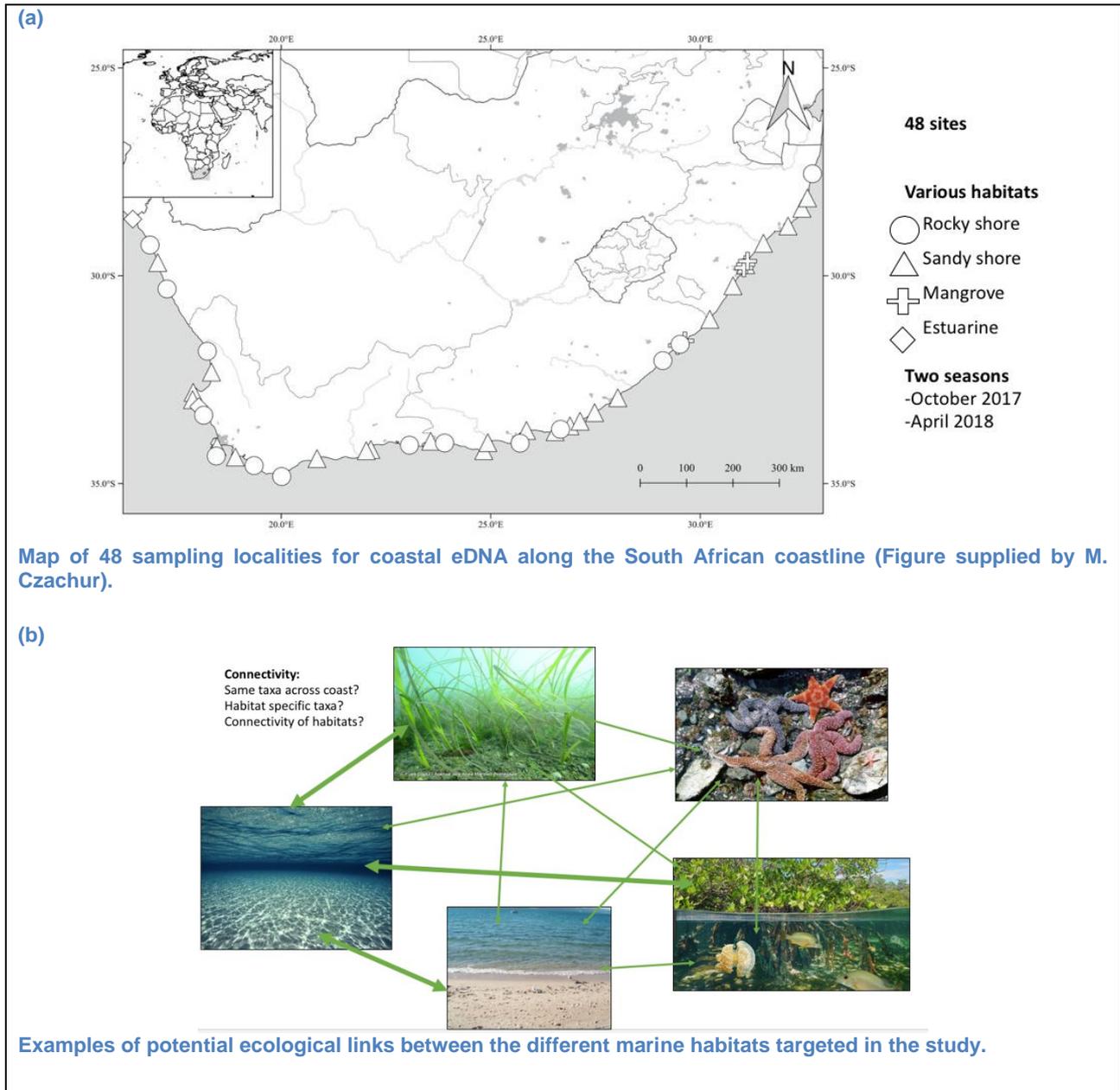
3.3. When or how often should diversity be monitored?

Monitoring can be considered in three temporal stages: short-term, medium-term and long-term. From an ecological perspective, monitoring has tended to follow particular timeframes. Short-term monitoring tends to look at potential trends within 10–20 years; medium-term monitoring between a 20–50 year timeframe; and long-term monitoring, over 50 years. However, from a genetics perspective, such timeframes do not necessarily work for all taxa. A simulated study found that the ability (i.e. power) at detecting change in genetic diversity within a population or species can only be achieved after at least seven generations post-decline (Hoban *et al.* 2014). This can differ considerably for species. For example, the African Elephant (*Loxodonta africana*) has a generation time of 25 years (Blanc 2008), the African Penguin (*Spheniscus demersus*) a generation time of 10 years (BirdLife International 2018), and amphibians vary between a few months to a few years.

Consequently, even a perceived long-term study (i.e., + 50 years) on the African Elephant will not likely detect any change in genetic diversity. It is therefore imperative that generation times be considered when designing and interpreting genetic monitoring studies. It might actually be more informative to classify genetic monitoring studies in terms of generation times with short-term monitoring defined as under 10 generations, medium-term between 10-20 generations, and long-term as greater than 20 generations. However, the implications of this would be that for larger organisms, which tend to possess longer generation times, long-term genetic monitoring studies could take hundreds of years, which is not practical.

Difficulties in obtaining long-term datasets, whether through the traditional or proposed (genetic) timeframe, likely explains why long-term datasets are lacking globally. This highlights the importance of historical genetic data, which may be acquired from museums and other natural history, as well as sediment and ice cores. There has been considerable difficulty in acquiring good quality DNA from ancient specimens. However, advances in genetic technology are making it much easier (for example, see Hofreiter *et al.* 2015; Basler *et al.* 2017; Paijmans *et al.* 2017). Species or populations of concern that are not well represented in historical collections and that have not been prioritized for immediate monitoring in the short-term should have samples biobanked for future genetic monitoring (see Jansen van Vuuren *et al.* 2019.

To increase the chances of detecting change in genetic diversity within the short-term, the species under consideration should typically have fairly short generation times, no overlapping generations, and be narrow endemics. These criteria would ensure that the species or populations under investigation are truly discrete entities. Such monitoring can assist with immediate management interventions when multiple lines of evidence indicate that the species is at high risk of extinction. *Capensibufo rosei* (Cape mountain toad or Rose's mountain toad) is a key example of a short-term genetic monitoring program currently taking place in South Africa (Box 3.1). The published baseline dataset will be compared against future data which is being gathered through an ongoing monitoring program for the species (da Silva & Tolley 2018). Another genetic monitoring program that has been initiated is for the Endangered Western Leopard Toad (*Amietophrynus pantherinus*), looking at data from 12 microsatellite data loci gathered from two time periods (2008 and 2018). In addition to the immediate management implications of studies such as these, all short-term monitoring can be considered a baseline for continued monitoring programmes regardless of the species being examined.

3.4.   How should genetic diversity be monitored?

3.4.1 What markers should be used?

Genetic diversity can be measured using a variety of molecular markers. Choosing the appropriate one depends on the question to be answered. For the purposes of genetic monitoring, the main question is often whether there has been any change in the genetic diversity within a population or species. Such a fine-scale assessment requires the use of highly sensitive markers able to detect differences between individuals, such as microsatellites and single nucleotide polymorphisms (SNPs). As mentioned in section 3.2.4, however, genetic monitoring can also be used to answer the question of what species are present within a community or ecosystem and how has this changed over time. This question typically relies on genetic differentiation estimates available at or above species-level (Faith 1992). Consequently, mitochondrial DNA (mtDNA) markers are often applied here.

Until about 2010, the majority of population genetic studies used a handful of mtDNA markers or approximately 10–20 microsatellite loci (Hunter *et al.* 2018). Since then there has been a shift to Next-Generation Sequencing (NGS), which allows for individuals and populations to be assessed at

thousands to millions of SNP loci or for the simultaneous sequencing (typically using mtDNA markers) of multiple individuals in a complex sample, known as metabarcoding (Taberlet *et al.* 2012; Thomsen and Willerslev 2015; Hunter *et al.* 2018). While NGS technology exists within South Africa, with respect to monitoring genetic diversity within populations, microsatellites are likely to remain the preferred marker in South Africa into the near future because of their affordability. However, as the affordability and available capacity to carry out NGS improves within South Africa, SNPs are likely to become the main genetic monitoring marker. For species and populations where initial microsatellite assessments have already been conducted, it is highly recommended that genetic monitoring programmes continue to utilise the same microsatellite loci, while, at the same time, developing a SNP dataset (Carroll *et al.* 2018). This would provide a way to transition between the two markers and potentially provide a comparative estimate of genetic diversity between them. Ideally, a SNP profile would be matched to a specific individual's microsatellite genotype (Carroll *et al.* 2018). The brown bear has been a model system illustrating this transition (e.g. De Barba *et al.* 2017; Norman *et al.* 2017).

A more detailed look at the three markers (mtDNA, microsatellites, SNPs) is provided below.

Mitochondrial DNA markers

Mitochondrial DNA (mtDNA) are circular haploid molecules that are maternally inherited and usually transmitted without recombination (Barr *et al*. 2005). In general, mtDNA has fairly high mutation rates compared to nuclear DNA markers (not microsatellites however), which allows for the reconstruction of evolutionary relationships between and within species (such as phylogenetic and phylogeographic events), often assisting in resolving taxonomic uncertainties (e.g. Avise *et al.* 1987). The mitochondrial gene Cytochrome Oxidase 1 (CO1) is a 600 base pair region most commonly used as a species identified or 'DNA barcode' for animals and some protists.

Microsatellite loci

Microsatellite markers, or Short Tandem Repeats (STR), are polymorphic DNA loci consisting of tandemly repeating mono-, di-, tri-, and tetranucleotide units, which are distributed throughout the genomes of most eukaryotic species. Within vertebrates, the dinucleotide repeats (GT and CA) are considered the most common microsatellites (Zardoya *et al.* 1996). Microsatellites are codominant, highly polymorphic, and Mendelian inherited, which make them suitable for studying population structure, parentage, and genetic differences among and within species (e.g. Avise *et al.* 1987; Schwartz *et al*. 2007).

Patterns of genetic variation between mtDNA and microsatellites are not always congruent, largely because of (i) differences in the selection intensities acting on each marker, (ii) different mutation rates between markers, and (iii) the effective population size for maternally-inherited markers, such as mtDNA, differ from those of microsatellites which are biparentally inherited (Johnson *et al*. 2003). Genetic diversity is also influenced by patterns of mating, sex-biased dispersal and other demographic parameters (Chesser & Baker 1996; Johnson *et al*. 2003; Yang & Kenagy 2009; de Oliveira Francisco *et al*. 2013; Kolleck *et al.* 2013), which can further contribute to the discrepancies in genetic diversity estimated between these markers. In general, microsatellites provide more fine-scale resolution of recent demographic events (e.g. Avise *et al*. 1987). Examined together, one may be able to ascertain a species' evolutionary change in time (see da Silva & Tolley 2017).

In South Africa, microsatellite markers have been, thus far, the only markers used for temporal genetic monitoring purposes (Box 3.1 and Box 3.2).

Single Nucleotide Polymorphisms (SNPs)

Single Nucleotide Polymorphisms (SNPs, pronounced 'snips') are the most common type of genetic variation among organisms. They are versatile and sensitive markers, evenly spread throughout the genome that have the potential to substantially expand the ability to analyse both coding and noncoding regions within populations, thereby providing a broader coverage than microsatellites (e.g. Morin *et al.* 2004; Ouborg *et al.* 2010). They arise as a result of mutations that produce base-pair differences among chromosome sequences. For example, a SNP may replace the nucleotide cytosine (C) with the nucleotide thymine (T) in a particular region of DNA. As such, they are biallelic markers. When compared against mutiallelic microsatellites, they would be considered inherently less informative for uses such as individual identification and parentage analysis (Glaubitz *et al.* 2003). However, their simpler mutational dynamics reduces the risk of homoplasy (shared character between two or more species that did not arise from a common ancestor) (Syvänen 2001; Vignal *et al.* 2002; Brumfield *et al.* 2003). Moreover, there are increasingly faster and more inexpensive methods available to screen thousands of SNPs per sample per population (e.g. Wang *et al.* 2009). Lastly, SNP genotypes tend to be universally comparable and do not require standardization across detection platforms. In contrast, it is difficult to compare microsatellite data sets produced by different laboratories, due to inconsistencies in allele size calling and misinterpretation of the electrophenograms (Vignal *et al.* 2002).

Overall, the high abundance of SNPs and genome-wide distribution make them a valuable source of genetic variation for studies on population demographics, adaptation, and genome evolution (Brumfield *et al.* 2003; Morin *et al.* 2004; Fuentes-Pardo & Ruzzante 2017; Perrier *et al.* 2017) and, subsequently, genetic monitoring. Indeed, such a genome-wide approach is particularly important for examining the adaptation of populations to environmental changes over time, especially in the long-term (Allendorf *et al.* 2010; Hoffmann *et al.* 2015).

Many studies employing SNPs for biodiversity assessments typically use tens of thousands SNPs; however, Hoban and colleagues (2014) observed that 2 500 SNPs were as effective as 250 microsatellites at detecting subtle demographic declines. Consequently, a great deal of precision can be achieved with far fewer markers than originally expected. Moreover, because of the increased precision provided by SNPs, fewer individuals may be required to obtain accurate measures of genetic diversity.

Recently, there has been growing interest in using SNPs for eDNA amplification (Nichols & Spong 2017; Carroll *et al.* 2018). Most eDNA studies have typically used species-specific or universal mitochondrial primers, such as COI or Cyt-b, to amplify DNA and generate barcodes (Bohmann *et al.* 2014). However, eDNA is often degraded and of low quantity, which results in poor amplification of larger markers (i.e. > 650 bp) (Carew *et al.* 2013; Nichols & Spong 2017). By using SNPs, which are shorter than other markers, these problems may be dramatically reduced (Nichols & Spong 2017).

3.4.2. What genetic measures or indicators should be utilised?

A variety of metrics have been used to quantify genetic diversity (see Table 3.3 for common examples). Some metrics, such as haplotype and nucleotide diversity, are restricted to a particular type of molecular data (i.e., sequence data); whereas others have been applied across markers. Of the metrics that appear more universal in application, no one has been singled-out as the most appropriate or superior for measuring changes in genetic diversity within a population or species. This is because the results, or rather the sensitivity of the metric, tend to differ depending on the molecular markers used to generate the data. Moreover, the accuracy of statistical analyses is closely correlated with the number of markers used and individuals examined, which may vary between studies. As such, researchers must be cautious about comparing results from different studies.

With that said, there have been concerted efforts made to identify the most sensitive metric for measuring changes in genetic diversity. For example, Hoban *et al.* (2014) conducted an in-depth assessment of six of the most common summaries of a population's genetic status based on microsatellite and SNP data: allelic richness (i.e., allelic diversity, allelic size range, observed heterozygosity, expected heterozygosity, the Garza-Williamson *M*-ratio bottleneck statistic, and Wright's inbreeding coefficient (F_{IS}). They tested these metrics under various temporal sampling protocols to determine which would be most sensitive to detecting genetic erosion in the short-term. The authors found that allelic richness outperformed all others, exhibiting the greatest power in detecting changes in genetic diversity across all scenarios tested. Despite this finding, the effectiveness of using allelic richness as a measure of genetic diversity has been questioned in studies using SNP data (see Carroll *et al.* 2018). This is because SNPs tend to have far fewer alleles (generally one or two) compared to microsatellite loci. Instead, estimates of heterozygosity (H_O, H_E) are thought to be more informative for SNP loci (see Doyle *et al.* 2016).

Yet another metric purported to be one of the best metrics for evaluating genetic erosion is effective population size (N_E), or more specifically contemporary N_E, which is essentially the number of individuals in a population who contribute offspring to the next generation (Wright 1931; Schwartz *et al*. 2007; Frankham *et al*. 2014; Carroll *et al*, 2018; Leroy *et al*. 2018). Effective population size (N_E) has been estimated from various molecular markers. However, estimates may vary between markers due to differences in mutation rates and modes of inheritance. Moreover, these estimates may be further compounded by sex-biased dispersal, which is characterized by one sex being philopatric (i.e. individuals of this sex stay or return to their natal site or group to breed), while the other is more prone to disperse. This is hypothesised to occur due to resource competition, inbreeding avoidance, and (in the case of animals) local-mate competition. In mammals and plants, for example, dispersal is often male-biased, with males having higher dispersal rates than females, whereas in birds the reverse pattern is generally found (Greenwood 1980; Dobson 1982; Clarke *et al.* 1997; Galloway 2005). The consequences of sex-biased dispersal are different levels of genetic diversity between males and females, which may translate into substantially different estimates of effective population size.

Given that, at present, there is no single measure that can best quantify changes in genetic diversity, where possible, a variety of metrics should be evaluated and their power or sensitivity tested and reported.

Table 3.3. Common genetic diversity metrics and the molecular data used to generate them.

Genetic diversity metrics	Molecular data
Haplotype diversity (*H*, also known as gene diversity)	Sequence (e.g., mtDNA)
Nucleotide diversity (π)	Sequence (e.g., mtDNA)
Allelic richness (A_R)	microsatellites, SNPs
Heterozygosity (Observed = H_O, Expected = H_E)	microsatellites, SNPs
Garza-Williamson Bottleneck statistic (*M*-ratio)	microsatellites, SNPs
Fixation indices (e.g., F_{ST}, F_{IS})	mtDNA, microsatellites, SNPs
Effective population size (N_e)	mtDNA, microsatellites, SNPs

3.5. The way forward: the future of genetic monitoring in South Africa

The overall paucity of genetic monitoring datasets should not squash any and all attempts at establishing genetic monitoring programs. If anything, it should strengthen our pursuit in recognizing and utilizing what tools are currently available, and identify tools that are needed and yet to be developed.

3.5.1. Utilizing existing tools

Existing resources

Indeed, the plethora of population genetic assessments already published provide a baseline of genetic diversity, upon which future short and long-term genetic monitoring studies could be based. In some instances, reorganizing datasets might even allow for initial short-term monitoring studies to be conducted without the addition of extra samples. Additionally, museums and other natural history collections have long been recognized and utilized as sources of historical genetic material (e.g. Roy *et al.* 1994; Bouzat *et al.* 1998; Ross *et al.* 2006; Martinez-Cruz *et al.* 2007; Wandeler *et al.* 2007; Hoeck *et al.* 2010; Ugelvig *et al.* 2011; Banhos *et al.* 2016; Dures *et al.* 2019). Furthermore, comprehensive tools exist that can aid researchers in the planning and design of genetic monitoring studies.

Repurposing published population genetic datasets

There are numerous population genetic studies published and datasets available. Some of these studies may have pooled data from multiple years for a given population or species in order to bolster sample sizes. Provided there are sufficient sample numbers per year, these datasets may be reorganized with the purpose of time series data to be available in order to analyse for changes in genetic diversity. Not all years would need to be included, as that would likely dilute any actual changes in diversity. It would be recommended to first compare the earliest and latest data for differences.

Although it is now highly encouraged that all datasets be made publically available, in order for such datasets to be rendered useful for repurposing, it is essential to provide as much detail as possible. For example, sample ID, location, date or year of collection, population assignment, and accession numbers (if available). Unfortunately, many datasets made publically available only tend to include information on the population or species under investigation and the specific genetic sequence or genotype associated with that individual. Details on when the samples were collected are often absent.

Museum and natural history collections

The inclusion of samples from museums and other natural history collections would be invaluable to monitoring programs as they compromise representatives of a species or population pre-demographic decline, which are often linked to human-influenced habitat loss and fragmentation (e.g., 1800s, 1900s). As such, they have the ability to act as a true baseline of genetic data. One of the potential drawbacks of ancient samples, however, would be in obtaining good quality DNA.

Molecular studies using samples from museums or other natural history collections are often constrained by the highly degraded DNA obtained from them and the high risk of (Paabo *et al.* 2004; Wandeler *et al*. 2007). This often makes sequence and genotype data prone to many errors, such as the insertion of incorrect bases which is prevalent in formalin-preserved specimens (e.g. Williams *et al.* 1999), and false alleles and allelic dropout (e.g. Morin *et al.* 2001; Wandeler *et al.* 2003; Wandeler *et al*. 2007). The former can look like new alleles or sequences and therefore lead to overestimation of the genetic diversity of past populations (Paabo *et al.* 2004; Sefc *et al*. 2007; Wandeler *et al*. 2007), while the latter typically result in the underestimation of genetic diversity for historical samples compared with modern samples (Wandeler *et al.* 2003; Wandeler *et al*. 2007).

In addition to these challenges, different preservation methods can negatively affect the ability to extract, amplify and sequence or genotype DNA (e.g. Schander & Halanych 2003; Hedmark & Ellegren 2005; Austin & Melville 2006). Consequently, caution should be taken in interpreting results from ancient DNA. To ensure reliable genetic data, repeated sequencing and genotyping from independent PCR products and the cloning of important sequences are the most commonly used practices (Sefc *et al.* 2003; Wandeler *et al*. 2007). However, ongoing technological innovations and the use of NGS have proven extremely valuable at acquiring quality DNA from ancient samples (e.g. Der Sarkissian *et al.* 2014; Hofreiter *et al.* 2015; Palencia-Madrid & de Pancorbo 2015; Basler *et al.* 2017; Paijmans *et al.* 2017; Dures *et al*. 2019).

Planning new genetic monitoring projects and programmes

A key problem identified with population genetic studies on a given taxon has been that there has been no consistent and comparable approach among them with respect to the number of sampling periods, sampling intervals, the type and number of genetic markers used, and the number of individuals sampled (Hoban *et al.* 2014). In future, the development of a National Genetic Monitoring Framework will help resolve these issues (see Section 3.5.3), however, until such time, researchers are encouraged to utilize sample planning tools currently available, such as that developed by ConGRESS (Conservation Genetic Resources for Effective Species Survival: http://www.congressgenetics.eu/ ; Hoban *et al*. 2013).

ConGRESS is an online tool that helps researchers determine how many individuals and genetic markers to include in their study to obtain genetic results that are reliable and useful for genetic monitoring and overall conservation purposes. Realising that not all research questions have the same sampling requirements, this tool is broken down into five modules: bottlenecks, connectivity, assignment, hybridization and temporal sampling. After you have chosen the appropriate module for your study, the user will choose possible sampling schemes and then receive clear information about the probability of success for the different sampling schemes.

3.5.2. Developing new tools

Standardized metric to compare outputs from different genetic markers

Since the discovery of the DNA double helix in 1953, there have been exceptional advancements in molecular technology (Table 3.4). Consequently, it would be impossible to say that the techniques used today would be representative of the techniques applied into the distant future. For population and conservation genetics, there has already been a noticeable shift from mitochondrial DNA to the more fine-scale microsatellite markers, and more recently to SNPs (Hunter *et al.* 2018). Such progression and transition may make people weary of initiating genetic monitoring programmes, since the results obtained today may not be applicable or comparable later on unless the same molecular markers and tools are carried forward. Although a valid concern, it cannot eclipse the urgent need to monitor genetic diversity. To overcome this challenge, there is a need for the development of a standardized metric that would enable the results from different genetic markers to be compared. This is not a little or simple task as there are multiple obstacles that would need to be considered and overcome, including, but not necessarily limited to, the different modes of inheritance among markers and their different mutation rates. If such a standardized metric could be developed, it would open up a world of genetic monitoring possibilities. Instead of the current paucity of genetic monitoring studies the world over, we may be able to transform available data and possibly even begin to report on long-term trends in the genetic diversity of several taxa.

Table 3.4. Timeline of principal discoveries in molecular biology that influenced the development of molecular diagnostics (modified from Patrinos & Ansorge 2005).

Year	Discovery
1953	DNA double helix
1958	Isolation of DNA polymerases
1966	Allozymes
1977	DNA sequencing
1983	First synthesis of oligonucleotides
1985	Restriction fragment length polymorphism (RFLP) analysis
1985	Invention of PCR
1988	Optimization of PCR
1989	Microsatellites
1992	Conception of real time PCR
2001	First draft versions of the human genome sequence
2003	SNPs

3.5.3 Establishing a Genetic Monitoring Framework for South Africa

Currently, a National Biodiversity Monitoring Framework (NBMF) is being developed for South Africa. This framework will speak to high-level reporting requirements for all levels of biodiversity, drawing from the CBD's targets, as well as South Africa's national biodiversity policies (namely NEMBA and the National Biodiversity Strategy and Action Plan [NBSAP]). A critical component of this framework will be identifying key monitoring indicators, which will be in line with international monitoring standards and guidelines, such as the Essential Biodiversity Variables being developed by GEO BON (https://geobon.org/ebvs/what-are-ebvs/). Embedded within this, will be a careful evaluation of existing gaps around these indicators.

A genetic monitoring framework (GMF) or guidance document will be a component of the NBMF. Ideally, the GMF will outline how genetic diversity can be monitored at a national, ecosystem and population level. Although a national or ecosystem measure of genetic diversity can be hard to contemplate, if one understands that at the heart of all biodiversity monitoring are population- and species-level assessments, such high-level metrics or indicators are not impossibilities. We may simply need time and data to realise or conceptualise what the best indicators could be. With that said, there are ways in which genetic diversity can be assessed across a landscape (for example, see Tolley & Šmíd 2019). With respect to population-level genetic monitoring, the content discussed in this Chapter 3 will help guide the GMF, with the aim of directing future genetic monitoring research.

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4. EVALUATING THE STATUS AND TRENDS OF LANDSCAPE LEVEL GENETIC DIVERSITY: A CASE STUDY USING SOUTH AFRICAN REPTILES

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Overview

Covers potential landscape level indicators for tracking 'genetic diversity', using reptiles as a case study.

4.1. Introduction

Ecosystems, species and genes are the fundamental components of biodiversity. Typically, ecosystems and species form the basis of local, national and global conservation plans and strategies for the protection of biodiversity, and for trends of biodiversity loss (e.g. Driver *et al.* 2012; Schmeller *et al.* 2015). Therefore, assessment of biodiversity status and trends classically focuses on threats to species richness and ecosystem function (Vane-Wright *et al*. 1991). Recently, suites of biodiversity indicators and essential biodiversity variables have been proposed to track biodiversity loss relating to species and ecosystems (Butchart *et al.* 2010; Pereira *et al*. 2013; Tittensor *et al.* 2014). Although genes are also recognised as a fundamental component of biodiversity, genes are typically not used to inform biodiversity planning because of the difficulty in quantifying genetic diversity on the landscape (Scholes *et al.* 2012). Temporal trends in genetic diversity are relatively straightforward to assess for target species (e.g. da Silva & Tolley 2018), but this does not easily translate to the landscape level because that approach is species specific, and does not cover assemblages of species. Therefore, caution should be taken if only using a few target species as proxies for genetic diversity trends across an ecosystem or entire landscape, unless larger sets of taxa can be evaluated simultaneously (e.g. Paz-Vinas *et al*. 2015; 2018). Perhaps more problematic is tracking trends in genetic diversity due to the timescale at which genes respond (evolutionary) in relation to the rapid timescale (generations) at which biodiversity is being lost. That is, populations or species could potentially decline or become extinct before the loss of genetic diversity is detected. Therefore, genetic indicators are not a direct proxy for other levels of biodiversity (*i.e.* species). Despite this, genetic diversity is clearly linked to ecosystem function, evolutionary potential and species resilience (Hughes *et al.* 2008; Cardinale *et al.* 2012), and should not be disregarded in biodiversity assessments.

Despite the challenges, safeguarding genetic diversity has now been built into major global and national initiatives such as the Convention on Biological Diversity (CBD) and the Group on Earth Observations, Biodiversity Observation Network (GEO BON). For example, the CBD Aichi Target 13 specifically states that genetic diversity should be maintained and safeguarded. South Africa, as a signatory to the CBD has an obligation to meet this target. Despite this, no planning or progress has been made toward this target. Essentially, there is a lack of protocols, methods and indicators for creating baselines and for tracking the status of genetic diversity.

To date, quantification of the effects of changes in genetic diversity has focussed primarily at the population level (i.e. Hughes *et al*. 2008; Hoban *et al*. 2014; da Silva & Tolley 2018; da Silva *et al*. 2019). Indeed, modern population genetics theory incorporates numerous metrics for quantifying genetic diversity at this level, several of which are useful to monitor changes in genetic diversity for populations

(Hughes *et al.* 2008; Hoban *et al.* 2014). These metrics are valuable for examining species of conservation concern, where low inter-specific genetic diversity is implicated in the retention of deleterious alleles with concomitant effects on the phenotype or physiology of the organism (*e.g*. Jansen van Vuuren *et al*. 2019). In particular, changes in allelic richness performs well as an indicator for tracking loss of genetic diversity because the metric responds quickly to loss of rare alleles (Hoban *et al.* 2014). A number of additional indicators have been proposed *e.g*. heterozygosity, M-ratio, F_{ST}, F_{IS}, among others (Feld *et al.* 2009; Hoban *et al.* 2014). Additional indicators proposed by GEO BON include relatedness and the number of individuals for livestock breeds and varieties (GEO BON Management Committee 2017). However, all these indicators relate to the monitoring of intraspecific diversity (within or between populations of a single species) of wild and domestic species (see da Silva *et al*. 2019 for full discussion of intraspecific indicators). Therefore, the use of these indicators can be used to detect losses in genetic diversity at the species at the population level (da Silva & Tolley 2018; da Silva *et al.* 2019). Such indicators are not suited to detect landscape level changes in diversity, because the landscape consists of entire assemblages of species, and to monitor indicators for all (or many) species or populations in an assemblage is logistically untenable.

While a targeted approach to conservation genetics provides insight for the management of individual species and populations (e.g. da Silva & Tolley 2018; Vinceti *et al*. 2013), quantifying the status and trends of genetic diversity over the landscape is elusive. Yet landscape level genetic diversity plays an important, if not central, role in creating species richness, as well as underpinning ecosystem function and species resilience (Hughes *et al.* 2008; Cardinale *et al.* 2012; Cadotte *et al*. 2012). Therefore, changes in genetic diversity across the landscape should be considered essential to assess and monitor, given the unprecedented anthropogenic impact on the landscape. This concept has simply not been addressed as a means for tracking genetic diversity over time primarily due to the inherent problems for assessing and monitoring changes in landscape level genetic diversity (Winter *et al.* 2013). The question then, is whether there are any useful surrogate metrics that would suffice to track genetic diversity over the landscape.

While most metrics of genetic diversity relate to intraspecific diversity, e.g. allelic richness or heterozygosity (da Silva *et al*. 2019), there are several metrics for establishing spatial patterns of 'genetic diversity' at the higher level, *i.e*. genera or families. For example, the widely used metric, phylogenetic diversity (PD), is excellent for ascertaining the spatial distribution of genetic richness within an entire taxonomic group and has been quantified on local and global scales (Forest *et al.* 2007; Winter *et al.* 2013; Frishkoff *et al.* 2014; Jetz *et al.* 2014). For a given taxonomic group with a comprehensive phylogeny, PD for a geographic region is simply the additive branch lengths of all taxa in that region, from the tips to the root of the phylogeny (Faith 1992; 2010). PD is robust to taxonomic uncertainty, because lineages need not be described species, but are simply distinct tips in the phylogeny (Mace *et al*. 2003). This makes the metric applicable for all phylogenetic depths and taxonomic ranks depending on the data available and question posed, *e.g*. genus-level phylogenies for the identification of global PD spatial patterns (Fritz & Rahbek 2012) despite an insufficient taxonomic framework (Rosauer *et al.* 2016; 2017).

 Phylogenetic Diversity (PD) has been recommended for inclusion in conservation planning to select priority areas (Rosauer *et al.* 2016, 2017) or to identify areas that have lost diversity due to anthropogenic impacts (Frishkoff *et al.* 2014).

There are quite a few additional genetic metrics that are useful for understanding the spatial distribution of important centres of genetic richness at the landscape level (Mishler *et al.* 2014; Mazel *et al.* 2016; Tucker *et al.* 2016). Below, in addition to PD, we focus on some of those more commonly used. All of these metrics relate to understanding the spatial pattern of phylogenetic diversity, or phylogenetic richness, at the interspecific (or intergeneric) level and changes in that pattern of time, or due to anthropogenic impacts on

the landscape. As such, they should not be confused with more typical genetic diversity measures at the intraspecific level such as allelic richness or heterozygosity. Despite that, these higher level metrics are still measures of the richness of genetic diversity, just not in the colloquial sense. Here, we adopt the term 'phylogenetic richness' when more specifically referring interspecific phylogenetic metrics (e.g. PD among others), as such measures are akin to species richness given that they measure the diversity of lineages over a landscape.

Evolutionary distinctiveness (ED) is a measure of uniqueness within a given phylogeny based on branch lengths (Vane-Wright *et al*. 1991) but can also be evaluated spatially (Jetz *et al.* 2014). Evolutionary distinctiveness (ED) therefore, is useful to identify regions that hold numerous unique taxa. For example, a global analysis of the Class Aves shows that high ED is not distributed randomly, but that there are clusters of unique lineages in isolated regions, *e.g*. Australia, New Zealand and Madagascar (Jetz *et al.* 2014). The EDGE metric (Evolutionarily Distinct and Globally Endangered) is an extension of ED that incorporates phylogenetic uniqueness (long branches in a phylogeny) with level of extinction risk (as assessed by the IUCN), and can be mapped spatially to examine whether certain regions have a high number of unique but threatened species (Isaac *et al.* 2007; Tonini *et al.* 2016). Weighted phylogenetic endemism (PE) incorporates both evolutionary history and spatial information, combining species range size (endemism) with phylogenetic diversity allowing for identification of areas that have spatially restricted, highly divergent species (Rosauer *et al.* 2009; Rosauer & Jetz 2015).

Each of these metrics is useful for evaluating biodiversity under different scenarios, and the choice of metric depends on the conservation objectives. Phylogenetic diversity, for example, assesses which geographic areas are genetically rich for a taxonomic group (Faith 2015; Tucker *et al.* 2016). Similarly, PE assesses the phylogenetic richness, but weights this with range size. The geographic areas that have a rich genetic assemblage of endemics will stand out. Evolutionary distinctiveness (ED) is instead used to identify species that are genetically different from other species. If a set of species with high ED (and EDGE) are evaluated spatially, this allows for a focus on conserving uniqueness and in the case of EDGE, conserving unique but threatened species (Faith 2015; Tucker *et al.* 2016).

These higher level metrics have the potential to be extremely useful for conservation planning because they can identify genetically rich areas (Rosauer *et al.* 2016; 2017), however, they are rarely applied (Winter *et al*. 2013; Santamaría *et al.* 2012). Conservation tends to focus on preservation of species and ecosystems, as these are tangible targets with concrete threats (Mace & Purvis 2008; Santamaría & Méndez 2012). For example, the Red List Index makes use of repeated IUCN Red List assessments of a group of species to track the proportion of species threatened with extinction over time (Butchart *et al.* 2007; 2010; Böhm *et al.* 2013; Tolley *et al*. 2019). In contrast, the landscape level genetic diversity metrics such as PD and ED cannot be used to directly track changes to diversity because such change is accumulated on an evolutionary time scale (centuries, millennia or longer), making them impractical for conservation. Despite this, these metrics are important because they relate directly to extinction risk and ecosystem function (Mace *et al*. 2003; Winter *et al.* 2013), as areas higher in genetic and functional diversity are considered more resilient (Hughes *et al.* 2008).

A potential solution is that of Frishkoff *et al*. (2014) who compared PD across natural and altered habitats by carrying out surveys for taxa inhabiting those habitat matrices and estimating the amount of PD contained in the set of taxa. Certainly, repeated surveys over time could reveal fluctuations in the amount of PD for habitat types, and this is a promising technique. However, the method requires directed surveys of specific localities and habitats, which will prove labour intensive especially because national scale

'indicators' of biodiversity status and trends are required in order to meet the Strategic Goals outlined in the Convention on Biological Diversity (https://www.cbd.int/sp/default.shtml).

We propose an approach to track changes in 'phylogenetic richness' over time at the landscape level using various high level metrics as proxies (e.g. PD, PE, ED, EDGE). The phrase 'phylogenetic richness' encompasses the application of any one, or combination, of phylogenetic based metrics or approaches, that would produce a spatial pattern of 'diversity' on the landscape, akin to species richness. For a given phylogeny, various such metrics can be estimated and using Geographic Information System software (GIS), the spatial distribution of the metrics overlain with spatial distribution of pressures. The pressure is then tracked over time, rather than the genetic metric. For example, the amount of phylogenetic richness contained in areas that are heavily transformed by human activities (e.g. agriculture, urban expansion and mining) would provide an indication of the pressure on areas of high phylogenetic richness, because we can assume that species become decline as their habitat is lost, becoming locally extinct. As species are lost to an area, the total phylogenetic richness of the area will decline, and this can be used as a proxy for 'genetic erosion' to the landscape. Given there is good access to current and past land use layers, the amount of phylogenetic richness 'lost' over time by these activities can be interrogated. Furthermore, when overlain with the distribution of Protected Areas (PAs), the amount of phylogenetic richness under protection, that is, the areas important for safeguarding phylogenetic richness at the higher taxonomic level can be identified (e.g. Pollock *et al*. 2015).

To accomplish these objectives, a comprehensive phylogeny for specific taxonomic groups is required, as are good distribution data and a time-series for land cover change. Given the wealth of publicly accessible genetic data (e.g. GenBank), near complete phylogenies can readily be estimated for many taxonomic groups. Species occurrence data, while available (e.g. Global Biodiversity Information Facility) is often scarce and inadequate in many areas (Tolley *et al.* 2016). However if approached wisely, distribution maps can be made through expert opinion or through the use of species distribution models (e.g. Mecenero *et al.* 2015) as long as primary distribution records are available to guide these approaches (*e.g*. iNaturalist, or the Virtual Museum for South African biota). Finally, for South Africa, a time-series of land cover is available from two time periods (1990 and 2013) to provide the spatial layer of pressure that is needed for this approach (Geo Terra Image 2015; 2016).

We examined the utility of this approach for tracking the impacts to landscape level phylogenetic richness in South Africa using reptiles as a case study. The country has a diverse assemblage of 406 reptile species, although 16 are considered peripheral or vagrants, and one is exotic (Appendix: Table A1). Of the remaining species, we generated a near-complete phylogeny using 378 species, harvesting sequence data from GenBank and supplementing the dataset with our own DNA sequencing. We then constructed species distribution models for each species and when combined with the phylogeny, we were able to map selected genetic diversity metrics (PD, PE) on the landscape. These spatial products were intersected with National Land Cover (NLC) datasets from 2013 and 1990 (Geo Terra Image 2015; 2016). The NLC layers have detailed spatial data on a number of land use categories (*i.e*. built-up, cultivated, mining, plantation, natural) derived from spectral modelling of Landsat images. The 23 year time-frame allowed us to interrogate areas where land cover change intersects with high phylogenetic richness at two diverse time points to determine (1) the areas with high phylogenetic richness (PE or PE) but that are impacted by land cover change i.e. 'genetic erosion', and (2) areas that show a trend for genetic erosion over the time period examined. We also intersected the spatial maps of the phylogenetic richness metrics with the National Protected Areas Network to examine whether areas of high phylogenetic richness are encompassed within Protected Areas (PAs).

4.2. Methods

4.2.1. Baseline taxonomy

To infer the phylogeny of all South African reptile species, we needed a matrix of genetic markers for the sampled taxa. As a first step, it was necessary to have a baseline taxonomy that would reflect current species composition of the South African reptiles. For this, we relied on the recent seminal work by Bates *et al.* (2014) and the regularly updated Reptile Database (Uetz *et al.* 2018). Since reptile taxonomy is a very dynamic field of research, several taxonomic changes have been made since Bates *et al.* (2014) and the last database release, which we took into account using the primary literature. In our analyses we focused only on the reptiles native to South Africa. Our list of taxa thus included the squamates (order Squamata) and chelonians (order Testudines). We excluded the marine sea snake, *Hydrophis platurus* (Elapidae), as it is considered a vagrant, as well as marine turtles (*Cheloniidae* and *Dermochelyidae*). We also excluded the Nile crocodile (*Crocodylus niloticus)*, as it is phylogenetically closer to birds than to other reptiles (even though 'reptiles' *per se* are not monophyletic and the position of chelonians is controversial; e.g. Chiari *et al.* 2012). This left 399 species from 102 genera and 19 families and of those, sequence data were available for 378 species (Appendix: Table A1, A2).

4.2.2. Sequence data acquisition

To gather DNA sequence data, we searched GenBank by the combination of "family name AND gene name or gene abbreviation" to identify candidate sequences. We targeted loci that are commonly used in reptile phylogenies and Geneious v.8 (Kearse *et al.* 2012) for running the queries. For each locus and family, we reconstructed a cursory NJ tree using Geneious tree builder to confirm that species clustered together. Apparently erroneous sequences that did not cluster with other samples of the same species were not used. The most complete sequence for each species was then selected. We compiled a matrix of 18 genetic markers, five mitochondrial (mtDNA) and 13 nuclear (Appendix: Table A2). The number of genes available for each taxon ranged between 1 and 13, with the mean of 5.2. The number of taxa sequenced for a particular gene ranged between 6 and 294, with the mean of 110. The mtDNA genes were obviously the most represented (141–294 taxa sequenced), of the nuclear ones the best represented were RAG-1 (sequenced for 196 taxa) and cmos (195 taxa).

4.2.3. DNA sequencing

Not all South African reptiles have yet been sequenced and placed in a phylogenetic framework. Of the taxa that could not be gleaned from GenBank we acquired tissue samples. Most of the tissue samples were housed at SANBI, some were collected during targeted field work, and some were acquired through collaborations for a total of 48 species of 28 genera from 12 families, which we sequenced for up to five genes (Appendix: Table A2). Genomic DNA was extracted according to a standard procedure with proteinase-K digestion followed by a salt extraction protocol (Aljanabi & Martinez 1997). To be able to place the newly generated sequences within the phylogenetic framework, for each sample we carefully selected markers and primers so the new sequences would match genes already available on GenBank (Appendix: Table A3). The amplified fragments were sequenced using the forward PCR primer at Macrogen (Amsterdam, the Netherlands). The amount of sequences generated for the new material ranged between 1 and 5 genes, with the mean of 3.3, depending on the quality of the DNA.

4.2.4. Analyses

We aligned all genes using MAFFT v.7 (Katoh & Standley 2013) with the 'auto' option. Although the Q-INS-i method would be preferable for the 12S and 16S genes as it considers the secondary structure of the RNA,

it could not be used because this method is limited to datasets with less than 200 sequences (Katoh & Toh 2008). The variable tRNAs that flank the mtDNA genes and which are problematic to align were excluded from the analysis. The genes that have been sequenced for the blind snakes (Typhlopidae) and were available on GenBank failed to amplify for one of the species, the Schinz's beaked blind snake *Rhinotyphlops schinzi*. Not having overlapping gene fragments for the blind snake and the rest of typhlopids would result in that the species would not cluster with the family, but instead forming a separate long-branch in the tree, which would increase the relative weight of this species in the downstream analyses. To avoid this problem, we retained the Brahminy Blind Snake (*Indotyphlops braminus*), in the analysis because its sequences overlapped with those of *R. schinzi* and with other typhlopids as well. This way we 'anchored' *R. schinzi* to the family in which it belongs, and subsequently pruned *Indotyphlops braminus* from the tree manually.

The final matrix contained sequences for 378 reptile species. The taxa were represented by a mean of 3402 bp with the range being between 304 bp (*Homoroselaps dorsalis*, Lamprophiidae) and 11,400 bp (*Stigmochelys pardalis*, Testudinidae). In terms of sampling, this constitutes 97% of the non-peripheral reptiles native to South Africa (following Bates *et al*. 2014). Species level taxonomic sampling for the individual families represented in the analysis were: Agamidae, Chamaeleonidae, Natricidae, Pelomedusidae, Pythonidae, Testudinidae, Typhlopidae, Varanidae, and Viperidae at 100%, Colubridae, Cordylidae, Elapidae, and Scincidae at 94%, Amphisbaenidae at 67%, Gekkonidae at 95%, Gerrhosauridae at 85%, Lacertidae at 97%, Lamprophiidae at 92%, Leptotyphlopidae at 82%. All higher taxa (families, genera) were included with two exceptions, which were the genera *Chirindia* (Amphisbaenidae) and *Montaspis* (Lamprophiidae). This dataset is by far the most complete overview of the taxonomic diversity and systematics of South African reptiles.

Phylogenetic inference was carried out using the maximum likelihood (ML) approach implemented in RAxML v.7.3 (Stamatakis 2006) through the CIPRES Science Gateway (Miller *et al*. 2010). The chelonians were used to root the tree.

4.2.5. Inferring species ranges

The distribution of South African reptiles is reasonably well documented thanks to the tremendous effort of many professionals (see Bates *et al*. 2014 for a review). However, the best available distribution data (Bates *et al.* 2014) is only referenced to the spatial accuracy of a quarter-degree (0.25° × 0.25°), which at the latitude of South Africa provided grid cells of about 676 km^2. Such a scale was too coarse for our purposes as it would not allow detection of fine-scale spatial nuances in the heterogeneous environment (see Cowling *et al.* 1989; Thuiller *et al.* 2006). Therefore, we modelled the ranges of all species using their environmental, land cover, and topographic preferences.

First, we compiled a dataset of distribution records that were based on Bates *et al*. (2014) data but also contained records from more recent years, for which we sourced published literature, unpublished observations and contributions made by citizen scientists to the Virtual Museum of the Animal Demographic Unit, University of Cape Town (http://vmus.adu.org.za/). This initial set contained over 140,000 records, which were carefully vetted by experts in different fields of reptile taxonomy and distribution for outlying records.

Some of the distribution data from Bates *et al*. (2014) could not be assigned to exact localities. These were assigned by those authors to the centroids of the grid cells from which they originated. We divided the dataset into two subsets: one included only the high resolution records with precise coordinates (GPS

precision), the other included the centroids. Only the former subset was used for the modelling described below.

Due to the uncertainty of the actual origin of records assigned to centroids this part of the dataset could not be used for the modelling. To include the centroids, which represented a considerable portion of the original data (39% of records were centroids), in the range estimations, we buffered each record in this subset with a radius of 0.125° (ca. 12 km in diameter).

To run the Species Distribution Modelling (SDM), we used the maximum entropy approach implemented in Maxent v.3.3 (Phillips *et al*. 2006) and mapped the potentially suitable habitat for each species. Because small sample size may negatively influence modelling success, we followed Rissler *et al.* (2006) and did not model species that had less than five unique locality points. Instead, records (including centroids) of these species (47 in total) were buffered by a 0.125° radius and the resulting 'distribution map' was used as a proxy for their distribution in mapping the phylogenetic diversity metrics. The spatial background defined for developing the models was delineated by the borders of the Republic of South Africa with Lesotho and Swaziland included.

We downloaded and generated using ArcGIS v.10.3 (ESRI 2011) a broad set of environmental and topographic variables to best capture the diverse habitat preferences present across species of such a heterogeneous group as reptiles. These were:

1) the recently updated 19 bioclimatic variables that include global monthly temperature and precipitation patterns for the time period 1979–2013 (Karger *et al.* 2017);
2) altitude, slope and aspect (the latter two were created from the altitude layer);
3) land cover data GlobalCover 2009 v.2.3 (European Space Agency; http://due.esrin.esa.int/page_globcover.php); and
4) Global Aridity Index, an index used to quantify precipitation availability over atmospheric water demand and calculated as the mean of mean annual precipitation divided by mean annual potential evapotranspiration over the period of 1950–2000 (Trabucco & Zomer 2009).

All layers had the resolution of 30 arc sec and the species records were filtered to remove duplicates from each such cell. Subspecies were modelled separately as they often show distinguished geographic distributions and thus, perhaps, adaptations to locally specific conditions.

We used ENMTools (Warren *et al*. 2010) to test for spatial autocorrelation between the 24 environmental variables, and of those that were strongly correlated (Pearson's $r > 0.75$) we retained the more biologically meaningful ones. The final set that was used for the modelling included these 14 variables: Bio3 – isothermality; Bio4 – temperature seasonality; Bio8 – mean temperature of wettest quarter; Bio9 – mean temperature of driest quarter; Bio10 – mean temperature of warmest quarter; Bio11 – mean temperature of coldest quarter; Bio15 – precipitation seasonality; Bio16 – precipitation of wettest quarter; Bio17 – precipitation of driest quarter; Bio19 – precipitation of coldest quarter; altitude; slope; aspect; land cover.

Presence-only species distribution modelling methods, such as that implemented in Maxent, are biased by sampling effort, when some areas are sampled more intensively than others (Phillips *et al.* 2009). This is typical for major cities that usually have detailed species inventories and locality data, while remote and difficult-to-access areas are only poorly sampled. To account for this bias we generated a Gaussian kernel density layer that was based on all GPS-precise species records and a search radius 0.1°, and which we used as a bias file for the modelling. We used the area under the receiver operating curve (AUC) as a measure of model accuracy of each replicate run and final models were averaged over the ten replicates. Models of species with the mean AUC < 0.8 were not used as the predictive accuracy of such models is not

trustworthy (Araújo *et al.* 2005). This applied to 15 species and, interestingly, it affected some large-ranging species distributed across the whole country and further north into Africa such as *Bitis arietans* (Viperidae), *Stigmochelys pardalis* (Testudinidae), *Trachylepis capensis* (Scincidae), perhaps due to their generalist habits. Ranges of these low-AUC species were drawn as 0.125° buffers around all their locality records. For each species, Maxent produces a continuous layer with cell values ranging between 0 and 1 indicating the probability of its presence in each cell. To obtain binary presence/absence maps, we reclassified the continuous predictive models using the maximum training sensitivity plus specificity threshold. This threshold maximizes the combined rate of correctly predicted presences (sensitivity) and absences (specificity) and is considered to most accurately predict the potential presence of a species (Worth *et al.* 2014). While Maxent predicts the potential species environmental niche, it is ignorant of other relevant biotic factors such as, for instance, species-specific dispersal limitations or interspecific interactions (Graham & Hijmans 2006; Peterson 2011). As a result, the predicted potential distributions are likely to be overestimates of the actual species' ranges (Vasconcelos *et al.* 2012). To account for the over-prediction caused by ignoring dispersal limitations, we generated minimum convex polygons for all species that encompassed all their records and were buffered by a radius of 50 km. The binary maps produced by the SDMs were then intersected with these 50km-buffered hulls and the ranges predicted outside the hulls were removed (i.e. we cookie-cut the SDMs out of the hulls). Final maps were created by merging the layers of the 0.125° buffered points (i.e. the centroids, species with less than five unique localities, and modelled species with low AUC) and predicted binary ranges into a single shapefile. Since we worked at the level of species in the downstream analyses we merged subspecies ranges into a species.

4.2.6. Phylogenetic richness metrics

To reduce computational burden, while retaining enough spatial resolution, maps of phylogenetic diversity (PD) were generated at the scale of 0.1° (ca. 10 × 10 km). We calculated PD of each grid cell using the *picante* package in R (Kembel *et al.* 2010) using the ultrametric maximum likelihood tree. We did not include the tree root in the PD calculation.

The spatial layer for phylogenetic endemism (PE) was calculated at the same scale as PD using the Biodiverse v2.0 package (Laffan *et al.* 2010). The species distributions from Maxent were used to create a presence-absence matrix for each grid cell for the Biodiverse input. The phylogenetic tree was imported and the analysis for PE was run. This produced a spatial layer with values of PE for each grid cell.

Evolutionary distinctiveness (ED) was estimated for each species with the Tuatara v1.0 module for Mesquite v3.5.1 (Maddison & Mooers 2007; Maddison & Maddison 2018). EDGE (evolutionary distinct and globally endangered) values were calculated following (Isaac *et al.* 2007) using the IUCN Threat status of each species as of 2018 as weightings. The resulting ED and EDGE scores were ranked from largest to smallest and the top 5% scoring species were identified for each metric. These SDMs were mapped using QGIS and a richness map was produced by joining the attributes by location, and then use R to sum the number of joins by each quarter-degree square (QDS). This output was joined back to the original QDS layer to map the richness of ED and EDGE species. Note that the Critically Endangered *Scelotes inornatus* and the Vulnerable *Scelotes bourquini* were missing from the phylogenetic analysis and were therefore not included in the EDGE calculations. All other Threatened species were included. Data Deficient and Not Evaluated species were coded as missing data for their extinction risk. Furthermore, two South African reptile species are classified by IUCN as Extinct (*Scelotes guentheri, Tetradactylus eastwoodae*), and were not included in any analyses.

4.2.7. Pressures mapping

To track the impacts of land use on phylogenetic diversity at the landscape level, change indices were derived for commonly used diversity metrics (i.e. PD, PE, ED, EDGE). These were projected spatially using QGIS (QGIS Development Team 2018) and values for each grid cell were extracted. The South African National Land Cover (NLC) spatial datasets from 1990 and 2013 were also projected at the same spatial resolution (Geo Terra Image 2015; 2016), and the proportion of transformed land cover for each cell was extracted for both time periods. For each time period, an index was calculated for every grid cell of the proportion of transformed land cover multiplied by the log (PD) or log (PE). These indices are termed 'phylogenetic richness indices' (PRI) and are distinguished with subscripts of the underlying phylogenetic metric (e.g. PRI_{PD} and PRI_{PE}). The indices were estimated for each cell at each time period, and these were mapped spatially. The maps then, can show areas that have high phylogenetic richness' and that are also prone to 'genetic erosion' due to loss of habitat for 1990 and for 2013. In contrast, the maps would also show areas of low phylogenetic richness with low habitat loss.

The index values for 1990 were then subtracted from the 2013 values to generate a phylogenetic richness *change* index for both PD and PE ($PRI_{(PDC)}$ and $PRI_{(PEC)}$). The *change indices* were mapped spatially, allowing for identification of areas with high PD or PE, but which also show the greatest *change* in land cover. Thus, the change indices denote areas of high phylogenetic richness, but with a trend for natural habitat loss in the last decades. The indices were derived and mapped for all reptiles combined and also for each reptile family to examine whether spatial or temporal patterns differ at the family level.

To investigate the level of protection for phylogenetic richness, PD and PE were intersected with the South African Protected Area (PA) network (Department of Environmental Affairs 2010). The top 10% of the highest PD and PE values were then mapped within the PAs. This allowed us to visually assess which PAs contain the highest reptile phylogenetic richness and are therefore essential to conserve into the long term. As an exploration, the change index for PD (denoted as $PRI_{(PDC)}$) was also intersected with the PA network. While we do not expect this change to reflect changes *within* the protected areas (presumably no land cover change within the PAs), those PAs that are in areas of high phylogenetic richness but have high land use change at their borders could be identified.

For ED and EDGE, phylogenetic richness maps were made (i.e. each grid cells value as the number of species that were in the 5% of ranked values for ED and EDGE) to identify priority areas in terms of extinction risk for evolutionarily unique species. That is, grid cells with multiple ED or EDGE species should be considered important for prioritising conservation. These metrics are not currently incorporated into the change indices nor the protected areas analyses.

4.3. Results

4.3.1. Landscape level phylogenetic patterns

Phylogenetic diversity for reptiles is highest in the north-eastern margin of South Africa (Figure 4.1). This is primarily due to the area being a contact zone for temperate/sub-tropical fauna mainly found to the south, and tropical species mainly found to the north. Similarly, northern KwaZulu-Natal Province also shows exceptionally high PD for reptiles. The arid interior shows the lowest PD for reptiles overall.
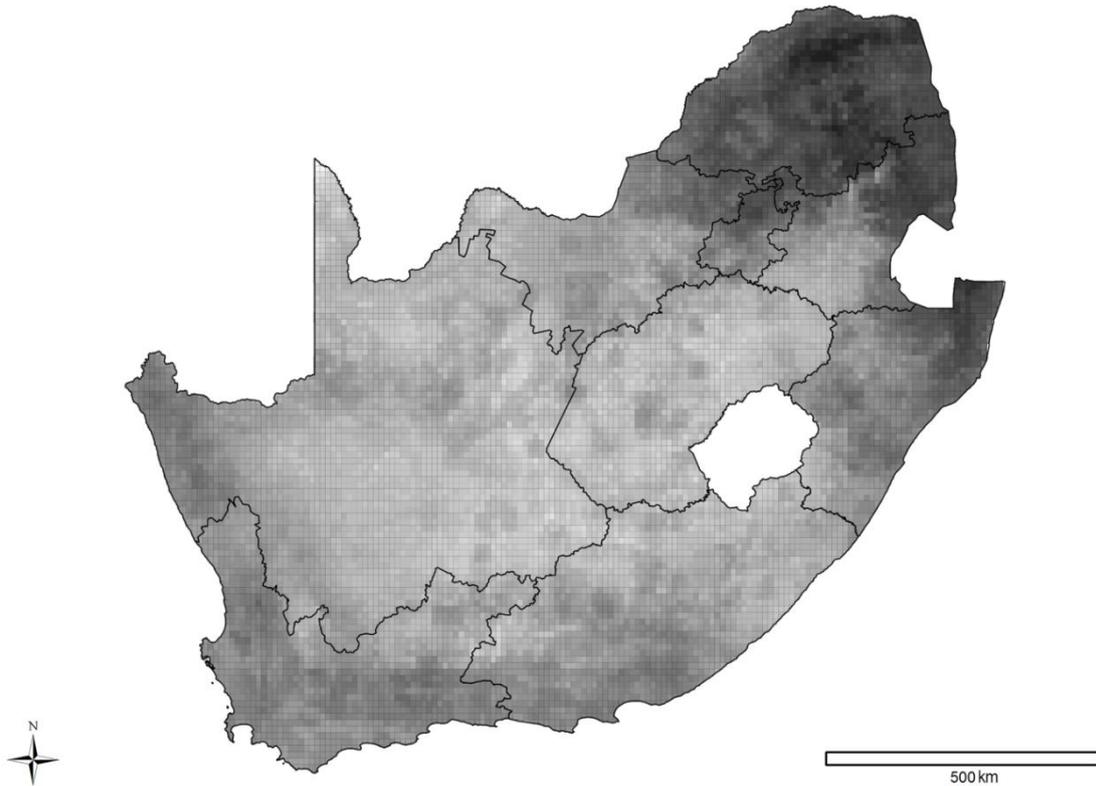
Figure 4.1. Phylogenetic diversity (PD) for reptiles from South Africa. Darker shading indicates areas with PD.

Similarly, phylogenetic endemism is high in the north-east, although the highest values occur in the north-west in concentrated areas centred in the Richtersveld National Park (Northern Cape Province), as well the northern edge of iSimangaliso Wetland Park, KwaZulu-Natal Province (Figure 4.2). These two protected areas therefore, hold the high phylogenetic endemism for South African reptiles. Other areas that are notable are the western margin of South Africa (both Western and Northern Cape provinces) and the Cape St. Francis area of the Eastern Cape. It should be noted that the high PE in the northeast is bias by the inclusion of widespread southern African species which have just a small range inside South Africa.

As PE accounts for range size (within South Africa), these species are weighted more heavily in the analysis and this will intensify the pattern of high PE in that area. Indeed, many or most species will be distributed across political borders and to some degree, any analysis using political rather than biological boundaries will have this issue. Regardless, from a South African perspective these species do have a small range nationally, so the pattern of higher PE in that area can be considered meaningful for a national analysis. This bias will be less important in the arid northwest centre of endemism, as fewer widespread species (from the north) are present there, and many of the species that drive this pattern are indeed endemics or near-endemics.
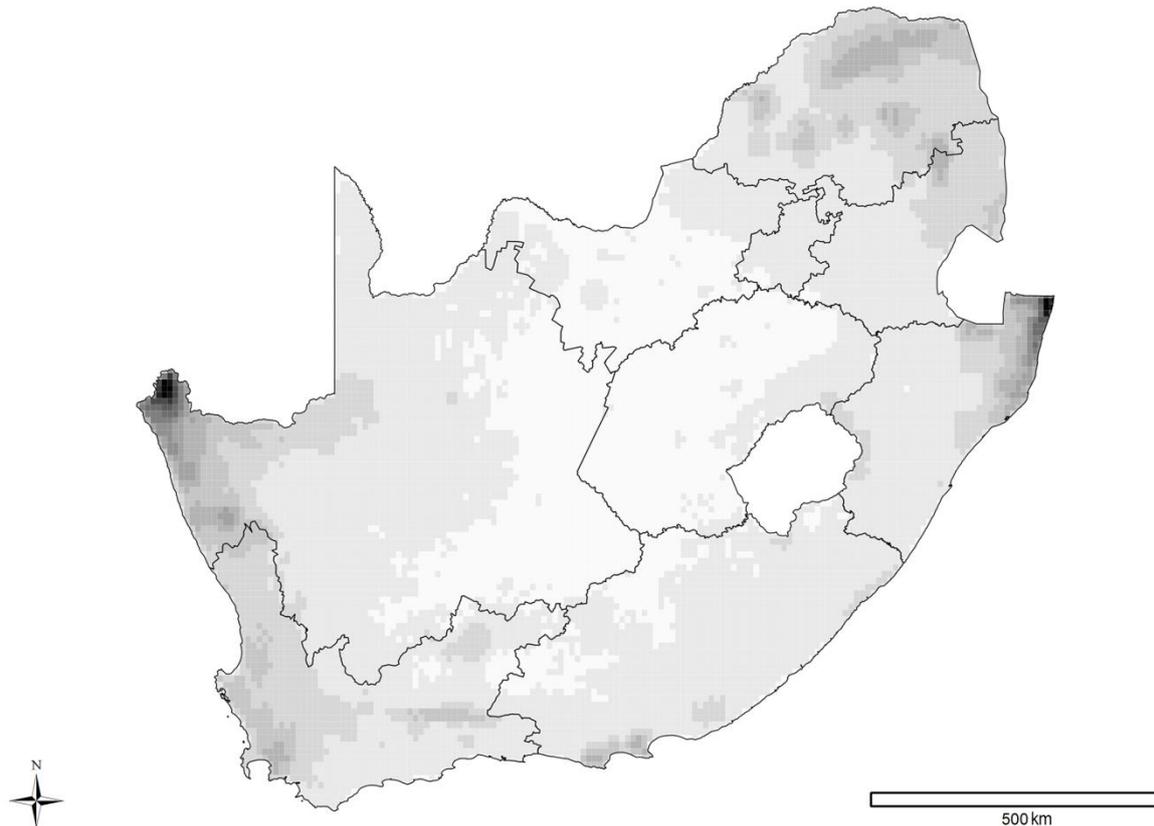
Figure 4.2. Phylogenetic endemism for reptiles from South Africa. Darker shading indicates areas with higher phylogenetic endemism.

4.3.2. Impacts on phylogenetic richness

The PRI$_{(PD1990)}$ and PRI$_{(PD2013)}$ for all reptiles combined shows that phylogenetic richness (using PD as a proxy) has been most impacted in the north-east and extreme south-west (Figure 4.3a & b). These areas can be considered as having undergone the most 'genetic erosion' as compared to the historical natural state. It should be noted that this map does not represent the true extent of genetic erosion, because many species persist in a partly transformed landscape. Without an accompanying survey of species composition and density, this issue cannot be avoided. At a minimum, these areas can be considered at risk of genetic erosion and highly sensitive to further changes relating to habitat loss. Most of the areas correspond with high land use or centres of population e.g. near Johannesburg, Pretoria, Cape Town and Durban. There are several additional regions that are notable for areas of potential genetic erosion, including central Limpopo Province, and northern Mpumalanga particularly near Sabi and Nelspruit.
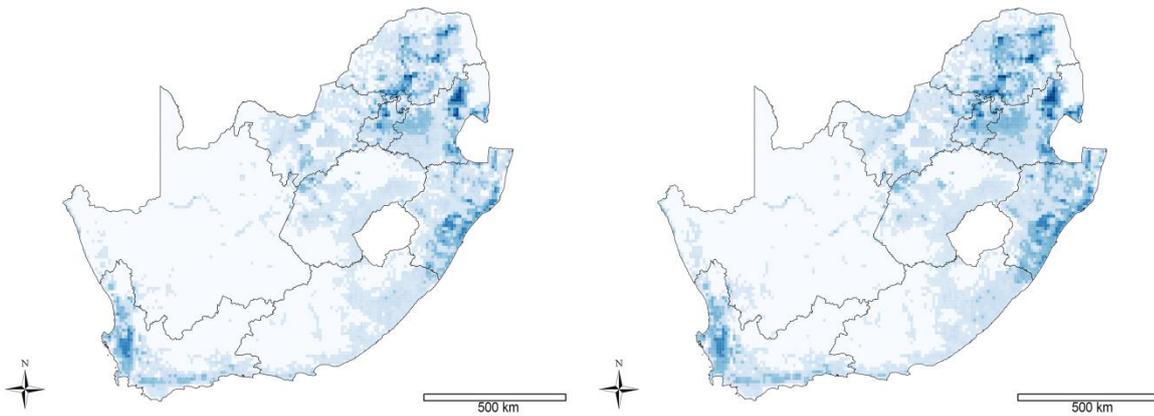
Figure 4.3. a) Spatial distribution of PRI(PD1990) for South African reptiles and b) spatial distribution of PRI(2013) for South African reptiles. Darker blue shading showing cells with the highest values.

The change index PRI $_{(PDC)}$ (the difference between the 1990 and 2013 time periods), was relatively minor overall (Figure 4.4; Appendix: Fig. A1 & A3), suggesting the greatest impacts to phylogenetic richness occurred prior to 1990 rather than between 1990 and 2013. However, there were some notable areas in which the PRI $_{(PDC)}$ was prominent. Principally, these are found in southern Limpopo or northern Gauteng provinces, as well as eastern Mpumalanga and KwaZulu-Natal provinces (Figure 4.4). Noteworthy, there are six obvious clusters of cells with high PRI $_{(PDC)}$. Cluster 1 is in the area of Shoshanguve and Hammanskraal in Gauteng Province. Cluster 2 (Matlerekeng area) and Cluster 3 (Sekhukhune District) are both in Limpopo Province. Cluster 4 is situated south of Komatipoort in Mpumalanga Province, whereas Cluster 5 is in northern KwaZulu-Natal Province in the area near Mkhuze or Ndumu, northwest of the iSimangaliso Wetland Park. Essentially, these areas could be considered as having a comparatively high rate of change, indicating where the current 'genetic erosion' is taking place.
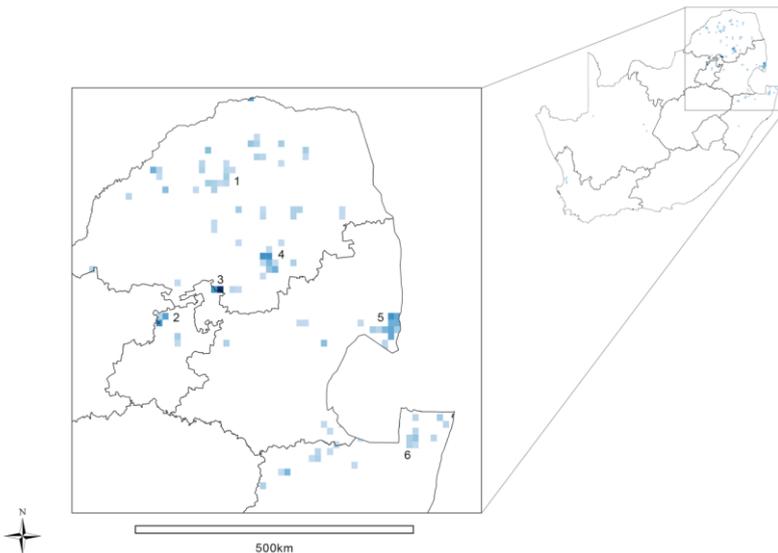
Figure 4.4. Spatial distribution of PRI $_{(PDC)}$ in north-eastern South Africa for reptiles. Cells with the highest 1% of values (i.e. most change between 1990 and 2013) are shaded, with darker shading showing cells with the highest values. The top clusters where change is highest are labelled (1-6). Inset: PRI $_{(PDC)}$ for reptiles across South Africa (see also Appendix, Figs. A1 & A3).

The proportion of each specific land use category that contributed to the six clusters was extracted for 1990 and 2013 to investigate which type of land use has been most impactful in terms of pressures to reptile phylogenetic richness (Figure 4.5). For Clusters 2 and 3 located in or near Gauteng Province, increasing urban expansion had the greatest effect. Increasing cultivation shows the greatest impact for

Clusters 1 & 4-6, although Cluster 5 in northern KwaZulu-Natal shows a mixture of land use types that affect phylogenetic richness, primarily urban expansion and cultivation. PRI $_{(PDC)}$ was mapped separately for each major reptile family, and this showed some variation in the geographic location for highest values of the change index (Appendix: Fig. A1). Although in most cases, the highest values remain in the north-eastern part of South Africa, there are notable exceptions. For example, high values are also concentrated in KwaZulu-Natal Province for *Agamidae, Chamaeleonidae, Gerrhosauridae, Colubridae, Lamprophiidae,* and *Viperidae,* although in most cases these values are still lower than that found in the northeast provinces (i.e. Limpopo, Mpumalanga and Gauteng).
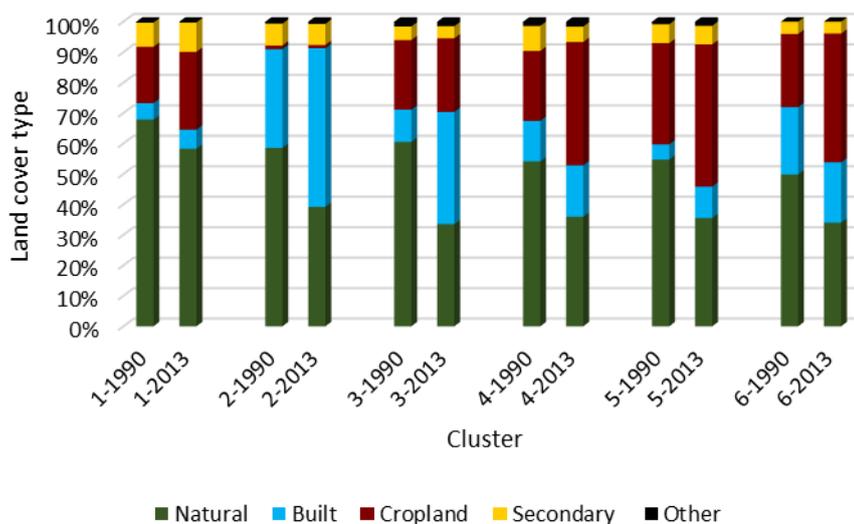
Figure 4.5. The proportion of each major land cover type for the six clusters that are in the top 1% for change index PRI $_{(PDC)}$ values. Land cover types are shown for 1990 and 2013. Clusters locations are shown in Figure 4.4.

4.3.3. Safeguarding phylogenetic richness with protected areas

The intersection between Protected Areas (PAs) and PD (e.g. phylogenetic richness) shows that PAs in the northeast capture the highest levels of richness (Figure 4.6). Indeed, the highest 10% of PD values are captured in PAs are in Limpopo and Mpumalanga provinces as well as northern KwaZulu-Natal Province. In particular, the PAs with the largest areas of high richness are Kruger National Park, Blouberg East, Wolkberg, Blyde River Canyon and Tembe Elephant Reserve. These PAs therefore should be regarded as particularly important in conserving the evolutionary potential and richness of South Africa's reptiles.
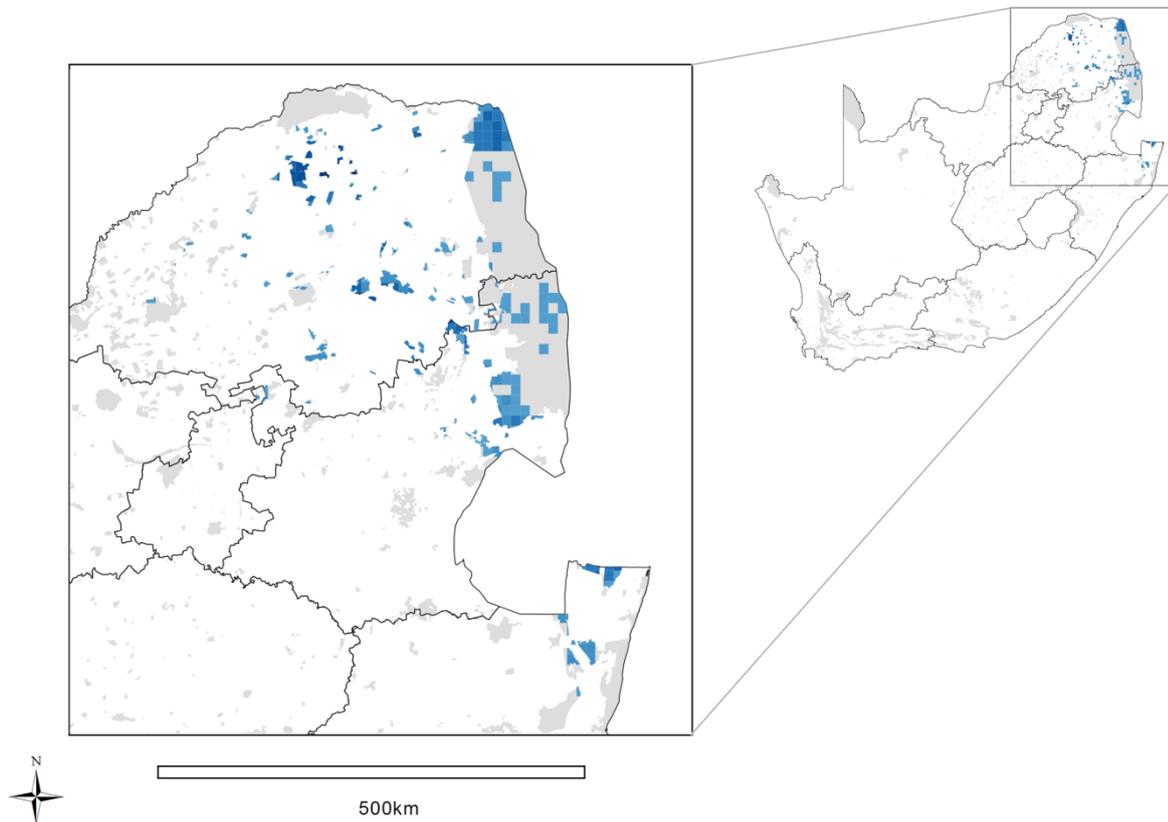
Figure 4.6. Intersect between Protected Areas (grey shading) and phylogenetic diversity (highest 10% of PD values), shaded in a blue gradient.

The intersect between PAs and the top 5% of PRI$_{(PDC)}$ however, show that some of these important PAs are likely influenced by land use change on their borders (Figure 4.7). There are a number of PAs in Limpopo and Mpumalanga provinces that hold high reptile phylogenetic richness (e.g. PD), but have substantial land transformation immediately outside their borders. Many of these are small private nature reserves, but some larger reserves also may be affected. For example, the southern borders of Kruger National Park, Ithala and iSimangaliso appear to be near areas of high land cover change, and possibly should be flagged for explorations on whether existing buffer zones are intact, or for setting up buffer zones where they do not exist.

a)
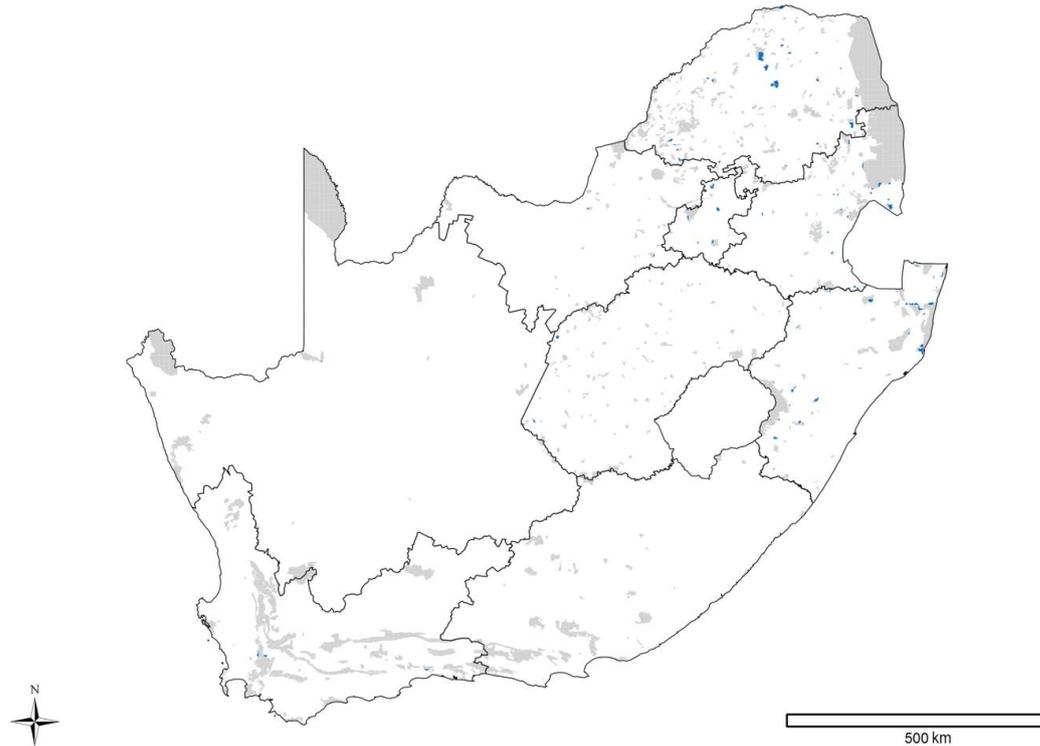
b)
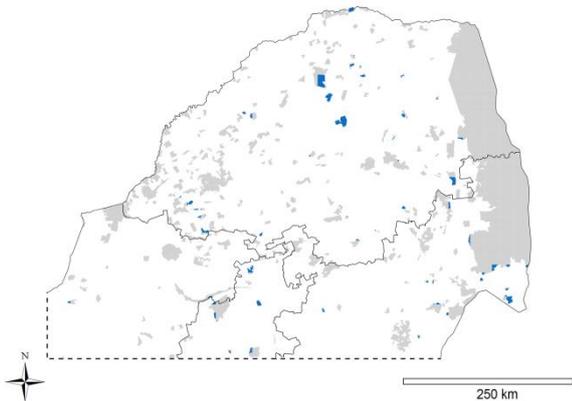
c)
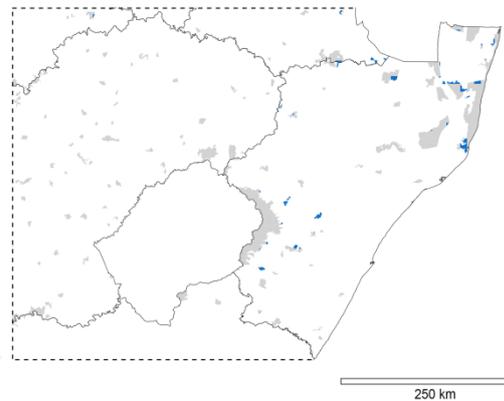
Figure 4.7. Intersection between Protected Areas (shown in grey) and highest 5% of values for PRI$_{(PDC)}$ shaded in blue for a) all South Africa, and enlarged for b) the northern provinces of Limpopo, Gauteng and Mpumalanga, and c) KwaZulu-Natal Province.

4.3.4. Impacts on Phylogenetic Endemism (PE)

The PRI indices for phylogenetic endemism (PRI $_{(PD1990)}$ and PRI $_{(PD2013)}$) for all reptiles combined show that phylogenetic endemism is most impacted in the north-east and extreme south-east at both time periods, similar to phylogenetic diversity (Figure 4.8 a & b). Although PE was highest in the north-west (Figure 4.2), that area shows essentially no land use change, as much of the area is within the Richtersveld National Park, and/or is otherwise low in human population density.

The cells with the highest values for the change index (GRI$_{PEC}$) are found in the north-east, similar to PD (Figure 4.9). The top 5% of cells however, are concentrated in north-eastern KwaZulu-Natal near northern section of iSimangaliso Wetland Park. This suggests that phylogenetic endemism for reptiles has undergone 'genetic erosion' primarily in this area. Thus, reptile species that have small ranges but are phylogenetically diverse are the most impacted in northern KwaZulu-Natal.

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a)
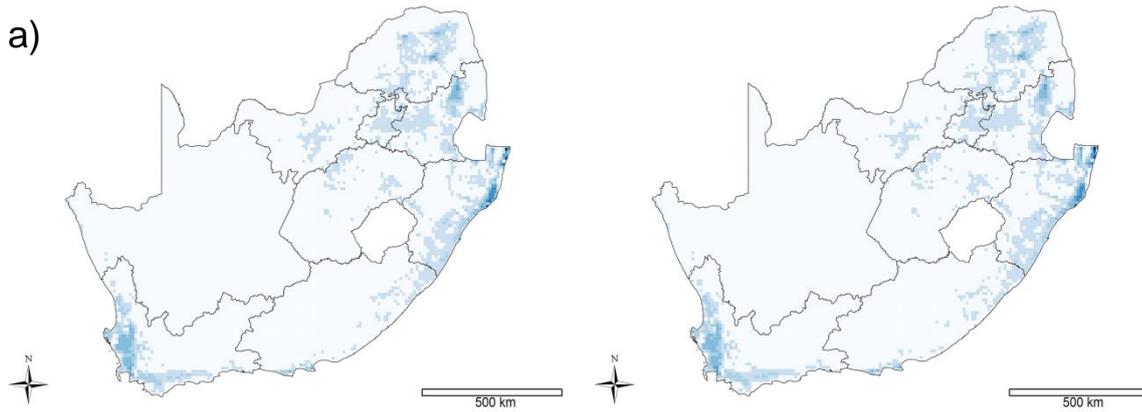
Figure 4.8. For South African reptiles, the a) spatial distribution of PRI(PE1990), and b) spatial distribution of PRI(PE2013). Darker blue shading shows cells with the highest values. The pattern for the two time periods is very similar (refer to Figure 10 which highlights differences in finer detail).
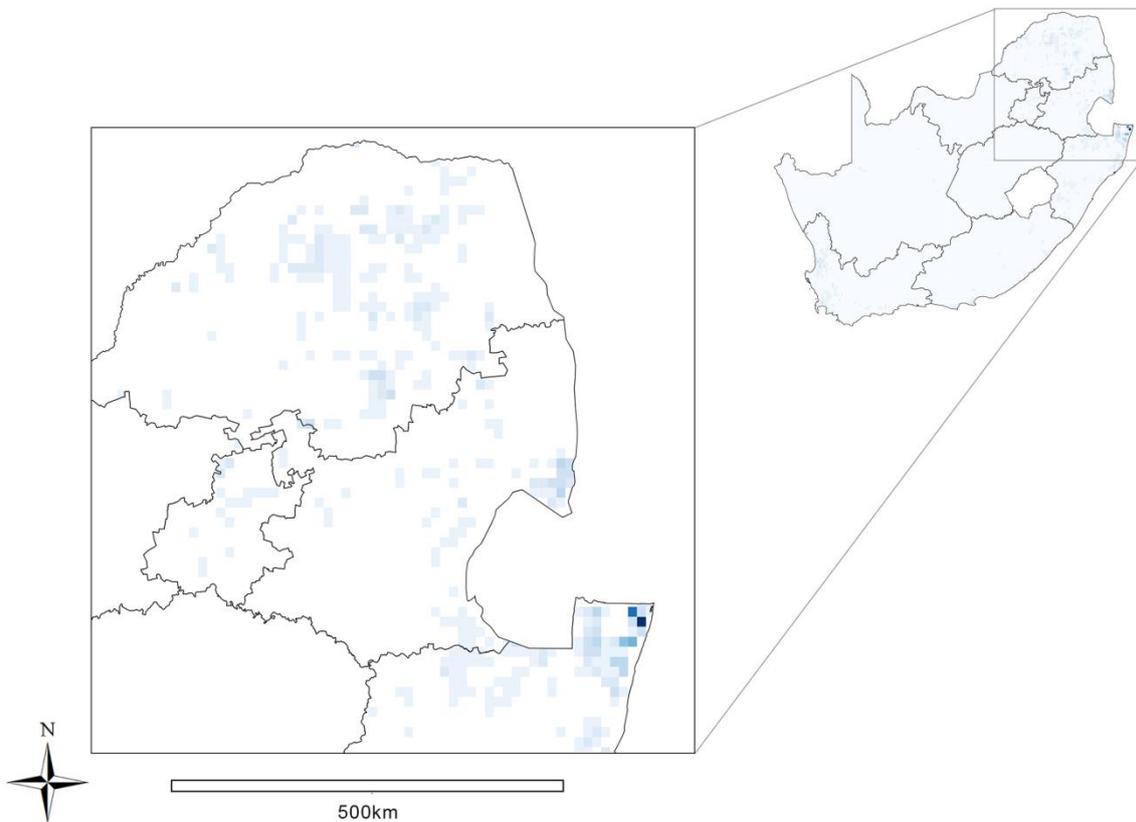
Figure 4.9. Spatial distribution of PRI (PEC) for South African reptiles. Cells with the highest 5% of values (i.e. most change between 1990 and 2013) are shaded, with darker shading showing cells with the highest values. Inset: PRI (PEC) for reptiles across South Africa.

4.3.5. Evolutionary Distinctiveness (ED)

All but one of the top 5% of ED reptiles are Least Concern species, although one species was Not Evaluated for IUCN as it is considered peripheral (Table 4.1). The list of the top 5% of EDGE species (weighted for threat status) did not correspond with the list of top ED species, although given that the ED species were all Least Concern, we would expect the two lists to be different. Of the 19 threatened reptiles in South Africa, only one Endangered species is not among the top EDGE species (*Bradypodion thamnobates*). The richness values of ED species ranged from 0 to 11 species per grid cell, with the richest grid cells in the north-east (Figure 4.10a). In contrast, there was a maximum richness (in a given grid cell) of just 4 EDGE species. Most of these grid cells with highest EDGE richness are along the north-western margin of South Africa where there is comparatively little habitat alteration, and in northern KwaZulu-Natal near Mkhuze (Figure 4.10b). Although three threatened species were not included in the phylogeny (Table 6. List of 5% top ranking species and scores for EDGE and ED, with their respective IUCN Threat status level as of 2018.), their distributions were included in the mapping of the top EDGE species.

Table 4.1. List of 5% top ranking species and scores for EDGE and ED, with their respective IUCN Threat status level as of 2018.

| EDGE species			ED species		
Species	**Score**	**IUCN**	**Species**	**Score**	**IUCN**
Psammobates geometricus	2.833	CR	*Ptenopus garrulus*	0.461	LC
Chersobius signatus	2.147	EN	*Rhinotyphlops schinzi*	0.287	LC
Chersobius boulengeri	2.146	EN	*Hemidactylus mabouia*	0.285	LC
Bradypodion caffer	2.135	EN	*Myriopholis longicauda*	0.273	LC
Bradypodion caeruleogula	2.133	EN	*Varanus albigularis*	0.247	LC
Bitis albanica	2.120	EN	*Varanus niloticus*	0.229	LC
Tetradactylus fitzsimonsi	1.484	VU	*Heliobolus lugubris*	0.215	LC
Smaug giganteus	1.483	VU	*Namibiana occidentalis*	0.212	LC
Pelusios castanoides	1.477	VU	*Chondrodactylus angulifer*	0.211	LC
Pelusios rhodesianus	1.477	VU	*Pachydactylus wahlbergii*	0.210	LC
Dendroaspis angusticeps	1.466	VU	*Phelsuma ocellata*	0.199	LC
Kinixys natalensis	1.452	VU	*Panaspis wahlbergi*	0.197	LC
Hemicordylus nebulosus	1.447	VU	*Acanthocercus atricollis*	0.197	LC
Kinixys lobatsiana	1.441	VU	*Panaspis maculicollis*	0.196	LC
Bitis armata	1.440	VU	*Pachydactylus rangei*	0.194	NE
Cryptactites peringueyi	0.829	NT	*Ichnotropis capensis*	0.186	LC
Bitis schneideri	0.766	NT	*Vhembelacerta rupicola*	0.183	LC
Psammobates tentorius	0.764	NT	*Mochlus sundevallii*	0.179	LC
Threatened species not in EDGE analysis					
Scelotes inornatus	NA	CR			
Scelotes bourquini	NA	VU			
Crocodylus niloticus	NA	VU			

a)   b)
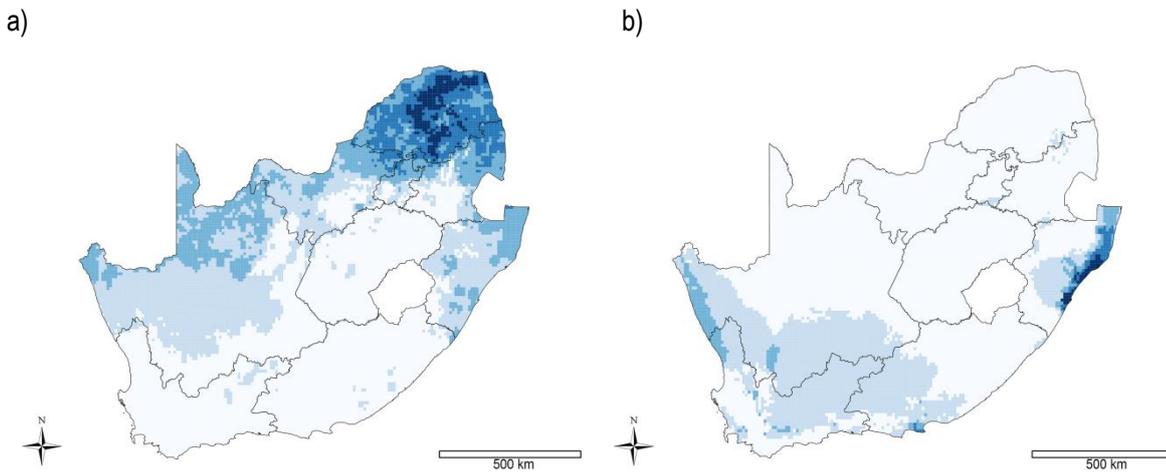
Figure 4.10. For South African reptiles, the a) Evolutionary distinctiveness (ED) richness, ranging from 0 to 11 species, and b) EDGE richness, ranging from 0 to 4 species. Richness gradient is indicated by the blue shading.

4.4. Discussion

4.4.1. Application of the indicators

Reptile phylogenetic diversity is highest in north-eastern South Africa, although the south-western margin of the country also has relatively high values. The high PD in the north-east is likely a result of the area being a contact zone between the temperate, sub-tropical and semi-arid South African species with the more tropically distributed species to the north (*e.g.* Mozambique, Zimbabwe). These groups have different evolutionary histories, resulting in longer summed branch lengths, which will generate higher PD values. That is, high PD in the area is the result of species assemblages that are dispersed in the phylogeny, some of which have larger ranges to the north. In contrast, the south-western part of the country is relatively isolated from the rest of the continent and is well known for the high number of endemic reptile species that do not occur anywhere else (Bates *et al.* 2014). Therefore, the high PD in this area is rather a result of high species richness of certain clades.

Approximately 20% of South Africa's land cover is transformed at present (2013 NCL), of which, ca. 2% was lost between 1990 and 2013 (Geo Terra Image 2015; 2016). Most of the land transformation in South Africa took place prior to 1990 (Skowno *et al*. 2019), and this was primarily in the north-east and the south-west (Appendix: Fig. A2). This corresponds with the expansive areas in the north-east showing high levels of PRI $_{(PD1990)}$. That is, the greatest impacts to 'genetic erosion' on the landscape occurred cumulatively throughout the history of human development of the South Africa landscape up until 1990. In some ways, both PRI $_{(PD1990)}$ and PRI $_{(PD2013)}$ spatial patterns closely match the national land cover (Fig. A2) which at a glance, might indicate that land cover drives the pattern rather than the underlying phylogenetic richness. However, high PD (and some areas of high PE) are indeed in the most transformed landscapes. So by intersecting these two layers, we find areas that are highest for both features. There are some areas with high habitat alteration, but low PD (*e.g*. eastern Free State Province, or southern Northwest Province) that do not feature on the PRI maps for PD. Similarly, the highest region for PE (Richtersveld National Park region) does not feature on the PRI maps for PE. These contrasts show that the method does highlight important areas of phylogenetic richness that are under impact from habitat alteration.

Moreover, the change index (difference between 1990 and 2013) shows that the trend for 'genetic erosion' continues in the north-east in several hotspots (Fig. 4 & Fig. A3). Conversely, there has been little change in

the south-west during this short time period (Fig. 4 & Fig. A3). The top scoring cells for the PRI $_{(PDC)}$ are found in six general clusters: three in Limpopo Province, and one in each of Gauteng, Mpumalanga and KwaZulu-Natal provinces. This analysis indicates that these areas have seen the largest increases on the impact to phylogenetic richness between 1990 and 2013, primarily due to increasing urbanisation and agriculture (Fig. 5).

When considering the PRI $_{(PDC)}$ for the diverse reptile families (Appendix: Fig. A1), most patterns are similar to the overall. However, land use change in KwaZulu-Natal seems to be of greater impact for chameleons, gerrhosaurids, lamprophiids and vipers possibly because PD is higher in that area for those families (e.g. Tolley *et al*. 2008). Therefore, while the overall pattern for reptiles is informative at a high level for identifying the status of genetic diversity as a whole, there are important nuances that are picked up when the data are interrogated at a finer level. For identification of conservation priorities, these nuances should not be overlooked.

The general pattern of the impact of land use for PE (Figs. 8 & 9) shows some distinct differences to that of PD, due to the very different baseline spatial distribution of PE and PD (Figs. 1 & 2). Although the greatest recent changes in the impact to PE are also in Limpopo, Mpumalanga and KwaZulu-Natal provinces, unlike PD, there is no substantial impact in the Gauteng Province most likely due to the area showing low to moderate PE (Fig. 2). The main hotspot of 'genetic erosion' for PE is not in protected areas, but is near the northern borders of iSimangaliso Wetland Park, Thembe Elephant Park, Ndumu Game Reserve and the uMkhuze Game Reserve. This same area was also flagged for 'genetic erosion' using PD as a proxy. As both PE and PD showed this general area as a hotspot, it suggests that it should be an area of high priority for attention to safeguarding genetic diversity. Furthermore, this result demonstrates the importance of including a number of different genetic diversity metrics to ensure that proper attention is given to different genetic features.

4.4.2. Safeguarding phylogenetic richness

There are large geographic regions with high phylogenetic richness that are found in Protected Areas (Fig. 6). In particular, the Kruger National Park (KNP) is nearly 2 million hectares in size and contains some of the highest PD values in the country, particularly in the northern section of the park (Appendix: Fig. A4). Therefore, this Park should be considered as a reservoir of genetic diversity that is essential for conserving the phylogenetic richness of reptiles at a landscape level. There are a number of smaller PAs that also have high phylogenetic diversity (Fig. 6) and could be equally relevant in terms of impact given that our analysis does not take PA size into account.

In particular, Blouberg East Nature Reserve in Limpopo (>33,000ha) as well as several other small nature reserves in the Soutpansberg area have high phylogenetic diversity (Luvhondo Nature Reserve, Happy Rest Nature Reserve and Roodewal Private Nature Reserve). Together, these areas should be flagged as important areas to safeguard genetic diversity. Most of these PAs however, do not encompass high PE, ED or EDGE. Indeed, PE is captured by a different set of PAs, primarily in the north-west (Fig. A6). Indeed, the Kruger National Park shows somewhat low PE in stark contrast to the PD that is captured there. Instead, PE is highest in the Richtersveld National Park in the extreme north-east of South Africa. Other areas with high PE (*i.e*. Soutpansberg, iSimangaliso region) are not captured by any substantial combination of Protected Areas. Evolutionary Distinctiveness (ED) species are mostly found in the northeast although PAs in the area do not capture the highest number of ED species, although Kruger National Park and Blouberg Nature Reserve appear to have some of the highest values. EDGE species appear to be not well protected, with the highest number of species captured only in a few very small nature reserves along the KwaZulu-Natal coast (e.g. uMlalazi, Ngoye, Enseleni, Roosfontein among others) ranging in size from ca. 100 to 4000 ha.

There are a number of PAs (Fig. 7) that border areas under high impact to phylogenetic richness. While the phylogenetic richness within the PAs should be relatively intact, the high PRI $_{(PCD)}$ values just outside these reserves could be a cause for concern if the impacts have the potential to spill-over into the protected areas themselves.

4.4.3. Caveats and assumptions

The approach proposed here is a test case in terms of methodology and taxonomic group, and should be regarded as a starting point for future work. There are a number of assumptions regarding the results that need to be considered carefully. For example, the method assumes that all species or lineages are affected similarly by land cover change, but this is clearly not the case. A potential solution is to weight each species in the analysis for their ability to tolerate the various land cover types. Similarly, the quality and intensity of land cover change is not incorporated. For example, land under agriculture is assumed to be 'transformed' and therefore not natural. However, there could be natural road verges remaining or natural habitat at farmsteads that serve as habitat for some species. Furthermore, in the analysis of protected areas, the size of the PA is not accounted for in terms of its' importance in capturing phylogenetic richness. This could be corrected with a weighting for the amount of phylogenetic richness per hectare of the protected area, providing an alternative view of the importance of certain protected areas. Other caveats relate to the variation of density of individuals over the natural landscape, which in reality tend to be clumped, particularly for habitat specialists. Yet the spatial distributions we used in our analyses assume individuals are distributed homogeneously.

It is also notable that the spatial patterns for each underlying phylogenetic metric (PD, PE, ED, EDGE) show very different patterns of phylogenetic richness, because each metric has a slightly different underlying assumption as to what is being quantified. For example, Phylogenetic Diversity (PD) provides a general assessment of phylogenetic richness with all species weighted equally in the analysis. Species that have long-branch lengths in the phylogeny (*i.e.* the most divergent) will contribute highly to PD for an area. Areas that have several such species will stand out as being genetically rich. In contrast, Phylogenetic Endemism (PE) weights species according to their range size. The result is that species with small ranges are weighted higher in the analysis, resulting in areas that have spatially restricted and highly divergent species standing out strongly. The drawback of this method is that while some species might be 'restricted' in South Africa, they are widespread elsewhere. In an overall sense, this puts an unfairly high weighting on such species, as their total range size could be large. The measure of Evolutionary Distinctiveness (ED) is driven by the few species that have the longest branch lengths in a phylogeny. Therefore, it can be thought of as a measure of the most 'unique' lineages in a phylogeny, whereas PD incorporates all branch lengths, not just the top few. Mapping out ED species highlights regions that have the most unique taxa. Evolutionary Distinctiveness (ED) can be further weighted by species that are the most unique but also are threatened (EDGE: Evolutionarily Distinct and Globally Endangered). Thus, mapping the distribution of EDGE species points to areas that have unique species that are under threat of extinction.

The use of different underlying phylogenetic richness metrics influences the spatial patterns of the change indices and protected area analyses. Compare for example, the PD and PE derived change indices (Fig. 7 versus Fig. 12). The former would point to prioritising conservation efforts for Limpopo, Mpumalanga, central and northern KwaZulu-Natal, and the west coast of the Western Cape, whereas the latter would point to northern KwaZulu-Natal and the extreme west coast of the Western Cape. Therefore, the choice of phylogenetic metric clearly would have an effect on prioritisation of conservation efforts, so the metric used should be considered carefully and be related to conservation needs. For this test case, we primarily

focussed on PD as the underlying phylogenetic metric because it represents the sum of the diversity across the phylogeny. Phylogenetic Endemism (PE) also has merits as it weights range restricted species more strongly, and such species will be more vulnerable to extinction. The drawback to PE, is that the weighting is bias for some species that are actually widespread regionally, but have a small distribution within South Africa. These species are treated as 'range restricted' in the analysis, and as a species, are not actually more vulnerable to extinction. However, within South African political boundary, they are restricted and could go locally extinct due to habitat loss, meaning that the species would be 'extinct' in South Africa. As such, the PE based analyses could in fact be the most valuable in terms of national conservation priorities. The two metrics based on distinctiveness (ED and EDGE) are probably less meaningful for a landscape level approach. Both metrics focus on a few distinctive species, rather than a geographic region that has a set of species that are highly diverse. Evolutionary Distinctiveness (ED) and EDGE might rather be more applicable for assisting to prioritise individual species conservation objectives. Indeed, the identification of EDGE species is an important aspect for understanding whether unique species are at risk of extinction (Isaac *et al*. 2007; Isaac & Pearse 2018). Our analysis extends the EDGE method by also identifying where such species are located on a national scale, and which land use might be impacting those species.

4.4.4. Conclusions

The various metrics interrogated generally indicate that the greatest historical impacts to phylogenetic richness for reptiles are the north-east (Limpopo, Mpumalanga, and Gauteng provinces), south-west (Western Cape Province) and the coastal margin of KwaZulu-Natal Province. There are several hotspots of increase rates of genetic erosion that appear in each of the mapping analyses. In particular, the UMkhuze/Ndumu region northwest of iSimangaliso Wetland Park (KwaZulu-Natal Province) is rich in genetic diversity overall (PD), rich in phylogenetically endemic species (PE) and also a high number of unique but Threatened reptile species (EDGE). The high PRI values of PD and PE for that area suggest that phylogenetic richness has declined on the landscape level. The Komatipoort area (Mpumalanga Province), and the Sekhukhune District (Limpopo Province) also feature in the analyses for both PD and PE. While these areas do not have an abundance of EDGE species, they have elevated losses of their rich genetic diversity. Northern Gauteng Province and the Soutpansberg area also hotspots for increasing erosion of PD and PE, respectively. While there are losses to reptile phylogenetic richness throughout South Africa (e.g. Fig. 3), these areas show the most change between 1990 and 2013.

4.4.5. The Way Forward

Losses in genetic richness are expected globally given current extinction risk levels (Huang *et al*. 2011). However, an actual assessment of losses and tracking trends in loss has been elusive to date. Our approach allows for identification of areas most impacted with regards to phylogenetic richness, and a means to track the trends. While we have focussed on only one taxonomic group, this model could be applied across taxonomic groups to better understand broad trends. For example, groups with nearly complete phylogenies and detailed distribution maps such as birds or mammals could be analysed readily. By examining congruent patterns of 'genetic erosion' across taxa, a more comprehensive understanding of the status and trends for phylogenetic richness could be gained. Such an analysis would be useful for informing the prioritisation of conservation efforts. The method could also be valuable in tracking whether 'genetic erosion' might occur at different rates in the future, or if the areas of greatest impact shift spatially as land use patterns change.

This approach is logistically bind financially feasible to apply, as much of the DNA sequence data already exists, and data gaps can be filled in with relatively little effort. There are reasonably good occurrence records for some taxonomic groups, which can be used to produce the use of species distribution models to

inform the distribution maps needed for this approach. Essentially, the approach provides an achievable means for tracking the status and trends to genetic diversity at the landscape level.

The method could also be extended easily to other geographic realms. For example, the marine realm has been extensively mapped for impacts in South Africa (Sink *et al*. 2019) and application of this method to some marine taxonomic groups could be informative as to areas of concern regarding 'genetic erosion' . Furthermore, the method could be used to understand whether South Africa's Critical Biodiversity Areas (CBAs), Marine Protected Areas (MPAs) or the South African National Protected Areas Expansion Strategy will be instrumental for safeguarding genetic diversity into the future.

An interesting possible extension to using the phylogenetic metrics of biodiversity to track changes over time across the landscape would be to employ species distribution modelling to project species distributions at future time periods. Similar approaches are commonly applied in estimating species range dynamics and response to future global climate changes (see Burrows *et al.* 2014; Garciá Molinos *et al.* 2016). These distribution models could be used to forecast changes to phylogenetic richness. A drawback however is that while models account for changes that relate to the climatic variables (e.g. mean annual temperature, precipitation, etc.) they do not account for projections of human-induced changes to habitats. This could prove difficult to predict, although rates of change have been estimated for South Africa (Skowno *et al*. 2019) and potentially could be extrapolated into the future.

In some cases, species ranges have well-recorded recent range shifts, expansions or contractions. For example, some avian fauna for South Africa (Hockey *et al.* 2011; http://sabap2.adu.org.za/) have clearly documented range shifts which would impact landscape genetic diversity patterns for birds. If historical ranges are known, and new ranges are documented, this presents an exciting opportunity to 'backcast' changes in landscape genetic diversity as well as monitor changes into the future. In some cases, vegetation shifts have been documented, particularly where desertification, bush encroachment or climate change has influenced species assemblages (*e.g*. Moncrieff *et al.* 2015; Slingsby *et al.* 2017). These types of distribution changes could have a profound impact on phylogenetic richness patterns, and the incorporation of recorded range shifts into these methods could be used to track the trends of e.g. phylogenetic diversity on the landscape (even from a time point in the past to the present day). Such ranges shifts contextualised in a phylogenetic framework as done here, could be used to document genetic erosion over the landscape where ranges have contracted, causing a loss of species in an area, or perhaps even *increases* in phylogenetic diversity where species distributions have expanded due to increases in species richness in an area. However, detailed data on past and present ranges, as well as species assemblage changes, would be needed for such an endeavour. Regardless, tracking biodiversity changes over time is methodologically more feasible when comparing the present status with the past, for which we may have real comparative data.

There are a number of very interesting extensions to our proposed methods for examining impacts to phylogenetic richness on a national or landscape level. With better data on distributions (either static or shifting distributions) and accompanying phylogenetic information, in combination with detailed maps and information on land cover changes, it could be possible to track these impacts over time, allowing for phylogenetic diversity/richness to become an important and informative feature for biodiversity assessments and planning.

Critical Gaps:

- Additional taxonomic groups for landscape level analyses.

- Testing of additional phylogenetic metrics.

- Additional analysis of pressures (land cover types) with other phylogenetic metrics.

- Analysis of protection status (e.g. Protected Areas) with additional phylogenetic metrics.

- Analysis of Critical Biodiversity Areas and the National Protected Areas Expansion Strategy as measures to safeguard genetic diversity.

- Investigate potential for using recorded range shifts, assemblage shifts, and/or species distribution. modelling to track trends of landscape level 'genetic erosion' (and *increases* in landscape level genetic diversity) or to project areas that might undergo genetic erosion in the future.

- Incorporation of landscape level genetic richness into biodiversity assessments and planning.

4.5. References

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4.6.   Appendices (Figures and Tables)

Figure A1. Maps showing the change index for South African reptile families. Areas with highest change index are in the darker shading. The name of the reptile family is given for each panel.

Chamaeleonidae

Cordylidae

Gekkonidae

Gerrhosauridae

Lacertidae

Scincidae

Snakes

Colubridae

Elapidae

Lamprophiidae

Leptotyphlopidae

Typhlopidae

Viperidae

Chelonians

Pelomedusidae

Testudinidae
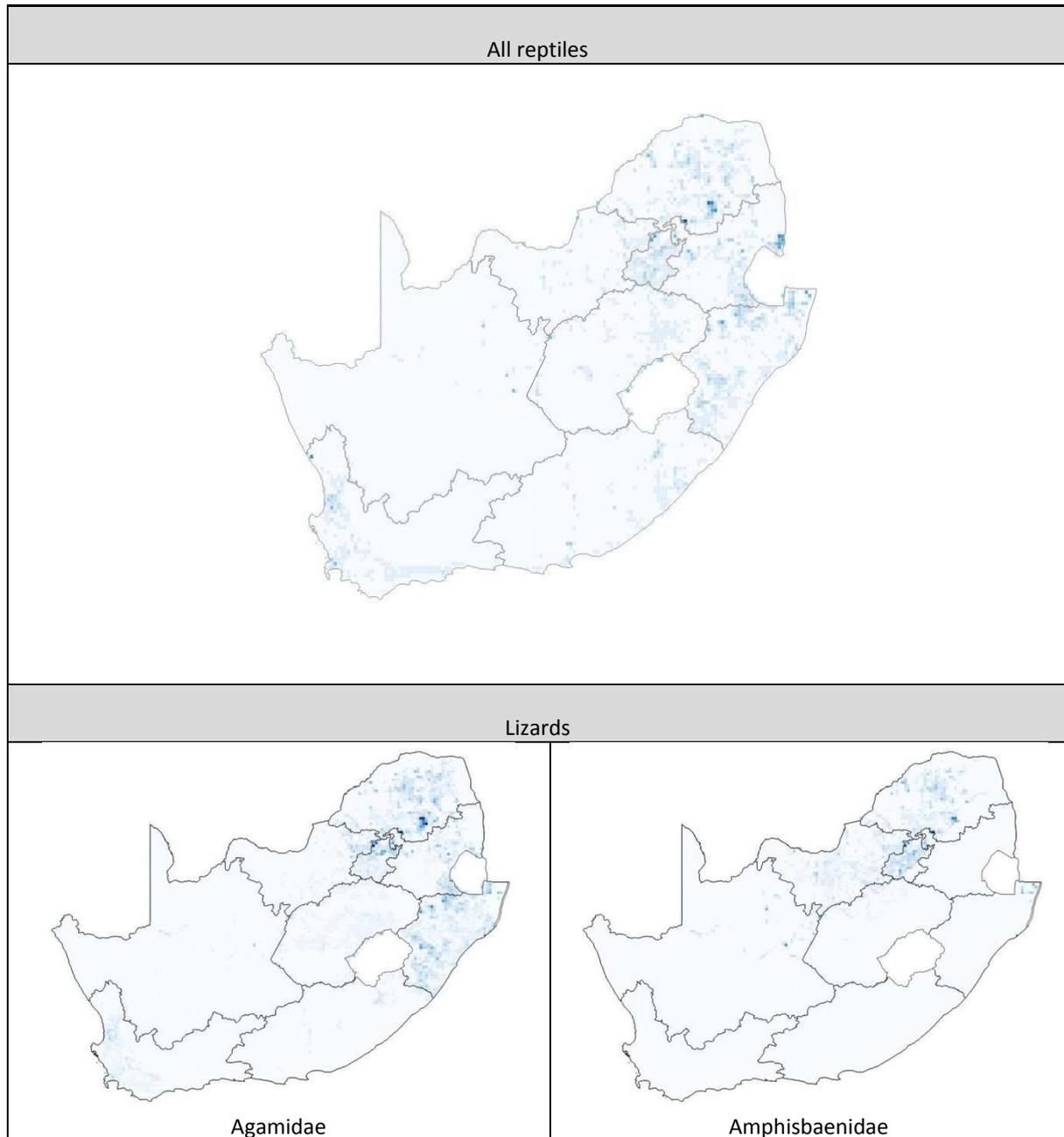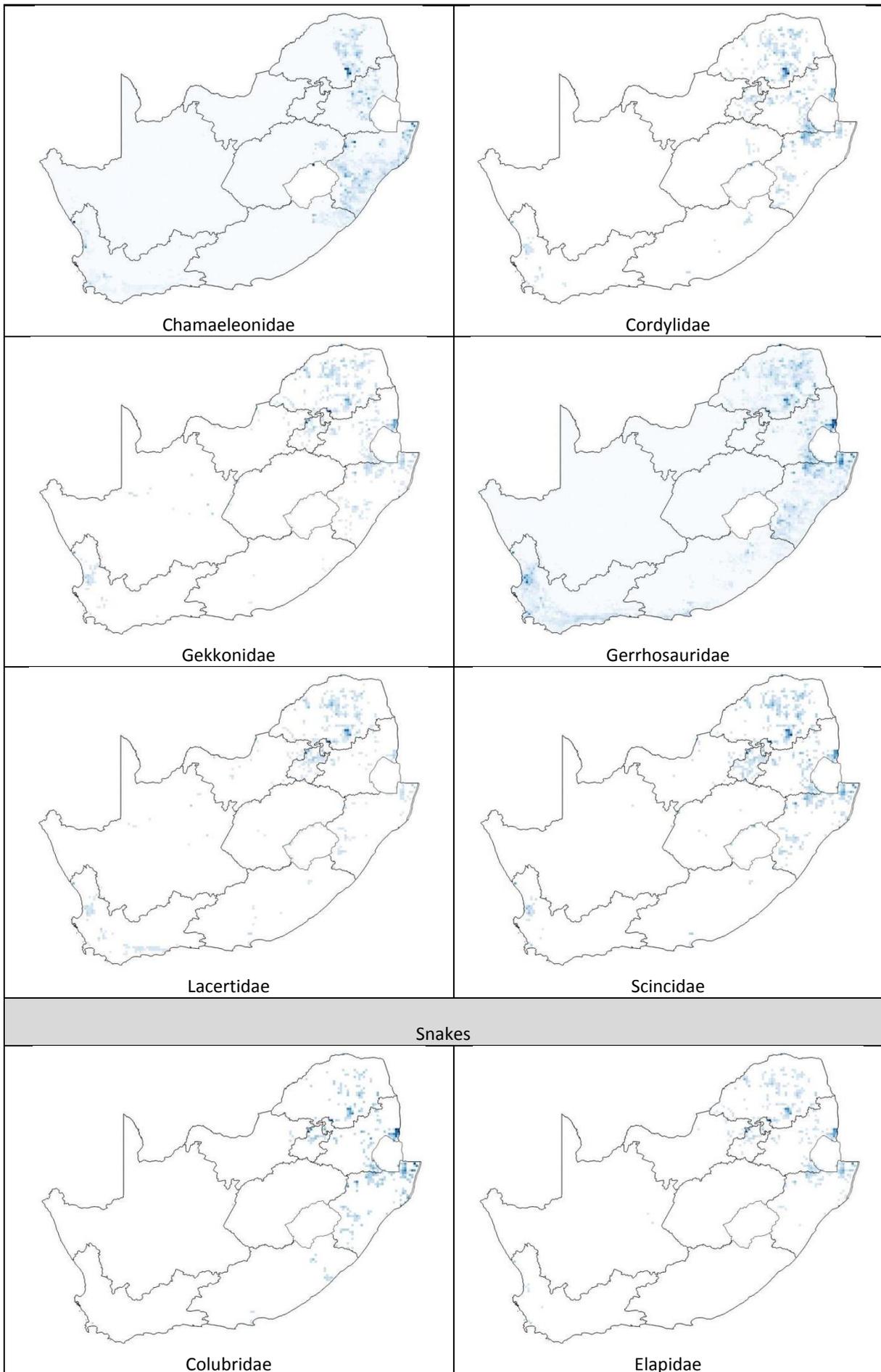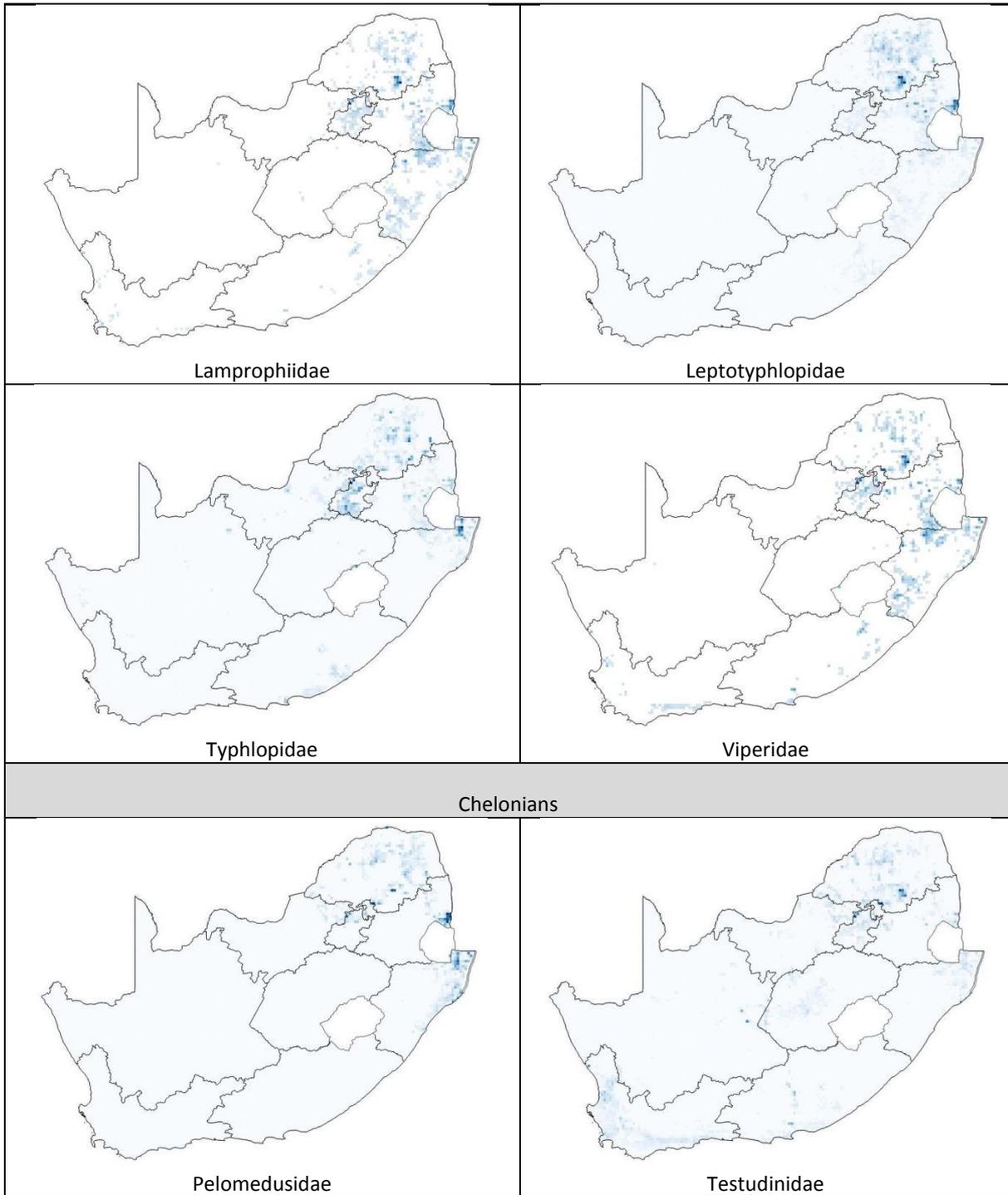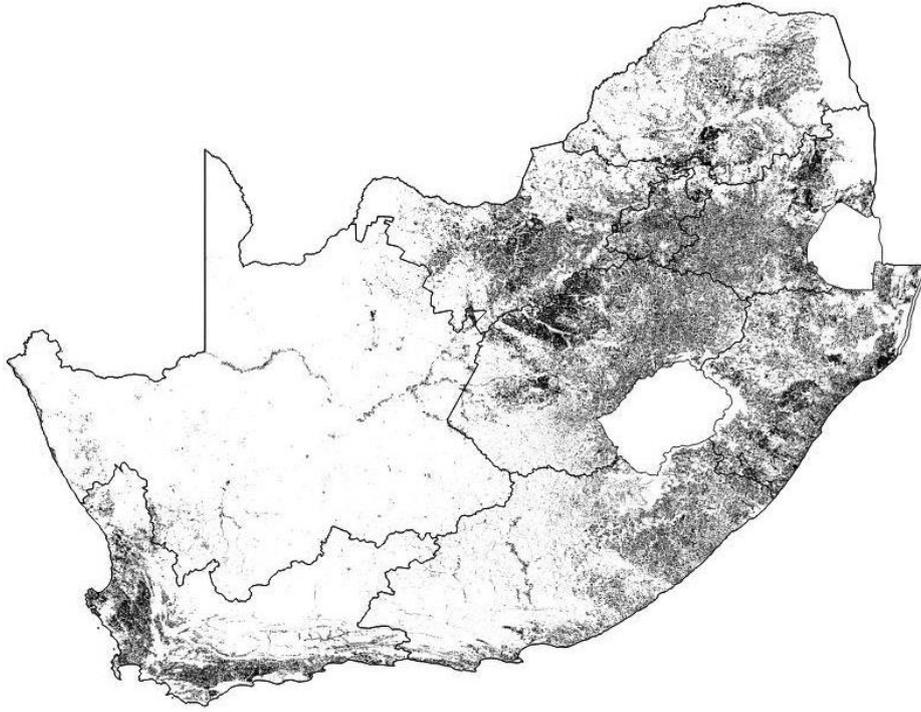
Figure A2. National land cover map for 2013. Shading indicates areas with no natural habitat remaining.
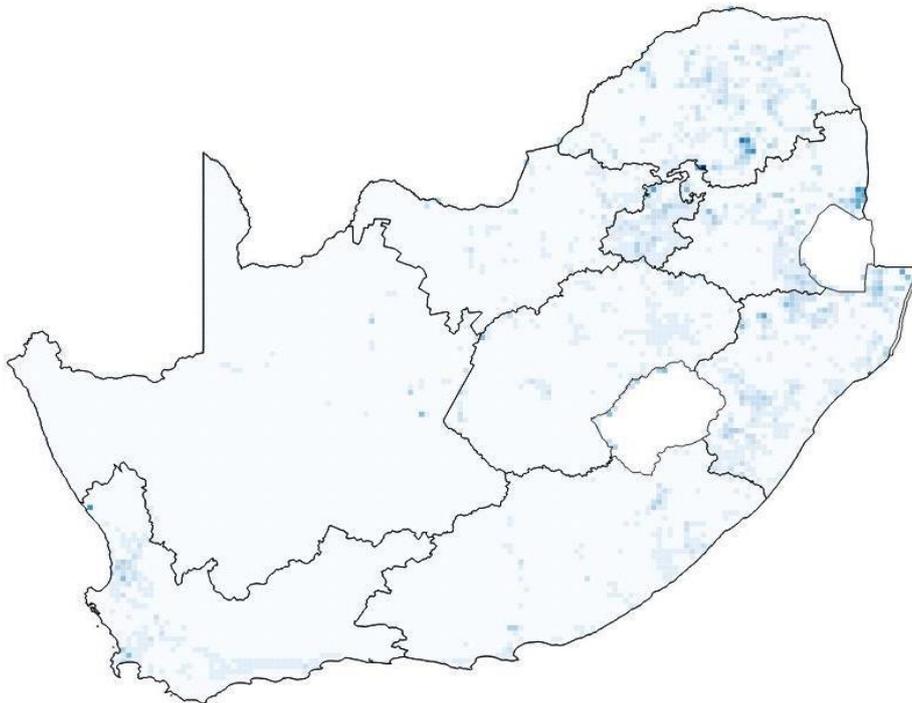
Figure A3. Enhanced view of the spatial distribution of PRI $_{(PDC)}$ of all values, for reptiles across South Africa.
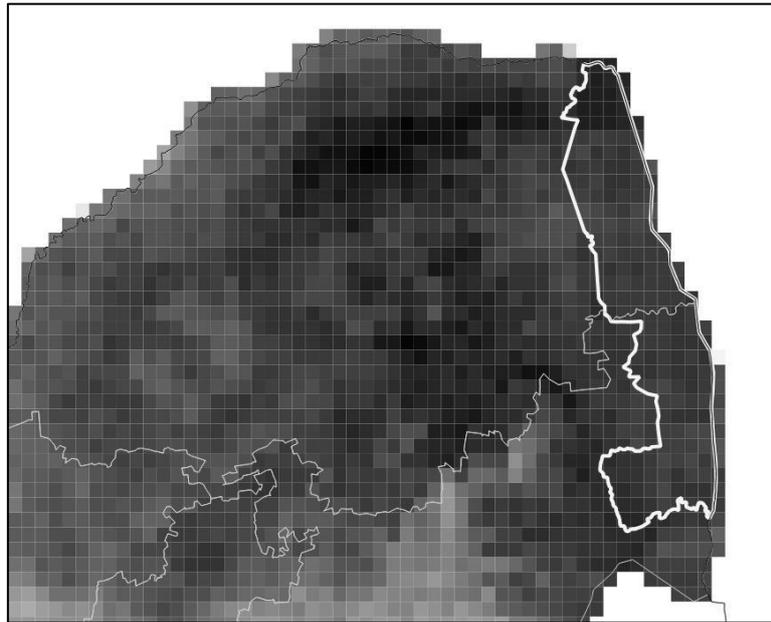
Figure A4. Phylogenetic diversity (PD) for reptiles from north-western South Africa. Darker shading indicates higher values of PD. The border of Kruger National Park is indicated by a thick white line. High values of PD can be found particularly in the northern section of the park.
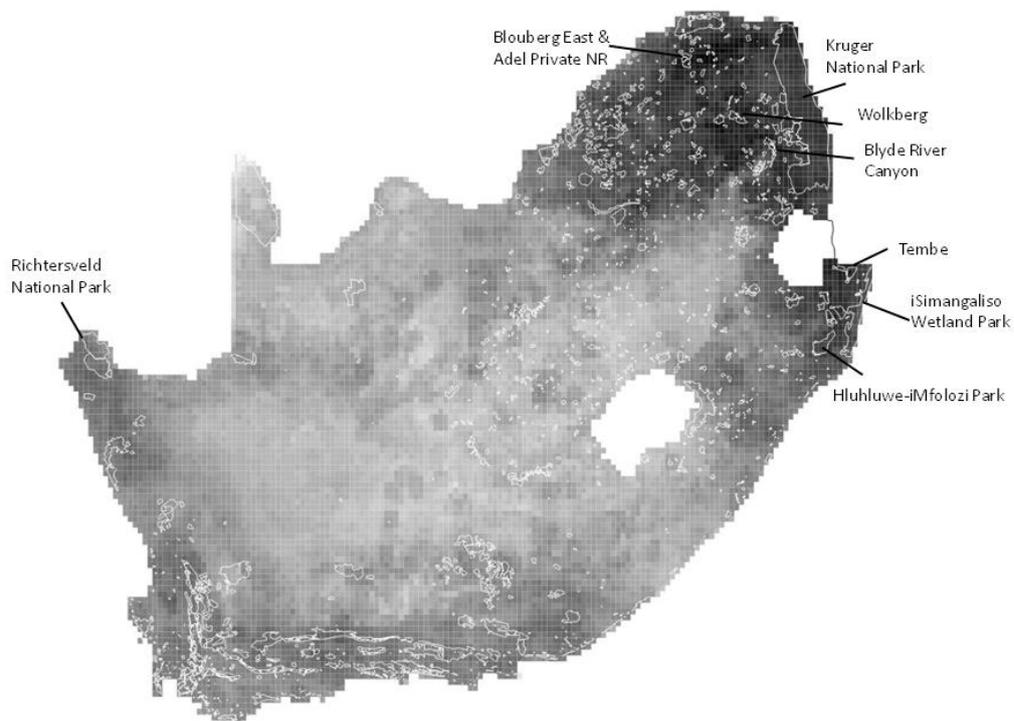
Figure A5. Location of important Protected Areas (PAs) for safeguarding phylogenetic diversity of reptiles in South Africa pointed out by the solid lines. Polygons show the outlines of all PAs.

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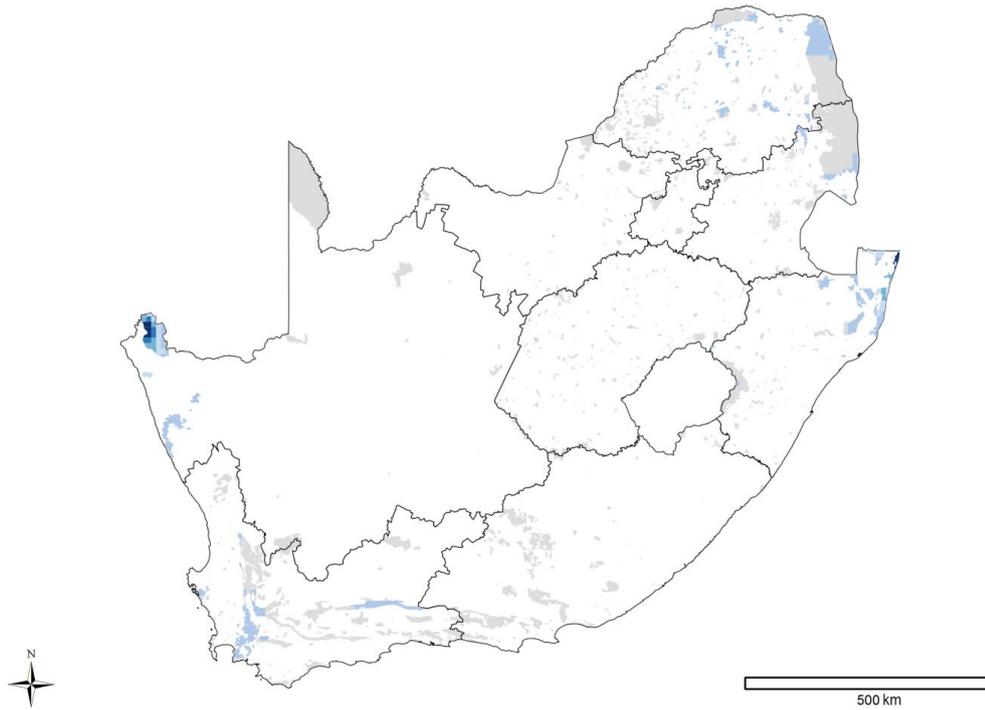
Figure A6. Network of Protected Areas (grey shading) in South Africa with areas of high phylogenetic endemism (PE) shown by the blue gradient shading.
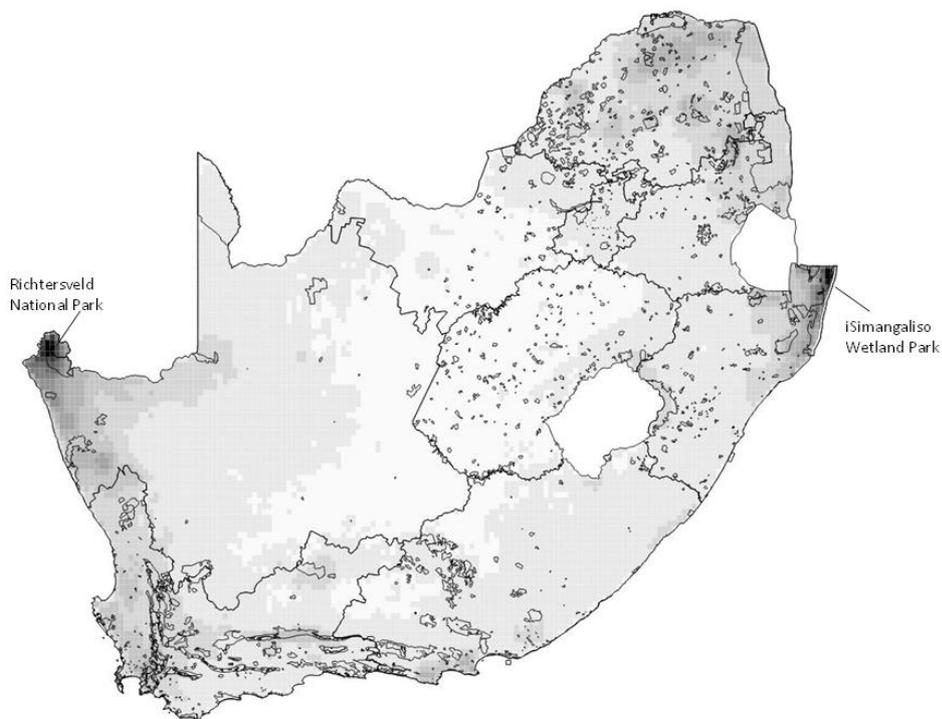
Figure A7. Location of important Protected Areas (PAs) for safeguarding phylogenetic endemism of reptiles in South Africa, pointed out by the solid lines. Polygons show the outlines of all PAs.
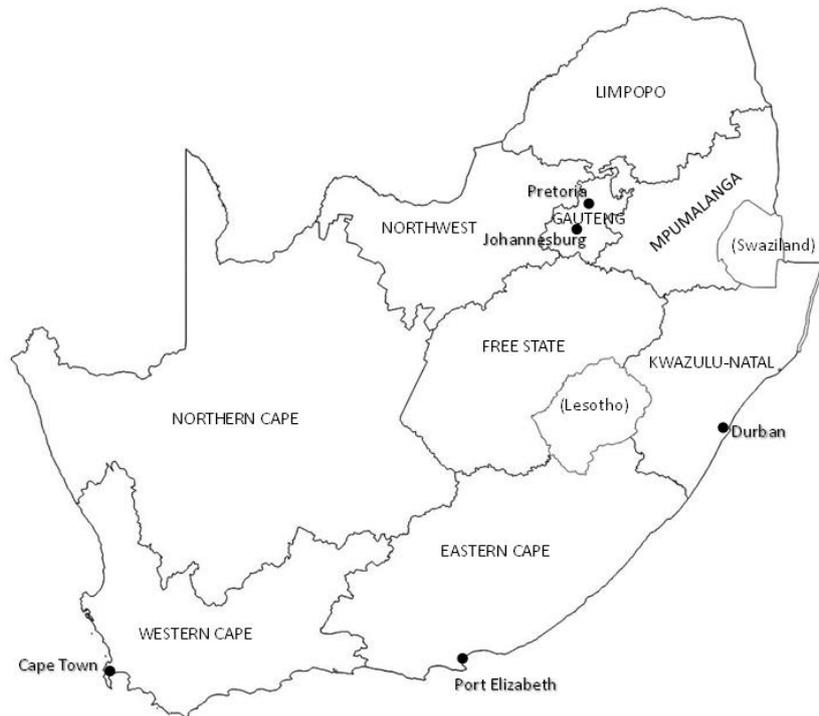
Figure A8. Provinces (in capital letters) and important urban centres (lower case letters) in South Africa. Other countries are in brackets.

Table A1. List of South African reptile species and their IUCN status as of November 2018. Those species included in the analysis are indicated.

| Group	Family	Species	IUCN 2018	Included in analysis
Chelonian	Cheloniidae	*Caretta caretta*	NE	No
Chelonian	Cheloniidae	*Chelonia mydas*	NE	No
Chelonian	Cheloniidae	*Eretmochelys imbricata*	NE	No
Chelonian	Cheloniidae	*Lepidochelys olivacea*	NE	No
Chelonian	Dermochelyidae	*Dermochelys coriacea*	NE	No
Chelonian	Pelomedusidae	*Pelomedusa galeata*	LC	Yes
Chelonian	Pelomedusidae	*Pelomedusa subrufa*	LC	Yes
Chelonian	Pelomedusidae	*Pelusios castanoides*	VU	Yes
Chelonian	Pelomedusidae	*Pelusios rhodesianus*	VU	Yes
Chelonian	Pelomedusidae	*Pelusios sinuatus*	LC	Yes
Chelonian	Pelomedusidae	*Pelusios subniger*	LC	Yes
Chelonian	Testudinidae	*Chersina angulata*	LC	Yes
Chelonian	Testudinidae	*Chersobius boulengeri*	EN	Yes
Chelonian	Testudinidae	*Chersobius signatus*	EN	Yes
Chelonian	Testudinidae	*Homopus areolatus*	LC	Yes
Chelonian	Testudinidae	*Kinixys lobatsiana*	VU	Yes
Chelonian	Testudinidae	*Kinixys natalensis*	VU	Yes
Chelonian	Testudinidae	*Kinixys spekii*	LC	Yes
Chelonian	Testudinidae	*Kinixys zombensis*	LC	Yes
Chelonian	Testudinidae	*Psammobates geometricus*	CR	Yes
Chelonian	Testudinidae	*Psammobates oculifer*	LC	Yes
Chelonian	Testudinidae	*Psammobates tentorius*	NT	Yes
Chelonian	Testudinidae	*Stigmochelys pardalis*	LC	Yes
Crocodilia	Crocodylidae	*Crocodylus niloticus*	LC	No
Lizard	Agamidae	*Acanthocercus atricollis*	LC	Yes
Lizard	Agamidae	*Agama aculeata*	LC	Yes
Lizard	Agamidae	*Agama anchietae*	LC	Yes
Lizard	Agamidae	*Agama armata*	LC	Yes
Lizard	Agamidae	*Agama atra*	LC	Yes
Lizard	Agamidae	*Agama hispida*	LC	Yes
Lizard	Amphisbaenidae	*Chirindia langi*	LC	No
Lizard	Amphisbaenidae	*Dalophia pistillum*	LC	Yes
Lizard	Amphisbaenidae	*Monopeltis capensis*	LC	Yes
Lizard	Amphisbaenidae	*Monopeltis decosteri*	LC	No
Lizard	Amphisbaenidae	*Monopeltis infuscata*	LC	Yes
Lizard	Amphisbaenidae	*Monopeltis leonhardi*	NE	No
Lizard	Amphisbaenidae	*Monopeltis mauricei*	LC	Yes
Lizard	Amphisbaenidae	*Monopeltis sphenorhynchus*	LC	Yes
Lizard	Amphisbaenidae	*Zygaspis quadrifrons*	LC	Yes
Lizard	Amphisbaenidae	*Zygaspis vandami*	LC	Yes
Lizard	Chamaeleonidae	*Bradypodion atromontanum*	LC	Yes

Lizard	Chamaeleonidae	*Bradypodion caeruleogula*	EN	Yes
Lizard	Chamaeleonidae	*Bradypodion caffer*	EN	Yes
Lizard	Chamaeleonidae	*Bradypodion damaranum*	LC	Yes
Lizard	Chamaeleonidae	*Bradypodion dracomontanum*	NT	Yes
Lizard	Chamaeleonidae	*Bradypodion gutturale*	LC	Yes
Lizard	Chamaeleonidae	*Bradypodion kentanicum*	NT	Yes
Lizard	Chamaeleonidae	*Bradypodion melanocephalum*	NT	Yes
Lizard	Chamaeleonidae	*Bradypodion nemorale*	NT	Yes
Lizard	Chamaeleonidae	*Bradypodion ngomeense*	NT	Yes
Lizard	Chamaeleonidae	*Bradypodion occidentale*	LC	Yes
Lizard	Chamaeleonidae	*Bradypodion pumilum*	NT	Yes
Lizard	Chamaeleonidae	*Bradypodion setaroi*	LC	Yes
Lizard	Chamaeleonidae	*Bradypodion taeniabronchum*	LC	Yes
Lizard	Chamaeleonidae	*Bradypodion thamnobates*	NT	Yes
Lizard	Chamaeleonidae	*Bradypodion transvaalense*	LC	Yes
Lizard	Chamaeleonidae	*Bradypodion ventrale*	LC	Yes
Lizard	Chamaeleonidae	*Chamaeleo dilepis*	LC	Yes
Lizard	Chamaeleonidae	*Chamaeleo namaquensis*	LC	Yes
Lizard	Cordylidae	*Chamaesaura aenea*	LC	Yes
Lizard	Cordylidae	*Chamaesaura anguina*	LC	Yes
Lizard	Cordylidae	*Chamaesaura macrolepis*	LC	No
Lizard	Cordylidae	*Cordylus aridus*	LC	Yes
Lizard	Cordylidae	*Cordylus cloetei*	LC	Yes
Lizard	Cordylidae	*Cordylus cordylus*	LC	Yes
Lizard	Cordylidae	*Cordylus imkeae*	LC	Yes
Lizard	Cordylidae	*Cordylus jonesii*	LC	Yes
Lizard	Cordylidae	*Cordylus macropholis*	LC	Yes
Lizard	Cordylidae	*Cordylus mclachlani*	LC	Yes
Lizard	Cordylidae	*Cordylus minor*	LC	Yes
Lizard	Cordylidae	*Cordylus niger*	LC	Yes
Lizard	Cordylidae	*Cordylus oelofseni*	LC	Yes
Lizard	Cordylidae	*Cordylus vittifer*	LC	Yes
Lizard	Cordylidae	*Hemicordylus capensis*	LC	Yes
Lizard	Cordylidae	*Hemicordylus nebulosus*	VU	Yes
Lizard	Cordylidae	*Karusasaurus polyzonus*	LC	Yes
Lizard	Cordylidae	*Namazonurus lawrenci*	LC	Yes
Lizard	Cordylidae	*Namazonurus peersi*	LC	Yes
Lizard	Cordylidae	*Ninurta coeruleopunctatus*	LC	Yes
Lizard	Cordylidae	*Ouroborus cataphractus*	LC	Yes
Lizard	Cordylidae	*Platysaurus attenboroughi*	LC	Yes
Lizard	Cordylidae	*Platysaurus broadleyi*	LC	Yes
Lizard	Cordylidae	*Platysaurus capensis*	LC	Yes
Lizard	Cordylidae	*Platysaurus guttatus*	LC	No
Lizard	Cordylidae	*Platysaurus intermedius*	LC	Yes
Lizard	Cordylidae	*Platysaurus lebomboensis*	LC	Yes

Lizard	Cordylidae	*Platysaurus minor*	LC	Yes
Lizard	Cordylidae	*Platysaurus monotropis*	NT	Yes
Lizard	Cordylidae	*Platysaurus orientalis*	LC	Yes
Lizard	Cordylidae	*Platysaurus relictus*	LC	Yes
Lizard	Cordylidae	*Pseudocordylus langi*	LC	Yes
Lizard	Cordylidae	*Pseudocordylus melanotus*	LC	Yes
Lizard	Cordylidae	*Pseudocordylus microlepidotus*	LC	Yes
Lizard	Cordylidae	*Pseudocordylus spinosus*	LC	Yes
Lizard	Cordylidae	*Pseudocordylus transvaalensis*	LC	Yes
Lizard	Cordylidae	*Smaug barbertonensis*	LC	Yes
Lizard	Cordylidae	*Smaug breyeri*	LC	Yes
Lizard	Cordylidae	*Smaug depressus*	LC	Yes
Lizard	Cordylidae	*Smaug giganteus*	VU	Yes
Lizard	Cordylidae	*Smaug vandami*	LC	Yes
Lizard	Cordylidae	*Smaug warreni*	LC	Yes
Lizard	Elapidae	*Hemachatus haemachatus*	LC	Yes
Lizard	Elapidae	*Hydrophis platurus*	NE	No
Lizard	Gekkonidae	*Afroedura amatolica*	LC	Yes
Lizard	Gekkonidae	*Afroedura broadleyi*	LC	Yes
Lizard	Gekkonidae	*Afroedura granitica*	DD	Yes
Lizard	Gekkonidae	*Afroedura haackei*	LC	Yes
Lizard	Gekkonidae	*Afroedura halli*	LC	Yes
Lizard	Gekkonidae	*Afroedura hawequensis*	LC	Yes
Lizard	Gekkonidae	*Afroedura karroica*	LC	Yes
Lizard	Gekkonidae	*Afroedura langi*	LC	Yes
Lizard	Gekkonidae	*Afroedura leoloensis*	LC	No
Lizard	Gekkonidae	*Afroedura maripi*	LC	Yes
Lizard	Gekkonidae	*Afroedura marleyi*	LC	Yes
Lizard	Gekkonidae	*Afroedura multiporis*	LC	Yes
Lizard	Gekkonidae	*Afroedura namaquensis*	LC	No
Lizard	Gekkonidae	*Afroedura nivaria*	LC	Yes
Lizard	Gekkonidae	*Afroedura pienaari*	LC	Yes
Lizard	Gekkonidae	*Afroedura pondolia*	LC	Yes
Lizard	Gekkonidae	*Afroedura pongola*	DD	Yes
Lizard	Gekkonidae	*Afroedura rondavelica*	DD	No
Lizard	Gekkonidae	*Afroedura rupestris*	DD	Yes
Lizard	Gekkonidae	*Afroedura tembulica*	LC	Yes
Lizard	Gekkonidae	*Afroedura transvaalica*	LC	Yes
Lizard	Gekkonidae	*Afroedura waterbergensis*	LC	Yes
Lizard	Gekkonidae	*Afrogecko porphyreus*	LC	Yes
Lizard	Gekkonidae	*Chondrodactylus angulifer*	LC	Yes
Lizard	Gekkonidae	*Chondrodactylus bibronii*	LC	Yes
Lizard	Gekkonidae	*Chondrodactylus turneri*	LC	Yes
Lizard	Gekkonidae	*Cryptactites peringueyi*	NT	Yes
Lizard	Gekkonidae	*Goggia braacki*	LC	Yes

Lizard	Gekkonidae	*Goggia essexi*	LC	Yes
Lizard	Gekkonidae	*Goggia gemmula*	LC	Yes
Lizard	Gekkonidae	*Goggia hewitti*	LC	Yes
Lizard	Gekkonidae	*Goggia hexapora*	LC	Yes
Lizard	Gekkonidae	*Goggia incognita*	LC	Yes
Lizard	Gekkonidae	*Goggia lineata*	LC	Yes
Lizard	Gekkonidae	*Goggia matzikamaensis*	LC	Yes
Lizard	Gekkonidae	*Goggia microlepidota*	LC	Yes
Lizard	Gekkonidae	*Goggia rupicola*	LC	Yes
Lizard	Gekkonidae	*Homopholis arnoldi*	LC	Yes
Lizard	Gekkonidae	*Homopholis mulleri*	LC	Yes
Lizard	Gekkonidae	*Homopholis wahlbergii*	LC	Yes
Lizard	Gekkonidae	*Lygodactylus bradfieldi*	LC	Yes
Lizard	Gekkonidae	*Lygodactylus capensis*	LC	Yes
Lizard	Gekkonidae	*Lygodactylus graniticolus*	LC	Yes
Lizard	Gekkonidae	*Lygodactylus incognitus*	LC	Yes
Lizard	Gekkonidae	*Lygodactylus methueni*	DD	Yes
Lizard	Gekkonidae	*Lygodactylus montiscaeruli*	LC	Yes
Lizard	Gekkonidae	*Lygodactylus nigropunctatus*	LC	Yes
Lizard	Gekkonidae	*Lygodactylus ocellatus*	LC	Yes
Lizard	Gekkonidae	*Lygodactylus soutpansbergensis*	LC	Yes
Lizard	Gekkonidae	*Lygodactylus stevensoni*	LC	Yes
Lizard	Gekkonidae	*Lygodactylus waterbergensis*	LC	Yes
Lizard	Gekkonidae	*Pachydactylus affinis*	LC	Yes
Lizard	Gekkonidae	*Pachydactylus amoenus*	LC	Yes
Lizard	Gekkonidae	*Pachydactylus atorquatus*	LC	Yes
Lizard	Gekkonidae	*Pachydactylus austeni*	LC	Yes
Lizard	Gekkonidae	*Pachydactylus barnardi*	LC	Yes
Lizard	Gekkonidae	*Pachydactylus capensis*	LC	Yes
Lizard	Gekkonidae	*Pachydactylus carinatus*	LC	Yes
Lizard	Gekkonidae	*Pachydactylus formosus*	LC	Yes
Lizard	Gekkonidae	*Pachydactylus geitje*	LC	Yes
Lizard	Gekkonidae	*Pachydactylus haackei*	LC	Yes
Lizard	Gekkonidae	*Pachydactylus kladaroderma*	LC	Yes
Lizard	Gekkonidae	*Pachydactylus labialis*	LC	Yes
Lizard	Gekkonidae	*Pachydactylus latirostris*	LC	Yes
Lizard	Gekkonidae	*Pachydactylus macrolepis*	LC	Yes
Lizard	Gekkonidae	*Pachydactylus maculatus*	LC	Yes
Lizard	Gekkonidae	*Pachydactylus mariquensis*	LC	Yes
Lizard	Gekkonidae	*Pachydactylus monicae*	LC	Yes
Lizard	Gekkonidae	*Pachydactylus montanus*	LC	Yes
Lizard	Gekkonidae	*Pachydactylus namaquensis*	LC	Yes
Lizard	Gekkonidae	*Pachydactylus oculatus*	LC	Yes
Lizard	Gekkonidae	*Pachydactylus punctatus*	LC	Yes
Lizard	Gekkonidae	*Pachydactylus purcelli*	LC	Yes

Lizard	Gekkonidae	*Pachydactylus rangei*	NE	Yes
Lizard	Gekkonidae	*Pachydactylus rugosus*	LC	Yes
Lizard	Gekkonidae	*Pachydactylus tigrinus*	LC	Yes
Lizard	Gekkonidae	*Pachydactylus vansoni*	LC	Yes
Lizard	Gekkonidae	*Pachydactylus visseri*	LC	Yes
Lizard	Gekkonidae	*Pachydactylus wahlbergii*	LC	Yes
Lizard	Gekkonidae	*Pachydactylus weberi*	LC	Yes
Lizard	Gekkonidae	*Phelsuma ocellata*	LC	Yes
Lizard	Gekkonidae	*Ptenopus garrulus*	LC	Yes
Lizard	Gekkonidae	*Ramigekko swartbergensis*	LC	Yes
Lizard	Gerrhosauridae	*Broadleysaurus major*	LC	Yes
Lizard	Gerrhosauridae	*Cordylosaurus subtessellatus*	LC	Yes
Lizard	Gerrhosauridae	*Gerrhosaurus auritus*	NE	Yes
Lizard	Gerrhosauridae	*Gerrhosaurus flavigularis*	LC	Yes
Lizard	Gerrhosauridae	*Gerrhosaurus intermedius*	LC	Yes
Lizard	Gerrhosauridae	*Gerrhosaurus typicus*	LC	Yes
Lizard	Gerrhosauridae	*Matobosaurus validus*	LC	Yes
Lizard	Gerrhosauridae	*Tetradactylus africanus*	LC	Yes
Lizard	Gerrhosauridae	*Tetradactylus breyeri*	LC	No
Lizard	Gerrhosauridae	*Tetradactylus eastwoodae*	EX	No
Lizard	Gerrhosauridae	*Tetradactylus fitzsimonsi*	VU	Yes
Lizard	Gerrhosauridae	*Tetradactylus seps*	LC	Yes
Lizard	Gerrhosauridae	*Tetradactylus tetradactylus*	LC	Yes
Lizard	Lacertidae	*Australolacerta australis*	LC	Yes
Lizard	Lacertidae	*Meroles ctenodactylus*	LC	Yes
Lizard	Lacertidae	*Meroles cuneirostris*	LC	Yes
Lizard	Lacertidae	*Meroles knoxii*	LC	Yes
Lizard	Lacertidae	*Meroles squamulosus*	LC	Yes
Lizard	Lacertidae	*Meroles suborbitalis*	LC	Yes
Lizard	Lacertidae	*Nucras caesicaudata*	NE	No
Lizard	Lacertidae	*Nucras holubi*	LC	Yes
Lizard	Lacertidae	*Nucras intertexta*	LC	Yes
Lizard	Lacertidae	*Nucras lalandii*	LC	Yes
Lizard	Lacertidae	*Nucras livida*	LC	Yes
Lizard	Lacertidae	*Nucras ornata*	LC	Yes
Lizard	Lacertidae	*Nucras taeniolata*	LC	Yes
Lizard	Lacertidae	*Nucras tessellata*	LC	Yes
Lizard	Lacertidae	*Pedioplanis burchelli*	LC	Yes
Lizard	Lacertidae	*Pedioplanis inornata*	LC	Yes
Lizard	Lacertidae	*Pedioplanis laticeps*	LC	Yes
Lizard	Lacertidae	*Pedioplanis lineoocellata*	LC	Yes
Lizard	Lacertidae	*Pedioplanis namaquensis*	LC	Yes
Lizard	Lacertidae	*Tropidosaura cottrelli*	LC	Yes
Lizard	Lacertidae	*Tropidosaura essexi*	LC	Yes
Lizard	Lacertidae	*Tropidosaura gularis*	LC	Yes

Lizard	Lacertidae	*Tropidosaura montana*	LC	Yes
Lizard	Lacertidae	*Vhembelacerta rupicola*	LC	Yes
Lizard	Lamprophiidae	*Gracililima nyassae*	LC	Yes
Lizard	Lamprophiidae	*Hemirhagerrhis nototaenia*	LC	Yes
Lizard	Scincidae	*Acontias albigularis*	DD	Yes
Lizard	Scincidae	*Acontias breviceps*	LC	Yes
Lizard	Scincidae	*Acontias cregoi*	LC	Yes
Lizard	Scincidae	*Acontias fitzsimonsi*	LC	Yes
Lizard	Scincidae	*Acontias gariepensis*	LC	Yes
Lizard	Scincidae	*Acontias gracilicauda*	LC	Yes
Lizard	Scincidae	*Acontias grayi*	LC	Yes
Lizard	Scincidae	*Acontias kgalagadi*	DD	Yes
Lizard	Scincidae	*Acontias lineatus*	LC	Yes
Lizard	Scincidae	*Acontias lineicauda*	LC	Yes
Lizard	Scincidae	*Acontias litoralis*	LC	Yes
Lizard	Scincidae	*Acontias meleagris*	LC	Yes
Lizard	Scincidae	*Acontias namaquensis*	LC	Yes
Lizard	Scincidae	*Acontias occidentalis*	LC	Yes
Lizard	Scincidae	*Acontias orientalis*	LC	Yes
Lizard	Scincidae	*Acontias parietalis*	LC	Yes
Lizard	Scincidae	*Acontias plumbeus*	LC	Yes
Lizard	Scincidae	*Acontias poecilus*	DD	Yes
Lizard	Scincidae	*Acontias richardi*	DD	Yes
Lizard	Scincidae	*Acontias rieppeli*	NT	Yes
Lizard	Scincidae	*Acontias tristis*	LC	Yes
Lizard	Scincidae	*Acontias wakkerstroomensis*	DD	Yes
Lizard	Scincidae	*Cryptoblepharus africanus*	NE	Yes
Lizard	Scincidae	*Mochlus sundevallii*	LC	Yes
Lizard	Scincidae	*Panaspis maculicollis*	LC	Yes
Lizard	Scincidae	*Panaspis wahlbergi*	LC	Yes
Lizard	Scincidae	*Scelotes anguineus*	LC	Yes
Lizard	Scincidae	*Scelotes arenicolus*	LC	Yes
Lizard	Scincidae	*Scelotes bidigittatus*	LC	Yes
Lizard	Scincidae	*Scelotes bipes*	LC	Yes
Lizard	Scincidae	*Scelotes bourquini*	VU	No
Lizard	Scincidae	*Scelotes caffer*	LC	Yes
Lizard	Scincidae	*Scelotes capensis*	LC	Yes
Lizard	Scincidae	*Scelotes fitzsimonsi*	LC	Yes
Lizard	Scincidae	*Scelotes gronovii*	LC	Yes
Lizard	Scincidae	*Scelotes guentheri*	EX	No
Lizard	Scincidae	*Scelotes inornatus*	CR	No
Lizard	Scincidae	*Scelotes kasneri*	NT	Yes
Lizard	Scincidae	*Scelotes limpopoensis*	LC	Yes
Lizard	Scincidae	*Scelotes mirus*	LC	Yes
Lizard	Scincidae	*Scelotes montispectus*	NT	Yes

Lizard	Scincidae	*Scelotes mossambicus*	LC	Yes
Lizard	Scincidae	*Scelotes sexlineatus*	LC	Yes
Lizard	Scincidae	*Scelotes vestigifer*	LC	Yes
Lizard	Scincidae	*Trachylepis capensis*	LC	Yes
Lizard	Scincidae	*Trachylepis damarana*	LC	Yes
Lizard	Scincidae	*Trachylepis depressa*	LC	Yes
Lizard	Scincidae	*Trachylepis homalocephala*	LC	Yes
Lizard	Scincidae	*Trachylepis laevigata*	DD	Yes
Lizard	Scincidae	*Trachylepis margaritifer*	LC	Yes
Lizard	Scincidae	*Trachylepis occidentalis*	LC	Yes
Lizard	Scincidae	*Trachylepis punctatissima*	LC	Yes
Lizard	Scincidae	*Trachylepis punctulata*	LC	Yes
Lizard	Scincidae	*Trachylepis sparsa*	LC	Yes
Lizard	Scincidae	*Trachylepis spilogaster*	LC	Yes
Lizard	Scincidae	*Trachylepis striata*	LC	Yes
Lizard	Scincidae	*Trachylepis sulcata*	LC	Yes
Lizard	Scincidae	*Trachylepis varia*	LC	Yes
Lizard	Scincidae	*Trachylepis variegata*	LC	Yes
Lizard	Scincidae	*Typhlosaurus caecus*	LC	Yes
Lizard	Scincidae	*Typhlosaurus lomiae*	LC	Yes
Lizard	Scincidae	*Typhlosaurus meyeri*	LC	Yes
Lizard	Scincidae	*Typhlosaurus vermis*	LC	Yes
Lizard	Varanidae	*Varanus albigularis*	LC	Yes
Lizard	Varanidae	*Varanus niloticus*	LC	Yes
Snake	Colubridae	*Crotaphopeltis hotamboeia*	LC	Yes
Snake	Colubridae	*Dasypeltis inornata*	LC	Yes
Snake	Colubridae	*Dasypeltis medici*	LC	Yes
Snake	Colubridae	*Dasypeltis scabra*	LC	Yes
Snake	Colubridae	*Dipsadoboa aulica*	LC	Yes
Snake	Colubridae	*Dispholidus typus*	LC	Yes
Snake	Colubridae	*Meizodon semiornatus*	LC	Yes
Snake	Colubridae	*Philothamnus angolensis*	LC	Yes
Snake	Colubridae	*Philothamnus hoplogaster*	LC	Yes
Snake	Colubridae	*Philothamnus natalensis*	LC	Yes
Snake	Colubridae	*Philothamnus occidentalis*	LC	Yes
Snake	Colubridae	*Philothamnus semivariegatus*	LC	Yes
Snake	Colubridae	*Telescopus beetzii*	LC	Yes
Snake	Colubridae	*Telescopus semiannulatus*	LC	Yes
Snake	Colubridae	*Thelotornis capensis*	LC	Yes
Snake	Elapidae	*Aspidelaps lubricus*	LC	Yes
Snake	Elapidae	*Aspidelaps scutatus*	LC	Yes
Snake	Elapidae	*Dendroaspis angusticeps*	VU	Yes
Snake	Elapidae	*Dendroaspis polylepis*	LC	Yes
Snake	Elapidae	*Elapsoidea boulengeri*	LC	Yes
Snake	Elapidae	*Elapsoidea sundevallii*	LC	Yes

Snake	Elapidae	*Naja annulifera*	LC	Yes
Snake	Elapidae	*Naja melanoleuca*	LC	Yes
Snake	Elapidae	*Naja mossambica*	LC	Yes
Snake	Elapidae	*Naja nigricincta*	LC	Yes
Snake	Elapidae	*Naja nivea*	LC	Yes
Snake	Gekkonidae	*Hemidactylus mabouia*	LC	Yes
Snake	Lacertidae	*Heliobolus lugubris*	LC	Yes
Snake	Lacertidae	*Ichnotropis capensis*	LC	Yes
Snake	Lamprophiidae	*Amblyodipsas concolor*	LC	Yes
Snake	Lamprophiidae	*Amblyodipsas microphthalma*	LC	Yes
Snake	Lamprophiidae	*Amblyodipsas polylepis*	LC	Yes
Snake	Lamprophiidae	*Amblyodipsas ventrimaculata*	NE	Yes
Snake	Lamprophiidae	*Amplorhinus multimaculatus*	LC	Yes
Snake	Lamprophiidae	*Aparallactus capensis*	LC	Yes
Snake	Lamprophiidae	*Aparallactus lunulatus*	LC	Yes
Snake	Lamprophiidae	*Atractaspis bibronii*	LC	Yes
Snake	Lamprophiidae	*Atractaspis duerdeni*	LC	Yes
Snake	Lamprophiidae	*Boaedon capensis*	LC	Yes
Snake	Lamprophiidae	*Dipsina multimaculata*	LC	Yes
Snake	Lamprophiidae	*Duberria lutrix*	LC	Yes
Snake	Lamprophiidae	*Duberria variegata*	LC	Yes
Snake	Lamprophiidae	*Homoroselaps dorsalis*	LC	Yes
Snake	Lamprophiidae	*Homoroselaps lacteus*	LC	Yes
Snake	Lamprophiidae	*Inyoka swazicus*	LC	Yes
Snake	Lamprophiidae	*Lamprophis aurora*	LC	Yes
Snake	Lamprophiidae	*Lamprophis fiskii*	LC	Yes
Snake	Lamprophiidae	*Lamprophis fuscus*	LC	Yes
Snake	Lamprophiidae	*Lamprophis guttatus*	LC	Yes
Snake	Lamprophiidae	*Limaformosa capensis*	LC	Yes
Snake	Lamprophiidae	*Lycodonomorphus inornatus*	LC	Yes
Snake	Lamprophiidae	*Lycodonomorphus laevissimus*	LC	Yes
Snake	Lamprophiidae	*Lycodonomorphus obscuriventris*	LC	No
Snake	Lamprophiidae	*Lycodonomorphus rufulus*	LC	Yes
Snake	Lamprophiidae	*Lycophidion capense*	LC	Yes
Snake	Lamprophiidae	*Lycophidion pygmaeum*	LC	No
Snake	Lamprophiidae	*Lycophidion variegatum*	LC	No
Snake	Lamprophiidae	*Macrelaps microlepidotus*	LC	Yes
Snake	Lamprophiidae	*Montaspis gilvomaculata*	DD	No
Snake	Lamprophiidae	*Prosymna bivittata*	LC	Yes
Snake	Lamprophiidae	*Prosymna frontalis*	LC	Yes
Snake	Lamprophiidae	*Prosymna janii*	LC	Yes
Snake	Lamprophiidae	*Prosymna lineata*	LC	Yes
Snake	Lamprophiidae	*Prosymna stuhlmanni*	LC	Yes
Snake	Lamprophiidae	*Prosymna sundevallii*	LC	Yes
Snake	Lamprophiidae	*Psammophis angolensis*	LC	Yes

Snake	Lamprophiidae	*Psammophis brevirostris*	LC	Yes
Snake	Lamprophiidae	*Psammophis crucifer*	LC	Yes
Snake	Lamprophiidae	*Psammophis jallae*	LC	Yes
Snake	Lamprophiidae	*Psammophis leightoni*	LC	Yes
Snake	Lamprophiidae	*Psammophis mossambicus*	LC	Yes
Snake	Lamprophiidae	*Psammophis namibensis*	LC	Yes
Snake	Lamprophiidae	*Psammophis notostictus*	LC	Yes
Snake	Lamprophiidae	*Psammophis subtaeniatus*	LC	Yes
Snake	Lamprophiidae	*Psammophis trigrammus*	LC	Yes
Snake	Lamprophiidae	*Psammophis trinasalis*	LC	Yes
Snake	Lamprophiidae	*Psammophylax rhombeatus*	LC	Yes
Snake	Lamprophiidae	*Psammophylax tritaeniatus*	LC	Yes
Snake	Lamprophiidae	*Pseudaspis cana*	LC	Yes
Snake	Lamprophiidae	*Rhamphiophis rostratus*	LC	Yes
Snake	Lamprophiidae	*Xenocalamus bicolor*	LC	Yes
Snake	Lamprophiidae	*Xenocalamus sabiensis*	NE	No
Snake	Lamprophiidae	*Xenocalamus transvaalensis*	LC	Yes
Snake	Leptotyphlopidae	*Leptotyphlops distanti*	LC	Yes
Snake	Leptotyphlopidae	*Leptotyphlops incognitus*	LC	Yes
Snake	Leptotyphlopidae	*Leptotyphlops jacobseni*	LC	Yes
Snake	Leptotyphlopidae	*Leptotyphlops nigricans*	LC	Yes
Snake	Leptotyphlopidae	*Leptotyphlops scutifrons*	LC	Yes
Snake	Leptotyphlopidae	*Leptotyphlops sylvicolus*	LC	Yes
Snake	Leptotyphlopidae	*Leptotyphlops telloi*	NE	No
Snake	Leptotyphlopidae	*Myriopholis longicauda*	LC	Yes
Snake	Leptotyphlopidae	*Namibiana gracilior*	LC	No
Snake	Leptotyphlopidae	*Namibiana occidentalis*	LC	Yes
Snake	Natricidae	*Natriciteres olivacea*	NE	Yes
Snake	Natricidae	*Natriciteres sylvatica*	LC	Yes
Snake	Pythonidae	*Python natalensis*	LC	Yes
Chelonian	Testudinidae	*Homopus femoralis*	LC	Yes
Snake	Typhlopidae	*Afrotyphlops bibronii*	LC	Yes
Snake	Typhlopidae	*Afrotyphlops fornasinii*	LC	Yes
Snake	Typhlopidae	*Afrotyphlops mucroso*	LC	Yes
Snake	Typhlopidae	*Afrotyphlops schlegelii*	LC	Yes
Snake	Typhlopidae	*Rhinotyphlops lalandei*	LC	Yes
Snake	Typhlopidae	*Rhinotyphlops schinzi*	LC	Yes
Snake	Viperidae	*Bitis albanica*	EN	Yes
Snake	Viperidae	*Bitis arietans*	LC	Yes
Snake	Viperidae	*Bitis armata*	VU	Yes
Snake	Viperidae	*Bitis atropos*	LC	Yes
Snake	Viperidae	*Bitis caudalis*	LC	Yes
Snake	Viperidae	*Bitis cornuta*	LC	Yes
Snake	Viperidae	*Bitis gabonica*	LC	Yes
Snake	Viperidae	*Bitis inornata*	DD	Yes

Snake	Viperidae	*Bitis rubida*	LC	Yes
Snake	Viperidae	*Bitis schneideri*	NT	Yes
Snake	Viperidae	*Bitis xeropaga*	LC	Yes
Snake	Viperidae	*Causus defilippii*	LC	Yes
Snake	Viperidae	*Causus rhombeatus*	LC	Yes

Table A2. List of samples included in the phylogeny, with corresponding GenBank accession numbers available online at: http://hdl.handle.net/20.500.12143/6591

Table A3. List of primers used for each gene region to generate additional sequence data for this study.

Genome	Gene	Primer	Direction	Sequence	Primer source
mtDNA	12S	12Sa	F	AAACTGGGATTAGATACCCCACTAT	Kocher *et al.* (1989)
mtDNA	12S	12Sb	R	GAGGGTGACGGGCGGTGTGT	Kocher *et al.* (1989)
mtDNA	12S	12S268	F	GTGCCAGCGACCGCGGTTACACG	Utiger *et al.* (2002)
mtDNA	12S	12S916	R	GTACGCTTACCATGTTACGACTTGCCCTG	Utiger *et al.* (2002)
mtDNA	16S	16Sa	F	CGCCTGTTTAACAAAAACAT	Palumbi (1996)
mtDNA	16S	16Sb	R	CCGGTCTGAACTCAGATCACGT	Palumbi (1996)
mtDNA	cytb	cytb1	F	CCATCCAACATCTCAGCATGATGAAA	Kocher *et al.* (1989)
mtDNA	cytb	cytb2	R	CCCTCAGAATGATATTTGTCCTCA	Kocher *et al.* (1989)
mtDNA	cytb	L14910	F	GACCTGTGATMTGAAAACCAYCGTTGT	Burbrink *et al.* (2000)
mtDNA	cytb	H16064	R	CTTTGGTTTACAAGAACAATGCTTTA	Burbrink *et al.* (2000)
mtDNA	ND2	L4437	F	AAGCTTTCGGGCCCATACC	Macey *et al.* (1997)
mtDNA	ND2	H5540	R	TTTAGGGCTTTGAAGGC	Macey *et al.* (1997)
mtDNA	ND4	ND4_f	F	CACCTATGACTACCAAAAGCTCATGTAGAAGC	Arévalo *et al.* (1994)
mtDNA	ND4	Leu (R)	R	CATTACTTTTACTTGGATTTGCACCA	Arévalo *et al.* (1994)
nDNA	cmos	Cmos-FUF	F	TTTGGTTCKGTCTACAAGGCTAC	Gamble *et al.* (2008)
nDNA	cmos	Cmos-FUR	R	AGGGAACATCCAAAGTCTCCAAT	Gamble *et al.* (2008)
nDNA	cmos	S77	F	CATGGACTGGGATCAGTTATG	Lawson *et al.* (2005)
nDNA	cmos	S78	R	CCTTGGGTGTGATTTTCTCACCT	Lawson *et al.* (2005)
nDNA	PRLR	PRLRf1	F	GACARYGARGACCAGCAACTRATGCC	Townsend *et al.* (2008)
nDNA	PRLR	PRLRr3	R	GACYTTGTGRACTTCYACRTAATCCAT	Townsend *et al.* (2008)
nDNA	rag1	L2408	F	TGCACTGTGACATTGGCAA	Vidal & Hedges (2004)
nDNA	rag1	H2920	R	GCCATTCATTTTYCGAA	Vidal & Hedges (2004)
nDNA	rag1	G396	F	TCTGAATGGAAATTCAAGCTGTT	Groth & Barrowclough (1999)
nDNA	rag1	G397	R	AAAGGTGGCCGACCGAGGCAGCATC	Groth & Barrowclough (1999)
nDNA	rag2	Rag2-PY1F	F	CCCTGAGTTTGGATGCTGTACTT	Gamble *et al.* (2008)
nDNA	rag2	Rag2-PY1R	R	AACTGCCTRTTGTCCCCTGGTAT	Gamble *et al.* (2008)

4.6.1. References for Table A3

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5. KEY FINDINGS AND RECOMMENDATIONS

5.1 Key findings

- To date, the vast majority of genetic studies and datasets within South Africa provide single point estimates of genetic diversity for a limited number of species. These studies do not focus on tracking genetic diversity over time and are insufficient for monitoring purposes. However, they may provide a baseline of genetic diversity, upon which future short- and long-term genetic monitoring studies could be based.

- New indicators to track and monitor the status of genetic diversity are being developed in South Africa. These indicators can assist in identifying areas essential for the maintenance of genetic diversity across the landscape.

5.2 Key message

- Genetic diversity should be maintained because it enables species to evolve and adapt within an ever-changing environment.

5.3 Priority actions

- Development of a National Genetic Diversity Monitoring Framework. This framework should outline how to strategically prioritise taxa for monitoring, identify appropriate genetic markers and metrics, and provide advice on the frequency of monitoring.
- Test additional landscape level metrics.
  - Conduct additional analysis of pressures (land cover types) with other phylogenetic metrics.
  - Conduct analysis of protection status (e.g. Protected Areas) with additional phylogenetic metrics across a range of taxonomic groups.
- Investigate the potential for using recorded range shifts, assemblage shifts, and/or species distribution modelling to track trends of landscape level 'genetic erosion' (and increases in landscape level genetic diversity) or to project areas that might undergo genetic erosion in the future.
- Incorporate landscape level genetic richness into biodiversity assessments and planning.
- Conduct analysis of Critical Biodiversity Areas and the National Protected Areas Expansion Strategy as measures to safeguard genetic diversity.

5.4 Knowledge gaps

- There is a lack of landscape genetic studies for most South African taxonomic groups.
- There is a lack of temporal genetic datasets, as well as a lack of genetic diversity indicators and thresholds, with which data can be compared.
- Measures of genetic diversity are lacking from biodiversity assessments and planning.