A genetic perspective on leopard (*Panthera pardus*) conservation units across southern Africa

By

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CONTENTS

ABSTRACT

Conservation units are a tool to guide policy such that conservation goals can be achieved. These units should ideally synthesise a wide array of data – genetic, ecological, demographic – to identify the appropriate scales at which conservation actions can then be directed. Despite being the most widespread of all felids in Africa, and facing numerous threats across its range, it has been proposed that the entirety of southern Africa be considered one conservation unit for leopards (*Panthera pardus pardus*). This proposal does not take into consideration the likelihood of existing population genetic structure across an increasingly fragmented landscape. Further complicating regional leopard conservation is the variability in conservation policies among the geopolitical leopard-range states. Within this single proposed conservation unit, the patchwork of different legislation does not support a unified policy for leopard conservation. Using a population genetic perspective, this study explores and tests the values and shortcomings of southern Africa as a single conservation unit, and explores the importance of leopard range states within the context of conservation units. Parallel investigations of leopard microsatellite genotype data within the framework of a genetic population study spanning eight countries across southern Africa were carried out. This study presents consilient evidence supporting the finding that southern Africa contains six clusters of unique genetic lineages, and as such does not constitute a single genetic unit. Furthermore, it is shown here that the spatial genetic structure that exists does not correlate with the separate geopolitical range states. Leopard range states within southern Africa instead capture varying levels of unique genetic structure and thus are not of equal value with respect to the conservation of genetic lineages. These findings have several implications for leopard conservation across the region. While the data presented here specifically consider a genetic element of conservation units, they do suggest shortcomings in adopting either the entirety of southern Africa as a single unit or separate geopolitical range states as conservation units. The variability in leopard conservation policy across southern Africa is unlikely to sufficiently protect their existing regional genetic structure. If conservation units are indeed a tool to guide conservation policy, then the southern Africa unit for leopards is potentially less effective than a smaller unit whose spatial scale more accurately captures the discrete variation in population genetic structure. Genetic diversity and population structure is an important component of conservation units and should not be neglected. Currently, however, an appropriate framework allowing for conservation policy to be informed at the necessary scale does not exist; although the establishment of Transfrontier Conservation Areas speaks to the growing acknowledgement that conservation needs to evolve beyond the historical confines of

geopolitical range states. The evidence presented here further supports the need for a rethinking of existing policy structures.

1. Introduction

Conservation biology is a crisis discipline (Soulé 1985; DeSalle & Amato 2004), one in which the limited resources available need to be efficiently allocated in the hopes of maximising conservation gains (Bottrill et al. 2008). The corollary of this is that conservationists must first be able to determine where resources need to be allocated (Freudenberger et al. 2013). Conservation has historically been considered in terms of species (consider the IUCN Red List, for example: IUCN 2021; Mace et al. 2008), yet there remain a plethora of unresolved questions surrounding – and new revisions being proposed to – the taxonomy of organisms (Padial et al. 2014; Fitzpatrick et al. 2015). This presents an ever-changing landscape across which conservation efforts need be applied (Garnett & Christidis 2017; Zachos 2018; Gippoliti 2019). Furthermore, many species have spatial or temporal distributions which preclude the possibility of applying targeted measures across the species' entire distribution (Dallimer & Strange 2015; Johnston et al. 2020).

Conservation units (CUs) are tools by which conservation efforts can be better directed (Ryder 1986; Moritz 1994; Funk et al. 2012). The power of CUs lies in their ability to identify intraspecific groupings of organisms whose conservation value may be disproportionate to conspecifics (Green 2005). Consider the evolutionary continuum that exists between populations and species (Coates et al. 2018) and the gradual differentiation and population structure that exist across a species' range (Rundle & Nosil 2005). Conservation value, then, may be considered in terms of population evolutionary history (Moritz 2002), ecological traits (Bolnick et al. 2003), and demographic parameters (Palsbøll et al. 2007). This can be conceptualised more broadly as having value in either genetic divergence or phenotypic differentiation (Figure 1; Coates et al. 2018). The precursor to the CU was the evolutionarily significant unit (ESU), itself a tool first suggested to guide *ex situ* conservation efforts by the American Association of Zoological Parks and Aquariums (Ryder 1986). ESUs were subsequently developed by the wider scientific community for the conservation of natural populations. In the years since, a number of competing definitions have been proposed (Waples 1991; Moritz 1994; Crandall et al. 2000; Fraser & Bernatchez 2001; Mee et al. 2015) but ESUs are broadly affirmed to be conspecific populations that possess a unique evolutionary lineage (Moraes-Barros et al. 2007).

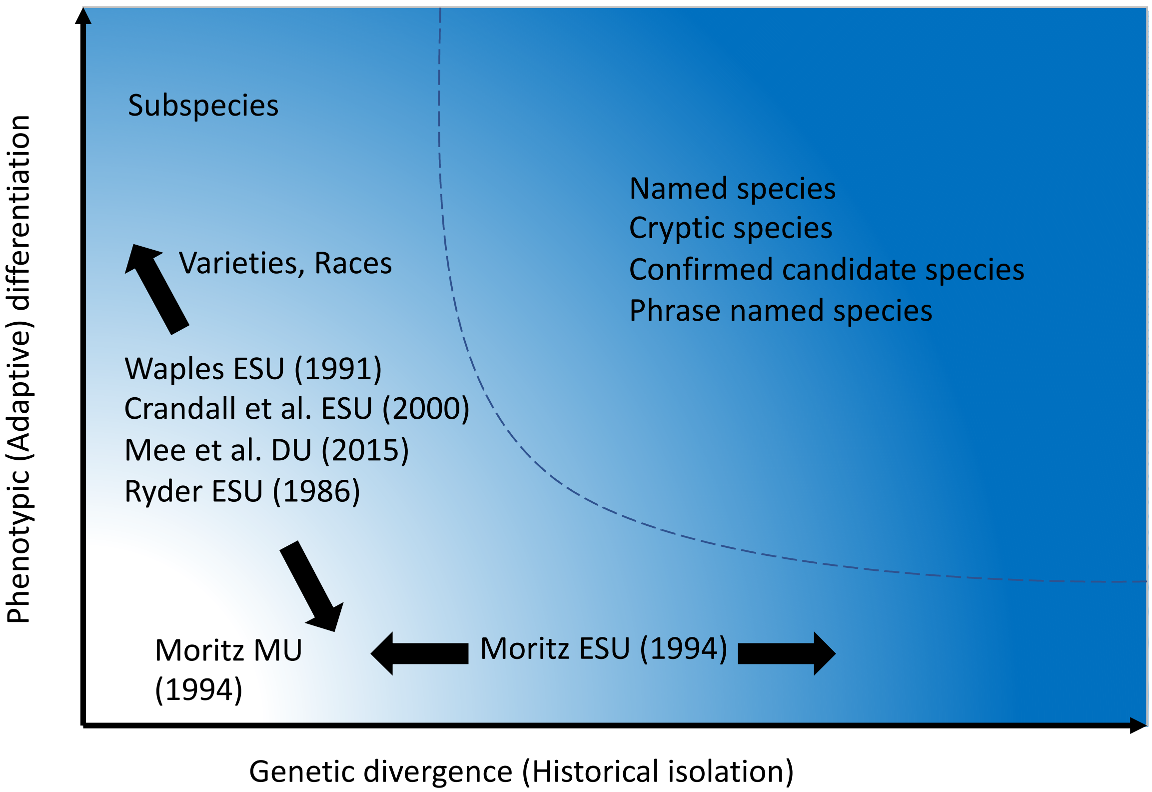

Figure 1: Conservation units (CUs) across a spectrum of genetic divergence and phenotypic divergence. Adapted from Coates et al. (2018)

ESUs are still widely used in conservation today (Jorge et al. 2020; Shaney et al. 2020), but they are now joined by many other CUs that have been defined and are employed around the world. Some (for example, Management Units, MUs) have sought to supplement ESUs (Moritz 1994, 2004); others – Distinct Population Segments (DPSs) and Designatable Units (DUs) – have sought to replace them (Green 2005; Mee et al. 2015). Specific CUs have also been designed for separate threatened taxa (Sanderson et al. 2002; IUCN SSC Cat Specialist Group 2006). As can be seen from Figure 1, the majority of CUs place an emphasis on the conservation of phenotypic or adaptive differentiation. This may, in part, be because genetic data, though highly informative for conservation (Frankham 2010; Allendorf 2017), are not available in many instances (Shafer et al. 2015). The use of subspecies as CUs, a logical conclusion when considering phenotypic differentiation (Figure 1), remains problematic (Kitchener & Yamaguchi 2010; Wilting et al. 2015), in no small part due to a lack of taxonomic clarity (Mayr 1976; Mallet 1995; Hey 2001; De Queiroz 2007). With respect to policy, CUs have been incorporated into legislation in both the United States (Rosen 2007) and Canada (Weckworth et al.

2018) to address declines in biodiversity; in contrast, EU legislation remains focused at the species level (Casacci et al. 2014). Presently, the legislation of Southern African Development Community (SADC) member states make no mention of CUs (Cirelli & Morgera 2010) either. Legislation ascertaining to the protection of wildlife in these countries instead remains focused towards the species level or above.

International treaties and conservation

The Convention on Biological Diversity (CBD) is one of the principal international instruments for enacting conservation (Coates et al. 2018). Since coming into force in 1993 and with 196 Parties to the agreement, the CBD has emphasised – inter alia – preserving biological diversity, defined as “diversity within species, between species and of ecosystems” (United Nations 1992). Genetic diversity may assist species in adapting to future climatic conditions (Hoban et al. 2020), and its loss has been shown to negatively affect the fitness of individuals (Blomqvist et al. 2010). As part of the strategic plan for 2011-2020, the Aichi Biodiversity targets explicitly called for the safeguarding of genetic diversity as part of this agreement, yet made no mention of intraspecific conservation units that might assist in achieving set targets (Coates et al. 2018). In order to satisfy the strategic goals as laid out by the CBD, Parties are expected to produce and carry out their own National Biodiversity Strategies and Action Plans. The result is that the convention affirms the sovereign rights of each Party, relying upon national rather than international action to conserve biodiversity, but has no regulatory mechanism to itself enforce compliance. To date, there has been a widespread failure by Parties to sufficiently document and monitor existing genetic diversity (Laikre et al. 2010; Díaz et al. 2020). With the release of the CBD’s draft post-2020 framework, the maintenance of genetic diversity is listed as one of four long-term goals for Parties to achieve by 2050 (Laikre et al. 2020). Yet, missing from this framework are any clear targets to guide Parties towards this goal. Hoban et al. (2020) offer clear recommendations for targets that could easily be implemented to help shepherd the conservation of genetic stock. But should the post-2020 goals and framework be ratified without any further amendments – which now appears likely – Parties are unlikely to deviate from their present course and genetic diversity shall continue to be overlooked within National Biodiversity Strategies and Action Plans.

Other Multinational Environmental Agreements (MEAs) at the forefront of international conservation – such as the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and the Convention on Migratory Species (CMS) – involve a greater degree of international cooperation and agreement among Parties than the CBD (Cooney 2001). Yet here too, MEAs are species-specific and do not consider conservation units below the species level (Cirelli & Morgera 2010). While – in theory – both MEAs consider the genetic diversity of species, the dearth of funding and experience among practitioners has made its consideration difficult, at best (Holderegger et al. 2019). As an MEA, CITES has widespread membership with 183 Parties and seeks to address the danger to species from unsustainable harvesting (Robinson & Sinovas 2018). Within southern Africa, all of the SADC states are currently Parties to the agreement – although, discontent and increasing scepticism towards CITES over recent decisions concerning the trade in ivory and rhino horns has led to reports that Zimbabwe and other SADC member states could leave the agreement (Hellinx 2020). CMS, on the other hand, has 132 Parties and seeks to address habitat loss and fragmentation between range states for species transcending international borders (Hensz & Soberón 2018). International treaties provide valuable frameworks upon which efforts to conserve species can be built – in particular, for charismatic carnivores which have large ranges and are frequently the targets of international trade (Hodgetts et al. 2018). However, whereas Parties to the CBD are under obligation to no-one but themselves to fulfil targets and goals, international treaties requiring cooperation and agreement are subject to the vagaries of international politics and competing self-interests. This can stymie efforts to achieve the stated aims of the MEA (Couzens 2014; Hodgetts et al. 2019).

Sub-Saharan Africa – future projections & associated consequences

Sub-Saharan Africa is expected to undergo dramatic demographic and environmental changes over the course of the 21st Century. Between now and 2050, the region will see the addition of more than a billion people (more than half of the world's population growth in this time); in contrast to other parts of the world, growth here is projected to continue throughout the century (United Nations 2019). This will lead to a concomitant increase in a need for resources (Boon 2011), as well as habitat loss and fragmentation (Seoraj-Pillai & Pillay 2016). At the same time, climatic projections indicate the region will experience a warming trend, be subject to more frequent occurrences of extreme heat events, increased aridity and reduced precipitation across southern Africa (Serdeczny et al. 2017). Much of rural Sub-Saharan Africa is dependent upon agriculture (Kotir 2011), making the region extremely vulnerable to these projected changes in climate. Crop failures and heightened livestock mortality are likely to drive urban expansion, further encroaching upon both natural habitats (Rouget et al. 2003) and species (Scheun et al. 2015). Human-wildlife conflict is also likely to be exacerbated (Mekonen 2020), whether it occurs for sustenance (Rentsch & Damon 2013) or profit (Hauenstein et al. 2019). Viewed as a whole then, many of the existing threats to wildlife across Sub-Saharan Africa are only likely to increase in their intensity. Predominantly through the 20th century, protected areas (PAs) and national parks were established across Africa following the traditional conservation paradigm of "fortress conservation" – that fauna and flora needed to be physically separated from malign human influences in order to be conserved (Büscher 2016). While there is a growing acceptance that such approaches have negatively impacted the rights of indigenous peoples (Sand 2012), PAs and National Parks have proved effective in maintaining populations of species that have otherwise been extirpated beyond their boundaries (Stephens 2015). Evidence demonstrating that PAs still do not insulate the species within from human-related extinctions (Brashares et al. 2001) is an ominous sign for the future.

Conservation paradigms today favour moving away from *sensu stricto* land sparing practices towards community-based conservation efforts (Hanks 2001). Conservation initiatives that involve, and are driven by, local communities are less likely to lead to conflict between stakeholders and wildlife (Blackburn et al. 2016). Megafauna require more space than can be set aside in a single PA (Graham et al. 2009), and so conservation efforts must consider landscape-scale solutions that extend beyond a single PA. Large mammals require large and well-connected patches of habitat within the landscape to survive (Courbin et al. 2014; Selier et al. 2015; de la Torre et al. 2017): island biogeography theory and species-area relationships dictate that species will be lost faster from smaller isolated patches than from larger ones (MacArthur & Wilson 2001). Isolated populations are more vulnerable to demographic stochasticity as a result of declining genetic diversity and reduced fitness (Westemeier et al. 1998; Wilder et al. 2020). Across southern Africa, fences and major infrastructure such as highways severely restrict the movement of migratory species within the landscape (Newmark 2008). There are two conservation strategies of note that have been pursued to address the connectivity of populations within landscapes – both of which seek to enhance the viability of populations by providing opportunities for gene flow and genetic rescue to occur (Hogg et al. 2006). Firstly, habitat corridors seek to provide a means through which individuals can readily traverse the landscape (Beier & Noss 1998). These can be natural, in the case of preserved or restored habitat (Chetkiewicz et al. 2006) – or artificial, such as wildlife overpasses (Corlatti et al. 2009). Secondly, Transfrontier Conservation Areas (TFCAs) are expansions upon the original PA design that have sought to acknowledge larger functional ecological systems (Tshipa et al. 2017). TFCAs are sections of a greater ecological area straddling the boundary of two or more countries' borders, and including within them one or more PAs and multiple resource use areas (Southern African Development Community 1999). TFCAs recognise that species do not recognise international borders, but have proven to be not without their own difficulties. TFCAs require mutual cooperation between border states, yet – as is the case with MEAs – such cooperation is subject to wider geopolitical considerations (consider the political complications for South Africa in partnering with Zimbabwe over the Great Limpopo Transfrontier park in the early 2000s following Zimbabwe's controversial policies and international pariah status; Ferreira 2004). While TFCAs have been heralded by global actors as the solution to trans-national environmental problems (Duffy 2006), they frequently fail to deliver on such expectations for these reasons.

Conservation of flagship species – Africa's leopard

Flagship species – normally charismatic mammals such as mega-herbivores or large carnivores (Smith et al. 2012) – have large landscape requirements (Ripple et al. 2014, 2015). Conservation strategies for these species must therefore take different approaches to other taxa – such as migratory birds which instead can be protected by the selection of specific sites for conservation (Kirby et al. 2008; Runge et al. 2015). Felids hold symbolic value among a number of human cultures (Herrmann et al. 2013; Naude et al. 2020a), and there remains strong interest in their continued persistence in the wild (Dickman et al. 2015). Felids are wide-ranging carnivores whose landscape needs are larger than a PA allows for (Zanin et al. 2015). Leopards (*Panthera pardus*) are the most widespread of all felids, occurring across a diverse array of habitats in 79 different countries throughout both Africa and Asia (Jacobson et al. 2016). Leopard taxonomy is still subject to revision, with either eight (Stein et al. 2020) or seven (Kitchener et al. 2017) subspecies recognised outside of Africa. Crucially however, only one subspecies – the nominate form *P. p. pardus* (Miththapala et al. 1996) – is recognised across the African continent (Kitchener et al. 2017).

Across their range, leopards are declining (Jacobson et al. 2016; Stein et al. 2020). As diverse as the habitats in which they are found, so too are the threats they face today: habitat fragmentation & loss, depletion of prey, conflict with humans, unsustainable harvesting & poaching, as well as indiscriminate killing (Jacobson et al. 2016). Globally, leopards have seen range declines of 63-75%; within Africa, range loss was between 48-67% (Jacobson et al. 2016) where loss has been spatially heterogeneous with much of the remaining range in southern and east Africa. In contrast, leopards have lost 94-99% of their historic range in northern Africa, and 86-95% in western Africa (Jacobson et al. 2016). Within southern Africa, the total extant range has been estimated at 2,872,000 km^2 (IUCN SSC Cat Specialist Group 2018), much of it outside the region's network of PAs (62%; Jacobson et al. 2016). Owing to a lack of data, robust population estimates for leopards are not available, and previous assessments of population size (Martin & De Meulenaer 1988) have been criticised for being unrealistic. Nonetheless, inferences from evidence of declines in prey (Wolf & Ripple 2016), habitat loss (Perrings & Halkos 2015) and declines in fellow large carnivore guild species (Bauer et al. 2015) suggest the population is indeed declining.

Leopards are listed by CITES in Appendix I, meaning that the international trade of leopards or their parts is prohibited with limited exemptions – such as for scientific research (Smith et al. 2011). Parties to CITES are allocated export quotas that allow in theory for the controlled trade of hunting trophies and skins. These quotas are set during the Conference of the Parties and should be derived from the size of member states' leopard populations (Trouwborst et al. 2020). It is largely understood that there is significant uncertainty in the population estimates of species, and so quotas are to be set following the precautionary principle (*In dubio pro natura*; Trouwborst 2007). Nonetheless, it has become apparent that many countries' quotas have grossly overestimated their leopard populations leading to inflated quotas (Strampelli et al. 2020) although most Parties do not meet these inflated quota allocations (Trouwborst et al. 2020). The illegal trade, on the other hand, remains a significant problem with leopard skins sought after for their status as symbols of pride and royalty (Naude et al. 2020a), and their body parts desired for traditional medicinal use (Nieman et al. 2019), among indigenous African cultures. Poaching, as well as retaliatory killings, has an indirect negative effect on leopard populations beyond the removal of individuals and genes. Leopards exhibit male-biased dispersal, with males normally dispersing away from their home range. Yet following the removal of males, younger males do not disperse away from their natal range and risk increasing inbreeding within the population (Naude et al. 2020b). Inbreeding reduces survival fitness, thus worsening the condition of the population and increasing extinction risk (Brook et al. 2002).

Leopards are instead listed by CMS under Appendix II, which means that all Parties to the agreement are encouraged to work towards improving the conservation status of leopards but are not prohibited from harvesting them (i.e., trophy hunting; Hensz & Soberón 2018). Leopards were only added to Appendix II in 2017 following a motion proposed by Ghana, Kenya, Saudi Arabia, and Iran; the motion passed by a vote after being opposed by Uganda, Tanzania, Zimbabwe and South Africa (Lewis & Trouwborst 2019). The stated reason for the objection was that leopards do not classify as migratory species, yet it is likely that there were deeper considerations. Tanzania, after all, raised no objections to adding chimpanzees (*Pan troglodytes*) to Appendix II at the same meeting. Listing a species in Appendix II may more readily pave the way for its listing in Appendix I at a later date, which would then prohibit the harvesting of the species across its range – and trophy hunting is a hugely important source of income for these countries (Lindsey et al. 2007). Habitat fragmentation is of serious concern for leopard conservation, as male leopards require vast contiguous habitat to traverse for reproduction (Balme et al. 2019). Unfortunately, the ability of CMS to foster international cooperation is stymied by the fact that there are countries within southern Africa with leopard populations – Namibia, Botswana, and Zimbabwe – that are not Party to CMS. A joint initiative – the African Carnivores Initiative, ACI – between CITES and CMS launched in 2017 to coordinate efforts to facilitate the conservation of leopards, as well as lions (*Panthera leo*), cheetah (*Acinonyx jubatus*) and African wild dogs (*Lycaon pictus*), may solve this concern, as these countries are all Parties to CITES (Hellinx 2020).

For the first meeting of the ACI in 2018, the IUCN's Species Survival Commission Cat Specialist group produced a roadmap for the conservation of leopards in order to guide and focus conservation decisions. As part of this roadmap, the group divided *P. p. pardus'* range into Conservation Units to more readily form specific Regional Conservation Strategies (IUCN SSC Cat Specialist Group 2018). The group proposed four regional CUs: West Africa, Central Africa, Southern Africa, and East Africa (as leopards have been all but extirpated from northern Africa, this was not considered a separate region). The Southern Africa CU consisted of the following 10 countries: Angola, Botswana, Lesotho, Malawi, Mozambique, Namibia, South Africa, eSwatini (formerly Swaziland), Zambia & Zimbabwe (IUCN SSC Cat Specialist Group 2018). The CUs sought to emulate past CUs designed for cheetah and

African wild dog (RWCP & IUCN SSC 2015) and to facilitate the development of Regional Conservation Strategies. Nonetheless, the designation of an entire region as a CU for leopards has not been previously attempted.

Leopard conservation units

Establishing a coherent and targeted CU for any species requires considering their life-history, the data available, and the wider geopolitical situation within which your conservation strategy will be carried out. This begs the question: for a large, solitary felid such as *P. p. pardus*, what is the most appropriate scale to consider conservation action?

MEAs like the CBD (United Nations 1992) have emphasised the sovereignty of nations to achieve conservation gains by developing and implementing their own national policies. Furthermore, countries are the signatory unit to MEAs. Species' populations existing entirely within one nation may be subject to different threats and solutions – not to mention different monitoring and reporting strategies. It may, therefore, make sense to consider countries as separate CUs – albeit as an arbitrary unit that has no ecological, demographic or genetic meaning.

The principal difficulty in treating countries as separate CUs is that wildlife doesn't recognise international borders (Trouwborst et al. 2016), and wide-ranging species – like leopards – may find themselves floating between two or more CUs. Transfrontier Conservation Areas (TFCAs) provide protection across entire landscapes beyond the confines of national borders, and the policies for species in these landscapes is made and agreed at the level of these TFCAs between national representatives (Hanks 2003). As such, these could instead be considered as a suitable CU for leopards (and one that actually has biological meaning). This has precedent too for felids – Landscape Conservation Units (LCUs) have been applied to snow leopards (*Panthera uncia*) across 12 countries (Li et al. 2020). LCUs were selected for by using the planning tool Zonation to identify priority areas for conservation (Lehtomäki & Moilanen 2013) and then by choosing those priority areas with contiguous areas greater than 10,000 km^2 (Li et al. 2020). A habitat suitability model was later run to identify the most important of the LCUs identified for snow leopard conservation. Similar conservation units have been used for tigers in south east Asia (Tiger Conservation Landscapes, formerly Tiger Conservation Units; Sanderson et al. 2010). Conceptually then, there is certainly a strong basis for considering landscapes as a viable CU for leopards across southern Africa. Practically, adoption of the CU by the TFCA can allow for ongoing monitoring and enforcement of conservation actions throughout that landscape. The trade-off here is that implementation of conservation strategies may be complicated as it will be necessary to obtain buy-in from all of the range countries involved in the TFCA. Conservation may thus become more exposed to political negotiations. If individual countries are the unit of conservation, the CU will instead only have to be formally recognised by that country. There are ten countries within the proposed regional southern Africa CU.

A final consideration might be conservation at a regional scale, as the IUCN SSC Cat Specialist Group has proposed (IUCN SSC Cat Specialist Group 2018). Conservation at a regional scale affords the opportunity to implement conservation strategies across a variety of ecoregions and biomes while engaging with a framework of stakeholders with shared goals and values (Soulé & Terborgh 1999) – in this case, SADC countries (IUCN SSC Cat Specialist Group 2018). Here, too, there is precedent for considering a region as a CU. Jaguar Conservation Units (JCUs) have been used to identify areas necessary for Jaguar (*Panthera onca*) within the Atlantic Forest in Brazil, Paraguay and Argentina (Paviolo et al. 2016) and contains a number of separate ecoregions (Cantidio & Souza 2019). JCUs are defined as areas that have either sufficient populations of prey and resident jaguars such that jaguars could be self-sustaining for the next 100 years, or areas containing fewer jaguars but with stable habitats and prey populations within such that jaguar numbers could increase should threats be alleviated (Sanderson et al. 2002). The previous regional CUs devised for cheetah and African wild dog also sought to identify viable populations for conservation within western, central and northern Africa by comparing range distributions, threats, habitat connectivity and ecoregions per region (RWCP & IUCN SSC 2015). This regional approach to CUs has also been applied to marine systems (Wallace et al. 2010). Conservation at a regional scale – at least, in a terrestrial environment – suffers from the same difficulties as the country and landscape CUs. Conservation action must now navigate an increased number of stakeholders as well as international politics, which may ultimately prove ruinous to conservation goals – except that it must now take place at an even greater scale. There is no overarching body to manage conservation that currently exists across an entire region. This would mean that either an outside organisation would be required to continually shepherd conservation goals among the ten countries comprising this region, or it would have to be hoped that the ten countries might set national policies that would complement each other. However, without buy-in on this idea from all ten countries from the outset, the idea of complementary national polices and legislation seems far flung. It remains to be seen, then, how effective a regional CU might actually be.

Increasing spatial scale – from country to landscape to region – allows for more ecological and demographic data to inform the conservation unit. Yet, dissonance between the spatial scale of the CU and the scale at which policy is dictated may limit the efficacy of the unit. CUs also need to consider genetic data in order to be effective (Crandall et al. 2000; Taylor et al. 2010; Coates et al. 2018). With respect to genetic metrics, the best CU will be one which balances genetic diversity and maintains gene flow among populations – while being mindful of the scales at which policy will be applied. The IUCN SSC's regional conservation plan for leopards makes several implicit assumptions about genetic diversity – habitat connectivity is needed for gene flow, for instance (IUCN SSC Cat Specialist Group 2018) – yet makes no attempt for any explicit consideration of genetic diversity.

Research Aims & Objectives

The overarching aim of this study is to interrogate how well the southern African region works as a single conservation unit for leopards with respect to conserving the distribution and structure of genetic variation in the region. Additionally, this study seeks to evaluate the relative contribution of individual leopard range states with respect to genetic variation and structure captured within their geo-political boundaries.

To understand the distribution and structure of genetic diversity in leopards across their southern African Conservation Unit, microsatellite genotype data for leopards sampled across the region were used to (1) investigate how genetic variation is distributed within and across the region, using both a non-spatial Bayesian clustering algorithm and a multivariate analysis of the data, (2) quantify the degree of genetic differentiation between leopard range-states, i.e. countries, using pairwise estimates of FST, and (3) determine how much of the region's genetic variation is captured in each range-state using both descriptive metrics and an Analysis of Molecular Variance.

2. Methods

2.1. Ethics statement

Approval from the University of Cape Town's Faculty of Science ethics committees was not required for this study, as the data analysed here did not require samples to be collected and/or genotyped. All data analysed here were collected and approved as part of a previous study (Naude 2020) and are used here with permission.

2.2. Study area and data collection

Data collection, extraction and amplification of DNA samples, and sample genotyping were all carried out previously during the course of the original PhD study and are recounted here as background for the present study. More detailed information can be found in Naude (2020).

Samples were provided by a network of collaborators from different backgrounds and fields, including – but not limited to – research institutions, National Parks authorities, eco-tourism operators, and citizen scientists, between 2015-2019. While the majority of samples were isolated from faecal material, tissue and blood samples were also collected. Storage of blood samples was in BD Vacutainer® EDTA tubes at 4 °C; tissue and faecal samples, on the other hand, were dry-stored on silica beads at -20 °C. Sample provenance was recorded with a GPS unit. All samples were collected under the appropriate local conservation authority permits (Naude 2020).

The samples analysed here represent 21 different areas across eight countries within southern Africa (Figure 2). Due to the nature of the data collection, the geographic spread and number of samples is unevenly distributed – the Democratic Republic of Congo (hereafter, just "Congo"), Botswana, and Zambia are particularly less represented than others.

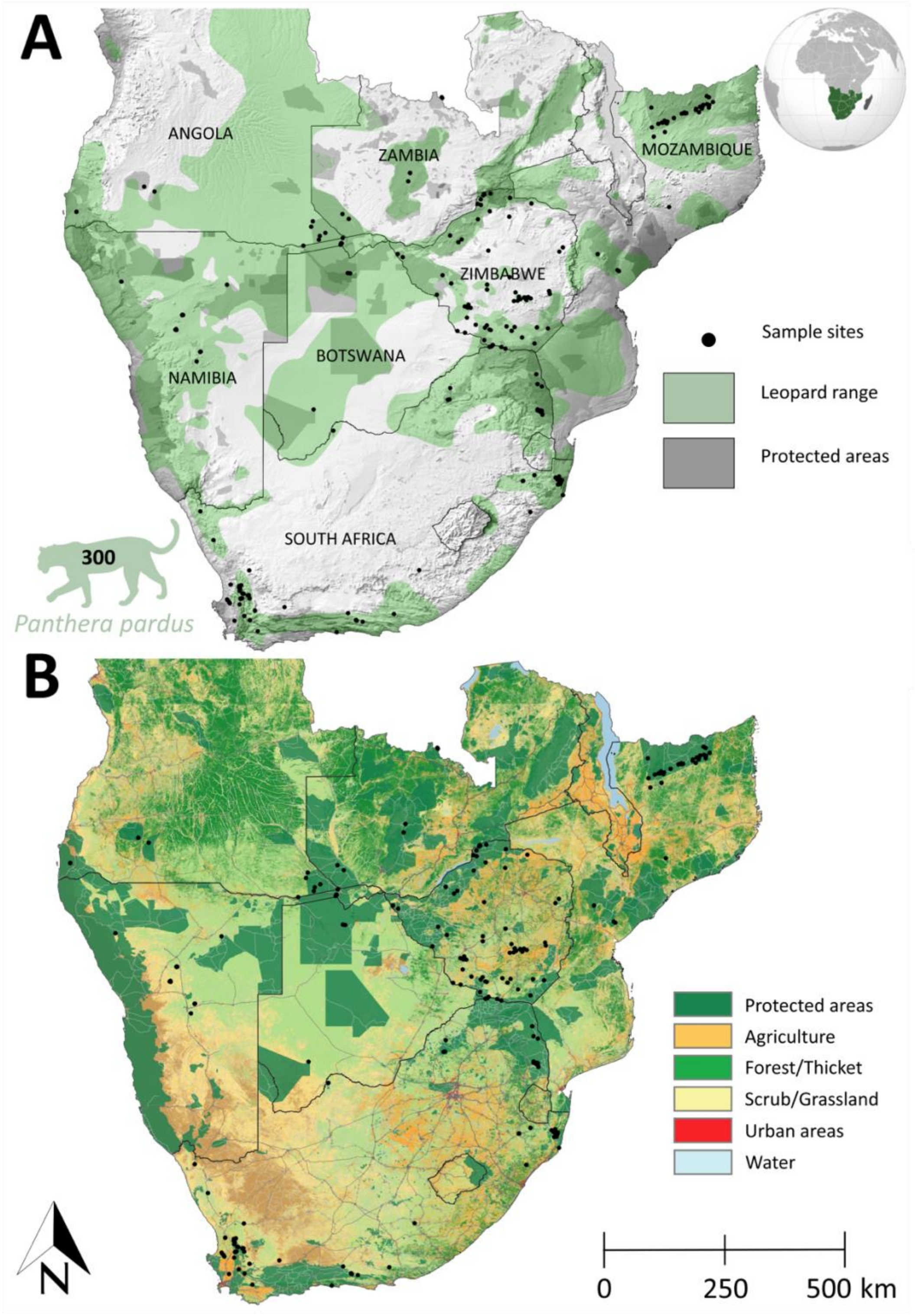

Figure 2: Sampling sites of leopards (*Panthera pardus*) across southern Africa. (A) Leopard range and protected areas across southern Africa. (B) Protected areas and land use types across southern Africa.

2.3. DNA extraction, PCR and genotyping

DNA was extracted from faecal samples using the QIAamp DNA Stool Mini Kit; the DNEasy Blood and Tissue Kit was instead used to extract DNA from tissue and blood samples (Qiagen, Inc., Valencia, CA, USA). All extractions followed the guidelines set out by the manufacturer and DNA samples were stored at -20 °C.

Samples were genotyped using 22 microsatellite loci that have been isolated from the *Felis catus* genome (Menotti-Raymond et al. 1999) and subsequently validated as polymorphic in leopards (Uphyrkina et al. 2001). Sex was determined through the use of a Zn-finger sex-linked marker (Pilgrim et al. 2005). DNA amplification was carried out by polymerase chain reaction (PCR) following the procedures described by Menotti-Raymond et al. (1999). In brief, PCR runs used 50-100 ng DNA, 200 ng bovine albumin serum, a locus-specific $MgCl_2$ concentration (between 1.5-2.5 mM), 2 µM of forward- and reverse-labelled primers, 5 µl DreamTaq™ Green PCR Master Mix (Thermo Scientific), and deionised water to give a total reaction volume of 25 µl. PCRs were carried out on an Applied Biosystems Veriti® Thermal Cycler. Sexing PCRs were run in triplicate for each sample, and only those samples with identical results for at least two independent reactions were considered reliable for sex identification. The quality of DNA samples extracted from faecal material was generally poor, so as a consequence all samples were amplified in singleplex reactions and replicated where necessary to ensure reproducibility and reliability.

Genotypes were analysed using a 3100-Avant Genetic Analyzer (Applied Biosystems) at the Central Analytical Facility at Stellenbosch University, South Africa. Genotypes were sized using the LIZ® 600 internal size standard, while alleles were scored using GENEIOUS R10 (Biomatters Limited). Automated allele calls were also checked manually for accuracy. In order to account for potentially misidentified faecal samples that might have been collected, NADH-5 mitochondrial sequences were compared with known leopard sequences on Genebank® (http://www.ncbi.nlm.gov/genebank) using NCBI BLASTN® and the megablast programme; all non-leopard samples were then excluded. All 22 microsatellite loci were tested and checked for null allele frequencies, and no null alleles were identified in the data set (Naude 2020). Finally, pairwise genotype comparisons were carried out using CERVUS 2.0 (Kalinowski et al. 2007) to determine the number of unique individuals within the data set and to remove duplicates.

2.4. Genetic diversity, Hardy-Weinberg equilibrium, and linkage disequilibrium

Indices of genetic diversity – including the number of alleles (N_A), private allelic richness (PA_R), and expected (H_E) and observed (H_O) heterozygosity – were calculated in GenAlEx version 6.5102 (Peakall & Smouse 2006, 2012). Rarefied allelic richness was not calculated as the smallest sample size was two, limiting interpretation across populations. Hardy-Weinberg equilibrium (HWE) was tested across loci for populations using the *pegas* package (version 0.14; Paradis 2010) in R, version 3.6.3 (R Core Team 2020); following Guo & Thompson (1992). Linkage disequilibrium was tested for in R with the package *poppr* (version 2.8.6; Kamvar et al. 2014).

2.5. Genetic structure

To assess population structure across southern Africa without *a priori* assumptions concerning scale, the data was first analysed using STRUCTURE version 2.3.3 (Pritchard et al. 2000; Falush et al. 2003). STRUCTURE employs non-spatial Bayesian clustering to probabilistically assign samples to a given number (*K*) of genetically distinct clusters or populations. *K*-values from between 2 and 10 were tested through STRUCTURE with a burn-in of 100,000 iterations followed by 100,000 subsequent Markov chain Monte Carlo iterations using the “Admixture” ancestry model. In order to assess the most likely value of *K*, STRUCTURE HARVESTER web v0.6.94 (Earl & vonHoldt 2012) was used to implement the Evanno ΔK method (Evanno et al. 2005).

A complementary investigation into population structure was also carried out using a multivariate approach. While STRUCTURE assumes HWE and linkage equilibrium within populations (Falush et al. 2003), a multivariate approach assesses the distribution of variance in multivariate space without any population genetic assumptions. The data were analysed using a Discriminant Analysis of Principal Components (DAPC; Jombart et al. 2010) with the package *adegenet* (version 2.1.3; Jombart 2008) in R (3.6.3; R Core Team 2020). DAPC is a multivariate method that identifies and describes clusters of similar individuals, using sequential *K*-means and model selection to infer genetic clusters in the data. The optimal *K* value identified by STRUCTURE was used to inform the number of clusters to be plotted in multivariate space, allowing for a comparison between different assessments of the genetic structure across southern Africa.

To interrogate the genetic structure captured by countries, Weir & Cockerham's (1984) pairwise F_{ST} values and 95% confidence intervals (from 10,000 bootstraps) were calculated using the package *hierfstat* (version 0.5; Goudet 2005) in R. The spatial scale at which genetic structure exists within countries was also assessed by conducting an analysis of molecular variance (AMOVA) using *poppr* (2.8.6; Kamvar et al. 2014) in R (3.6.3; R Core Team 2020).

3. Results

3.1. Microsatellite genotyping

Samples were genotyped for 300 individual leopards across eight countries in southern Africa (Angola [*n*=13], Botswana [*n*=4], Congo [*n*=2], Mozambique [*n*=47], Namibia [*n*=25], Republic of South Africa (hereafter, South Africa) [*n*=137], Zambia [*n*=6], and Zimbabwe [*n*=66]). Of these, 168 were male and 123 female; nine individuals could not be sexed.

3.2. Genetic diversity, Hardy-Weinberg Equilibrium, and Linkage Disequilibrium

Overall, the heterozygosity of leopards in countries across southern Africa was lower than expected under the Hardy-Weinberg Equilibrium model ($p < 0.001$), with mean observed heterozygosity (H_O) of 0.61 ± 0.02, less than that of the expected heterozygosity (H_E) of 0.67 ± 0.02 (Table 1).

South Africa and Zimbabwe both have a larger number of alleles (N_A of 10.27 and 10.77, respectively) and a larger number of unique alleles (PA_R of 15.00 and 19.00, respectively) than the mean ($N_A = 7.19 \pm 0.38$; $PA_R = 6.25 \pm 2.30$). Heterozygosity in Zimbabwe ($H_O = 0.52$) was much lower than expected ($H_E = 0.82$), and I found support that the country was not in HWE ($p < 0.001$). There is also evidence that South Africa deviated from HWE ($p = 0.03$). In both countries, there was not enough evidence to reject the null hypothesis of no linkage among microsatellite markers (South Africa: $\bar{r}_d = 0.13$, $p = 0.001$; Zimbabwe: $\bar{r}_d = 0.03$, $p = 0.001$).

Mozambique and Namibia, on the other hand, have very few unique alleles (PA_R of 4.00 and 6.00, respectively) despite having a large number of alleles in total (N_A of 10.68; 10.27). Namibia also displays much lower heterozygosity ($H_O = 0.57$) than expected ($H_E = 0.85$), but there was not enough evidence to support the country deviating from HWE ($p = 0.07$). Mozambique also did not deviate from HWE ($p = 0.11$). There was no evidence for linkage disequilibrium among loci in either country.

The low sample sizes from Angola, Botswana, Congo, and Zambia make it difficult to draw too many conclusions about genetic diversity in these countries. Heterozygosity appears to be greater than expected in Botswana ($H_O = 0.61$, $H_E = 0.46$), Congo ($H_O = 0.45$, $H_E = 0.31$) and Zambia ($H_O = 0.72$, $H_E = 0.56$), and less than expected in Angola ($H_O = 0.52$, $H_E = 0.81$). In Angola, there was no evidence the country deviated from HWE, but there was insufficient evidence to reject the null hypothesis of no linkage among markers. Neither Botswana or Zambia deviated from HWE, and there was no evidence to support linkage disequilibrium. The small sample size of the Congo samples meant that there was missing data from eight loci (FCA126, FCA008, FCA097, FCA096, FCA026, FCA075, FCA094, FCA678); however, there was no evidence to suggest from the remaining 14 loci that the country deviated from HWE or that there was linkage disequilibrium.

Table 1: Summary statistics of genetic diversity. The number of alleles (N_A), private allelic richness (PA_R), observed (H_O) and expected (H_E) heterozygosity of leopards across southern Africa. * indicate countries where interpretation of results is difficult owing to their small sample sizes. H_O values that are underlined indicate countries significantly out of HWE ($\alpha = 0.05$)

Country	**n**	**N_A**	**PA_R**	**H_O**	**H_E**
Angola*	13	8.00	3.00	0.52	0.81
Botswana*	4	2.73	1.00	0.61	0.46
Democratic Republic of Congo*	2	1.50	2.00	0.45	0.31
Mozambique	47	10.68	4.00	0.76	0.79
Namibia	25	10.27	6.00	0.57	0.85
Republic of South Africa	137	10.27	15.00	**0.70**	0.77
Zambia*	6	3.27	0.00	0.72	0.56
Zimbabwe	66	10.77	19.00	**0.52**	0.82
Total Mean	-	7.19	6.25	0.61	0.67
SE	-	0.38	2.30	0.02	0.02

3.3. Genetic structure

The output from the STRUCTURE analysis (Evanno ΔK; see Appendix Figure A1 for how alternate K values performed) supported six clusters across southern Africa, with different levels of admixture among sampling areas (Figure 3A). Three of these clusters were distributed throughout southern Africa in different proportions ("Cluster 1", "Cluster 2" and "Cluster 5"). One cluster was present in Zimbabwe and central Mozambique ("Cluster 3"), while another characterises the South African provinces of KwaZulu-

Natal and Limpopo (‘Cluster 4”). The last cluster corresponded broadly to southwestern Africa, being present in Namibia and Angola (“Cluster 6”).

Countries contained different proportions of these genetic lineages (Figure 3B). Namibian and Angolan samples were principally defined by Cluster 6. Zambian and Congolese samples were largely represented by Cluster 2. Within Botswana, the dominant clusters were those of Cluster 1 and Cluster 5 in the south, and Cluster 2 and 5 in the north. These same clusters – Cluster 1 and Cluster 5 – were well represented in Mozambican samples also, although samples from central Mozambique also held large proportions of the Cluster 3 lineage. Cluster 3 was strongly represented in southern and north-east Zimbabwe, yet was less represented in central – and hardly at all in western – Zimbabwe; western Zimbabwean samples instead were strongly representative of the Cluster 1 lineage. South African samples were strongly differentiated by region. The predominant lineage in the Western Cape was Cluster 2. Samples from the Northern and Eastern Capes were instead mostly representative of Cluster 5. Cluster 4 was the dominant lineage in KwaZulu-Natal; in Limpopo, Clusters 4 and 5 were instead both well represented.

The distribution of genetic clusters identified in the STRUCTURE analysis was further supported by the DAPC analysis. The distributions of the six clusters in multivariate space (Figure 4A) correspond to the spatial structure suggested by the STRUCTURE analysis – namely, that three clusters (Cluster 3, Cluster 4 and Cluster 6) exhibit distinct variation while the three remaining clusters overlap in multivariate space (Figure 4B).

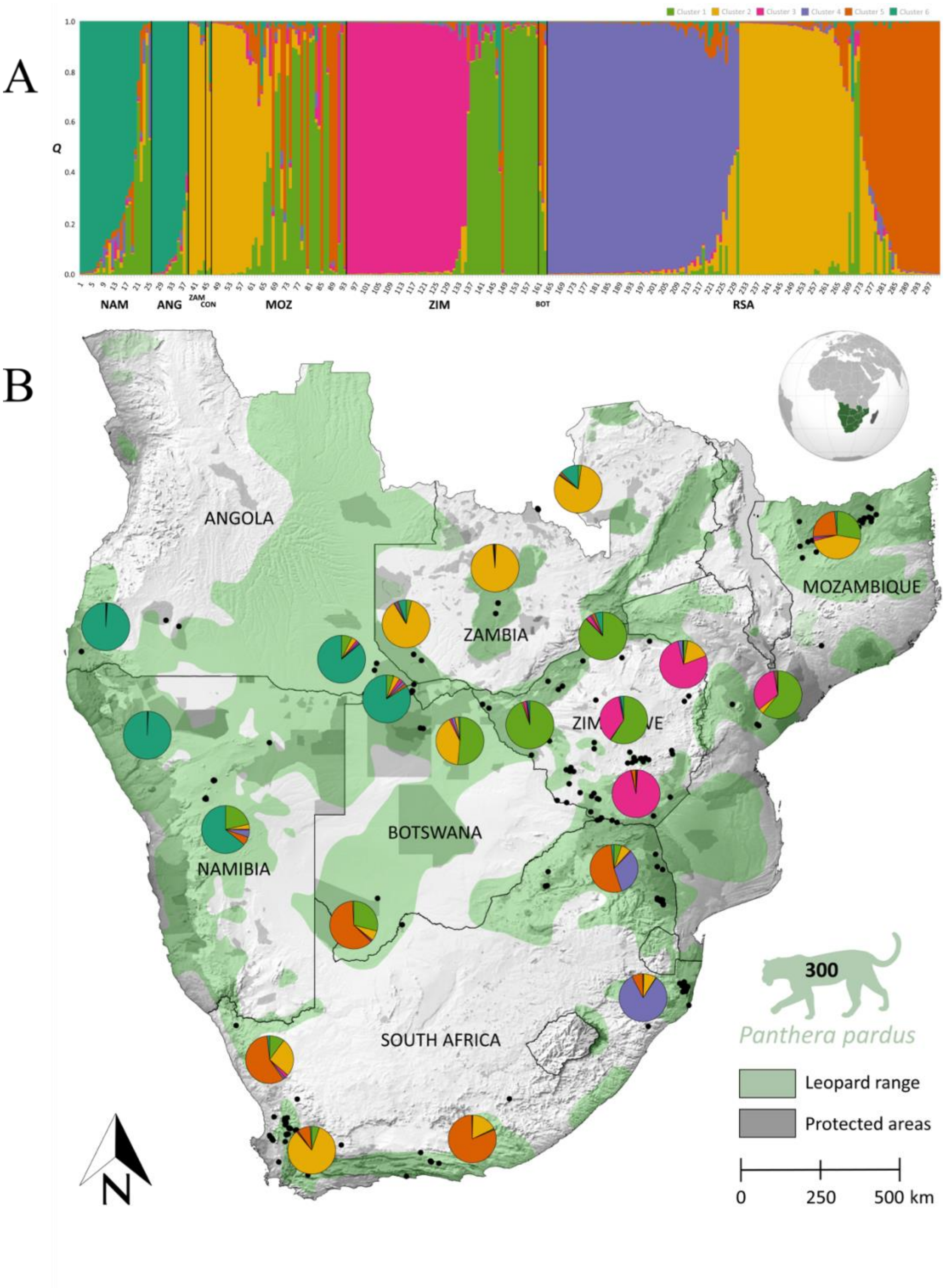

Figure 3: Genetic structure of leopards across southern Africa. (A) STRUCTURE plot where the vertical bars represent individuals grouped by country. Bars are colour coded by the percentage of membership (Q) to the clusters (K) identified. (B) Sampling locations across southern Africa. Pie charts represent the proportions of Q.

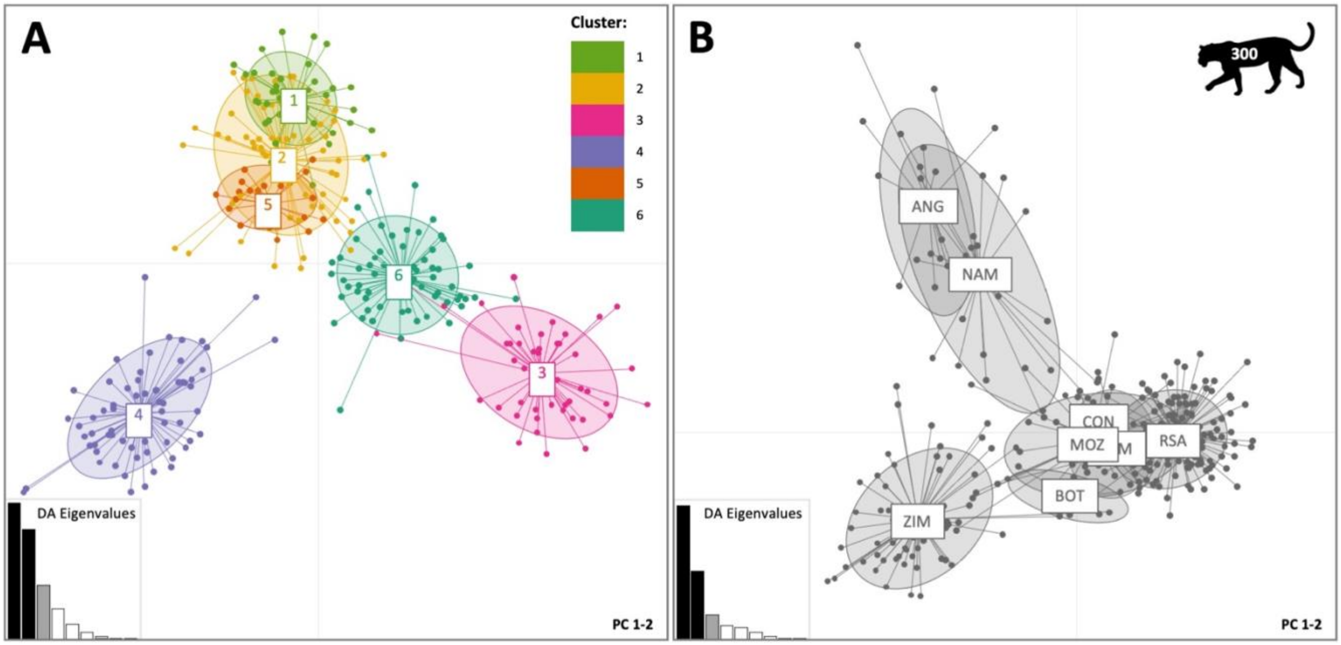

Figure 4: Discriminant analysis of Principal Components (DAPC) based on southern African genotypes. (A) DAPC clusters using an *a priori* number of defined clusters following the STRUCTURE analysis (*K* = 6). Data points represent individual genotypes. The first two principal components are visualised here (B) DAPC clusters by country of sample origin.

Pairwise F_{ST} values indicate low to moderate genetic differentiation between countries (Table 2). The largest values (0.17 – 0.19) occur between countries with a paucity of samples, and so may not be indicative of the true differentiation between countries. The F_{ST} values between 0.03 – 0.10 among South Africa, Mozambique and Zimbabwe suggest low differentiation – likely caused by gene flow between these three adjacent countries. Interestingly, the pairwise F_{ST} values between Namibia and Mozambique (0.06) and Namibia and Zimbabwe (0.07) also suggest relatively low differentiation, despite not being contiguous. Countries as sampling units themselves explained very little of the total genetic variation found within leopards across southern Africa (AMOVA, 0.31% of variation, $\varphi = 0.00$, $p = 0.24$). More variation can instead be attributed to between sampling areas (5.58% of variation, $\varphi = 0.06$, $p = < 0.001$) and between individuals within sampling areas (26.10% of variation, $\varphi = 0.28$, $p = < 0.001$). The majority of the variation in the genetic data arose from individuals (68%, $\varphi = 0.32$, $p = < 0.001$).

Table 2: Pairwise F_{ST} estimates with 95% confidence intervals (10,000 bootstraps) by country. * indicate countries where interpretation of results is difficult owing to their small sample sizes.

	Angola*	**Botswana***	**Congo***	**Mozambique**	**Namibia**	**South Africa**	**Zambia***	**Zimbabwe**
Angola*	-	-	-	-	-	-	-	-
Botswana*	**0.17** (0.13 - 0.21)	-	-	-	-	-	-	-
Congo*	**0.03** (0.00 - 0.08)	**0.09** (0.00 - 0.21)	-	-	-	-	-	-
Mozambique	**0.11** (0.08 - 0.14)	**0.11** (0.06 - 0.16)	**0.02** (0.00 - 0.06)	-	-	-	-	-
Namibia	**0.04** (0.02 - 0.06)	**0.12** (0.07 - 0.17)	**0.00** (0.00 - 0.04)	**0.06** (0.04 - 0.08)	-	-	-	-
South Africa	**0.14** (0.11 - 0.16)	**0.13** (0.07 - 0.19)	**0.04** (0.00 - 0.09)	**0.03** (0.02 - 0.05)	**0.07** (0.06 - 0.09)	-	-	-
Zambia*	**0.18** (0.13 - 0.22)	**0.19** (0.09 - 0.28)	**0.00** (0.00 - 0.00)	**0.09** (0.06 - 0.12)	**0.12** (0.08 - 0.16)	**0.12** (0.07 - 0.15)	-	-
Zimbabwe	**0.08** (0.06 - 0.12)	**0.13** (0.09 - 0.17)	**0.04** (0.01 - 0.08)	**0.07** (0.05 - 0.09)	**0.07** (0.05 - 0.09)	**0.10** (0.07 - 0.13)	**0.15** (0.11 - 0.18)	-

4. Discussion

Studies of leopard population genetic structure with good sampling coverage across Africa are limited (McManus et al. 2015; Ropiquet et al. 2015; Pečnerová et al. 2021). This is the first study to describe contemporary genetic variation and assess population-level structure across the breadth of the south-central and southern African region. The main aims of the study were (i) an assessment of how effective a single regional conservation unit is at conserving the distribution and structure of contemporary genetic variation, and (ii) an evaluation of the relative amount of genetic variation and structure captured within the geo-political boundaries of countries in the study region. The results presented here provide consilient evidence for a degree of regional structure, revealing several spatially distinct genetic clusters within southern Africa's leopards. Bayesian clustering analysis supports the presence of six genetic clusters in the region, and was supported by a multivariate DAPC analysis (see Appendix Figure A2). Together these analyses identify that Angola/ Namibia and Zimbabwe contain genetic clusters largely unique to these countries, and that South Africa also contains a unique regional genetic cluster. The remaining three genetic clusters occur in different proportions throughout Botswana, Zambia, Congo, Mozambique, Zimbabwe and South Africa. The results also demonstrate that countries in the study area vary in the amount of genetic variation they capture, and this has implications for the effectiveness of range states as contributary conservation units.

Comparisons with published data

Additional support for the presence of meaningful spatial genetic structure in southern Africa's leopards presented here comes through comparisons with the handful of previous microsatellite studies on the subject. Ropiquet et al. (2015) used a small microsatellite data set of eight genetic markers and 145 samples from South Africa and Mozambique (and one additional sample from southern Zimbabwe), and found support for two STRUCTURE-informed clusters – one cluster for all the individuals from South Africa's KwaZulu-Natal area, and one cluster to which all other individuals sampled were assigned. The identification of a single unique cluster in KwaZulu-Natal in Ropiquet et al's study (2015) concurs with the results presented here indicating a differentiated signal from KwaZulu-Natal, while clustering analysis also indicated that South African samples from both the Western and Eastern Capes and Limpopo share the same (two) lineages as found within Niassa province in northern Mozambique (Ropiquet et al.'s 2015 Mozambican samples were also sourced from Niassa province; see Figure 3B). It should be noted, however, that the limited sampling from the Ropiquet et al. (2015) analysis could be obscuring additional population structure. The absence of sampling in Zimbabwe (with the exception of a single sample from the south) in particular means that caution need to applied when interpreting these results.

McManus et al. (2015) also used a small microsatellite data set of 14 genetic markers and 40 individuals to conduct an even finer-scale spatial genetic analysis of population structure across two provinces within South Africa, the Western and the Eastern Cape. Using Bayesian clustering the authors identified three distinct 'genetic populations' – one that spanned the two provinces, and two smaller ones within the Western Cape (McManus et al. 2015). The current study reports that South Africa contains a high diversity of STRUCTURE-informed microsatellite clusters/ lineages, with the Western and Eastern Cape dominated by three different lineages, one of which is unique to the Western Cape (Cluster 1; Figure 3B). Additional sampling in these provinces is required to provide a more informative finer-scale picture of South African leopard population genetic variation as attempted by McManus et al. (2015).

Most recently, (Pečnerová et al. 2021) report a degree of population structure across the African continent by analysing a spatially limited genomic data set from 53 leopards representing ten locations in Ghana, Namibia, Tanzania, Uganda and Zambia. A Principal Components Analysis differentiated the Namibian and Zambian individuals, also reflected in the current study. The authors also estimated individual genomic admixture proportions for their samples, concluding that Namibia represented a unique ancestral lineage, and that Zambia was a mixture of the Namibian and Tanzanian lineages. Caution is required when considering these results given the small sample sizes (Namibia, n = 13; Zambia, n = 17; western Tanzania , n = six; eastern Tanzania, n = 3) and spatially restricted sampling, however the results are intriguing given that the current study identifies a proportion of the Namibian/ Angolan lineage in the western Zambian samples (Figure 3B); unfortunately samples from Tanzania were not available for the current study.

The findings presented here provide the most detailed view to date of the southern African landscape with respect to leopard population genetic structure. The degree of admixture (i.e. shared ancestry) presented in Figure 3B suggests a lack of large landscape-scale barriers to gene flow within the region and a spatial landscape resistance analysis of the data is likely to provide valuable insight to how leopards perceive barriers to their movement across both small and large scales. The FST analysis clearly suggests that at certain spatial scales mechanisms are acting to promote sufficient genetic drift to genetically differentiate populations. Isolation-by-distance (IBD) is one possible mechanism behind this differentiation (Meirmans 2012). Male leopards can be “mega-dispersers” (Fattebert et al. 2013), and capable of persisting within landscapes heavily modified by human activity (Odden et al. 2014; Devens et al. 2021). Evidence for IBD among African leopard populations has previously been reported by Ropiquet et al. (2015) using a subset of the data analysed here – and it is for this reason that the present study did not test for IBD. Further analysis to confirm Ropiquet et al.’s (2015) findings using a larger data set, to quantify gene flow between populations and assess whether IBD is the principle mechanism behind population differentiation, would be highly valuable to disentangling the processes responsible.

The implications for the southern African region as a single CU for leopards

In summary, the results presented here suggest a degree of genetic heterogeneity of ancestral lineages throughout the sampled landscape. This has important implications when considering the southern African region as a single CU. While it is true that a single CU would encompass all these lineages – vis-à-vis an ‘umbrella’ conservation effect - the substantial variation among country-specific conservation policies in the region (see Table 3) is likely to stymie regional-scale conservation goals. Individual countries are guided by different principles with respect to biodiversity conservation and pursue divergent policies towards leopards. This would be of less concern under a scenario of minimal to no genetic population structure – in that instance, with respect to genetic metrics, all countries would be managing a large, shared proportion of the regional gene pool and as such the failure of one country to protect variation could be offset by the successes of another. However, with the presence of distinct genetic lineages that are not uniformly represented by countries, such redundancy does not exist. Mozambique and Zimbabwe are the only source of Cluster 3 throughout the landscape, yet both have declining leopard population trends (Jacobson et al. 2016). Should all leopards within southern Africa be managed under a single CU, then these leopards would have the same conservation value as leopards in Namibia, where leopard trends are increasing but belong to an entirely separate genetic lineage. This clearly makes little sense and defeats the purpose of the designated conservation unit (Mee et al. 2015).

Table 3 – Conservation status of leopards across southern Africa (summarised from IUCN SSC Cat Specialist Group 2018; Jacobson et al. 2016)

Country	Legal protections
Angola	- Extant range of 678,000 km^2 (8.9% protected) - Population trend: Declining - Party to CITES and CMS - Legal protection: N/A - Trophy hunting: prohibited - 2021 Export quota: N/A
Botswana	- Extant range of 367,200 km^2 (29.3% protected) - Population trend: Unknown - Party to CITES - Legal protection: partial; can be hunted with a license or permit; or in defence of a human life or property - Trophy hunting: suspended as of 2013 - 2021 Export quota: 130
Lesotho	- Extant range is unknown; possibly extinct - Party to CITES - Legal protection: N/A - Trophy hunting: N/A - 2021 Export quota: N/A
Malawi	- Extant range of 11,100 km^2 (69.7% protected) - Population trend: Unknown - Party to CITES and CMS - Legal protection: N/A - Trophy hunting: prohibited - 2021 Export quota: 50
Mozambique	- Extant range of 457,000 km^2 (14.6% protected) - Population trend: declining - Party to CITES and CMS - Legal protection: N/A - Trophy hunting: Allowed between April – November - 2021 Export quota: 120
Namibia	- Extant range of 568,900 km^2 (18.8% protected) - Population trend: stable and increasing - Party to CITES

	- Legal protection: protected; can be hunted with a permit; or in defence of a human life or livestock - Trophy hunting: allowed - 2021 Export quota: 250
South Africa	- Extant range of 401,300 km^2 (8.4% protected) - Population trend: declining - Party to CITES and CMS - Legal protection: protected; require permit to hunt, kill, import or export - Trophy hunting: allowed - 2021 Export quota: 150
eSwatini (formerly Swaziland)	- Extant range of 10,100 km^2 (4.5% protected) - Population trend: Unknown - Party to CITES and CMS - Legal protection: N/A - Trophy hunting: prohibited - 2021 Export quota: N/A
Zambia	- Extant range of 218,000 km^2 (24.1% protected) - Population trend: declining - Party to CITES - Legal protection: N/A - Trophy hunting: allowed - 2021 Export quota: 300
Zimbabwe	- Extant range of 160,000 km^2 (16.7% protected) - Population trend: declining - Party to CITES and CMS - Legal protection: N/A - Trophy hunting: prohibited - 2021 Export quota: 500

With respect to conservation policy, leopards occurring in separate countries can be considered as discrete units that are subject to different management strategies and protections (Kennedy et al. 2009). Yet, the findings presented here (both Figures 3 and 4) clearly demonstrate how, in the case of leopards, countries should not be considered as discrete units if the conservation of genetic variation (among other metrics used to ensure the persistence of the species) is considered. The AMOVA and pairwise F_{ST} results provide further support for this argument: countries as sampling units did not capture a large proportion of the genetic diversity present (in fact, countries captured very little of the total genetic variation – 0.31%). To this end, while some countries may present more enticing opportunities for conservation than others (see H_O in Table 1), countries cannot be considered as units for conservation as genetic variation does not correlate to geopolitical boundaries. Lineages, therefore, overlap multiple countries, and will be subject to a patchwork of policies as applied at the country level. If genetic goals are included in regional conservation efforts this is clearly far from ideal.

Developing a more nuanced leopard CU informed by multiple metrics of current status

In light of the genetic lineages distributed through southern Africa, conservation units should aim to preserve as many of these as possible. Of these lineages, three are spatially explicit within southern Africa (Clusters 3, 4, and 6); the other three (Clusters 1, 2 and 5) have overlapping spatial extents. A leopard CU that incorporated genetic metrics should therefore seek to consider various combinations of these lineages to maximise the number of lineages conserved.

Conservationists pursue two main approaches when developing conservation units – they either place a premium on genetic divergence or on phenotypic variation (Figure 1). The former approach requires a combination of population genetic, phylogeographic and phylogenetic analyses , and so shall only be briefly discussed. The data presented here (Figure 3) demonstrates that leopards from Namibia share a greater degree of ancestry with those from Angola and leopards from South Africa. Likewise, leopards through Mozambique, Zimbabwe, Botswana and South Africa all have more shared ancestry than leopards from Zambia. Phylogenetic and phylogeographic analyses could further be conducted to consider the evolutionary histories, processes and relationships between lineages and use this information to aid in prioritising lineages for conservation.

CU approaches that place less emphasis on genetic divergence can also be considered from the results presented here. Subspecies are the natural progression to consider for CU delineation when considering phenotypic variation (Figure 1). Notionally, conservation considered at the subspecies level would be an improvement as it would allow for more targeted conservation of the genetic diversity existing within a species (Hoban et al. 2020; although, again, issues with taxonomic classification -

Garnett & Christidis 2017). Molecular phylogenetics has discovered cryptic evolutionary partitions in species of conservation interest before (Karl & Bowen 1999), and it is quite possible that there might exist cryptic subspecies within Africa – leopards in South Africa's Western Cape have long been suspected as being taxonomically distinct (Martins & Martins 2006) although genetic data have not supported this (Ropiquet et al. 2015). Nonetheless, while it is easy to delineate conservation units by subspecies existing in distinct habitats – Javan leopards (*P. p. melas*), for example – it is far more difficult to do so when subspecies are either not recognised or share overlapping geographical ranges (Uphyrkina et al. 2001). In these instances, CU delineation is made by habitat or land use type (Sanderson et al. 2002). These non-taxonomic CUs will always lead to questions surrounding their suitability, and how well they might capture gene flow and migration (Wultsch et al. 2016).

It can be seen from Figure 3 how lineages transcend international borders – a stark reminder that landscapes are biologically more relevant than countries. TFCAs represent an attractive vehicle in this case for achieving conservation of southern African leopard lineages. Within southern Africa, there are five distinct landscapes containing leopards which traverse international borders (as defined by Jacobson et al. 2016; Table 4) and a number of these transfrontier leopard habitat patches could provide cover for multiple lineages – the Central southern Africa and coastal Namib patch, for example. Further extending the sampling coverage will undoubtedly improve our understanding of whether genetic clines exist between current sampling locations – such as between central Namibia and northern South Africa. The largest TFCA in southern Africa, the Kavango-Zambezi (Dures et al. 2019), would provide coverage for Clusters 1, 2 and 6; the Great Limpopo TFCA could potentially provide coverage for Clusters 3, 4 and 5 although it is not clear whether how well some of these lineages (Cluster 4, for example) might be represented. While there is precedent for using landscape-scale CUs for wide-ranging felids (Sanderson et al. 2002, 2010; Li et al. 2020), consideration of TFCAs as CUs in Africa is not without its own issues. Namely, the policy framework of TFCAs remains an ongoing question mark as to the efficacy of dictating and enacting policies that require multiple geopolitical actors to sign off on (Cumming 2011; Linell et al. 2019). TFCAs are, however, limited by their geographical coverage – and TFCAs cannot provide coverage for central Zimbabwe, for example.

Table 4 – Transboundary leopard habitat patches within southern Africa (Jacobson et al. 2016)

Patch name	List of countries covered by patch
Central southern Africa and coastal Namib	Angola, Botswana, Democratic Republic of Congo, Mozambique, Malawi, Namibia, South Africa, Zambia & Zimbabwe
Marromeu and central Mozambique	Mozambique & Zimbabwe
Matopos and south western Zimbabwe	Botswana & Zimbabwe
Kruger and eastern southern Africa	Botswana, Mozambique, South Africa, Eswatini (Swaziland) & Zimbabwe
Drakensberg mountains	Lesotho & South Africa

Identifying a role for TFCAs in the development of a more nuanced CU approach for leopards would allow for CUs to conserve spatial units that are informed by multiple ecological and evolutionary processes. Nevertheless, CUs are constrained by reality and as such the issue to consider here is less to do with ecology and evolution, than it is to do with geopolitical realities.

Practical considerations for leopard conservation

Quite simply, the policy framework needed to conserve the different lineages of southern African leopards does not exist. It is also – perhaps crucially – not likely to materialise in the near future. This leaves conservationists with a choice, one all too familiar when having to navigate scientific facts with geopolitical realities (Hodgetts et al. 2018): which solution offers the least worst outcome and that is workable within the existing policy framework?

TFCAs may provide good coverage of landscape ecological functions, but they are ultimately hamstrung by virtue that they exist at the nexus of competing geopolitical interests. These interests can be seen in full display through the submission and/ or defeat of motions during the Conference of the Parties to MEAs such as CITES and CMS. TFCAs require unanimous consent for policies to be enacted (Linell et al. 2019) and a clear-eyed view of the political landscape would suggest that TFCA CUs would likely be victim to the same recriminations that arise from politically contentious conservation policies based on science (i.e., the decision in 2017 by Uganda, Tanzania, Zimbabwe & South Africa to oppose listing the leopard under CMS Appendix II; Lewis & Trouwborst 2019).

The reality is that countries – despite capturing very little of the total genetic variation of leopards across southern Africa – represent a far better CU for leopards than either the entire southern African region or even TFCAs. This is a practical reality, rather than a scientific one – the ability for countries to be able to dictate, monitor, and enforce a single policy across a unit outweighs the costs that will come from being unable to provide coverage for entire lineages.

Within southern Africa, it is possible to consider the relative value of individual countries as CUs based upon indices of genetic diversity (Table 1), their extant leopard habitat (Table 3), and the number of lineages captured by the country. Angola and Namibia, for example, despite having unique genetic diversity and large amounts of extant leopard range, are not in and of themselves particularly diverse. Mozambique has large amounts of leopard range, with 14.6% of this protected, has high genetic diversity (H_O of 0.76) and represents four of the six lineages. South Africa has less suitable leopard range than Mozambique, yet also has high diversity (H_O of 0.70, despite not being in HWE) and four lineages represented. Zimbabwe has less extant leopard range and is significantly out of HWE (H_O of 0.52, deviating from an H_E of 0.82). That the country is a stronghold for Cluster 3 suggests the country should be considered a priority for leopard conservation given that the leopard populations inside the country appear to be struggling.

Lastly, regional and local leopard conservation is fundamentally influenced by the reality that 62% of leopard habitat lies outside of PAs (Jacobson et al. 2016). While this fact is often used to underscore the need for landscape-scale conservation, the corollary to this is often overlooked: leopard habitat within PAs is a constant amid an uncertain and rapidly changing landscape driven by global change and a growing human population (Serdeczny et al. 2017; United Nations 2019). PAs are designated by Governments (McNeely 1994), and countries within southern Africa have a disproportionate responsibility for leopard range compared to the rest of Africa (Jacobson et al. 2016; IUCN SSC Cat Specialist Group 2018). Within each country, a different proportion of leopard habitat is captured by PAs – and the landscapes covered by these PAs are also very different (Naude 2020; Appendix Figure A3). Namibia and Angola, for example, are widely divergent in the proportion of leopard range protected by PAs. While 46% of leopard range is protected in Namibia, only 9% is protected in Angola (Naude 2020). In Zambia, 75% of leopard range is protected. Interestingly, while South Africa is often touted as a fortress for leopard conservation (Balmford 2003; Balme et al. 2014), the country actually captures very little leopard range within PAs. A comparison of the protective value of relative landscapes is presently lacking from the current debate around leopard conservation. As countries are considered as the forefront option for leopard CUs, this is a key gap in our current knowledge of leopard conservation.

Conclusions

An understanding of the genetic diversity and structure underpinning populations is crucial in order to both contribute to their likely persistence into the future and contribute to defining biologically meaningful conservation units. This study identified that within southern African leopards, there is a degree of unique spatial genetic structure consisting of six distinct genetic populations. This structure suggests that the entirety of the southern African region is not appropriate as a conservation unit; because policy is dictated by the smaller geopolitical range states in the region, leopard conservation management will be heterogenous across the conservation unit . This study also provides evidence that some range states are characterised by moderate genetic differentiation, such that they do not each capture the same proportion of the regional variation currently present in southern Africa’s leopards. From an ecological perspective, Transfrontier Conservation Areas (TFCAs) would be valuable as leopard conservation units, following in the footsteps of other felids. Nonetheless, it is also argued here that the application of policy across TFCA conservation units – which likely would provide the best coverage for the different genetic lineages identified in this study – is likely to be a cause for some concern given geopolitical tensions over leopard conservation (Lewis & Trouwborst 2019). Conservation units are as much a recognition of the political and social conditions impacting biodiversity as they are the biological ones. In light of the reality that an existing framework across which biologically-meaningful leopard conservation policy can be applied does not exist, this study concludes that countries are likely to have the most success as leopard conservation units within southern Africa when including a genetic perspective in their development and application.

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APPENDIX

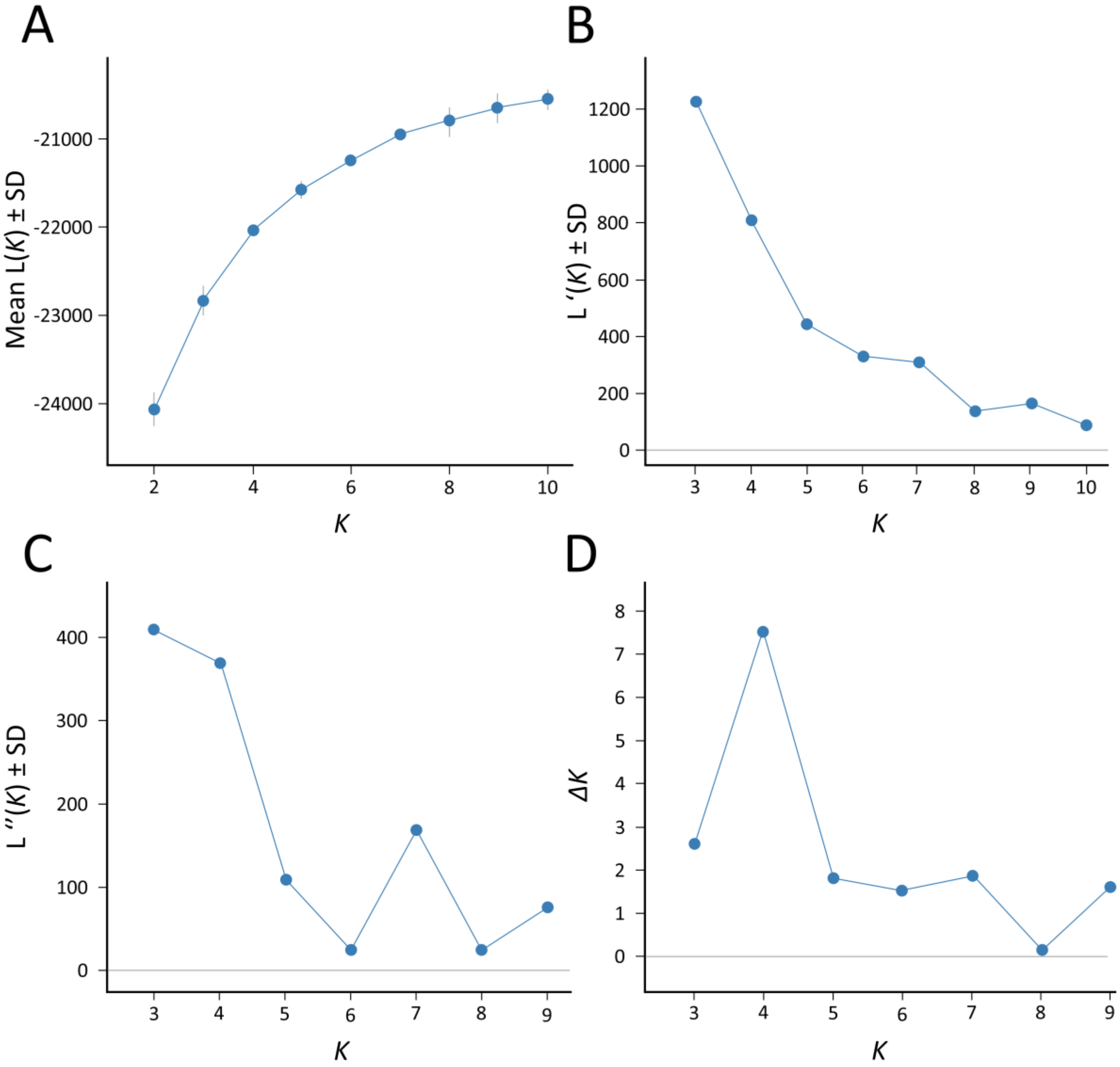


Figure A1: STRUCTURE HARVESTER outputs. Plots showing: (A) Mean likelihood (*K*); (B) rate of change of likelihood; (C) absolute values of the 2nd order rate of change of the likelihood ;(D) Δ*K*.

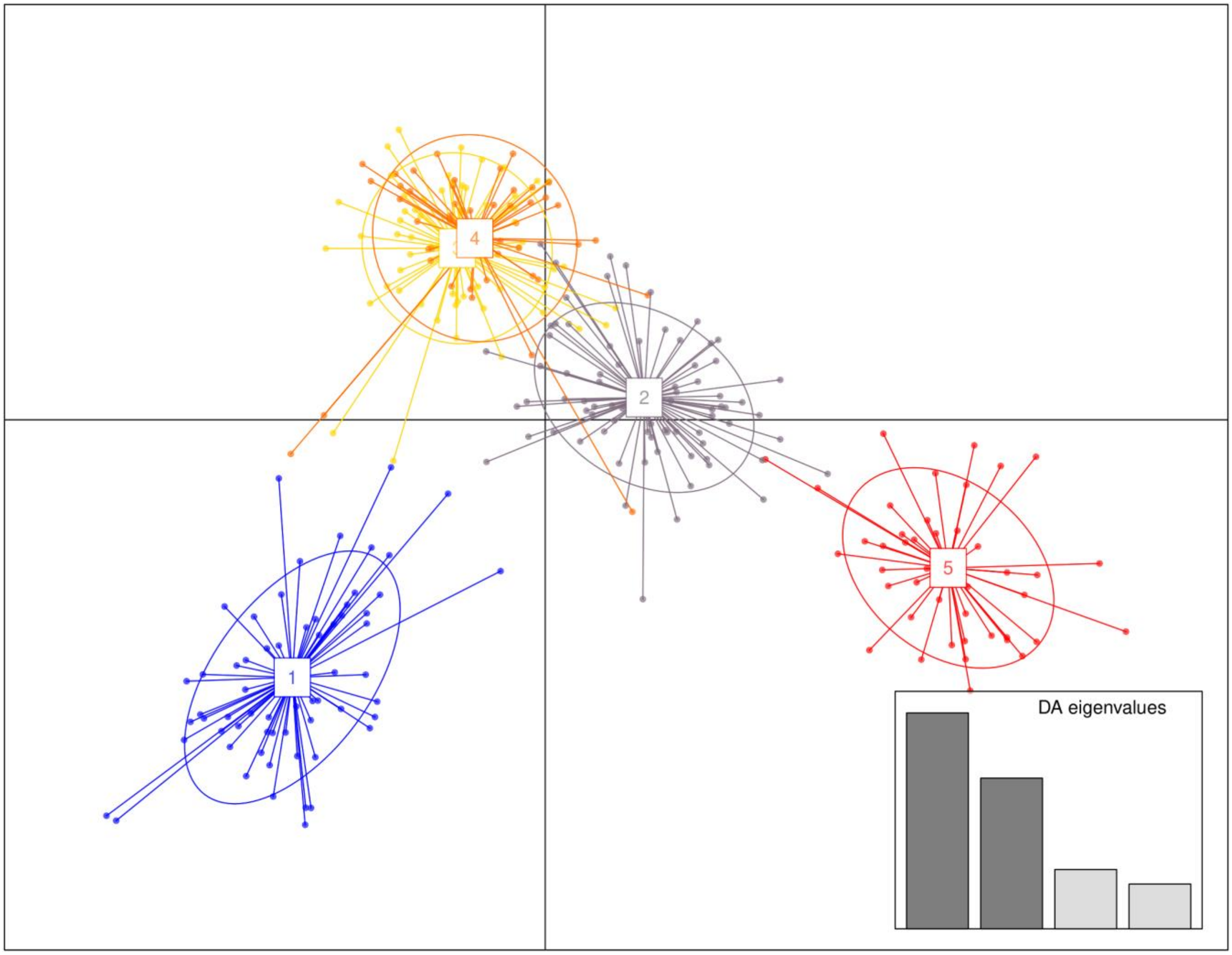
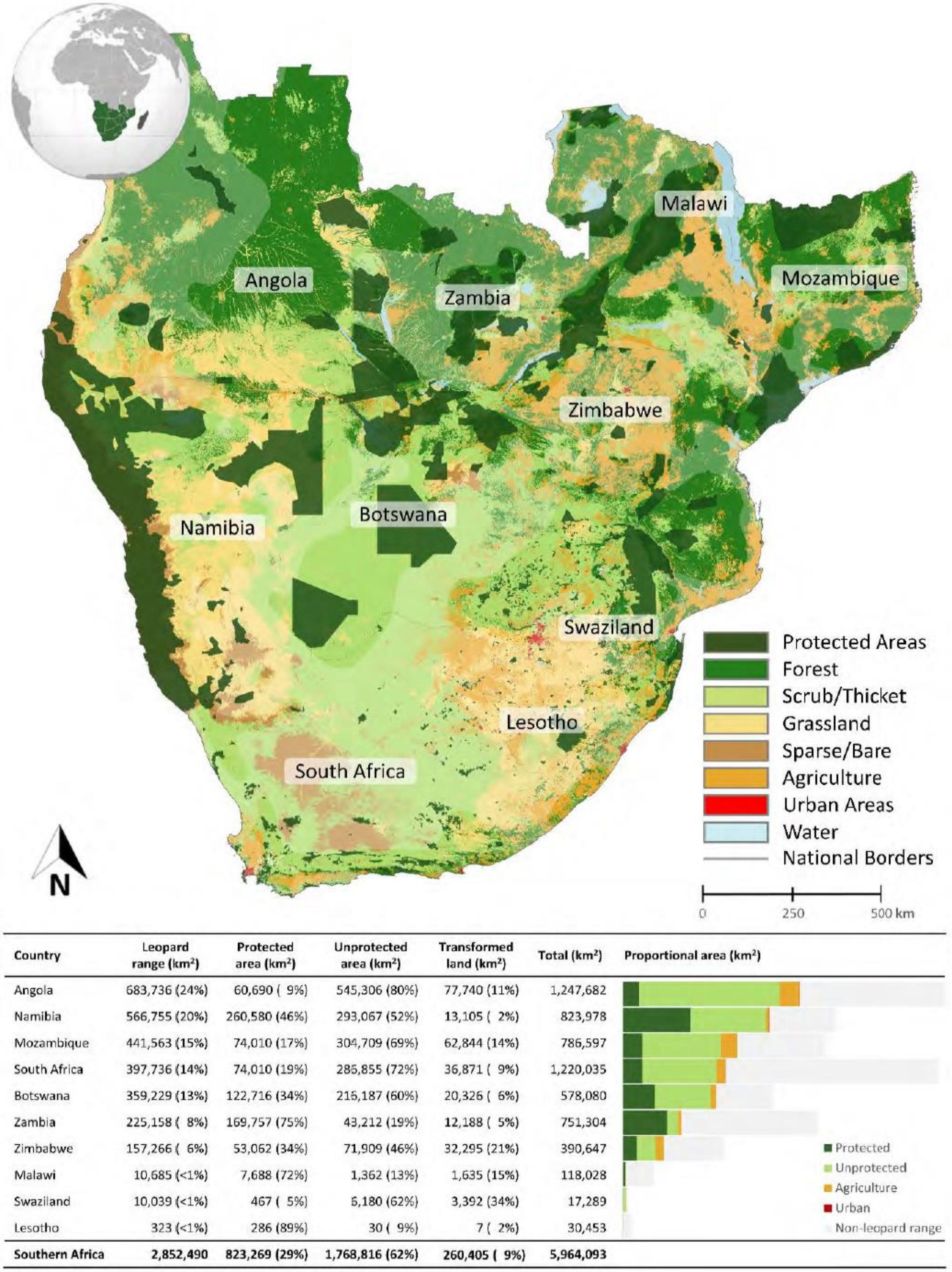


Figure A2: DAPC analysis with 5 clusters. Individual assignments confirmed that Clusters 1, 2 and 5 still conformed to Clusters 4, 6 and 3 from the DAPC plot with 6 clusters.



Country	Leopard range (km²)	Protected area (km²)	Unprotected area (km²)	Transformed land (km²)	Total (km²)	Proportional area (km²)
Angola	683,736 (24%)	60,690 (9%)	545,306 (80%)	77,740 (11%)	1,247,682	
Namibia	566,755 (20%)	260,580 (46%)	293,067 (52%)	13,105 (2%)	823,978	
Mozambique	441,563 (15%)	74,010 (17%)	304,709 (69%)	62,844 (14%)	786,597	
South Africa	397,736 (14%)	74,010 (19%)	285,855 (72%)	36,871 (9%)	1,220,035	
Botswana	359,229 (13%)	122,716 (34%)	216,187 (60%)	20,326 (6%)	578,080	
Zambia	225,158 (8%)	169,757 (75%)	43,212 (19%)	12,188 (5%)	751,304	
Zimbabwe	157,266 (6%)	53,062 (34%)	71,909 (46%)	32,295 (21%)	390,647	
Malawi	10,685 (<1%)	7,688 (72%)	1,362 (13%)	1,635 (15%)	118,028	
Swaziland	10,039 (<1%)	467 (5%)	6,180 (62%)	3,392 (34%)	17,289	
Lesotho	323 (<1%)	286 (89%)	30 (9%)	7 (2%)	30,453	
Southern Africa	**2,852,490**	**823,269 (29%)**	**1,768,816 (62%)**	**260,405 (9%)**	**5,964,093**	

Figure A3: Leopard habitat within southern Africa ranked by country, and the proportion covered by land use (Taken from Naude, 2020; used here with permission).