Filling the sampling gaps: conservation genetics of the leopard tortoise (*Stigmochelys pardalis*)

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Introduction

With 20 currently recognized tortoise species, sub-Saharan Africa harbours an exceptional chelonian richness and diversity which has not yet been fully explored (Branch 2007; Fritz & Havas 2007; Kindler et al. 2012; Hofmeyr et al. 2017). In South Africa, tortoises represent an important part of the local fauna (Branch 1998; Boycott & Bourquin 2000). The most common and the largest tortoise species in sub-Saharan Africa inhabiting a wide range of environmental conditions (Fig. 1), is the leopard tortoise (*Stigmochelys pardalis* Bell, 1828). The species' distributional range extends from the Horn of Africa across eastern Africa to the Republic of South Africa, Namibia and southernmost Angola (Fig. 1; Iverson 1992; Ernst et al. 2000; Fritz & Havas 2007; Fritz et al. 2010; Spitzweg et al. 2019). Across its range, *S. pardalis* is heavily affected by extensive habitat modification, destruction and pollution. In addition, it is frequently collected for human consumption and commercial trade (Evans 1988). The once widespread species is now considered threatened and has already disappeared in some areas of South Africa (Hofmeyr & Baard 2015). Bush fires (Kabugumila 2001), severe droughts and road mortality are the biggest contributors to their injuries and population decline (Boycott & Bourquin 2000). Its highly domed shell makes this species particularly vulnerable to electric fencing (Fig. 2; Beck 2010), which is common in farms to control predation on livestock (Burger & Branch 1994; Arnot & Molteno 2017; Macray 2017). The majority of these tortoises occur on fenced-up privately owned farm land, national parks and reserves. South Africa has an estimated 90,000km of game fencing, the bulk of which lies in the provinces of North West, Limpopo, Mpumalanga and Natal (Falkena & Van Hoven 2000). Thus, electric fences represent a major threat and driver of population decline in leopard tortoises (KZN Wildlife 2004).

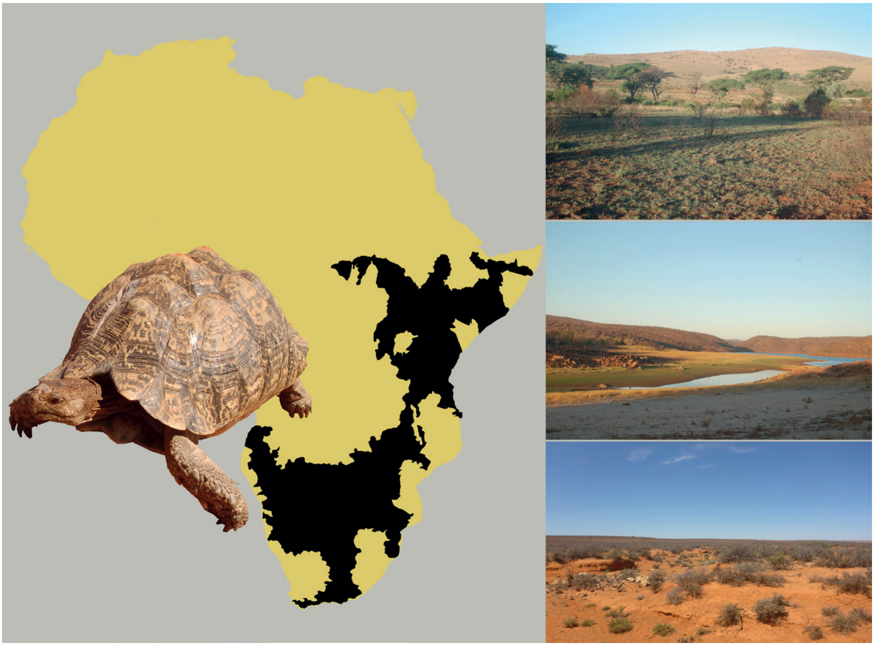

Fig. 1. Left: distribution range (black) of the leopard tortoise *Stigmochelys pardalis* according to TTWG (2017) and modified after Spitzweg et al. (2019). Right: *Stigmochelys* habitats in South Africa. All photos by M. Vamberger.

The leopard tortoise expresses considerable geographical variation in body size, shell shape and colouration (Fig. 3). Loveridge and Williams (1957) attributed these differences to subspecies variation, while Lambert et al. (1998) suggested phenotypic plasticity as a response to local environmental conditions. The traditional assignment to two distinct subspecies, namely *S. p. pardalis* (Bell 1828), restricted to southwestern Africa, and *S. p. babcocki* (Loveridge 1935) inhabiting the rest of the distributional range, was initially supported by a molecular genetic study of mitochondrial markers (Le et al. 2006). However, Fritz et al. (2010) demonstrated that Le et al. (2006) aligned a chimerical sequence of a pseudogene, which was erroneously identified as *S. p. babcocki*. Fritz et al. (2010) identified seven genetically distinct lineages, of which five occur in the southern portion of the species distributional range, hypothesizing that the species originated in southern Africa. The distributions of these seven lineages do not match the recognized subspecies nor does it reflect the geographic size variation. The division into two subspecies thus became no longer tenable (Fritz et al. 2010). These findings stress a considerable lack of knowledge regarding the evolutionary history of *S. pardalis*, which is a fundamental basis for taxonomic amendments and thus

Fig. 2. Electric fences represent a major threat for leopard tortoises. A) Living tortoise trying to cross an electric fence. B) Shell of electric fence killed individual. Pictures taken at the Addo Elephant National Park, South Africa.

Fig. 3. Left: adult male leopard tortoise measuring 46cm in carapace length (CL) with a uniformly coloured shell from Graaff-Reinet. Right: adult male (32cm in CL) displaying a vivid colouration pattern from the Kgaswane Nature Reserve.

conservation management actions. They also demonstrate the importance of the application of conservation genetic techniques in preserving the genetic diversity of a species. Furthermore, the release of confiscated tortoises is not permitted in state protected areas of many South African provinces, but confiscated leopard tortoises are nonetheless frequently released to private

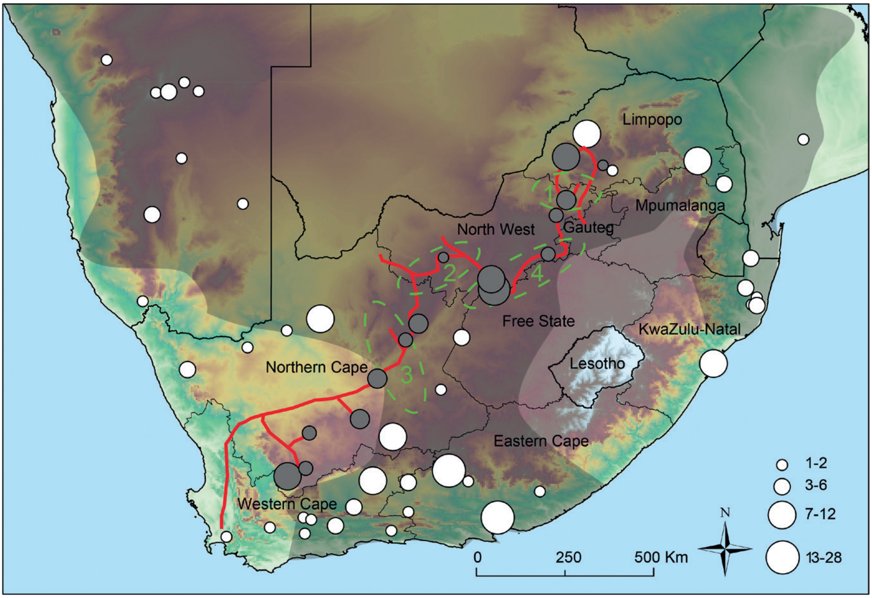

Fig. 4. Green areas marked with numbers: location of the selected study sites covering the sampling gaps from Spitzweg et al. (2019) in northern South Africa. White dots: samples available from Spitzweg et al. (2019). Grey dots: samples from the field trip. Red lines: approximate travel route from our field work supported by BCG and DGHT.

reserves without a documented release protocol (Wimberger et al. 2010), a practice that is questionable due to genetic variation among populations (Fritz et al. 2010; Wimberger et al. 2011).

In a recent study using nuclear microsatellite loci, Spitzweg et al. (2019) focussed on leopard tortoises from southern Africa in order to unravel recent gene flow among genetically distinct lineages and to establish a basis for conservation genetics. However, the study lacked sufficient data from northern central South Africa to fully cover the whole distribution range. The purpose of our study was to complete the existing dataset of Spitzweg et al. (2019), and to achieve the required coverage by closing sampling gaps in northern South Africa. Here, we deliver the essential background needed to develop appropriate guidelines for conservation release and relocation of the leopard tortoise in South Africa.

Methods

Study area

In order to complete the existing set of genetic samples published by Spitzweg et al. (2019) and to achieve a country-wide coverage we targeted the sampling gaps in northern South Africa (Fig. 4). The first study area covers the vicinity

Fig. 5. Field work methods for searching for leopard tortoises in South Africa. Searching for tortoises on foot (top left) or by car (top right), in the bush (bottom left) and near water (bottom right).

of Bela-Bela and Sun City, the second site covers the surroundings of Vryburg and Kuruman. Study area three stretches from Upington to Britstown and study area four covers the area around Benfontein and Johannesburg. Every tortoise encountered while travelling between the selected study sites was also processed.

Sample collection

Tortoises usually maintain a crepuscular daily activity, this being highest in the early morning (~6am-9am) and late afternoon (~4pm-7pm) depending on the weather conditions. Therefore, searches for tortoises were conducted in the early morning and late afternoon on foot and by car in November. However, to maximize success, we also searched during the day in the bushes where tortoises hide, and for drinking tortoises, near suitable water bodies (Fig. 5). For genetic analyses, a small amount of blood or tissue was collected from live tortoises in accordance with methods approved by the Ethics Committee of the University of the Western Cape (ethical clearance number (AR 19/4/1). Genetic samples have also been collected from dead specimens killed by motor vehicles and from dry shells encountered in the field. In addition, GPS coordinates were recorded, and standardized photographs were collected as

Fig. 6. Threats for leopard tortoises observed in South Africa. A) Habitat destruction by pollution in Limpopo Province. B) Leopard tortoise as a pet in a village in North West Province. C) Bush fire in Northern Cape Province. D) Bush fire victim from Bloemhof. E) Living leopard tortoise crossing a road in Limpopo Province. F) Road killed female in the Western Cape Province.

photo-vouchers. Each live tortoise was individually marked using a permanent marker and released at the exact locality where captured. All genetic samples were exported to Germany with the required documents in September 2019 and stored at -20°C. Fieldwork and sampling in South Africa was permitted by the Limpopo Provincial Government (ZA/LP/91608), the Department of Environmental Affairs, Biodiversity Northern Cape Province (245/2015), and Biodiversity North West Province (NW 6124/10/2018).

Table 1. Samples collected during the field trip supported by the BCG and DGHT.

Site	Number of individuals
South Africa: Northern Cape: Williston: Smitty Farm	6
South Africa: Northern Cape: Bovlei Farm	3
South Africa: Northern Cape: Southerland: Kanariesfontein	8
South Africa: Northern Cape: Carnarvorn: near Blaaukrantz Farm	7
South Africa: Northern Cape: Carnarvorn: Prieska	7
South Africa: Northern Cape: Griekwastad: Koekais Guest Farm	5
South Africa: Northern Cape: Papkuil: Gers Safari Lodge	6
South Africa: North-West: North of Vryburg	2
South Africa: North-West: Schweizer-Reneke	9
South Africa: North-West: Bloemhof Nature Reserve	11
South Africa: North-West: Highveld Nature Reserve	3
South Africa: North-West: Kgaswane Nature Reserve	2
South Africa: North-West: Vaalkop Dam Nature Reserve	6
South Africa: Limpopo: Reyneke Farm	10
South Africa: Limpopo: road to Mokolo Dam Nature Reserve	1

* For conservation purpose coordinates are not given.

Results and discussion

With 86 new samples of leopard tortoises from 15 localities, we successfully completed the sampling gaps of Spitzweg et al. (2019) in northern central South Africa (Table 1; Fig. 4). Despite suboptimal weather conditions, with no rain for months or even years in some localities, we have been able to obtain samples in more areas than originally proposed, especially in the southern part of the Northern Cape Province, and the western Limpopo (Fig. 4). Because of the unusually hot weather, we found most leopard tortoises near water bodies or hiding under the bushes (Fig. 5). We observed several of the mentioned threats for the tortoises (Fig. 6). For example, in the North West Province, a local village shaman and healer kept a leopard tortoise as a pet (Fig. 6B). Tortoises are spiritual creatures and play an important role in the mythology and religion of diverse cultures (Garfield 1986), including Africa. Furthermore, during the field work, we encountered six leopard tortoises killed by cars (Fig. 6E & F) and electric fences (Fig. 2), confirming that road mortality and electric fences are threats for this species (Boycott & Bourquin

2000; Beck 2010). We found four of what we believed to have been naturally dead tortoises in Northern Cape Province, possibly due to the extreme hot weather conditions with a lack of drinking water or other unknown causes. In addition to direct threats, severe droughts are considered responsible for population decline in this species (Boycott & Bourquin 2000). We found four burned leopard tortoises (Fig. 6D), three in the North West Province and one in Northern Cape. It is known that bush fires cause most injuries in this species (Kabugumila 2001).

For conservation genetics purposes, a dataset of 286 samples of leopard tortoises could be gathered for South Africa, including the existing data from Spitzweg et al. (2019). This comprehensive dataset now covers their whole distribution range in South Africa (Fig. 4) and can now be used in wildlife forensics of translocated or seized leopard tortoises in southern Africa, an important step towards successful conservation of this species. In the past, genetic assessments have been successfully used in other turtle and tortoise species, either to monitor translocation programmes (bog turtles *Glyptemys muhlenbergii*; Dresser et al. 2017), or to reveal genetic differentiation within a species (geometric tortoise *Psammobates geometricus*; Cunnimngham et al. 2002). With our study, we achieved the proposed goals, and delivered an essential framework for conservation management and preservation of genetic diversity of *S. pardalis*. In addition, we have been already able to establish a cooperation with the South African National Biodiversity Institute (SANBI) and the Gauteng Department of Agriculture and Rural Development (GDARD). Based on our comprehensive sampling of leopard tortoises from the field, we are in the process of setting up a joint collaboration, aimed at assigning confiscated tortoises to their respective geographic area of origin.

Additional output

In addition to our proposed project on *Stigmochelys*, we also gathered important new distribution data on the genus *Pelomedusa*. We provided the first evidence for the occurrence of the helmeted terrapin (*Pelomedusa galeata*) in the central part of the Northern Cape Province of South Africa, which was believed to be a natural distribution gap for this species (Vamberger et al. 2019). We published the data in Amphibian & Reptile Conservation in 2019 and acknowledged the BCG for the support.

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