Phylogenetic assessment and historical biogeography of the *Psammophis leightoni* complex

By

Jody M. Taft

Supervisor: **Dr Bryan Maritz**[1]

Co-supervisor: **Prof. Krystal A. Tolley**[2]

[1]Department of Biodiversity and Conservation Biology, University of the Western Cape, Bellville, Cape Town, South Africa

[2]South African National Biodiversity Institute, Kirstenbosch Research Centre, Private Bag X7, Claremont, 7735, South Africa

January 2018

DECLARATION

I, Jody M. Taft, declare that "**Phylogenetic assessment and historical biogeography of the *Psammophis leightoni* complex**" is my own work, that has not been submitted for any degree or examination at any other university, and that all the sources I have used or quoted have been indicated and acknowledged by means of complete reference
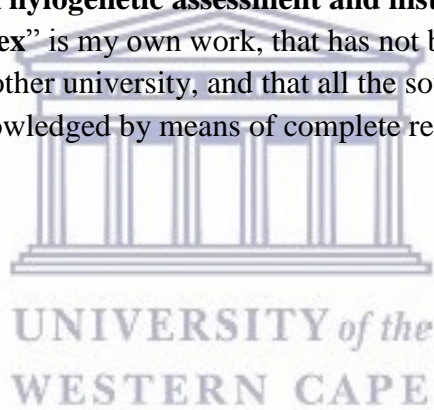
January 2018

ABSTRACT

Stable and accurate taxonomy remains a primary component of conservation. By overlooking taxonomic disorder, conservation management strategies may ineffectively distribute resources. The *Psammophis leightoni* species complex is one such example where taxonomic confusion may have an influence on the conservation of threatened species. *Psammophis leightoni*, *P. namibensis* and *P. trinasalis* were all elevated to specific rank on the basis of ecological differences largely attributed to where these taxa occur. A molecular revision of Psammophiinae highlighted that *P. leightoni* and *P. namibensis* show levels of intraspecific divergence; however, this was based on single representatives per putative species. *Psammophis leightoni* is currently considered Threatened and is listed as Vulnerable [B1ab(iii)], but the taxonomic uncertainty surrounding the *P. leightoni* complex influences how these taxa should be regarded in a conservation context. To remedy this, I aim to validate the taxonomic status of members of the *P. leightoni* complex using phylogenetic analyses and species distribution modelling (SDM) techniques. Maximum likelihood and Bayesian inference approaches were applied to ascertain the genetic relationships within the *P. leightoni* complex. Uncorrected p-distances were generated to assess the level of divergence between taxa of the *P. leightoni* complex relative to its African congeners. Furthermore, a General Mixed Yule Coalescent model and its Bayesian implementation were used as tree-based methods for species delimitation. Species distribution models were carried out using a maximum entropy approach that estimated the climate suitability for these putative taxa defining their distributions during the last glacial maximum, mid-Holocene and under current climatic conditions. The phylogenetic analyses recovered all individuals of the *P. leightoni* complex in a monophyletic clade. Furthermore, the study taxa show intraspecific level divergences between taxa of the *P. leightoni* complex and are suggested to collectively represent a single taxon based on the species delimitation analyses. Additionally, the species distribution models showed no difference between these taxa's spatial distribution suggesting that taxa of the *P. leightoni* complex are not ecologically distinct. Assuming the *P. leightoni* complex represents a single species, meeting the prerequisites of the unified general species concept, a taxonomic revision is necessary to assign the appropriate taxonomic rank. Both *P. namibensis* Broadley, 1975 and *P. trinasalis* Werner, 1902 should be considered synonyms of *P. leightoni* Boulenger, 1902. As a result, *P. leightoni* should be considered widespread and in need of a conservation reassessment, potentially removing its current threat status.

ACKNOWLEDGEMENTS

CONTENTS

LIST OF FIGURES

Figure 1.1: The distribution of the *Psammophis leightoni* species complex from Southern Africa extrapolated from Broadley (2002) and SARCA databases (see chapter 3 for details). *Psammophis leightoni* is highlighted in green, *P. namibensis* in blue, *and P. trinasalis* in yellow.

Figure 1.2: Morphology of the *Psammophis leightoni* complex compared to its sister species, *P. notostictus*. Body patterning: A – *P. leightoni*, B – *P. namibensis*, C – *P. trinasalis*, and D – *P. notostictus*. Head scales and patterning: E – *P. leightoni*, F – *P. namibensis*, G – *P. trinasalis*, and H – *P. notostictus*.

Figure 2.1: *Psammophis* species are morphologically conservative, evident by the slender body (A-F), head features (G-O) and patterning (P-R). Full body: A – *P. crucifer*, B – *P. brevirostris*, C – *P. notostictus*, D – *P. leightoni*, E – *P. namibensis*, F – *P. trinasalis*. Head morphology: G – *P. mossambicus*, H & K – *P. crucifer*, I & J – *P. brevirostris*, L – *P. notostictus*, M – *P. leightoni*, N – *P. namibensis*, O – *P. trinasalis*. Top-down: P – *P. brevirostris*, Q – *P. notostictus*, R – *P. crucifer*.

Figure 2.2: Sampling localities for all target taxa. Taxa are grouped by colour: Green – *Psammophis leightoni*, Blue – *P. namibensis*, Pink – *P. notostictus*, Yellow – *P. trinasalis*. Sub-clades are grouped by shape: Square – *P. leightoni* (South Africa), Circle – *P. namibensis* (Namibia), Diamond – *P. notostictus*, Triangle – *P. trinasalis*. . (i) – Study region depicted within Africa.

Figure 2.3: Bayesian Inference 50% majority-rule consensus phylogram from the concatenated dataset. Node support (ingroup only) is in the format: Bayesian posterior probability/ML bootstrap. Nodes not supported by either Bayesian or Maximum Likelihood analyses are denoted with a hyphen (-).

Figure 2.4: Frequency of uncorrected p-distances based on ND4 gene region for *Psammophis*. White bars: intraspecific uncorrected p-distances. Black bars: interspecific uncorrected p-distances. A – Variation between *P. leightoni* and *P. namibensis* (South Africa). B – Variation between *P. leightoni* and *P. namibensis* (Namibia). C – Variation between *P. leightoni*, *P. namibensis* and *P. trinasalis*. D – Variation between *P. leightoni*, *P. namibensis*, *P. trinasalis* with the closest sister, *P. notostictus*. Grey bar displays the barcoding gap transitioning between intra- and intraspecific distances. Dotted line indicates intraspecific cut-off.

Figure 2.5: Frequency of uncorrected p-distances based on Cyt-b gene region for *Psammophis*. White bars: intraspecific uncorrected p-distances. Black bars: interspecific uncorrected p-distances. A – Variation between *P. leightoni* and *P. namibensis* (South Africa). B – Variation between *P. leightoni* and *P. namibensis* (Namibia). C – Variation between *P. leightoni*, *P. namibensis* and *P. trinasalis*. D – Variation between *P. leightoni*, *P. namibensis*, *P. trinasalis* with the closest sister, *P. notostictus*. Grey bar displays the

barcoding gap transitioning between intra- and intraspecific distances. Dotted line indicates intraspecific cut-off.

Figure 2.6: *Psammophis* phylogeny with corresponding species delimitation methods results indicated to the right. A: Barcoding results based on both mtDNA gene regions. B: GMYC result based on mtDNA dataset. C: bGMYC delimited taxa based on probabilities < 0.95. Supported nodes in the phylogeny denoted with black circles. Each colour represents a species based on the applied model. Colours that are broken up indicate splitting by the applied model.

Figure 3.1: The geographic extent of the study area. (a): All models are restricted to South Africa, Namibia and Botswana. Palaeoclimate models included exposed land during the Last Glacial Maximum. (b): The extent of the study area within Africa.

Figure 3.2: The occurrence data for the *Psammophis leightoni* complex from Broadley (2002) and SARCA (see text for details) overlaid onto a digital elevation model. *Psammophis leightoni* - green, *P. namibensis* - blue, *P. trinasalis* - yellow.

Figure 3.3: Predicted climate envelopes for the *Psammophis leightoni* complex, as separate taxa and single taxon treatments, both current and mid-Holocene time slices. (i) & (ii): detailed view of the area of connectivity between taxa of the *P. leightoni* complex during respective time slices. Climate suitability scale shown in legend. Current climate models are shown for (a) all taxa of the *P. leightoni* complex, (b) STT1 and (c) STT2. Palaeoclimate models during mid-Holocene are shown for (d) all taxa of the *P. leightoni* complex, (e) STT1 and (f) STT2.

Figure 3.4: Predicted climate envelopes for the *Psammophis leightoni* complex, as separate taxa and single taxon treatments during Last Glacial Maximum. Climate stability maps for respective taxa depict possible climate refugia. (i): Overlapping climate envelopes of the *P. leightoni* complex. Palaeoclimate models during the Last Glacial Maximum are shown for (a) all taxa of the *P. leightoni* complex, (b) STT1 and (c) STT2. Climate suitability and stability scale shown in legend. Areas of stable climate are shown for (d) all taxa of the *P. leightoni* complex, (e) STT1 and (f) STT2.

LIST OF TABLES

Table 2.1: Uncorrected p-distance matrix for ND4 and Cyt-b gene regions of the *Psammophis leightoni* complex, with comparison values for its closest sister taxon, *P. notostictus*. Cyt-b p-distances (%) are on the bottom matrix. ND4 p-distances (%) are on the top matrix. Maximum within taxon distance (%) (Cyt-b/ND4) falls along the diagonal.

Table 3.1: Summarised performance scores (AUC and TSS) for each taxon and taxon treatment (STT1 & STT2) model.

Supplementary Table

Table S1: Sequence sources for the analyses presented in this study. All additional sequences are denoted with an 'x'.

UNIVERSITY *of the*
WESTERN CAPE

LIST OF ACRONYMS

AIC	Akaike Information Criterion
AUC	Area under the curve
bGMYC	Bayesian implementation of the General Mixed Yule-Coalescent model
BI	Bayesian Inference
BWA	Botswana
CCE	Current climate envelope
CI	Confidence interval
cmos	Oocyte maturation factor Mos
Cytb	Cytochrome b
DNA	Deoxyribonucleic acid
DNTP	Deoxynucleotide Triphosphate
ENM	Ecological niche modelling
EOO	Extent of occurrence
ESS	Effective Sample Size
GCM	General Circulation model
GMYC	General Mixed Yule-Coalescent model
GSC	Unified (General) Species Concept
IUCN	International Union for Conservation of Nature
LGM	Last Glacial Maximum
MCMC	Markov Chain Monte Carlo
ML	Maximum likelihood
mtDNA	Mitochondrial Deoxyribonucleic acid
NAM	Namibia
NC	Northern Cape
ND4	NADH dehydrogenase subunit 4
nDNA	Nuclear Deoxyribonucleic acid
PCR	Polymerase chain reaction
PP	Posterior probabilities
ROC	Receiver operating characteristic
RSA	Republic of South Africa
SARCA	South African Reptile Conservation Assessment
SDM	Species distribution modelling
STT	Single taxon treatment
TSS	True skill statistic
WC	Western Cape

GENERAL INTRODUCTION

1.1 The association between taxonomy and conservation

Acknowledging the influences of taxonomy on conservation is not a novel standpoint (McNeely 2002; Dubois 2003; Golding and Timberlake 2003; Mace 2004; Khuroo et al. 2007). Taxonomy lies at the core of conservation, specifically through the appropriate identification of organisms (Rojas 1992; Morrison et al. 2009). By neglecting taxonomic resources, conservation strategies may collapse. This is due to certain limitations such as a lack of site-specific data on the species composition, caused by the absence of taxonomic precision (Kim and Byrne 2006). To avoid such limitations on conservation, basic criteria need to be established when deciding which taxa need to be conserved and how these taxa as units are defined (Golding and Timberlake 2003; Mace 2004; Samper 2004).

Typically, the identification of an organism requires a scientific name to be allocated to the unit of interest, i.e., a species name (Dubois 2007). However, Morrison et al. (2009) refer to a lack of explicit criteria necessary to assign taxon ranks (e.g., species or subspecies) or form species boundaries between taxa. Describing species and the application of a species concept is dependent on a range of explicit and implicit factors. Discussions around the fundamental requirements necessary to define species still persist, especially when those units become integral to conserving and maintaining biodiversity (Rojas 1992; McNeely 2002; De Queiroz 2007; Redford et al. 2011). With over two dozen variations of what defines a species, the various species concepts recognise that species are real and discrete entities in nature (Hey 2001). Mace (2004) further explains that these entities represent evolving lineages within which the diversity, and what we hope to conserve, is categorised.

The conservation of species and biodiversity emerged as a result of increasing concerns regarding the global loss of endangered species and significant ecosystems, such as tropical forests and coral reefs, along with the realization of how particular anthropogenic influences negatively impact the globe (Redford et al. 2011). In retrospect, considering that conservation biology was presented as a mission-orientated and crisis-driven discipline (Meine et al. 2006), it is not surprising that conservation has primarily concentrated on charismatic species or key vegetation types (McNeely 2002). There is, however, no association between saving species and describing or deriving relationships between them (Mace 2004). The same applies to conservation guides and full species lists, which alone cannot conserve species. Without the appropriate knowledge and description, it is not possible for management strategies and procedures to progress any further, which is necessary for adequate species conservation (Rojas 1992; Samper 2004).

1.2 Influences of taxonomy on conservation

The impact of taxonomic decisions on conservation action is rarely without consequence (Price and Hayes 2009). Prioritising conservation effort around specific taxa is often heavily dependent on correct systematics. Additionally, accurate taxonomic classification allows for valid species to be assessed correctly, ultimately being assigned with an appropriate conservation status (Hayes 2006; Morrison et al. 2009). Threatened species are often severely affected incorrect taxonomy (Dulvy and Reynolds 2009), as management priorities are frequently determined by taxonomic classification, where the neglect of distinct taxa may lead to their possible extinction (Daugherty et al. 1990).

Flawed taxonomy regularly has a negative effect on conservation (Morrison et al. 2009). Discussions pertaining to the impact of incorrect taxonomy on conservation have been acknowledged by numerous papers addressing systematics (Funk et al. 2002), cryptic species (Russello et al. 2005), and effective conservation practices (McNeely 2002; Mace 2004; Casciotta et al. 2013). However, the definition of what constitutes correct or incorrect taxonomy is poorly defined (Morrison et al. 2009).

Taxonomic revisions often spark increase efforts in conservation, especially when dealing with cryptic or loosely delineated species. For example, the Chiricahua leopard frog (*Lithobates chiricahuensis*) and *L. pipiens* were split in a taxonomic revision leaving two species: a widespread Least Concern *L. pipiens* (Humphrey and Fox 2002; Rorabaugh 2002) and a restricted Vulnerable (A2ace) *L. chiricahuensis* (Clarkson and Rorabaugh 1989). In response to the revised listing based on updated taxonomy, the Malpai Borderlands Group was formed which has worked to protect over 30,350 ha of private land by enlisting private landowners and over 12 public institutions to cooperate as part of this conservation group (Glick 2005).

Taxonomic change is unfortunately not always taken into consideration when applying conservation efforts to species. Public appeal often plays an influential role in the assignment of conservation resources as some charismatic species are afforded resources based on appeal, not on threat (e.g., *Canis rufus*: Nowak 2002, 2003, *Ursus maritimus*: Talbot and Shields 1996). The green turtle (*Chelonia mydas*) is considered Endangered due to decreasing population sizes (IUCN), however, a molecular study revealed no significant distinction between the green turtle and the black turtle (*Chelonia agassizii*; Karl and Bowen 1998). Despite being regarded as a single species, resources put toward green turtle conservation have been continuing as debates surrounding the taxonomy remain unresolved (Morrison et al. 2009).

Because the classification and description of taxa are extremely valuable for successful conservation planning and execution, incorrect taxonomy can increase the rate at which species are lost (Daugherty et al. 1990; May 1990; Murphy et al. 2013). We cannot assume to conserve organisms that we cannot recognise, and our efforts to comprehend the significance of environmental alteration are reduced if we cannot completely recognise and describe components of natural ecosystems (Mace 2004; Evenhuis 2007).

2

1.3 Taxonomic changes–a blessing or a curse for conservation

Even though taxonomy is not considered the most appealing discipline, the development of analytical tools (e.g., molecular genetics) has led to the increased detection of new species (Wilson 2004; Köhler et al. 2005). Among vertebrates, the number of recognised amphibian species has increased 44 % since 1992 (AmphibiaWeb 2017; Duellman 1993). Nonetheless, amphibians are suffering serious declines with at least 32.5 percent of amphibian species globally threatened (Köhler et al. 2005). Isaac et al. (2004) suggested that an increase in the number of recognized species is due to taxonomic inflation, where subspecies are elevated to full species status as a result of a change in species concept, instead of new discoveries. It is vital to identify and validate 'real' species based on accepted, peer-reviewed information relating to definitive characteristics (e.g., genetic differences and/or morphology) before recognising them as conservation units. Although, the idea of taxonomic inflation cannot be applied to all taxa at the risk of simply neglecting the need for taxonomic exploration (Köhler et al. 2005).

Species boundaries are hypotheses, subject to change with new and more sophisticated ways to distinguish differences between organisms (Rylands and Mittermeier 2014). This allows for possible change which is not only prompted by using different species concepts, but also by a grey zone in defining what is and is not an important difference when recognising species. Primate taxonomy, as an example, has had considerable taxonomic inflation over the past decades, specifically with the move to using the phylogenetic species concept (Isaac et al. 2004). Lemurs in particular have seen significant increases (20 recognised species in 1931 to 97 species in 2010) (Tattersall and DeSalle 2007; Rylands and Mittermeier 2014). Such newly designated species as a result of inflation call into question the suitability of Red List assessments and how species are categorised under national laws and international agreements (Zachos 2013).

The International Union for Conservation of Nature (IUCN) Red List of Threatened Species is the most inclusive resource describing the global conservation status of plants and animals (Rodrigues et al. 2006). The Red List as a conservation tool integrates the classification of species within a particular threat category, with data and expert opinion used to support these assessments. Taxa included on the Red List are formally described species, subspecies, and varieties (only for plants). Submissions for the Red List now require a justification for listing, which is supported by data on range size, population size and trend, distribution, habitat preferences, altitude, threats, and conservation actions in place or needed (Rodrigues et al. 2006; Bates et al. 2014).

The Red List is a necessity to guide conservation efforts focused on a species of concern. The threat categories facilitate guiding priorities for conservation investment between species (Collar 1996), though in relation to additional information (such as cost and feasibility) (Possingham et al. 2002). The Red List assessments often produce recommendations for conservation action (Rodrigues et al. 2006). For example, 5500 conservation actions were identified for approximately 1000 globally threatened birds during the year 2000. These recommendations provided a reference to measure conservation responses, and with

considerable success, two-thirds of threatened bird species had some of form of conservation action implemented by 2004 (BirdLife International 2004). Without appropriate taxonomy, listings cannot be supported which leads to unwarranted conservation efforts. For instance, regional diversity estimates can be over- or underestimated if the taxonomy is inaccurate leading to resources being arbitrarily utilised (Chaitra et al. 2004).

1.4 Bridging inconsistencies between taxonomy and conservation

Three main reasons are likely responsible for the apparent gap between taxonomy and conservation (Golding and Timberlake 2003). Firstly, taxonomy as a science is often referred to as traditional in practice, and until fairly recently (e.g., Wheeler 2004; Kipling et al. 2005), has been slow to change at risk of becoming reduced to a "feeble" branch of science. Secondly, conservation-orientated agencies do not explicitly have measures or targets for taxonomic performance, either by recognising the use of specific taxonomic ranks or not. Lastly, taxonomy is considered immaterial by some, where the importance of taxonomic status when identifying conservation objectives is disregarded. For example, concerns relating to the various genetic forms of the African elephant (*Loxodonta africana*) were raised as this may affect the number of extant species, subspecies and hybrids (Comstock et al. 2002). The Convention on International Trade in Endangered Species list elephant subspecies, but the taxonomic uncertainty of *L. africana* impedes effort surrounding its conservation. If these difficulties can greatly affect charismatic species, how are species not as appealing affected?

Currently, a total of 10499 reptile species are described (Uetz, 2017), and new molecular evidence continues to reveal cryptic species previously undetected by morphological analyses (e.g., de Oliveira et al. 2016; Dowell et al. 2015; Nagy et al. 2012). Nonetheless, reptiles remain underrepresented on the IUCN Red List of Threatened Species, with a mere 35% of described species evaluated (Bohm et al. 2013). Despite Africa having a considerable proportion of the world's reptiles, the taxonomy of these taxa remain poorly documented. However, South Africa is relatively well explored, with a relatively well documented catalogue of herpetofauna supported by two of the largest herpetological collections on the African continent (Böhm et al. 2013; Tolley et al. 2016). Furthermore, in terms of conservation efforts, the conservation statuses of 405 taxa of the reptiles occurring within South Africa, including Lesotho and Swaziland, have been assessed (Bates et al. 2014). Work done at smaller, local levels has been noted to have greater success in informing actual conservation implementation as opposed to global conservation prioritizations (Mace et al. 2000; Brummitt and Lughadha 2003) largely because biodiversity and threats are not evenly distributed. Though, operating at the local level still involves separate processes (i.e., accurate taxonomy) which are necessary to meet actual conservation targets and priorities at these finer scales (Brooks et al. 2006).

1.5 Study species: the *Psammophis leightoni* species complex

Although evaluated in the recent conservation assessment of South African reptiles (Bates et al. 2014), the southern African *Psammophis leightoni* complex remains one of taxonomic concern. Historically *P. trinasalis*, *P. namibensis* and *P. leightoni* were considered subspecies of *P. leightoni*. Broadley (2002) raised *P. leightoni*, *P. namibensis*, and *P. trinasalis* to full species status, recognising them as good evolutionary species which show ecological differences. However, Kelly et al. (2008) showed (albeit based on limited sampling) that the elevation from sub-specific to species level was not supported by molecular evidence. Thus, the species boundaries associated with the *P. leightoni* complex are not clearly defined with specific interest in whether *P. namibensis* and *P. leightoni* are different species. This is of specific interest as *P. leightoni* is currently listed as Vulnerable [B1ab(iii)] according to the most recent IUCN Red List assessment, while *P. namibensis* and *P. trinasalis* are listed as Least Concern (Bates et al. 2014).

Psammophis leightoni, *P. namibensis* and *P. trinasalis*, were historically associated with the *P. notostictus* species complex (Broadley 2002), a complex which primarily inhabits arid regions (specifically the Namib Desert and Kalahari) of southern Africa. *Psammophis leightoni* inhabits the most mesic habitats of the group, and is restricted to Fynbos biome (particularly associated with West Coast Renosterveld and Sand Plain Fynbos). *Psammophis leightoni*, the Cape whip snake, is the only South African endemic of the family Psammophiidae, and is restricted to the western regions of the Western Cape. The species' limited distribution (extent of occurrence (EOO) < 20 00 km^2) is within an area characterised by high levels of habitat transformation. Additionally, these habitats associated with *P. leightoni* have seen a loss in particular vegetation types (e.g. Renosterveld) with inferred declines of up to 80% in extent (Rouget et al. 2003; Maritz 2014). Very few large undisturbed habitat patches which occur in the few protected areas remain for *P. leightoni*. With the fragmentation of remaining habitats, it is possible that majority of the populations are isolated and although *P. leightoni* is capable of long distance movement, altered habitats and roads may act as barriers (Maritz 2014).

The ecological differences used by Broadley (2002) to distinguish taxa of the *Psammophis leightoni* complex are solely based on where they occur. *Psammophis leightoni* and *P. namibensis* do not display overlapping distributions (assumed allopatry) and there is an apparent gap between the two species geographic distributions in the north-western parts of the Western Cape Province (Figure 1.1). *Psammophis trinasalis* has a distribution which is further inland and to the east of its congeners in this species complex, with partial overlap with *P. namibensis* in Namibia (Broadley 2002). The genetic relationship of *P. trinasalis* with *P. leightoni* and *P. namibensis* is currently unknown as it was absent from the phylogeny of Kelly et al. (2008). *Psammophis leightoni* and *P. namibensis* show intraspecific variation between them; however, this information is based on one individual per putative species (Kelly et al. 2008).

These taxa form the *Psammophis leightoni* species complex as they are morphologically conservative, with overlapping scale counts and maximum snout-vent lengths (Broadley

5

2002). Additionally, the dorsal patterns on the head consist of longitudinal pale stripes anteriorly and pale transverse markings on the back of the head for all three species (Broadley 1977). Broadley (2002) noted that *P. leightoni* has similar colouration to individuals in the southern end of *P. namibensis* distribution, which then breaks up into a black and yellow speckled pattern further north (common in Namibia). P*sammophis trinasalis* is unique in that the coloured lateral stripe extends across the temporal scales, continuing as the dorsolateral stripe along the body (Broadley 2002). At present, the lateral stripe of *P. trinasalis* is the only defining character distinguishing it from *P. leightoni* and *P. namibensis* (Figure 1.2). *Psammophis leightoni* and *P. namibensis* share morphological traits and can only be distinguished based on where they occur.
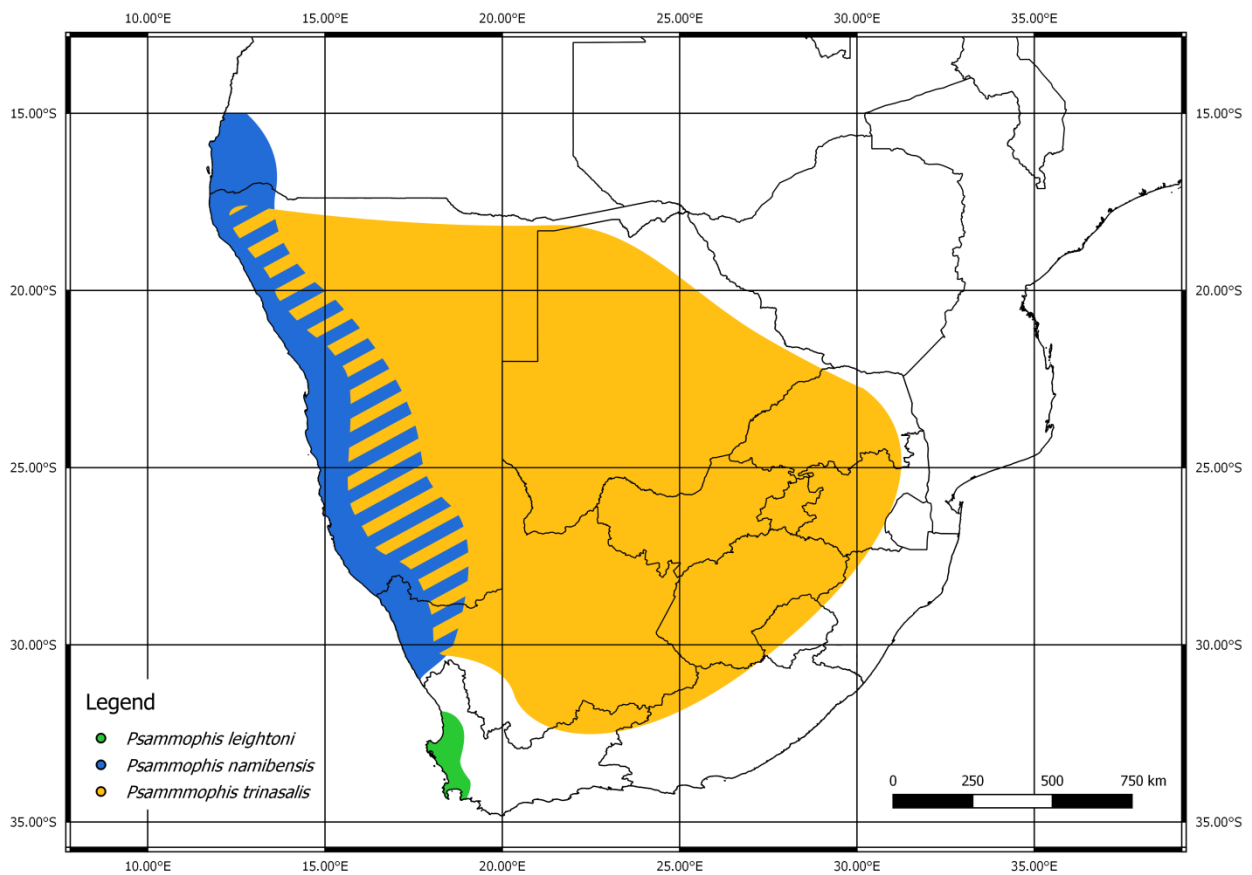
Figure 1.1: The distribution of the *Psammophis leightoni* species complex from Southern Africa extrapolated from Broadley (2002) and SARCA databases (see chapter 3 for details). *Psammophis leightoni* is highlighted in green, *P. namibensis* in blue, and *P. trinasalis* in yellow.

Figure 1.2: Morphology of the *Psammophis leightoni* complex compared to its sister species, *P. notostictus*. Body patterning: A – *P. leightoni*, B – *P. namibensis*, C – *P. trinasalis*, and D – *P. notostictus*. Head scales and patterning: E – *P. leightoni*, F – *P. namibensis*, G – *P. trinasalis*, and H – *P. notostictus*.

1.6 Problem statement

The known distributions of closely related *Psammophis namibensis* and *P. leightoni* do not overlap in the north western parts of the Western Cape (Figure 1.1). However, this region is not densely sampled, and this gap between the two known geographic distributions could be an artefact of limited sampling. Given this, the potential exists for *P. leightoni* and *P. namibensis* to simply be clinal forms of a single species.

7

The conservation status of *Psammophis leightoni* (Vulnerable [B1ab(iii)]) is largely dependent on the taxonomy being valid (Maritz 2014), assessing the sampling gap and addressing the lack of a comprehensive genetic analysis which has confounded the taxonomy. Ultimately, should *P. leightoni* and *P. namibensis* represent one species, the species would need to be reassessed and would likely be considered Least Concern as it would not meet any of the criteria for listing a species as threatened. However, should *P. leightoni* and *P. namibensis* indeed represent two species, conserving the existing habitat of *P. leightoni* should be prioritised along with assessing this snakes occurrence within transformed areas.

1.7 Overarching Hypotheses

Psammophis leightoni is now monotypic given the elevation of *P. l. namibensis* and *P. l. trinasalis* to full species status (Broadley 2002). Although these changes are questionable, Kelly et al. (2008) were only able to include single representatives of *P. leightoni* and *P. namibensis*, with no sample of *P. trinasalis* in their revision of Psammophiidae. I test the hypothesis that the three described species (*P. leightoni*, *P. namibensis* and *P. trinasalis*) are phylogenetically distinct. Furthermore, I predict that there are differences in the climate envelopes of *P. leightoni*, *P. namibensis* and *P. trinasalis* and this would in part, define and limit their distributions. Given these hypotheses, I aim to validate the taxonomic status of the *P. leightoni* complex using phylogenetic analyses and species distribution modelling (SDM) techniques in order to better inform conservation assessments for a potentially threatened taxon.

1.8 Approach

Kelly et al. (2008) discussed how the low molecular distance between *Psammophis leightoni* and *P. namibensis* would suggest intraspecific rather than interspecific divergence. However, further molecular work on these species is necessary to investigate the existence and limits of putative species boundaries within this complex. Chapter two centres around defining the phylogenetic relationships between all members of this species complex using species delimitation analyses to better elucidate boundaries within this group. With the addition of *P. trinasalis*, phylogenetic relationships between these species are compared within a generic and family level context.

Species distribution modelling (SDM), also referred to as ecological niche modelling (ENM), is a popular tool used to build spatially explicit predictions demonstrating environmental suitability for species (Guisan and Thuiller 2005; Phillips and Dudík 2008; Elith and Leathwick 2009). Chapter three of this thesis focuses on identifying the climate envelopes of *Psammophis leightoni* and *P. namibensis*. Environmental variables across the last glacial period (22 000 years ago to present) are used to map the shifts in these snakes climate space. This method has been used to highlight species range shifts relative to climate change (Cooper et al. 2016), identifying areas of climate stability (e.g. da Silva & Tolley 2017), and shifting hybrid zones between closely related species (Taylor et al. 2015).

8

CHAPTER TWO
EVOLUTIONARY RELATIONSHIPS OF THE *PSAMMOPHIS LEIGHTONI* SPECIES COMPLEX

2.1 Introduction

Long-term conservation success has been defined as the maintenance of species traits which facilitated a species preservation until the present, with the potential to express these characteristics in the future (Redford et al. 2011). Conservation plans do not take these traits into account and are often biased toward the conservation of areas prioritising the proportion of endemic species (Whittaker and Fernandez-Palacios 2007) and subspecies within an area (Phillimore and Owens 2006) instead of species-specific action. Given that conservation plans prioritise the number of species over ecological function, accurate delineation of taxa with the appropriate taxonomic status becomes essential. Accurately delimiting species can be problematic as the criteria to do so remain controversial, especially when dealing with cryptic taxa (De Queiroz 2007; Reid and Carstens 2012).

Concerns with accurately defining species boundaries become increasingly complex when dealing with cryptic taxa and even more so when existing taxonomic literature which is unreliable (Hoagland 1996). Phylogenies, in particular, can provide insights into taxonomy as the evolution of characters may be tracked, with the long-term patterns of adaptation and divergence used as evidence to accurately describe species traits on a genetic level (Brooks and Mclennan 1991; Harvey and Pagel 1991). Molecular data used within a phylogenetic framework provides a rich source of information when analysing relationships between taxa and, within limits, can also estimate divergence levels of species (Hillis and Moritz 1990). If the relevant genes of valid species are sequenced then proving the relationships between taxa will become easier, however, this is not currently possible as most groups of organisms are not yet sequenced (Hughes et al. 2001; Hebert et al. 2003; Hajibabaei et al. 2007; Murphy et al. 2013).

While species delimitation remains a complex matter, the Unified (General) species concept (GSC) define species as separately evolving metapopulation lineages (Hey 2001; De Queiroz 2007). Phylogenetic hypotheses can be used to assist in describing species under the GSC because the approach recognises historical progression of lineage divergence (Nielsen and Wakeley 2001). Of the approaches using genetic data to clarify species boundaries, thresholds based on pairwise sequence distances between individuals are generally applied to allow the grouping of samples into putative species (Pons et al. 2006; Ratnasingham and Hebert 2007; Aliabadian et al. 2009; Vieites et al. 2009; Yang and Rannala 2010). However, this distance threshold method has been criticized for not taking evolutionary processes into account (Hickerson et al. 2006). In addition, there is concern around the level of uncertainty in establishing appropriate thresholds based on the gap between intraspecific and interspecific sequence distances (Meyer and Paulay 2005; Nielsen and Matz 2006; Meier et al. 2008).

Pons et al. (2006) presented a tree-based approach to species delimitation as an alternative to distance threshold methods. The General Mixed Yule-coalescent (GMYC) model takes a phylogenetic tree estimated from sequence data and assumes that the branching nodes in the tree are a result of either divergence events between species-level taxa, or the variation within species at a population level (Pons et al. 2006). GMYC can be used to find the point of the rate shift between these branching events by assuming the rate of coalescence within species is greater than the rate of cladogenesis (Michonneau 2015). Reid & Carstens (2012) highlighted concerns with GMYC and proposed a Bayesian implementation to this model (bGMYC), which accounts for the uncertainty related to phylogenetic inference (Lecocq et al. 2014).

An integrative approach of applying multiple lines of evidence to species delimitation is important in understanding the process of species genetic differentiation (Nagy et al. 2007; Pinho et al. 2007; Petit and Excoffier 2009). Furthermore, incorporating genetic data with additional information (i.e., morphology, behaviour, habitat specificity, etc.) offers a more holistic approach when distinguishing taxa (Harris et al. 1998; Rubinoff and Holland 2005; De Queiroz 2007). However, this is only achievable if the data used to clarify species boundaries are reliable for all taxa of interest.

Members of the Psammophiinae occurs throughout Africa, southern Europe, the Middle East, across south-central Asia, as well as Madagascar, and currently including eight genera and about 50 species (Kelly et al. 2008). Accurately delineating species within the type genus *Psammophis* Boie, 1825 has been troublesome because a number of these species are morphologically conserved (Loveridge 1940; Broadley 1966, 1977; Hughes 1999; Kelly et al. 2008) (Figure 2.1). Kelly et al. (2008) sought to address the taxonomic disorder of *Psammophis* with the phylogenetic revision of Psammophiinae. Kelly et al.'s (2008) main objective was to use extensive taxon sampling representing all taxa of Psammophiinae to provide provisional DNA-based hypotheses of species limits within *Psammophis*. Although this contribution resolved numerous taxonomic issues within the family, they also highlighted uncertainty regarding species boundaries for the *P. leightoni* complex.

Historically, *Psammophis trinasalis*, *P. namibensis* and *P. leightoni* were considered subspecies of *P. leightoni*. These taxa were elevated to full species rank based on ecological differences and morphological variation (Broadley 2002). However, Kelly et al. (2008) showed that the specific rank assigned to *P. leightoni* and *P. namibensis* is not supported by molecular evidence, suggesting the genetic distance between these taxa are within intraspecific levels (e.g., sequence divergence of 0.39% compared to other species with values > 3.75%, generated using pooled Cytb and ND4 data). The genetic relationship of *P. trinasalis* within *Psammophis* is not clear because it was not included in the revision of Psammophiinae. Therefore, the species boundaries associated with taxa of the *P. leightoni* complex remain clouded. *Psammophis leightoni* is currently listed as Vulnerable [B1ab(iii)], threatened by increased habitat transformation and fragmentation of quality habitat. However, the taxonomic confusion around the *P. leightoni* complex may be limiting the way conservation resources are applied to *P. leightoni*.

Given that the conservation status for *Psammophis leighton*i relies on clarification of the current taxonomy, species delimitation analyses (i.e. divergence threshold and tree-based methods) may be applied to inform how the taxa of the *P. leightoni* complex are delimited based on the amount of genetic variation. Because of the low divergence between taxa included in previous phylogenies, I hypothesise that the three described taxa (*P. leightoni*, *P. namibensis* and *P. trinasalis*) are conspecific and are not distinct taxa. For this reason, I aim to validate the taxonomic status of the *P. leightoni* complex within a phylogenetic framework using several species delimitation methods.

UNIVERSITY *of the* WESTERN CAPE

Figure 2.1: *Psammophis* **species are morphologically conservative, evident by the slender body (A-F), head features (G-O) and patterning (P-R). Full body: A –** *P. crucifer***, B –** *P. brevirostris***, C –** *P. notostictus***, D –** *P. leightoni***, E –** *P. namibensis***, F –** *P. trinasalis***. Head morphology: G –** *P. mossambicus***, H & K –** *P. crucifer***, I & J –** *P. brevirostris***, L –** *P. notostictus***, M –** *P. leightoni***, N –** *P. namibensis***, O –** *P. trinasalis***. Top-down: P –** *P. brevirostris***, Q –** *P. notostictus***, R –** *P. crucifer***.**

2.2 Material & methods

2.2.1 Samples, amplification and sequencing

Samples from the three target species were sequenced, with additional sequences downloaded from GenBank (Table S1). In total, there were 144 individuals covering 32 species (excluding outgroups). Multiple samples of the target taxa were included: *Psammophis leightoni* (N = 12), *P. namibensis* (N = 3), *P. trinasalis* (N = 10) and *P. notostictus* (N = 15) from 23 unique localities (Figure 2.2). Morphological characters were used to identify *P. trinasalis* and *P. notostictus* in the field, specifically the lateral line through the temporal scales of *P. trinasalis* and the undivided anal shield in *P. notostictus*. Identification of *P. leightoni* and *P. namibensis* were solely based on location. For all new material (Table S1), total genomic DNA was extracted by means of salt extractions (Aljanabi & Martinez 1997) from the liver, tail tip, or muscle tissue, as well as blood samples from the collected specimens. Two mitochondrial (mtDNA) genes, NADH dehydrogenase subunit 4 (ND4) (with flanking tRNA) and cytochrome-b (Cyt-b), as well as the nuclear (nDNA) gene oocyte maturation factor Mos (cmos) were amplified by polymerase chain reactions (PCR). Primers ND4-R4/ ND4-F3 and Leu-tRNA (Arévalo et al. 1994) were used to amplify ND4, and Cyt-b was amplified with primers L14910/H16064 (Burbrink et al. 2000). Cmos was amplified using the primers S77/S78 (Lawson et al. 2005). Amplification was carried out in a 25 µl reaction mixture consisting of 2.5µl reaction buffer, 2.5 mM MgCl2, 2 µM of each primer, 0.2 mM DNTP solution, 0.5 U/µl Taq Polymerase (SuperTherm), and 30-50 ng/µl of DNA template.

PCR cycling conditions included an initial denaturation step at 95°C for 4 min, followed by 38 cycles of denaturation at 94°C for 30 s, annealing at 55–60°C for 30 s, and extension at 72°C for 1 min for ND4; and 35 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 45 s, and extension at 72°C for 1 min for Cyt-b, with a final extension at 72°C for 10 min. PCR products were visually inspected on 1% agarose gel electrophoresis stained ethidium bromide, and products were sent to Macrogen Inc. (Amsterdam, Netherlands) for sequencing.

2.2.2 Data matrix and alignment

In addition to the 144 ingroup taxa, 13 individuals were included as outgroup taxa (*Aspidelaps scutatus*, *Atractaspis bibronii*, *Buhoma procterae*, *Duberria lutrix*, *Lamprophis guttatus*, and *Pseudaspis cana*), with a total of 2575 base pairs per individual. Sequences were aligned using Geneious 10.2.3 (https://www.geneious.com, Kearse et al. 2012) using the MUSCLE alignment tool (Edgar 2004), with final adjustments done by eye. The ND4 and Cyt-b regions were translated to amino acid sequences to check for premature stop codons and validate whether the amino acid reading frame was maintained.

2.2.3 Phylogenetic analyses

Phylogenetic analyses were performed to estimate the evolutionary relationships within the *Psammophis leightoni* complex relative to Psammophiinae using maximum likelihood (ML) and Bayesian inference (BI) approaches. Evolutionary models that best fit the data were

estimated separately for each marker using jModelTest v.2.1.4 (Posada 2008). By inspecting the Akaike Information Criterion (AIC), GTR + I + G was the model which best fit ND4+tRNA and Cyt-b, and GTR for cmos. Data were then concatenated and partitioned by marker (ND4 + tRNA, Cyt-b and cmos) with the appropriate model. ML analyses were run using RAxML v.8 (Stamatakis 2014). Nodes with bootstrap values ≥ 70% were considered well supported (Hillis and Bull 1993). BI was performed under a Bayesian Markov Chain Monte Carlo (MCMC) framework with MrBayes v.3.2.2 (Ronquist and Huelsenbeck 2003). The MCMC chains were run for 10 million generations and sampled at every 1000th generation; the first 10% of the sampled trees were discarded as burn-in. The effective sampling size (ESS) of all parameters (ESS > 200) was assessed using TRACER v.1.6 (Rambaut et al. 2015). A 50% majority consensus tree was derived from the remaining trees. Nodes were considered supported with posterior probabilities ≥ 0.95 (Wilcox et al. 2002).

2.2.4 Species delimitation analyses

In order to ascertain the level of sequence divergence between taxa of the *Psammophis leightoni* complex, a barcoding gap approach was used. Uncorrected p-distances were estimated using Species Identifier v.1.8 (Meier et al. 2006) for all African representatives of *Psammophis* using the mtDNA dataset. This method estimates a threshold (given as a barcoding gap) between pairwise intra- and interspecific distances. All African *Psammophis* were included in this analysis, including the target taxa as currently described. This was done to ascertain if the target taxa fell below the intraspecific threshold compared to other species in *Psammophis*. The frequency of p-distances were then plotted as a histogram with the given barcoding gap for each mtDNA marker (Figure 2.4 & 2.5).

The General Mixed Yule-coalescent (GMYC) model approach (Pons et al. 2006) and its Bayesian implementation (bGMYC) (Reid and Carstens 2012) were used for tree-based delimitation using a single locus (usually mitochondrial: Esselstyn et al. 2012; Fujisawa and Barraclough 2013). To reduce compression of coalescent events towards the tips of the trees, the outgroup taxa were removed prior to running GMYC (Michonneau 2015). An ultrametric tree for the combined mtDNA data was constructed with BEAST 2.4.7 (Bouckaert et al. 2014) using a relaxed lognormal molecular clock (Walther et al. 2016). Chains were run for 100 million generations with the first 10% of the generations discarded as burn-in. The log files generated from the BEAST analysis were inspected using TRACER v.1.6 (Rambaut et al. 2015) to assess the accuracy of the parameters based on estimated sample sizes (ESS > 200). The GMYC analysis was run using the maximum clade credibility tree from the BEAST analysis created with TREEANNOTATOR 2.1.2 (Bouckaert et al. 2014) setting the posterior probability limit to 0 (Lecocq et al. 2014). The SPLIT (Fujisawa and Barraclough 2013) and bGMYC (Reid and Carstens 2012) R packages (R Core Team, 2013) were used to run the respective GMYC and bGMYC analyses.

14

The bGMYC analysis was run using an ultrametric tree for the combined mtDNA dataset, which included all African taxa within *Psammophis*. This tree was constructed with BEAST 2.4.7 (Bouckaert et al. 2014) using a relaxed lognormal molecular clock (Walther et al. 2016). Chains were run for 100 million generations as well, with the first 10% of the generations discarded as burn-in. The log files generated from the BEAST analysis were inspected using TRACER v.1.6 (Rambaut et al. 2015) where the estimated sample sizes (ESS > 200) were assessed. For the bGMYC analysis, the MCMC sampler was run for 1 million generations, discarding the first 100 000 generations as burn-in and sampling every 1000 generations. Groups with probabilities 0.95–1 are considered conspecific with strong support, while groups with probabilities ≤ 0.05 are not conspecific (Reid and Carstens 2012). Outputs from the analyses were assessed by inspecting the distribution of ratios of coalescence to speciation events to ensure that these ratios were well above 0, with no negative values. The log ratios of coalescence to speciation events were also evaluated to ensure these were not less than 0, as this would suggest the frequency of speciation events is higher than divergence rates at a population level (Reid and Carstens 2012).

Figure 2.2: Sampling localities for all target taxa. Taxa are grouped by colour: Green – *Psammophis leightoni*, Blue – *P. namibensis*, Pink – *P. notostictus*, Yellow – *P. trinasalis*. Sub-clades are grouped by shape: Square – *P. leightoni* (South Africa), Circle – *P. namibensis* (Namibia), Diamond – *P. notostictus*, Triangle – *P. trinasalis*. (i) – Study region depicted within Africa.

15

Figure 2.3: Bayesian Inference 50% majority-rule consensus phylogram from the concatenated dataset. Node support (ingroup only) is in the format: Bayesian posterior probability/ML bootstrap. Nodes not supported by either Bayesian or Maximum Likelihood analyses are denoted with a hyphen (-).

16
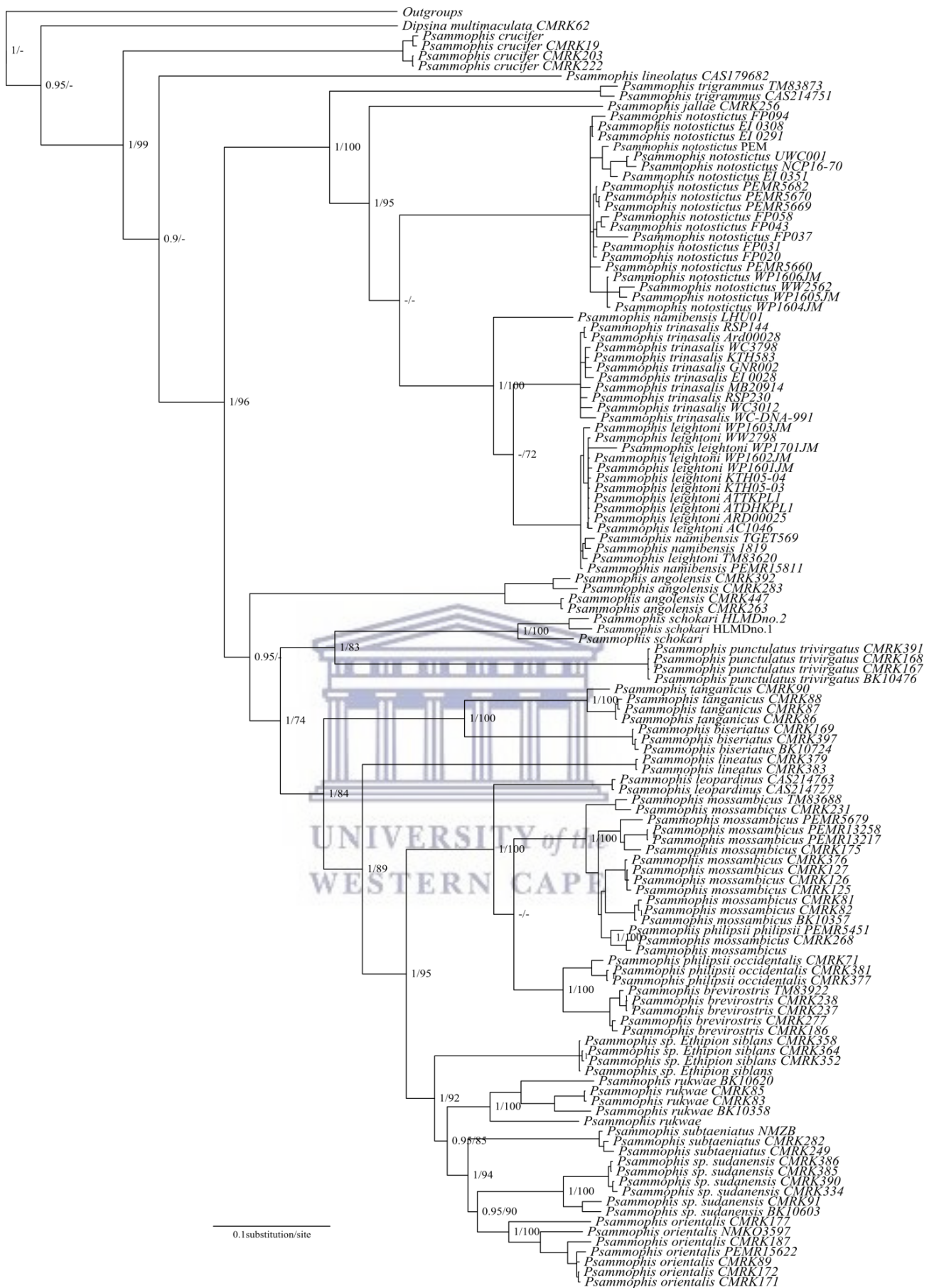
2.3 Results

2.3.1 Phylogenetic analyses

ML and BI analyses produced trees with the same basic topology (Figure 2.3). All individuals of the *Psammophis leightoni* complex form a well-supported monophyletic clade, although within it, there are shallow divergent clades that correspond with the geography. The pattern of these subclades shows that *P. leightoni* and *P. trinasalis* are sister to each other, with *P. namibensis* sister to them. Additionally, there are individuals identified as *P. namibensis* within South Africa that fall within the *P. leightoni* subclade.

2.3.2 Species Delimitation Analyses

Levels of sequence divergence within the *Psammophis leightoni* complex fall well below the intraspecific thresholds based on uncorrected p-distances (Figure 2.4 & Figure 2.5). The maximum observed p-distances for ND4 and Cyt-b for taxa of the *P. leightoni* complex ranged from 1.56% to 5.75% and 0.87% to 6.31%, respectively (Table 2.1). *Psammophis namibensis* had the most within taxon divergence: 6.31% (Cyt-b), followed by *P. trinasalis*: 5.33 (ND4). Taxa of the *P. leightoni* complex are more than 8% (Cyt-b) divergent to its nearest congener, *P. notostictus* (Table 2.1).

The GMYC model estimates a total of 30 taxa (confidence interval, CI: 29-39) for *Psammophis*, and separates the *P. leightoni* complex into separate taxa corresponding with their geographic location. Also, this model has split an additional six recognised species into separate taxa as well. This method, however, is known for over splitting taxa (Esselstyn et al. 2012; Paz and Crawford 2012; Fujisawa and Barraclough 2013; Talavera et al. 2013). *Psammophis namibensis* identified from South Africa is still positioned within the *P. leightoni* subclade. The bGMYC analysis indicates all taxa within *P. leightoni* complex form a single taxon (posterior probability, PP >0.95) (Figure 2.6). Although, a number of species were split when PP: 0.90 – 0.95 (e.g., *P. angolensis* and *P. crucifer*).

17

Table 2.1: Uncorrected p-distance matrix for ND4 and Cyt-b gene regions of the *Psammophis leightoni* **complex, with comparison values for its closest sister taxon,** *P. notostictus*. **Cyt-b p-distances (%) are on the bottom matrix. ND4 p-distances (%) are on the top matrix. Maximum within taxon distance (%) (Cyt-b/ND4) falls along the diagonal.**

	Psammophis leightoni	*Psammophis namibensis*	*Psammophis trinasalis*	*Psammophis notostictus*
Psammophis leightoni	0.87/1.56	0.12	4.36	9.46
Psammophis namibensis	0.22	6.31/5.75	4.27	9.45
Psammophis trinasalis	4.23	4.21	1.09/5.33	9.45
Psammophis notostictus	8.67	9.12	12.01	3.71/3.17

Figure 2.4: Frequency of uncorrected p-distances based on ND4 gene region for *Psammophis*. **White bars: intraspecific uncorrected p-distances. Black bars: interspecific uncorrected p-distances. A – Variation between** *P. leightoni* **and** *P. namibensis* **(South Africa). B – Variation between** *P. leightoni* **and** *P. namibensis* **(Namibia). C – Variation between** *P. leightoni*, *P. namibensis* **and** *P. trinasalis*. **D – Variation between** *P. leightoni*, *P. namibensis*, *P. trinasalis* **with the closest sister,** *P. notostictus*. **Grey bar displays the barcoding gap transitioning between intra- and intraspecific distances. Dotted line indicates intraspecific cut-off.**

18

Figure 2.5: Frequency of uncorrected p-distances based on Cyt-b gene region for *Psammophis*. **White bars: intraspecific uncorrected p-distances. Black bars: interspecific uncorrected p-distances. A – Variation between** *P. leightoni* **and** *P. namibensis* **(South Africa). B – Variation between** *P. leightoni* **and** *P. namibensis* **(Namibia). C – Variation between** *P. leightoni*, *P. namibensis* **and** *P. trinasalis*. **D – Variation between** *P. leightoni*, *P. namibensis*, *P. trinasalis* **with the closest sister,** *P. notostictus*. **Grey bar displays the barcoding gap transitioning between intra- and intraspecific distances. Dotted line indicates intraspecific cut-off.**

Figure 2.6: *Psammophis* **phylogeny with corresponding species delimitation methods results indicated to the right. A: Barcoding results based on both mtDNA gene regions. B: GMYC result based on mtDNA dataset. C: bGMYC delimited taxa based on probabilities ≤ 0.95. Supported nodes in the phylogeny denoted with black circles. Each colour represents a species based on the applied model. Colours that are broken up indicate splitting by the applied model.**

20

2.4 Discussion

The currently described species within the *Psammophis leightoni* complex are most likely a single taxon, and this is supported by multiple species delimitation methods. With this, the current taxonomy should be revised as there is little doubt they are conspecific. Recognising the *P. leightoni* complex as a single taxon is supported not only by the genetic species delimitation methods, but by their analogous morphological traits as well. There are some ecological differences, but this taxon is widespread, interacting with various habitats in which they have potentially become locally adapted. Furthermore, the level of genetic divergence between them is low and is similar to population level divergence. The barcoding graphs (Figure 2.4 & 2.5) suggest there may be differences in what is considered true *P. namibensis*, and individuals from South Africa are most likely misidentified *P. leightoni* (grouped within *P. leightoni* subclade: Figure 2.3). However, total evidence still suggests a single taxon. Should the taxonomy be revised, *P. namibensis* Broadley, 1975 and *P. trinasalis* Werner, 1902 would become synonyms of *P. leightoni* Boulenger, 1902.

Classifying the *Psammophis leightoni* complex as a single species is only acceptable with the appropriate species concept. The Unified (General) species concept (GSC) requires multiple lines of evidence in order to appropriately delineate true species (De Queiroz 2007). To meet the requirements of the GSC, conditions of the phylogenetic species concept were used to validate results of this chapter as lines of evidence. The clade recovering all taxa of the *P. leightoni* complex is monophyletic, demonstrated by the results of the phylogenetic analyses (Rosen 1979; Donoghue 1985; Mishler 1985). Moreover, results of the Bayesian and barcoding delimitation analyses suggest this clade is distinct from closely related congeners (Nelson and Platnick 1981; Cracraft 1983; Nixon and Wheeler 1990). Therefore, taxa of the *P. leightoni* complex comply with prerequisites of the phylogenetic species complex for delimiting species. In addition, taxa of the *P. leightoni* complex have been inferred to interact as conspecific organisms utilising similar niche space and adaptive zones (chapter 3), meeting delimitation requirements of the ecological species concept (van Valen 1976; Andersson 1990). Taking this into consideration, the *P. leightoni* complex may be regarded as a single species under the general (unified) species concept, meeting the requirements of both the phylogenetic and ecological species concepts validating the GSC.

Field identification of taxa within the *Psammophis leightoni* complex has been problematic because of their morphological similarity. This similarity, mirrored by their genetics, provides further support for this species complex representing a single taxon. Assuming *P. leightoni* is a single widespread taxon, there are several important morphological features that unify the complex for identification. *Psammophis leightoni* has 17 scale rows and eight supralabials, with the fourth and fifth labial entering the eye. Furthermore, they have one preocular scale and the posterior nasal scale is divided (Branch 1998). The cloacal shield is also divided. In terms of coloration, this is variable as with most species of *Psammophis* (Broadley 2002), however, the interior populations (previously *P. trinasalis*) displays a lateral stripe present on the temporal scales continuing as a dorsolateral stripe along the entire body (discussed in chapter 1). Taking *P. leightoni* as a single taxon allows these traits to be used when separating congeners that overlap with their distribution. For example, *P. crucifer* is the

21

only congener in the region with 15 scale rows, and *P. trigrammus* is the only southern African *Psammophis* with nine supralabials, with the fifth and sixth labial entering the eye (Broadley 2002). Defining *P. leightoni* from its sister taxon, *P. notostictus*, can be done using the cloacal shield as *P. notostictus* is the only *Psammophis* with an entire cloacal shield (Broadley 1977, 2002). These morphological characters allow for clear distinction from congeners, and can be used to validate the *P. leightoni* complex specific rank in conjunction with the results presented in this study.

Given the subclades shown within the *Psammophis leightoni* complex (Figure 2.3), there is reason to believe there may be population level structure across their geographic range. Within South Africa, the Northern Cape population of *P. namibensis* could be considered as an extension of the *P. leightoni* Western Cape population. This is based on these individuals level of divergence and being recovered within the *P. leightoni* subclade. The Namibian population of *P. namibensis* shows the greatest range of divergence within this complex. However, this is based on single representative which still falls below intraspecific thresholds for this genus. Assuming the *P. leightoni* complex is a single taxon, the variation seen between individuals is potentially a result of its wide geographic range and being influenced by a range of selection pressures. Even with this variation, both the bGMYC and barcoding analyses suggest the *P. leightoni* complex represents a single taxon, although, the GMYC result does contradict this claim (Figure 2.6.B). However, the GMYC has been noted to render well-supported clades of haplotypes as independent lineages leading to overestimation of species (Reid and Carstens 2012; Fujisawa and Barraclough 2013; Talavera et al. 2013). This overestimation of species is largely attributed to the number of individuals used in the analysis as this increases the likelihood of recovering rare and divergent haplotypes by the analysis which may be interpreted as additional species (Michonneau 2015). Specified parameters used in GMYC analyses have not been established, largely due to the unknown variation within datasets (Reid and Carstens 2012). For this reason, GMYC analyses should be used as part of a suite of tools in order to accurately delimit species.

Even though *Psammophis leightoni* would be considered widespread, assuming the species complex is a single taxon (distribution range: ca. 2 200 000 km^2), much of the South African range (especially along the west coast) is over highly transformed areas and may be inaccessible (Maritz 2014). Formally protected land spans less than 3000 km^2 on the west coast of South Africa where *P. leightoni* is known to occur, the majority of which is the Richtersveld Transfrontier Park (1624 km^2) found in the north-western corner of the Northern Cape (NPAES 2009). For comparison, in Namibia, the majority of the previously considered *P. namibensis* known range falls within the 130 000 km^2 formally protected regions (SPAN 2010). *Psammophis leightoni*, in the Western Cape, South Africa, is only formally protected within a limited region which highlights concern around potential losses of this peripheral population at local scales. Currently, the most significant cause of biodiversity loss in the Western Cape is habitat transformation, with agriculture, infrastructure development and urban expansion resulting in considerable losses of natural habitat, especially evident along the west coast (Turner 2012; Meyer and Maree 2013).

There is evidence of declines in populations of reptiles on a global scale (Gibbons et al. 2000; Reading et al. 2010; Sewell et al. 2012; Böhm et al. 2013; Tolley et al. 2016). Similarly, these declines have been attributed to a number of causes which include habitat loss or transformation (Gibbons et al. 2000), and climate change (Walther et al. 2002; Loarie et al. 2009; Ihlow et al. 2012). Snakes are considered top predators and a decline in the quality of their habitat can lead to a reduction in their numbers resulting in serious consequences concerning ecosystem functioning (Ostfeld and Holt 2004; Sergio et al. 2008). Evidence suggesting that snake populations are in decline are limited relative to other reptiles (Gibbons et al. 2000; Wake and Vredenburg 2008; Alroy 2015) however, there is a consensus that snakes may be disappearing globally (Seigel and Mullin 2009). There is a considerable lack of long-term individual-based studies of snake populations, accurate taxonomic works and ecological knowledge base, especially within an African context (Tolley et al. 2016). Although the causes of these declines are currently unknown, it is suspected that they are multi-faceted with a common root cause (e.g. global climate change) (Reading et al. 2010).

2.5 Conclusion

In summary, these molecular results suggest that the *Psammophis leightoni* complex represents a single species, requiring a complete taxonomic revision. Furthermore, this would necessitate a reassessment for the IUCN Red List as a single species. Additionally, my prediction that these taxa are conspecific is supported by the level of intraspecific divergence between them, as well as congruence across all delimitation analyses. Given that the conservation status for *P. leightoni* relies on clarification of the current taxonomy, this result may provide foundational evidence for a conservation reassessment which may remove its current threat status. Should the taxonomy be revised, *P. namibensis* Broadley, 1975 and *P. trinasalis* Werner, 1902 would be become synonyms of *P. leightoni* Boulenger, 1902. Recommending that these taxa be considered a single species would be welcomed in light of reports which state snake populations are in declining (Reading et al. 2010). Unfortunately, reversing the trend of declining snake populations in the future will be a lengthy process, only reiterating the need for accurate species delineation emphasising threatened species truly in need of conservation resources.

23

CHAPTER THREE

HISTORICAL BIOGEOGRAPHY OF THE *PSAMMOPHIS LEIGHTONI* SPECIES COMPLEX

3.1 Introduction

Species occurrences are typically restricted by mechanical or environmental constraints on the organism (Sexton et al. 2009). Although distributions are often defined by hard boundaries (e.g., coastlines), many species occur in several different environments or even across environmental gradients (Hargreaves et al. 2014). Identifying the ecological drivers outlining where species occur can be problematic because several drivers could be working in synergy and are thus difficult to tease apart. Interpretation of occurrences using environmental variables could identify primary drivers of species presence/absence with regards to their distributions (Holt 2003).

Predicting shifts in geographic distributions (e.g., expansions, contractions, and fragmentation), particularly as a result of climate change (Davis and Shaw 2001; Sinervo et al. 2010), is becoming increasingly important for conservation planning (Elith et al. 2006; Williams et al. 2008; Chen et al. 2011; Hargreaves et al. 2014). With the increasing availability and access to species occurrence data (through museum, herbarium collections or bioinformatic databases: Jetz et al. 2012; Phillips & Dudík 2008) an opportunity has developed to utilise these datasets effectively to support conservation efforts. However, occurrence data typically provides information on localities known to be occupied by species but does not include information on where species may be absent. Inaccurate occurrence data (with vague locality descriptors or low accuracy) can greatly affect conservation planning and its outcomes (Larsen and Rahbek 2005; Rondinini et al. 2006).

Effective management decisions can be greatly improved through the projection of species distribution as a number of problems can be anticipated and accommodated for beforehand, particularly when applying models incorporating occurrence records and spatial environmental data to predict environmental suitability (Guisan et al. 2013). Problems that can be predicted include shifts in suitable habitat for a number of species due to climate change (Araújo et al. 2011), areas that are possibly be invaded by a pest species (Thuiller et al. 2005), and the identification of conflict areas where species are unable to migrate across human-modified landscapes (Guisan et al. 2013). Species distribution modelling (SDM; also referred to as ecological niche modelling, ENM) is a popular tool used to build spatially explicit predictions estimating environmental suitability for species (Guisan and Thuiller 2005; Phillips and Dudík 2008; Elith and Leathwick 2009). These models are often substantiated through statistical relationships between species occurrence and environmental descriptors, although other approaches do exist (Guisan et al. 2013). Alternative approaches include engaging with expert opinion and using mechanistic modelling, which integrate processes which limit distributions (Kearney et al. 2010).

24

Identifying conservation problems and applying appropriate responses are important actions within a global conservation-driven culture (Naidoo et al. 2008; Guisan et al. 2013). Potentially, SDMs have a role to play in identifying likely shifts in suitable habitat (altering suitability as a result of climate change) of threatened species (Walther et al. 2002; Van Der Putten et al. 2010). In addition to identifying areas of overlap between closely related species (e.g., forming temporary hybrid zones: Taylor et al. 2015), SDMs may allow detection of areas where species may, or may not, be able to migrate through human-modified landscapes (Butler et al. 2005). Furthermore, the way species are spatially distributed greatly influences a species' genetic characteristics, largely affecting interplay between genetic drift, gene flow and natural selection (Eckert et al. 2008; Kozak et al. 2008). These influences dictate how species adapt, develop, and evolve under changing pressures, when only restricted by the species capability to adjust.

Understanding a taxon's evolutionary history and spatial distribution can assist in identifying genuinely threatened species as well as defining species boundaries. For example, the evolutionary history of modern tiger (*Panthera tigris*) populations was assessed phylogeographically based on geographically referenced specimens (or at least specimens from known putative subspecies; Cooper et al. 2016). However, because of limited sampling across the fragmented populations, the gaps in data restricted the conclusions that could be drawn (Luo et al. 2004; Waltari et al. 2007; Kozak et al. 2008; Driscoll et al. 2009). Cooper et al. (2016) highlighted that to understand the current phylogeographical patterns, there was a need for a geographically explicit understanding of the expansion and contraction of tiger ranges during glacial/interglacial cycles. Understanding the evolutionary history of tigers was important to successfully conserve this Endangered species. The findings of Cooper et al. (2016) supported the idea of unimpeded gene flow between all populations (as a result of the continuous modelled distribution of tigers in mainland Asia). As a result, a more targeted approach regarding conservation management was advised for mainland tiger populations, as it is likely that only recent anthropogenic changes caused the current disjunction between populations.

Similarly, confusion regarding the species boundaries of the *Psammophis leightoni* species complex plays a role in affecting conservation action of this group. *Psammophis namibensis* and *P. trinasalis* were recognised subspecies of *P. leightoni* prior to their taxonomic rank change based on ecological differences (Broadley 2002). With this taxonomic change, *P. leightoni* is now only known from a restricted region within the Western Cape, South Africa. According to the most recent IUCN Red List assessment (Maritz 2014), *P. leightoni* is listed as Vulnerable [B1ab(iii)] due to its limited distribution, habitat transformation and fragmentation. However, whether or not conservation effort is fitting for *P. leightoni* is largely dependent on the validity of the current taxonomic arrangement (Maritz 2014). There is reason to believe, with the morphological evidence and updated phylogeny presented in chapter 2, that these taxa may indeed represent a single species. Given that the taxa are marginally different in terms of their ecology, and exhibit overlapping morphological characters, it may be that *P. trinasalis* is simply displaying a unique phenotype of the same species (e.g. uniformly coloured *P. crucifer*: Broadley 1977; Branch 1998). In some cases,

25

morphological characteristics of species are often attributed to where they occur (Wüster et al. 2005; Breitman et al. 2015), and with the potential changes in species ranges due to climate change, certain morphological traits may be conserved or lost as a result (Thomas et al. 2006; Chen et al. 2011).

To shed light on the extent at which these species are spatially restricted, SDMs could be used to predict climatic suitability and infer the geographic ranges of these species to test whether 1) these species might be allopatric at present, and 2) if they might have been in contact historically. Given their current distributions, I predict that there are no differences in the climate envelopes of *P. namibensis*, *P. leightoni*, and *P. trinasalis* and this would not (in part) define and limit their distributions. I aim to map the climate envelopes of these taxa using a species distribution model approach by applying a maximum entropy framework.

Legend
RSA .... South Africa
NAM .... Namibia
BWA .... Botswana
LSO .... Lesotho
WC .... Western Cape
NC .... Northern Cape
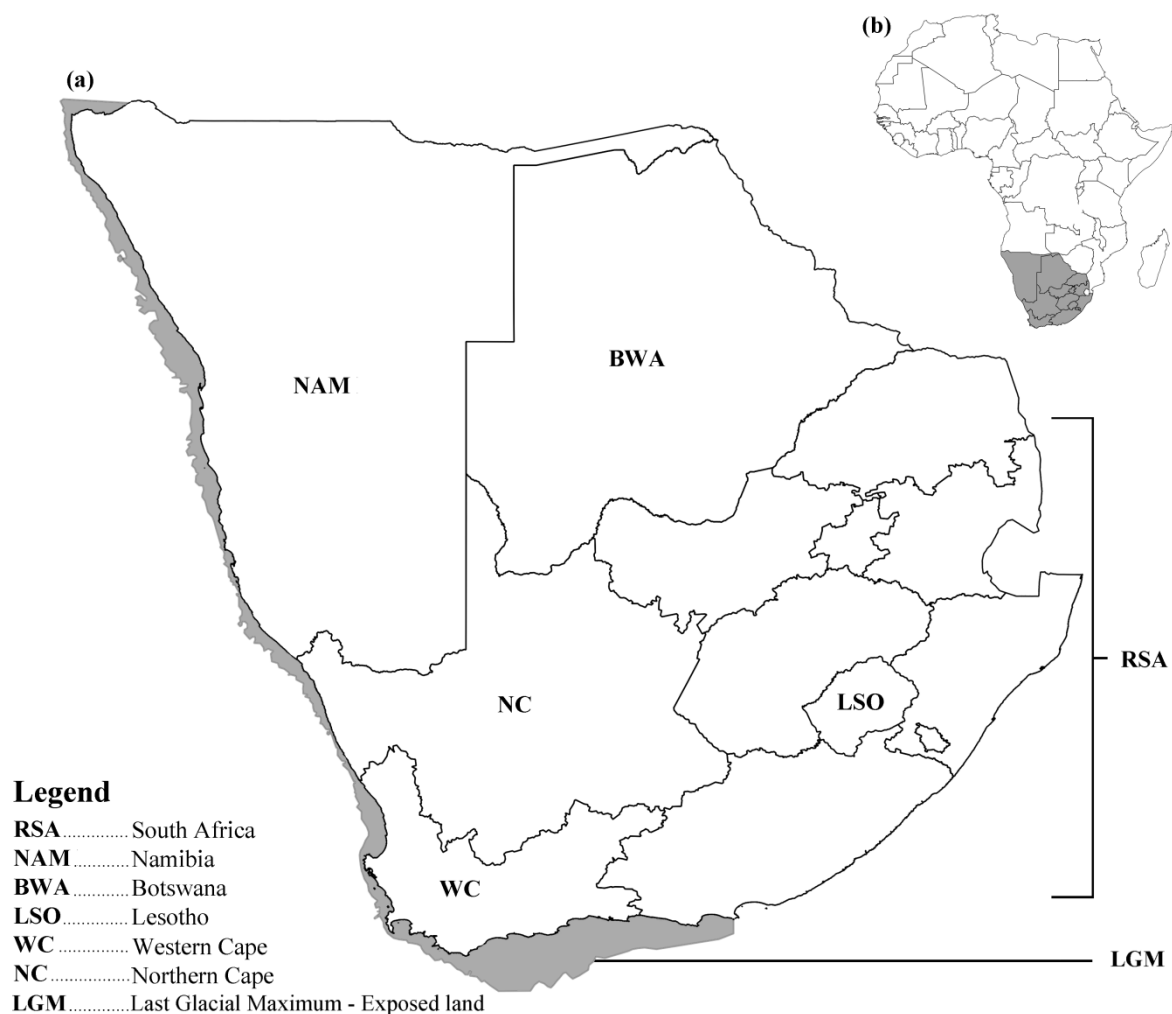**LGM** .... Last Glacial Maximum - Exposed land

Figure 3.1: The geographic extent of the study area. (a): All models are restricted to South Africa, Namibia and Botswana. Palaeoclimate models included exposed land during the Last Glacial Maximum. (b): The extent of the study area within Africa.

26

3.2 Methods

3.2.1 Current climatic envelope

Species distribution modelling was carried out using the maximum entropy approach in Maxent v3.3.3 (Phillips et al. 2006; Phillips and Dudík 2008). Spatial extent crucially affects model predictions (Anderson and Raza 2010; Barve et al. 2011), therefore all models were restricted to regions where these taxa are known to occur (*Psammophis leightoni*: Western Cape, South Africa, *P. namibensis*: South Africa & Namibia, *P. trinasalis*: South Africa, Namibia & Botswana; Figure 3.1 & Figure 3.2). The distributions were modelled separately for each species and as a single taxon under two treatments. The first single taxon treatment (STT1) assumes that *P. leightoni* and *P. namibensis* are a single species (inferred by Kelly et al. 2008) to the exclusion of *P. trinasalis*, while the second treatment (STT2) assumes all three taxa are a single species (inferred in chapter 2). All available occurrence records (52 *P. leightoni*, 132 *P. namibensis* and 253 *P. trinasalis*) were compiled from the South African Reptile Conservation Assessment (SARCA: Bates et al. 2014) supplemented with museum records collated in Broadley (2002). Maximum entropy was the preferred approach, as it performs better than other methods, especially when using a low number of occurrence localities (Elith et al. 2006; Pearson et al. 2007). Nineteen bioclimatic variables were downloaded from the WorldClim database (Hijmans et al. 2005) at a resolution of 2.5 arc minutes and trimmed to Namibia, Botswana and South Africa in QGIS 2.14.15 (Open Source Geospatial Foundation Project, 2017). Additional variables (e.g. vegetation type, soil type, land-use, and infrastructure) were not used in these models as they are not projectable to palaeoclimatic periods. Autocorrelation between the bioclimatic variables was examined using ENMtools 1.4.3 (Warren et al. 2010) with a pairwise Pearson correlation, and all highly correlated variables ($r \leq |0.75|$) were removed (Cooper et al. 2016; Dagnino et al. 2017).

Preliminary models were run to assess which environmental variables and parameters most influenced the climatic envelope of each species and single taxon treatments. The occurrence records used in these models were rarified using ArcGIS 10.4.1 (Esri 2012, Redlands, CA, USA), at a distance of 10 km between unique localities to limit the spatial autocorrelation within the occurrence records. Within Maxent, changes to the regularization multiplier and feature types were tested on the dataset to improve model performance (Merow et al. 2013; Radosavljevic and Anderson 2014; Morales et al. 2017) however, the default settings best suited the data of *P. namibensis* and *P. trinasalis* when using a bias grid file. Maxent assumes that occurrence data were spatially unbiased when sampled. To account for sampling bias, the Gaussian kernel density of sampling localities tool was used from the SDMToolbox in ArcGIS (Brown 2014). This tool produced a bias grid file which increases the weight of occurrence points with fewer neighbours across the landscape when used in Maxent. Modelling *P. leightoni* required no bias file (as occurrence data showed no dense clusters after being rarified) and the regularization multiplier was set at 1.5 while default feature types were used.
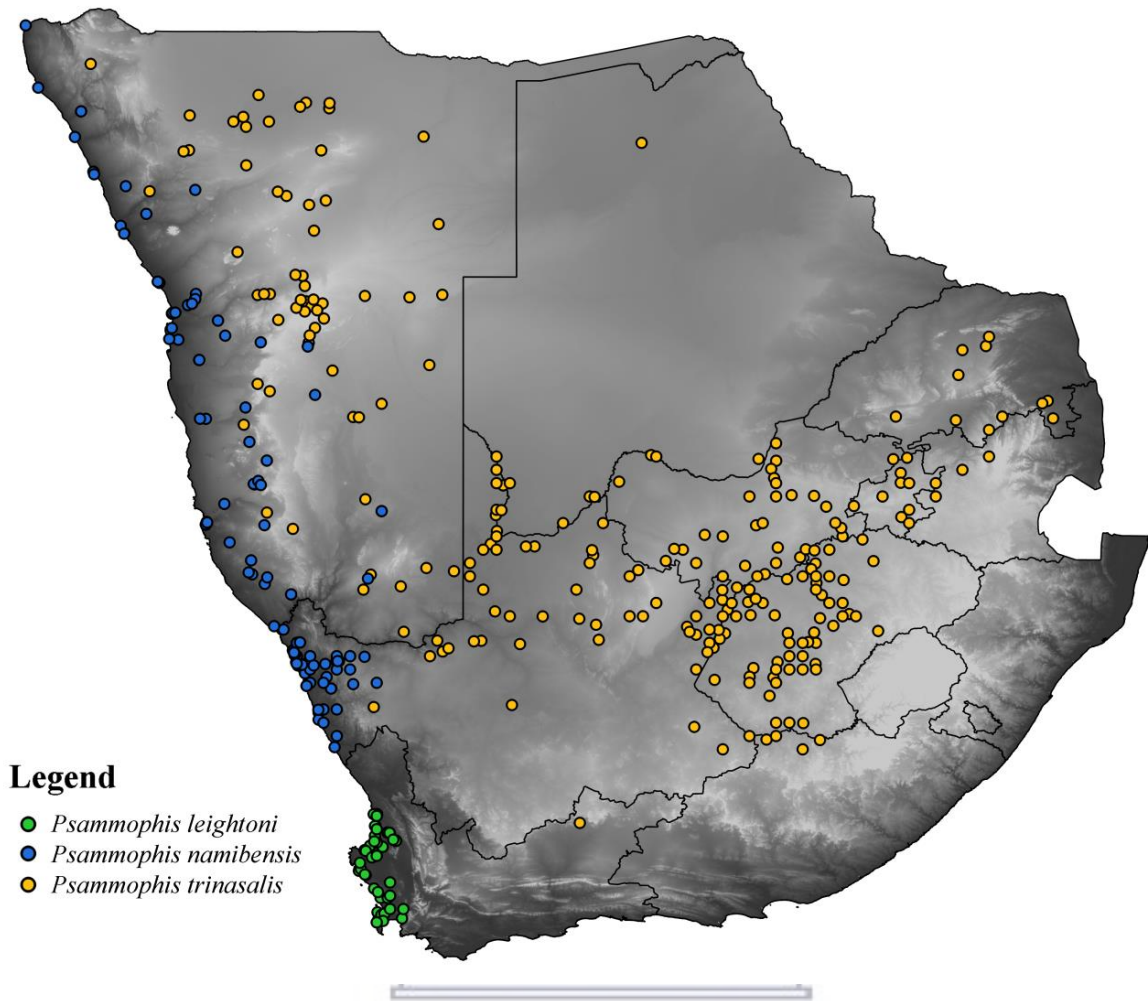
Figure 3.2: The occurrence data for the *Psammophis leightoni* complex from Broadley (2002) and SARCA (see text for details) overlaid onto a digital elevation model. *Psammophis leightoni* - green, *P. namibensis* - blue, *P. trinasalis* - yellow.

Successive models were run using the adjusted settings for 100 replicates. Each replicate had a 25% random test subsample to ascertain the model's predictive power on the locations used. Maximum iterations were increased to 5000 to allow the models to converge. Results of all replicates were inspected reviewing the test area under the curve (AUC) statistic of the receiver operating characteristic (ROC) plots, the table of variable contributions, and the three jackknife tests (regularized training gain, test gain, and AUC). In order to refine and select variables for further models, bioclimatic variables that contributed < 1% to the model performance when considering the permutation importance and (when present) resulted in no change in model predictive performance (according to at least one of the jackknife tests) were removed.

28

Models were rerun using the reduced set of bioclimatic variables, with variables removed until the remainder only improved the models' predictive power. The outputs from the last 100 replicates were evaluated with the final set of variables (nine bioclimatic variables). AUC measures are known to be correlated with area size, as well as being sensitive to occurrence data spatial density (Lobo et al. 2008). For this reason, the AUC scores were supplemented with the true skill statistic (TSS: Allouche et al. 2006) when assessing final model performance. The replicate with highest TSS value, assessed with the degree of agreement (Monserud and Leemans 1992), was selected for further processing. When mapping final model predictions (as present/absent: QGIS), the 10 percentile training presence logistic threshold was used. This threshold best suited the data as it is less sensitive to outliers and reduces over-predictions made by the model (Ficetola et al. 2009; Hu and Jiang 2010).

3.2.2 Palaeoclimatic envelope

Fluctuations in climate are known to affect species distributions (Ikeda et al. 2016; Rosenzweig et al. 2008; Taylor et al. 2015), with the most recent large scale climatic shifts being at the last Glacial Maximum and then lessened during mid-Holocene. Therefore, predicted distributions for the study taxa at these time periods were modelled using palaeoclimate environmental variables downloaded from WorldClim (Palaeoclimate Modelling Intercomparison Project Phase II [PMIP2]: Braconnot et al. 2007), derived from the general circulation models (GCMs; CCSM-4, MIROC-ESM, and MPI-ESM-P: Hijmans et al. 2005) based on CMIP5 (Taylor et al. 2012) data. These data are widely used when constructing palaeoclimate models incorporating climate cycles (e.g., Brown and Knowles 2012; Edwards et al. 2012; Alvarado-Serrano and Knowles 2014; Ornelas et al. 2015; da Silva and Tolley 2017). Suitable climate during the LGM and mid-Holocene were predicted by projecting the reduced set of bioclimatic variables from the optimised present day model. LGM variables are only available at a resolution of 2.5-arc minutes (Braconnot et al. 2007) and to maintain uniformity, mid-Holocene variables were downloaded at this resolution. As with the current climate predictions, a 10% training presence logistic threshold was used when identifying suitable and non-suitable habitat. When mapping the palaeoclimatic predictions, models for each GCM were reclassed and combined using ArcGIS and then classed for climate suitability (grid cells predicted present in all GCMs - High suitability, present in two GCMs - moderate suitability, present in one GCM - low suitability, not present in any GCM - not suitable).

3.2.3 Detecting refugia with climatic stability

To identify potential refugia, areas with high climatic stability over time were identified. The suitability maps for each time period (LGM, Holocene, and Present) were reclassed to binary (suitable and non-suitable climate) for each taxon (including STT1 and STT2). Climate stability was then estimated by summing the binary maps in QGIS, with four different possibilities for each grid cell (suitable in all periods=3, suitable in two periods =2, suitable in one period =1, not suitable in any period=0). Climatically stable grid cells therefore, would have high suitability across all three time slices (e.g. da Silva & Tolley 2017). Conversely, low stability would be reflected by a lack of suitability across the time slices.

3.3 Results

All processed models maintained acceptable levels of performance considering both AUC and TSS scores (Table 3.1). The overall TSS scores were > 0.10 lower than the respective AUC for all species, except *Psammophis leightoni*. *Psammophis leightoni* maintained scores > 0.99 for both AUC and TSS. Models which were projected over a wider geographic area had the greatest range of scores between AUC and TSS (i.e. *P. trinasalis* and STT2).

Table 3.1: Summarised performance scores (AUC and TSS) for each taxon and taxon treatment (STT1 & STT2) model.

		Psammophis leightoni		*Psammophis namibensis*		*Psammophis trinasalis*		STT1		STT2	
		AUC	TSS	AUC	TSS	AUC	TSS	AUC	TSS	AUC	TSS
Current		0.99	0.993	0.965	0.871	0.89	0.690	0.948	0.847	0.868	0.651
Mid Holocene	**CCSM-4**	0.996	0.994	0.94	0.853	0.899	0.632	0.92	0.8	0.864	0.655
	MIROC-ESM	0.996	0.992	0.958	0.866	0.895	0.681	0.951	0.864	0.875	0.648
	MPI-ESM-P	0.995	0.993	0.962	0.831	0.869	0.635	0.934	0.833	0.863	0.629
LGM	**CCSM-4**	0.994	0.991	0.961	0.888	0.881	0.659	0.89	0.829	0.853	0.631
	MIROC-ESM	0.995	0.994	0.961	0.861	0.859	0.683	0.935	0.816	0.869	0.621
	MPI-ESM-P	0.997	0.992	0.956	0.847	0.826	0.728	0.945	0.859	0.874	0.641

3.3.1 Current climatic envelope

The model predicted the current climate envelope (CCE) of *Psammophis namibensis* extending further south into the Western Cape than currently understood (Figure 3.3.a.i). *Psammophis leightoni* has a comparatively restricted CCE, which is somewhat fragmented in the western parts of the Western Cape (Figure 3.3). *Psammophis trinasalis* CCE as the largest extent, however it only overlaps with *P. namibensis* in Namibia (Figure 3.3.a). This model predicts potential climate pockets along the coastal regions of South Africa. The STT1 model predicts a continued envelope along the west coast, with an extension farther inland along the southern end of the Northern Cape (Figure 3.3.b), while the CCE predicted for STT2 is divided into two main areas (Figure 3.3.c). The first area of suitability for STT2 occurs through the southern end of the Kalahari Basin and further east to central South Africa. The second region extends along the west coast, expanding in central Namibia and western parts of South Africa.

30

Figure 3.3: Predicted climate envelopes for the *Psammophis leightoni* complex, as separate taxa and single taxon treatments, both current and mid-Holocene time slices. (i) & (ii): detailed view of the area of connectivity between taxa of the *P. leightoni* complex during respective time slices. Climate suitability scale shown in legend. Current climate models are shown for (a) all taxa of the *P. leightoni* complex, (b) STT1 and (c) STT2. Palaeoclimate models during mid-Holocene are shown for (d) all taxa of the *P. leightoni* complex, (e) STT1 and (f) STT2.

31

3.3.2 Palaeoclimatic envelope

During the mid-Holocene, the models suggest the palaeoclimate climate envelope of *Psammophis namibensis* extended further south than present (Figure 3.3.d.ii). Similarly, the palaeoclimate envelope of *P. leightoni* suggests that the species may have been more restricted and pushed southwards (Figure 3.3.d). The *P. trinasalis* model had high-moderate climate suitability through central regions of South Africa and parts of southern Botswana and central Namibia (Figure 3.3.d). There are two additional disjunct regions predicted, the first in the east of South Africa and the second along the west coast overlapping with envelopes of *P. namibensis* and *P. leightoni*.

The STT1 model shows high suitability through the southern half of the predicted envelope at the mid-Holocene but the northern half is somewhat fragmented, particularly along the mid-Namibian coastal regions (Figure 3.3.e). The STT2 model suggests connectivity through South Africa, Botswana and Namibia with high-moderate regions being maintained throughout (Figure 3.2.f). Along the west coast, suitability is highest in the north and in the south, with intermediate regions lower in suitability overall. However, connectivity could have been maintained through these lower areas, which are interspersed with small patches of high suitability.

For the Last Glacial Maximum, the model shows the climate envelope for *Psammophis namibensis* likely persisted on the west coast, occupying land exposed by sea level retractions (high suitability for this species); while extending southward into South Africa (Figure 3.4.a). The LGM model under predicted suitable regions for *P. leightoni* with an envelope with low suitability restricted to 310 km^2 (on land exposed due to sea level retractions) which overlapped with the region of low suitability for *P. namibensis* (Figure 3.4.a.i). *Psammophis trinasalis* had predicted envelope further north into Botswana and was fragmented in South Africa, however there was a suitable area of moderate-low suitability in the south west (Figure 3.4.a).

The STT1 model predicted two highly suitable isolated regions (one in central-Namibia and the other in the south western South Africa) at the LGM (Figure 3.4.b). There are predicted areas of moderately suitable climate but these are discontinuous, especially in southern Namibia. The STT2 model suggests there are three disjunct areas of suitability. The largest is centrally located, primarily over Botswana whereas the others are along the coast, situated to the north (Namibia) and south (South Africa) (Figure 3.4.c).

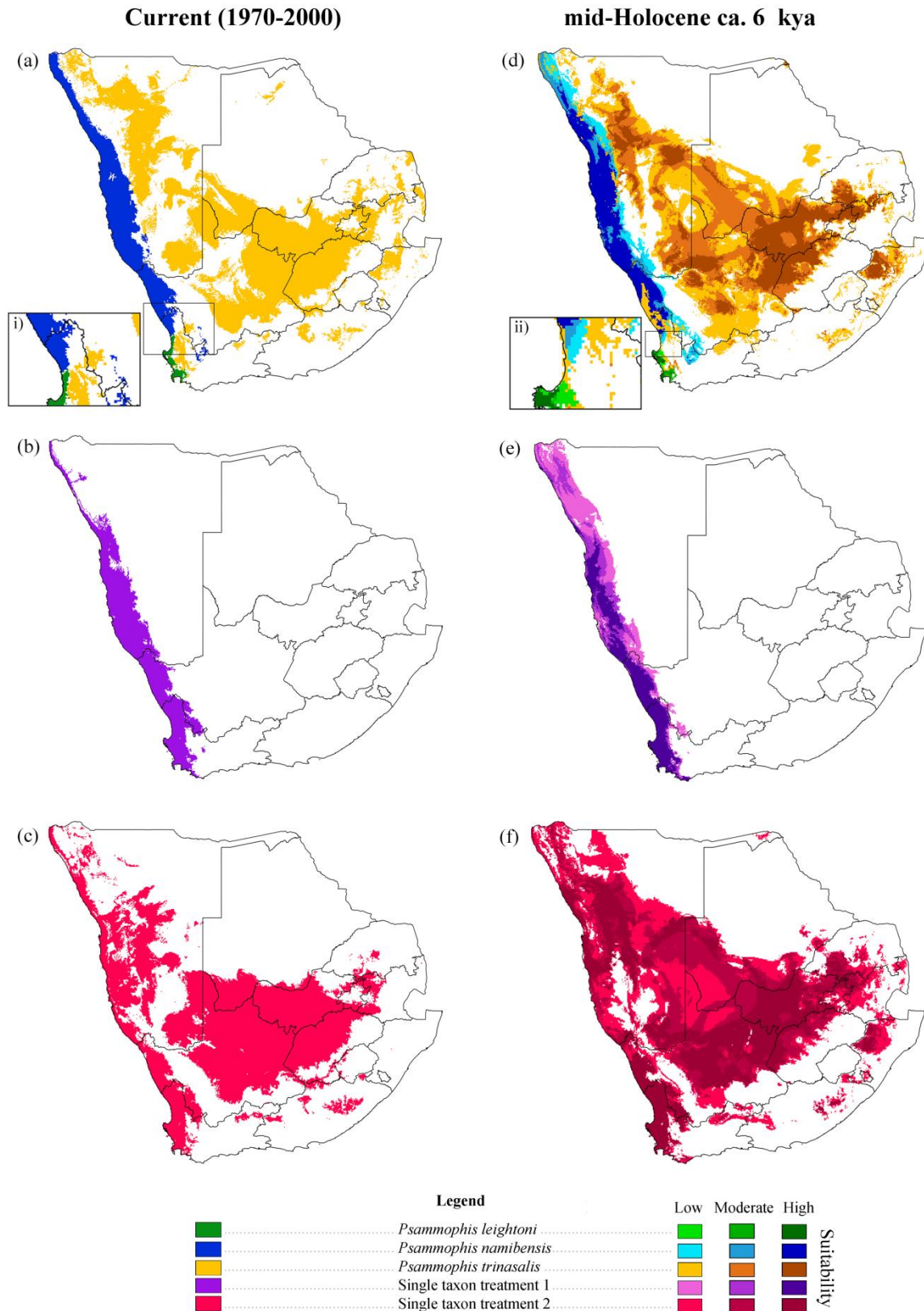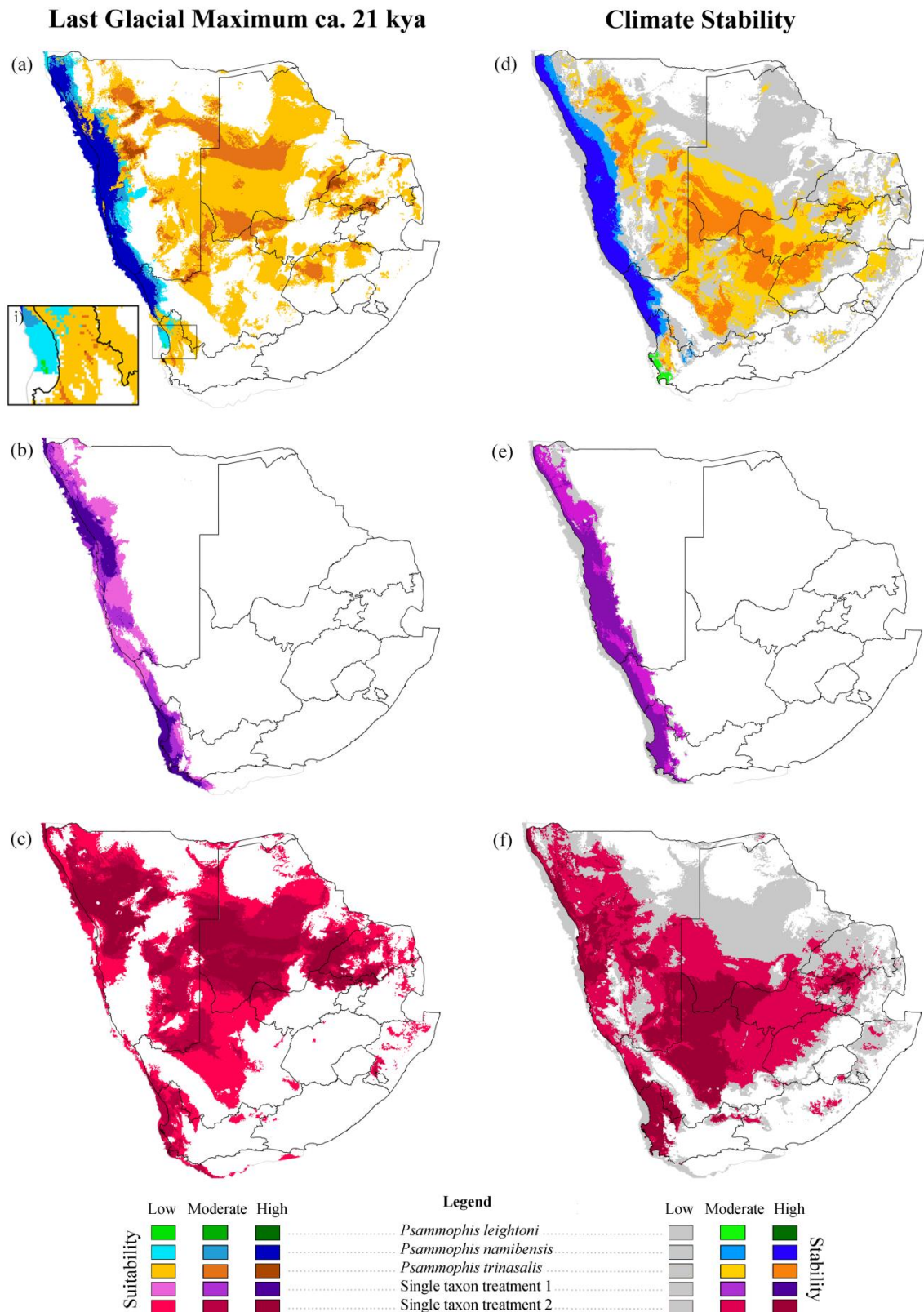**Figure 3.4: Predicted climate envelopes for the *Psammophis leightoni* complex, as separate taxa and single taxon treatments during Last Glacial Maximum. Climate stability maps for respective taxa depict possible climate refugia. (i): Overlapping climate envelopes of the *P. leightoni* complex. Palaeoclimate models during the Last Glacial Maximum are shown for (a) all taxa of the *P. leightoni* complex, (b) STT1 and (c) STT2. Climate suitability and stability scale shown in legend. Areas of stable climate are shown for (d) all taxa of the *P. leightoni* complex, (e) STT1 and (f) STT2.**

33

3.3.3 Climate stability

When treated as separate taxa, the models showed areas of high climate stability for *Psammophis namibensis* and *P. trinasalis*, but not for *P. leightoni* (Figure 3.4.d). The most stable areas for *P. namibensis* correspond to the present day models. Areas of high stability for *P. trinasalis* are fragmented throughout the central regions. Stable climate space for STT1 along the west coast persists from Namibia into South Africa (Figure 3.4.e). STT2 has three main areas of high stability connected by intermediate regions with moderately stable climates. Most notably, highly stable climate continues along the west coast from South Africa to Namibia (Figure 3.4.f).

UNIVERSITY *of the*
WESTERN CAPE

34

3.4 Discussion

Contrary to current thinking based on observations and locality records, the species distribution models suggest there is unlikely to be a gap between the geographic distributions of *Psammophis namibensis* and *P. leightoni* (Figure 3.3.a). More importantly, these results do not support the taxonomic adjustments made by Broadley (2002) that were based on these taxa being ecologically distinct. The current climate models suggest that STT1 forms a well-supported distribution along the coast in support of chapter 2. The palaeoclimate models for STT1 show that there is reason to believe there was historical disjunction between Namibia and South Africa corresponding with chapter 2 but not as currently interpreted in the literature (Kelly et al. 2008). Moreover, the palaeoclimate models for STT2 show that the complex may have, very early on, been split into an interior morph (*P. trinasalis*) and a coastal morph (*P. leightoni*/*P. namibensis*). The continuing stable climate bridging the current distributions of these taxa would suggest the currently described species within the *P. leightoni* complex could be co-occurring in certain regions with genetic exchange taking place. This is further justified by the consistent overlap of envelopes by the separate taxon models as well as the intraspecific variation presented in chapter 2. Furthermore, the assumptions made by Kelly et al. (2008), where *P. leightoni* and *P. namibensis* show intraspecific genetic variation between them, are well supported by these models.

The South African population of the currently described *Psammophis namibensis* is suggested to share climate space more often with the *P. leightoni* Western Cape population than with the Namibian *P. namibensis* population, supporting the genetic variation discussed in chapter 2. The predicted climate envelope of *P. namibensis* overlapping with *P. leightoni* could indicate that their distributions are not allopatric. This would suggest there have never been substantial periods of separation between them and this supports the idea that they are one taxon, genetically identical, with a continuous distribution. The fragmented climate envelope for *P. trinasalis* across South Africa would suggest that this taxon is not explicitly limited by climate. The occurrence of *P. trinasalis* estimated by the models suggest they may be partially restricted by altitude and/or substrates, as the separation between suitable climates is disconnected by the Cape Fold Belt, the Greater Escarpment and the Drakensberg. However, this is refuted since a representative of *P. trinasalis* sampled in the Karoo National Park, Western Cape (chapter 2) would suggest the disjunct predicted climate regions for this taxon may be real and that these snakes are able to migrate through unfavourable climate space.

Assuming the *Psammophis leightoni* complex is a single taxon, STT2 supports the level of intraspecific divergence presented in chapter 2. Additionally, the molecular divergence of currently described *P. trinasalis* and its fluctuating climate envelope (cycling between fragmentation and reconnection) suggests the unique lateral stripe displayed by *P. trinasalis* is maintained since contact with the populations of *P. leightoni* or *P. namibensis* is limited to specific contact zones. This is supposedly why this defining character is evident throughout the distribution of *P. trinasalis*. When the study taxa are modelled separately there is still overlap during certain time periods allowing for contact between all three taxa before separating into three distinctive refugia during periods of colder climate. Similarly, a number

35

of species are inferred to have undergone changes in their ranges due to climate changes since the LGM, with the development of unfavourable climate conditions outside of species' physiological tolerances (Erasmus et al. 2002; Hewitt 2004; Waltari et al. 2007). The period where the study taxa are separated may have resulted in the genetic and morphological differences (contributing to the previous impression of unique species); however, this period of separation was not long enough to speciate. Overall, the historical range contractions of these three study taxa have historical refugia that do not correspond with our current understanding of their species boundaries. Taking this into consideration, species delineation of these taxa would need to be revised.

Species distribution models are useful in predicting the extent of (and detecting shifts in) species distributions, and is highly favoured as a tool amongst researchers (Zimmermann et al. 2010; Schorr et al. 2012). There are, however, concerns regarding modelling performance and predictive power (Elith et al. 2006; Austin and Van Niel 2011). In terms of modelling the study taxa accurately, a primary concern is the way in which the spatial extent of the models plays a role in model performance (Lobo et al. 2008). Therefore, the spatial extent for each model was restricted in such a way to reduce the likelihood of exaggerating AUC scores (Anderson and Raza 2010; Barve et al. 2011). This emphasised a need for supplementation of an additional model performance measure when assessing models as well as restricting model areas to the extent surrounding occurrence points of each taxon (including STT1 and STT2). When modelling *Psammophis leightoni*, as it is recorded within a limited geographic range (Branch 1998; Bates et al. 2014), in addition to occurrence records being clustered prior to being spatially rarified. There is often a difference in the area of the species distribution and the total study area modelled. If the ratio between these two areas is small, then the number of absences increases, making the absence data environmentally distant from the areas where species are actually present. This results in rare species or species with limited occurrence data being generally "better predicted" than widespread species when assessed using their AUC scores (Brotons et al. 2004; Arntzen 2006; Hernández et al. 2006; Mcpherson and Jetz 2007; Lobo et al. 2008). Taking into account how these factors influence model performance, the models presented still suggest that the study taxa could potentially occur outside their known range and therefore increase the EOO. This should, however, be explored further with fine-scaled models using additional variables (e.g. vegetation type, soil type, land-use, and infrastructure), accurate population estimates, and ground truthing.

In view of global climate change, identifying potentially threatened taxa and detecting drivers of risk in order to minimise species extinction rates are essential to the conservation of species (Darrah et al. 2017). The extent to which threats are spread geographically is a major part of assessing extinction risk and of IUCN Red List assessments (Gaston 1991; Purvis et al. 2000; Syfert et al. 2014). In light of these modelling results, the distributions of the study taxa are called into question and may require verification on the ground. Extent of occurrence (EOO) is an estimate of risk, defined by the area that lies within the outermost limits of known or inferred locations which measures the total geographic spread of localities for a particular species (Gaston & Fuller 2009; IUCN 2017). Therefore, EOO needs to be as accurate as possible as it is often used when assessing the threat status of reptiles (e.g. Bates

et al. 2014). With the uncertainty around the *Psammophis leightoni* species complex actual distributions and SDMs may assist with informing a more accurate EOO for a reliable estimate of extinction risk. The IUCN (2017) recommends assessment of species' threat status to be data-orientated. Syfert et al. (2014) demonstrated that the EOOs estimated from SDMs can be more informative than EOOs based solely on a small number of specimen localities. Moreover, by constraining SDM predictions to the geographic shape of the point-based EOO seems to offer a conservative approach to identifying suitable environments where a species might occur but have not been surveyed (Syfert et al. 2014).

A taxon's suitable environmental space predicted by SDMs allow for an alternate and objective way of identifying resistance in the landscape without information on dispersal routes and how taxa may use available habitats (Koen et al. 2012; Razgour et al. 2014). The SDMs presented here essentially model the climate tolerance of these taxa across geographical space to better predict their spatial distribution (Guisan and Zimmermann 2000). As such, these predictions do not explicitly model physical barriers to dispersal but rather estimate where suitable environmental conditions for the species occur. Nevertheless, with the ability to travel long distances, limits to the dispersal of these taxa, in theory, are unrestricted within suitable climate space. Further limitations on SDMs are that anthropogenic influences on biological systems are often excluded from these models, despite being an important element (Leemans and Serneels 2004; Guisan and Thuiller 2005). Most SDMs predicting adaptive change under climate scenarios make assumptions without considering trends in human demography and land use. Accounting for future clearing, agricultural or urban intensification is recognisably difficult, however this can lead to over- or under-estimating species ranges across varying landscapes (Rouget et al. 2003; Leemans and Serneels 2004). It has been suggested that patterns of habitat fragmentation and connection are likely to have similar impacts on species and how they are distributed as climate change in the mid-long term (Jetz et al. 2007).

A number of SDMs have successfully incorporated interspecies interactions, anthropogenic influences, and habitat use, as well as climate change to estimate species ranges under various environmental changes (Leathwick and Austin 2001; Leathwick 2002; Heikkinen et al. 2007; Rödder and Lötters 2010; da Silva and Tolley 2017). However, a few studies have suggested these interactions are best accounted for through models with mechanistic elements, instead of SDMs using correlation alone (Sutherst et al. 2007; Keith et al. 2008). There is a knowledge gap regarding the important interactions for many species which is often unclear only with spatial data (Sutherst et al. 2007). It seems unlikely that SDMs incorporating interspecific interactions could be routinely produced for most species soon (Sinclair et al. 2010), and therefore should be implemented as part of a suite of tools in order to better estimate how species are distributed throughout the landscape under changing conditions.

3.5 Conclusion

In summary, these models suggest that 1) these three *Psammophis* taxa are likely to be parapatric or sympatric, not allopatric as previously thought based on existing records, and 2) they show no disjunction during interglacial periods but do show fragmentation during LGM with potential refugia in separate stable regions. Moreover, my prediction that there are differences in the climate envelopes of *P. namibensis* and *P. leightoni* was not borne out by the modelling. *Psammophis namibensis* (specifically from the Northern Cape) is predicted to maintain connectivity with *P. leightoni* in all modelled scenarios. The models also suggest that the *P. leightoni* complex represents a single species as the individual taxa share overlapping climate space facilitating gene flow in support not only of the phylogeny by Kelly et al. 2008, but also the more expanded phylogenetic analysis in chapter 2. Furthermore, it appears that SDMs do indeed provide an opportunity to present estimates for species occurrences based on the relationship between species and their environment (Sangermano and Eastman 2012). Also, the IUCN Red List assessments may benefit from the inclusion of SDMs as objective evidence without biases from experts (Fourcade et al. 2013), and can be improved if SDMs are used in combination with additional lines of evidence when assessing species (Marcer et al. 2013; Syfert et al. 2014; Parusnath et al. 2017).

CHAPTER FOUR

GENERAL CONCLUSION

The primary aim of this thesis was to validate the taxonomic status of the *Psammophis leightoni* complex using phylogenetic and species distribution modelling (SDM) techniques. In order to achieve this, two hypotheses were tested. The first was to ascertain whether the three described species (*P. leightoni, P. namibensis* and *P. trinasalis*) are phylogenetically distinct (chapter 2). The second hypothesis involved predicting the differences in the climate envelopes of *P. leightoni*, *P. namibensis*, and *P. trinasalis*, which would in part, define and limit their distributions (chapter 3). To answer these hypotheses, a mixed method approach was used incorporating phylogenetic analyses, focused on species delimitation, and SDM using a maximum entropy framework. The findings in chapter 2 suggest the *P. leightoni* complex does represent a single lineage with intraspecific level divergence between the taxa of this complex. Moreover, chapter 3 discusses the how these taxa share climate space and are not ecologically distinct in terms of their distribution.

The findings of this thesis dispute each taxon's specific rank claimed by Broadley (2002), and supports the intraspecific inference made by Kelly et al. (2008). With this, a formal taxonomic revision is necessary in order to appropriately reclassify these taxa (details in chapter 2). Similarly, an IUCN Red List reassessment is also needed in order to evaluate the conservation status of *Psammophis leightoni*, which is to be considered widespread. However, there is concern regarding the Western Cape population of *P. leightoni* which could be at risk of local extinction, if not by anthropogenic threats (Maritz 2014) then by climate change (due to a lack of stable climate refugia) (details in chapter 3). Taking this into consideration raises a challenge facing the conservation of widespread and common species as to whether they should be conserved at local levels or not.

A primary component of conservation is to support the long-term persistence of species across a number of habitats, particularly under environmental change (Meffe and Carroll 1994; Trombulak et al. 2004; Taylor et al. 2013). This causes an issue when appropriately conserving widespread species as the extent of their geographic distribution may shroud their security in a conservation framework (Brito 2010). As a result of occurring across various environments, widespread species may contain a number of evolutionary important lineages and phenotypes, potentially exposing them to localised threats across the species' range (Taylor et al. 2013). If species are to be adequately conserved, an understanding of how these species are likely to change under a range of pressures (e.g., habitat transformation and climate change) is essential. This, however, is poorly understood within a South African context and has regularly been excluded from conservation planning at a regional level (Erasmus et al. 2002).

Whether the majority of species are able to adapt to a rapidly changing climate remains a global concern (Dawson et al. 2011). Even more so within South Africa as the effect of climate change on the flora is predicted to result in substantial biodiversity losses in the

39

southwest of the country in terms of the number of species dependant on specific habitat features, and that species loss from local protected areas will be significant (McDonald and Midgley 1996; Rutherford et al. 1999; Erasmus et al. 2002). Even though *Psammophis leightoni* would be considered widespread, the population within the Western Cape is still the most at risk of extinction due to habitat transformation and climate change. Consideration in conserving this population may, however, prove imperative with further investigation, especially in light of widespread species declines (Seigel and Mullin 2009; Sewell et al. 2012; Ceballos et al. 2015; Inger et al. 2015).

Widespread species play particularly important roles in ecosystems and how these systems function (e.g., maintaining predator-prey relations and food-web structure) (Dickman and Steeves 2004; Gregory et al. 2005; Nowak et al. 2008; Gaston 2010). Snakes in particular are often the most common predators in an ecosystem, and in a variety of habitats maintain the role of top predator (Sun et al. 2002; Brischoux et al. 2007; Nowak et al. 2008). However the extent to which the presence of snakes affect ecosystem functioning is relatively unknown, except for cases of introductions into non-native snake free systems (Savidge 1987; Sun et al. 2002; Wüster et al. 2005; Rodda and Savidge 2007). Common species may be those that are most likely to best adapt to rapid climate change, snakes in particular (Hewitt 2004; Nowak et al. 2008), and could be most practical in terms of ecological monitoring (Devictor et al. 2007; Steffen et al. 2009; McComb et al. 2010).

Taxa of the *Psammophis leightoni* complex may display unique characteristics in how they utilise specific habitats. More specifically, these taxa are known from three core areas which, given the correct climatic conditions, can become isolated from the rest allowing for maintenance of distinct features undetectable by the methods used in this thesis. Furthermore, connectivity between core areas is only maintained in two regions, which may be due to specific physiological tolerances restricting the direction in which peripheral populations expand. This warrants further investigation into the physiological tolerances of individuals from each of the core areas, which could explain why connectivity between each area is restricted to specific corridors.

Species inhabiting a wider geographic range are generally exposed to variable habitats and habitat quality across the species' distribution. Variation in both quality and type of habitat can affect the abundance and demography of local populations (Brown 1984), which influences gene flow, genetic drift, and intraspecific genetic variation (Slatkin 1987). Prominent differences can occur between populations inhabiting more stable, well-connected, optimal habitat of the main regions of the species' distribution compared to populations found along the periphery. Peripheral populations are often patchily distributed, occasionally isolated from the main region of the species' distribution, and greater habitat and environmental variability (Brown 1984; Eckert et al. 2008). The differences between main and peripheral populations affect how a species is able to adapt to stochastic events. Main populations are more resilient to these events because of the population's greater evolutionary potential as a result of high levels of genetic diversity due to larger population sizes (Frankham 1996). In contrast, peripheral populations have an increased risk of inbreeding, genetic drift, and an inability to adapt because of limited genetic variability due to smaller

40

population sizes (Vucetich and Waite 2003). The genetic characteristics associated with peripheral populations make them more likely to become locally extinct than main populations (Lesica and Allendorf 1995).

The genetic differences between main and peripheral populations have been explored in a number of wide-ranging species as well as those restricted in distribution (Eckert et al. 2008). Some studies have shown that genetic variation is highest within main populations (Dolan 1994; Lammi et al. 1999; Garner et al. 2003), while others have suggested that the highest diversities are in populations occupying transitional habitats (Rowe et al. 2006; Kark et al. 2008). Garner et al. (2004) observed a directional gradient in diversity, decreasing away from glacial refugia suggesting population expansion. Assessing genetic diversity within *Psammophis leightoni* (now considered wide-ranging) is beyond the scope of this thesis, largely due to the limited number of samples from potentially larger, stable populations (i.e., in Namibia and Botswana). This type of information could prove useful to ascertain the degree and direction of gene flow between populations, allowing the monitoring of at risk populations more accurate if necessary.

With the number of contemporary threats occurring more frequently, populations lacking genetic diversity are at the greatest risk of local extinction (Rogell et al. 2010). Climate change and habitat loss/fragmentation remain prominent threats associated with observed population declines and a primary driver of species extinction (Thomas et al. 2004; Mantyka-pringle et al. 2012). A number of species, including snakes, are greatly affected by changes in habitat quality (Reading et al. 2010; Hargreaves et al. 2014), and if not properly monitored can result in considerable losses at both global and local scales (Huey 1991; Heard et al. 2004; Stümpel et al. 2016).

Whether or not species are conserved should rely on priority, with resources dedicated to taxa most at risk demonstrated by evidence of direct threats (IUCN 2017). This, however, is not always the case as a number of charismatic species are prioritised above those legitimately threatened (Small 2012). An accurate conservation assessment for taxa at risk is increasingly important considering how resources are often inappropriately distributed (Morrison et al. 2009). Assigning an appropriate conservation status to a particular taxon requires knowledge of its diversity, distribution, biology and habitat requirements, as well as evidence of the environmental and anthropogenic threats that it faces (Branch 2014). A primary requirement of conservation assessments are stable taxonomy, or at least an awareness of its limitations (Branch 2014). The findings of this thesis suggest the *Psammophis leightoni* complex represents a single widespread species with population specific features. A taxonomic revision and conservation reassessment is necessary, with a potential outcome removing the threatened status of *P. leightoni*.

REFERENCES

Alexander GJ, Marshall CL. 1998. Diel activity patterns in a captive colony of. *African Journal of Herpetology* 47: 29–32.

Aliabadian M, Kaboli M, Nijman V, Vences M. 2009. Molecular identification of birds: Performance of distance-based DNA barcoding in three genes to delimit parapatric species. *PLoS ONE* 4.

Aljanabi SM, Martinez I. 1997. Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. *Nucleic acids research* 25: 4692–4693.

Allouche O, Tsoar A, Kadmon R. 2006. Assessing the accuracy of species distribution models: Prevalence, kappa and the true skill statistic (TSS). *Journal of Applied Ecology* 43: 1223–1232.

Alroy J. 2015. Current extinction rates of reptiles and amphibians. *Proceedings of the National Academy of Sciences* 112: 13003–13008.

Alvarado-Serrano DF, Knowles LL. 2014. Ecological niche models in phylogeographic studies: Applications, advances and precautions. *Molecular Ecology Resources* 14: 233–248.

Anderson RP, Raza A. 2010. The effect of the extent of the study region on GIS models of species geographic distributions and estimates of niche evolution: Preliminary tests with montane rodents (genus Nephelomys) in Venezuela. *Journal of Biogeography* 37: 1378–1393.

Andersson L. 1990. The Driving Force : Species Concepts and Ecology. *Taxon* 39: 375–382.

Araújo MB, Alagador D, Cabeza M, Nogués-Bravo D, Thuiller W. 2011. Climate change threatens European conservation areas. *Ecology Letters* 14: 484–492.

Arévalo E, Sites JW, Davis SK, Arévalo E. 1994. Mitochondrial DNA sequence divergence and phylogenetic relationships among eight chromosome races of the sceloporus grammicus complex (phrynosomatidae) in central mexico. *Systematic Biology* 43: 387–418.

Arntzen J. 2006. From descriptive to predictive distribution models : a working example with Iberian amphibians and reptiles. *Frontiers in Zoology* 3: 1–11.

Austin MP, Van Niel KP. 2011. Improving species distribution models for climate change studies: Variable selection and scale. *Journal of Biogeography* 38: 1–8.

Barve N, Barve V, Jiménez-Valverde A, Lira-Noriega A, Maher SP, Peterson AT, Soberón J, Villalobos F. 2011. The crucial role of the accessible area in ecological niche modeling and species distribution modeling. *Ecological Modelling* 222: 1810–1819.

Bates MF, Branch WR, Bauer AM, Burger M, Marais J, Alexander GJ, De Villiers MS. 2014. *Atlas and Red List of the Reptiles of South Africa, Lesotho and Swaziland* (1st edn). South African National Biodiversity Institute, Pretoria: Suricata.

BirdLife International. 2004. *State of the world's birds: indicators of our changing world.* Cambridge, UK: BirdLife International.

Böhm M, Collen B, Baillie JEM, Bowles P, Chanson J, Cox N, Hammerson G, Hoffmann M, Livingstone SR, Ram M, Rhodin AGJ, Stuart SN, van Dijk PP, Young BE, Afuang LE, Aghasyan A, García A, Aguilar C, Ajtic R, Akarsu F, Alencar LRV, Allison A, Ananjeva N, Anderson S, Andrén C, Ariano-Sánchez D, Arredondo JC, Auliya M, Austin CC, Avci A, Baker PJ, Barreto-Lima AF, Barrio-Amorós CL, Basu D, Bates MF, Batistella A, Bauer A, Bennett D, Böhme W, Broadley D, Brown R, Burgess J, Captain A, Carreira S, Castañeda M del R, Castro F, Catenazzi A, Cedeño-Vázquez JR, Chapple DG, Cheylan M, Cisneros-Heredia DF, Cogalniceanu D, Cogger H, Corti C, Costa GC, Couper PJ, Courtney T, Crnobrnja-Isailovic J, Crochet PA, Crother B, Cruz F, Daltry JC, Daniels RJR, Das I, de Silva A, Diesmos AC, Dirksen L, Doan TM, Dodd CK, Doody JS, Dorcas ME, Duarte de Barros Filho J, Egan VT, El Mouden EH, Embert D, Espinoza RE, Fallabrino A, Feng X, Feng ZJ, Fitzgerald L, Flores-Villela O, França FGR, Frost D, Gadsden H, Gamble T, Ganesh SR, Garcia MA, García-Pérez JE, Gatus J, Gaulke M, Geniez P, Georges A, Gerlach J, Goldberg S, Gonzalez JCT, Gower DJ, Grant T, Greenbaum E, Grieco C, Guo P, Hamilton AM, Hare K, Hedges SB, Heideman N, Hilton-Taylor C, Hitchmough R, Hollingsworth B, Hutchinson M, Ineich I, Iverson J, Jaksic FM, Jenkins R, Joger U, Jose R, Kaska Y, Kaya U, Keogh JS, Köhler G, Kuchling G, Kumlutaş Y, Kwet A, La Marca E, Lamar W, Lane A, Lardner B, Latta C, Latta G, Lau M, Lavin P, Lawson D, LeBreton M, Lehr E, Limpus D, Lipczynski N, Lobo AS, López-Luna MA, Luiselli L, Lukoschek V, Lundberg M, Lymberakis P, Macey R, Magnusson WE, Mahler DL, Malhotra A, Mariaux J, Maritz B, Marques OAV, Márquez R, Martins M, Masterson G, Mateo JA, Mathew R, Mathews N, Mayer G, McCranie JR, Measey GJ, Mendoza-Quijano F, Menegon M, Métrailler S, Milton DA, Montgomery C, Morato SAA, Mott T, Muñoz-Alonso A, Murphy J, Nguyen TQ, Nilson G, Nogueira C, Núñez H, Orlov N, Ota H, Ottenwalder J, Papenfuss T, Pasachnik S, Passos P, Pauwels OSG, Pérez-Buitrago N, Pérez-Mellado V, Pianka ER, Pleguezuelos J, Pollock C, Ponce-Campos P, Powell R, Pupin F,

Quintero Díaz GE, Radder R, Ramer J, Rasmussen AR, Raxworthy C, Reynolds R, Richman N, Rico EL, Riservato E, Rivas G, da Rocha PLB, Rödel MO, Rodríguez Schettino L, Roosenburg WM, Ross JP, Sadek R, Sanders K, Santos-Barrera G, Schleich HH, Schmidt BR, Schmitz A, Sharifi M, Shea G, Shi HT, Shine R, Sindaco R, Slimani T, Somaweera R, Spawls S, Stafford P, Stuebing R, Sweet S, Sy E, Temple HJ, Tognelli MF, Tolley K, Tolson PJ, Tuniyev B, Tuniyev S, üzüm N, van Buurt G, Van Sluys M, Velasco A, Vences M, Veselý M, Vinke S, Vinke T, Vogel G, Vogrin M, Vogt RC, Wearn OR, Werner YL, Whiting MJ, Wiewandt T, Wilkinson J, Wilson B, Wren S, Zamin T, Zhou K, Zug G. 2013. The conservation status of the world's reptiles. *Biological Conservation* 157: 372–385.

Bouckaert R, Heled J, Kühnert D, Vaughan T, Wu CH, Xie D, Suchard MA, Rambaut A, Drummond AJ. 2014. BEAST 2: A Software Platform for Bayesian Evolutionary Analysis. *PLoS Computational Biology* 10.

Braconnot P, Otto-Bliesner B, Harrison S, Joussaume S, Peterchmitt J-Y, Abe-Ouchi A, Crucifix M, Fichefet T, Hewitt CD, Kageyama M, Kitoh A, Loutre M-F, Marti O, Merkel U, Ramstein G, Valdes P, Weber L, Yu Y, Zhao Y. 2007. Coupled simulations of the mid-Holocene and Last Glacial Maximum: new results from PMIP2. *Climate of the Past Discussions* 2: 1293–1346.

Branch W. 1998. *Field guide to snakes and other reptiles of southern Africa* (3rd edn). Cape Town: Struik.

Branch WR. 2014. Conservation status, diversity, endemism, hotspots and threats. In: Bates MF, Branch WR, Bauer AM, Burger M, Marias J, Alexander GJ, De Villiers MS (eds), *Atlas and Red List of the Reptiles of South Africa, Lesotho, and Swaziland*. South African National Biodiversity Institute, Pretoria: Suricata 1. pp. 22–50.

Breitman MF, Bonino MF, Sites JW, Avila LJ, Morando M. 2015. Morphological Variation, Niche Divergence, and Phylogeography of Lizards of the *Liolaemus lineomaculatus* Section (Liolaemini) from Southern Patagonia. *Herpetological Monographs* 29: 65–88.

Brischoux F, Bonnet X, Shine R. 2007. Foraging ecology of sea kraits Laticauda spp. in the Neo-Caledonian Lagoon. *Marine Ecology Progress Series* 350: 145–151.

Brito D. 2010. Overcoming the Linnean shortfall: Data deficiency and biological survey priorities. *Basic and Applied Ecology* 11: 709–713.

Broadley D. 1966. A review of the African Stripe-bellied Sand-snakes of the genus Psammophis. National Museums of Southern Rhodesia.

Broadley D. 1977. A review of the genus Psammophis

in southern Africa (Serpentes: Colubridae). National Museums and Monuments of Rhodesia.

Broadley DG. 2002. A review of the species of Psammophis Boie found south of Latitude 12° S (Serpentes: Psammophiinae). *African Journal of Herpetology* 51: 83–119.

Brooks DR, Mclennan DA. 1991. Phylogeny, Ecology, and Behavior. Chicago, Illinois: University of Chicago Press.

Brooks TM, Mittermeier RA, da Fonseca GAB, Gerlach J, Hoffmann M, Lamoreux JF, Mittermeier CG, Pilgrim JD, Rodrigues ASL. 2006. Global Biodiversity Conservation Priorities. *Science* 313: 58–61.

Brotons L, Thuiller W, Arau MB, Hirzel AH. 2004. Presence-absence versus presence-only modelling methods for predicting bird habitat suitability. *Ecograpy* 27: 437–448.

Brown JH. 1984. On the Relationship between Abundance and Distribution of Species. *The American Naturalist* 124: 255–279.

Brown JL. 2014. SDMtoolbox : A python-based GIS toolkit for landscape genetic , biogeographic and species distribution model analyses. *Methods in Ecology and Evolution* 5: 694–700.

Brown JL, Knowles LL. 2012. Spatially explicit models of dynamic histories: Examination of the genetic consequences of Pleistocene glaciation and recent climate change on the American Pika. *Molecular Ecology* 21: 3757–3775.

Brummitt N, Lughadha EN. 2003. Biodiversity : Where' s Hot and Where' s Not. *Conservation Biology* 17: 1442–1448.

Burbrink FT, Lawson R, Slowinski JB. 2000. Mitochondrial Dna Phylogeography of the Polytypic North American Rat Snake (Elaphe Obsoleta): a Critique of the Subspecies Concept Mitochondrial Dna Phylogeography of the Polytypic North American Rat Snake (Elaphe Obsoleta): a Critique of the Subspeci. *Evolution* 54: 2107–2118.

Butler H, Malone B, Clemann N. 2005. Activity patterns and habitat preferences of translocated and resident tiger snakes (*Notechis scutatus*) in a suburban landscape. *Wildlife Research* 32: 157.

Casciotta J, Almirón A, Sánchez S, Iwaszkiw J, Bruno MC. 2013. The genus Gymnotus (Gymnotiformes: Gymnotidae) in Argentina. How bad taxonomy results in poor regulations and no conservation. *Journal of Applied Ichthyology* 29: 208–212.

Ceballos G, Ehrlich PR, Barnosky AD, García A, Pringle RM, Palmer TM. 2015. Accelerated modern human – induced species losses: entering the sixth mass extinction. *Sciences Advances* 1: 1–5.

Chaitra MS, Vasudevan K, Shanker K. 2004. The

43

biodiversity bandwagon: The splitters have it. *Current Science* 86: 897–899.

Chen IC, Hill JK, Ohlemüller R, Roy D., Thomas CD. 2011. Rapid range shifts of species associated with high levels of climate warming. *Science* 333: 1024–1026.

Clarkson RW, Rorabaugh JC. 1989. Status of Leopard Frogs (Rana pipiens Complex : Ranidae) in Arizona and Southeastern California. *The Southwestern Naturalist* 34: 531–538.

Collar NJ. 1996. The reasons for Red Data Books. *Oryx* 30: 121.

Comstock KE, Georgiadis N, Pecon-Slattery J, Roca AL, Ostrander EA, O'Brien SJ, Wasser SK. 2002. Patterns of molecular genetic variation among African elephant populations. *Molecular Ecology* 11: 2489–2498.

Cooper DM, Dugmore AJ, Gittings BM, Scharf AK, Wilting A, Kitchener AC, Elith J. 2016. Predicted Pleistocene - Holocene range shifts of the tiger (Panthera tigris). *Diversity and Distributions* 22: 1199–1211.

Cracraft J. 1983. Species concepts and speciation analysis. *Current ornithology* 1: 159–187.

Dagnino D, Minuto L, Casazza G. 2017. Divergence is not enough: The use of ecological niche models for the validation of taxa boundaries. *Plant Biology*.

Darrah SE, Bland LM, Bachman SP, Clubbe CP, Trias-blasi A, Gardens RB. 2017. Using coarse-scale species distribution data to predict extinction risk in plants: 435–447.

Daugherty CH, Cree a., Hay JM, Thompson MB. 1990. Neglected taxonomy and continuing extinctions of tuatara (Sphenodon). *Nature* 347: 177–179.

Davis MB, Shaw RG. 2001. Range shifts and adaptive responses to Quaternary climate change. *Science* 292: 673–679.

Dawson TP, Jackson ST, House JI, Prentice IC, Mace GM. 2011. Beyond Predictions: Biodiversity Conservation in a Changing Climate. *Science* 332: 53–58.

Devictor V, Godet L, Julliard R, Couvet D, Jiguet F. 2007. Can common species benefit from protected areas? *Biological Conservation* 139: 29–36.

Dickman CR, Steeves TE. 2004. Use of habitat by mammals in eastern Australian forests: are common species important in forest management? *Conservation of Australia's Forest Fauna*: 761–773.

Dolan RW. 1994. Patterns of isozyme variation in relation to population size, isolation, and phytogeographic history in royal catchfly (Silene regia; Caryophyllaceae). *American Journal of Botany* 81: 965–972.

Donoghue MJ. 1985. A Critique of the Biological Species Concept and Recommendations for a Phylogenetic Alternative. *The Bryologist* 88:

172–181.

Driscoll CA, Yamaguchi N, Bar-Gal GK, Roca AL, Luo S, Macdonald DW, O'Brien SJ. 2009. Mitochondrial phylogeography illuminates the origin of the extinct caspian tiger and its relationship to the amur tiger. *PLoS ONE* 4.

Dubois A. 2003. The relationships between taxonomy and conservation biology in the century of extinctions. *Comptes Rendus Biologies* 326: 9–21.

Dubois A. 2007. Phylogeny, taxonomy and nomenclature: The problem of taxonomic categories and of nomenclatural ranks. *Zootaxa*: 27–68.

Duellman WE. 1993. *Amphibian species of the world : Additions and corrections*. Lawrence : University of Kansas,.

Dulvy NK, Reynolds JD. 2009. Biodiversity: skates on thin ice. *Nature* 462: 417.

Eckert CG, Samis KE, Lougheed SC. 2008. Genetic variation across species' geographical ranges: The central-marginal hypothesis and beyond. *Molecular Ecology* 17: 1170–1188.

Edgar RC. 2004. MUSCLE : multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32: 1792–1797.

Edwards DL, Keogh JS, Knowles LL. 2012. Effects of vicariant barriers, habitat stability, population isolation and environmental features on species divergence in the south-western Australian coastal reptile community. *Molecular Ecology* 21: 3809–3822.

Elith J, H. Graham C, P. Anderson R, Dudík M, Ferrier S, Guisan A, J. Hijmans R, Huettmann F, R. Leathwick J, Lehmann A, Li J, G. Lohmann L, A. Loiselle B, Manion G, Moritz C, Nakamura M, Nakazawa Y, McC. M. Overton J, Townsend Peterson A, J. Phillips S, Richardson K, Schachetti-Pereira R, E. Schapire R, Soberón J, Williams S, S. Wisz M, E. Zimmermann N. 2006. Novel methods improve prediction of species' distributions from occurrence data. *Ecography* 29: 129–151.

Elith J, Leathwick JR. 2009. Species Distribution Models: Ecological Explanation and Prediction Across Space and Time. *Annual Review of Ecology Evolution and Systematics* 40: 677–697.

Erasmus BFN, van Jaarsveld a S, Chown SL, Kshatriya M, Wessels KJ. 2002. Vulnerability of South African taxa to climate change. *Global Change Biology* 8: 679–693.

Esselstyn JA, Evans BJ, Sedlock JL, Anwarali Khan FA, Heaney LR. 2012. Single-locus species delimitation: a test of the mixed Yule-coalescent model, with an empirical application to Philippine round-leaf bats. *Proceedings of the Royal Society B: Biological Sciences* 279: 3678–3686.

44

Evenhuis N l. 2007. Helping solve the 'other' taxonomic impediment: Completing the Eight Steps to Total Enlightenment and Taxonomic Nirvana. *Zootaxa* 12: 67–68.

Ficetola GF, Thuiller W, Padoa-schioppa E. 2009. From introduction to the establishment of alien species : bioclimatic differences between presence and reproduction localities in the slider turtle. *Diversity and Distributions* 15: 108–116.

Fourcade Y, Engler JO, Besnard AG, Rödder D, Secondi J. 2013. Confronting expert-based and modelled distributions for species with uncertain conservation status : A case study from the corncrake (Crex crex). *Biological Conservation* 167: 161–171.

Frankham R. 1996. Relationship of genetic variation to population size in wildlife. *Conserv Biol* 10: 1500–1508.

Fujisawa T, Barraclough TG. 2013. Delimiting species using single-locus data and the generalized mixed yule coalescent approach: A revised method and evaluation on simulated data sets. *Systematic Biology* 62: 707–724.

Funk V a, Sakai a K, Richardson K. 2002. Biodiversity: the interface between systematics and conservation. *Systematic biology* 51: 235–237.

Garner TWJ, Angelone S, Pearman PB. 2003. Genetic depletion in Swiss populations of Rana latastei: Conservation implications. *Biological Conservation* 114: 371–376.

Garner TWJ, Pearman PB, Angelone S. 2004. Genetic diversity across a vertebrate species' range: A test of the central-peripheral hypothesis. *Molecular Ecology* 13: 1047–1053.

Gaston KJ. 1991. How large is a species' geographic range? *Oikos* 61: 434–438.

Gaston KJ. 2010. Valuing Common Species. *Science* 327: 154–155.

Gaston KJ, Fuller RA. 2009. The sizes of species ' geographic ranges. *Journal of Applied Ecology* 46: 1–9.

Gibbons JW, Scott DE, Ryan TR, Buhlmann KA, Tuberville TD, Metts BS, Greene JL, Mills T, Leiden Y, Poppy S, Winne CT. 2000. The Global Decline of Reptiles, Déjà Vu Amphibians. *BioScience* 50: 653–666.

Glick D. 2005. *Back from the brink*.

Golding JS, Timberlake J. 2003. How Taxonomists Can Bridge the Gap between Taxonomy and Conservation Science. *Conservation Biology* 17: 1177–1178.

Gregory RD, van Strien A, Vorisek P, Gmelig Meyling AW, Noble DG, Foppen RPB, Gibbons DW. 2005. Developing indicators for European birds. *Philosophical Transactions of the Royal Society B: Biological Sciences* 360: 269–288.

Guisan A, Thuiller W. 2005. Predicting species distribution: Offering more than simple habitat models. *Ecology Letters* 8: 993–1009.

Guisan A, Tingley R, Baumgartner JB, Naujokaitis-Lewis I, Sutcliffe PR, Tulloch AIT, Regan TJ, Brotons L, Mcdonald-Madden E, Mantyka-Pringle C, Martin TG, Rhodes JR, Maggini R, Setterfield SA, Elith J, Schwartz MW, Wintle BA, Broennimann O, Austin M, Ferrier S, Kearney MR, Possingham HP, Buckley YM. 2013. Predicting species distributions for conservation decisions. *Ecology Letters* 16: 1424–1435.

Guisan A, Zimmermann NE. 2000. Predictive habitat distribution models in ecology. *Ecological Modelling* 135: 147–186.

Hajibabaei M, Singer GAC, Hebert PDN, Hickey DA. 2007. DNA barcoding: how it complements taxonomy, molecular phylogenetics and population genetics. *Trends in Genetics* 23: 167–172.

Hargreaves AL, Samis KE, Eckert CG. 2014. Are species' range limits simply niche limits writ large? A review of transplant experiments beyond the range. *The American naturalist* 183: 157–73.

Harris DJ, Arnold EN, Thomas RH. 1998. Relationships of lacertid lizards (Reptilia : Lacertidae) estimated from mitochondrial DNA sequences and morphology. *Proceedings of the Royal Society of London Series B-Biological Sciences* 265: 1939–1948.

Harvey PH, Pagel MD. 1991. *The comparative method in evolutionary biology*. Oxford: Oxford University Press Inc.

Hayes WK. 2006. The urgent need for conservation taxonomy in the Bahamas: New bird species as an example. *Bahamas Naturalist & Journal of Science* 1: 12–24.

Heard GW, Black D, Robertson P. 2004. Habitat use by the inland carpet python (Morelia spilota metcalfei: Pythonidae): Seasonal relationships with habitat structure and prey distribution in a rural landscape. *Austral Ecology* 29: 446–460.

Hebert PDN, Cywinska A, Ball SL, deWaard JR. 2003. Biological identifications through DNA barcodes. *Proceedings of the Royal Society B: Biological Sciences* 270: 313–321.

Heikkinen RK, Luoto M, Virkkala R, Pearson RG, Körber JH. 2007. Biotic interactions improve prediction of boreal bird distributions at macro-scales. *Global Ecology and Biogeography* 16: 754–763.

Hernández PA, Graham CH, Master LL, Albert DL. 2006. The effect of sample size and species characteristics on performance of different species distribution modeling methods. *Ecography* 29: 773–785.

Hewitt GM. 2004. Genetic consequences of climatic oscillations in the Quaternary. *Philosophical Transactions of the Royal Society B: Biological Sciences* 359: 183–195.

45

Hey J. 2001. The mind of the species problem. *Trends in Ecology & Evolution* 16: 326–329.

Hickerson MJ, Meyer CP, Moritz C, Hedin M. 2006. DNA Barcoding Will Often Fail to Discover New Animal Species over Broad Parameter Space. *Systematic Biology* 55: 729–739.

Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A. 2005. Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* 25: 1965–1978.

Hillis DM, Bull JJ. 1993. An Empirical Test of Bootstrapping as a Method for Assessing Confidence in Phylogenetic Analysis. *Systematic biology* 42: 182–192.

Hillis DM, Moritz C. 1990. *Molecular systematics*. Sinauer.

Hoagland K. 1996. The taxonomic impediment and the convention on biodiversity. *Association of Systematics Collections Newsletter*.

Holt RD. 2003. On the evolutionary ecology of species' ranges. *Evol. Ecol. Res.* 5: 159–178.

Hu J, Jiang Z. 2010. Predicting the potential distribution of the endangered Przewalski's gazelle. *Journal of Zoology* 282: 54–63.

Huey RB. 1991. Physiological Consequences of Habitat Selection. *The American Naturalist* 137: S91–S115.

Hughes B. 1999. Critical review of a revision of Psammophis (Linnaeus 1758)(Serpentes, Reptilia) by Frank Brandstätter. *African Journal of Herpetology* 48: 63–70.

Hughes JB, Hellmann JJ, Ricketts TH, Bohannan BJM. 2001. Counting the Uncountable: Statistical Approaches to Estimating Microbial Diversity. *Applied and environmental microbiology* 67: 4399–4406.

Humphrey J, Fox V. 2002. *U.S. Fish and Wildlife Service Lists: Chiricahua Leopard Frog as a Threatened Species*. Phoenix, USA: Arizona Ecological Services Field Office.

Ihlow F, Dambach J, Engler JO, Flecks M, Hartmann T, Nekum S, Rajaei H, Rödder D. 2012. On the brink of extinction? How climate change may affect global chelonian species richness and distribution. *Global Change Biology* 18: 1520–1530.

Inger R, Gregory R, Duffy JP, Stott I, Voříšek P, Gaston KJ. 2015. Common European birds are declining rapidly while less abundant species' numbers are rising. *Ecology Letters* 18: 28–36.

Isaac NJB, Mallet J, Mace GM. 2004. Taxonomic inflation: Its influence on macroecology and conservation. *Trends in Ecology and Evolution* 19: 464–469.

IUCN Standards and Peitions Subcommittee. 2017. Guidelines for Using the IUCN Red List Categories and Criteria 13.

Jetz W, McPherson JM, Guralnick RP. 2012. Integrating biodiversity distribution knowledge: Toward a global map of life. *Trends in Ecology and Evolution* 27: 151–159.

Jetz W, Wilcove DS, Dobson AP. 2007. Projected impacts of climate and land-use change on the global diversity of birds. *PLoS Biology* 5: 1211–1219.

Kark S, Hadany L, Safriel UN, Noy-Meir I, Eldredge N, Tabarroni C, Randi E. 2008. How does genetic diversity change towards the range periphery? An empirical and theoretical test. *Evolutionary Ecology Research* 10: 391–414.

Karl SA, Bowen BW. 1998. Evolutionary Signfficant Taxonomy : Molecular systematcs of an endangered sea turtle (genus Chelonia). *Conservation Biology* 13: 990–999.

Kearney MR, Wintle BA, Porter WP. 2010. Correlative and mechanistic models of species distribution provide congruent forecasts under climate change. *Conservation Letters* 3: 203–213.

Kearse M, Moir R, Wilson A, Stones-havas S, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A. 2012. Geneious Basic : An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28: 1647–1649.

Keith DA, Akcakaya HR, Thuiller W, Midgley GF, Pearson RG, Phillips SJ, Regan HM, Araujo MB, Rebelo TG. 2008. Predicting extinction risks under climate change: coupling stochastic population models with dynamic bioclimatic habitat models. *Biology Letters* 4: 560–563.

Kelly CMR, Barker NP, Villet MH, Broadley DG, Branch WR. 2008. The snake family Psammophiidae (Reptilia: Serpentes): Phylogenetics and species delimitation in the African sand snakes (Psammophis Boie, 1825) and allied genera. *Molecular Phylogenetics and Evolution* 47: 1045–1060.

Khuroo AA, Dar GH, Khan ZS, Malik AH. 2007. Exploring an inherent interface between taxonomy and biodiversity: Current problems and future challenges. *Journal for Nature Conservation* 15: 256–261.

Kim KC, Byrne LB. 2006. Biodiversity loss and the taxonomic bottleneck: Emerging biodiversity science. *Ecological Research* 21: 794–810.

Kipling W, Mishler BD, Wheeler QD. 2005. The Perils of DNA Barcoding and the Need for Integrative Taxonomy. *Systematic Biology* 54: 844–851.

Koen EL, Bowman J, Walpole AA. 2012. The effect of cost surface parameterization on landscape resistance estimates. *Molecular Ecology Resources* 12: 686–696.

Köhler J, Vieites DR, Bonett RM, García FH, Glaw F, Steinke D, Vences M. 2005. New amphibians and global conservation: a boost in species discoveries in a highly endangered vertebrate

group. *BioScience* 55: 693–696.

Kozak KH, Graham CH, Wiens JJ. 2008. Integrating GIS-based environmental data into evolutionary biology. *Trends in Ecology and Evolution* 23: 141–148.

Lammi A, Siikamäki P, Mustajärvi K. 1999. Genetic diversity, population size, and fitness in central and peripheral populatins of a rare plant Lychnis viscaria. *Conservation Biology* 13: 1069–1078.

Larsen FW, Rahbek C. 2005. The influence of spatial grain size on the suitability of the higher-taxon approach in continental priority-setting. *Animal Conservation* 8: 389–396.

Lawson R, Slowinski JB, Crother BI, Burbrink FT. 2005. Phylogeny of the Colubroidea (Serpentes): New evidence from mitochondrial and nuclear genes. *Molecular Phylogenetics and Evolution* 37: 581–601.

Leathwick JR. 2002. Intra-generic competition among Nothofagus in New Zealand's primary indigenous forests. *Biodiversity and Conservation* 11: 2177–2187.

Leathwick JR, Austin MP. 2001. Competitive interactions between tree species in New Zealand old-growth indigenous forests. *Ecology* 82: 2560–2573.

Lecocq T, Dellicour S, Brasero N, Meulemeester T De, Michez D, Dellicour S, Lhomme P, Jonghe R De. 2014. An integrative taxonomic approach to assess the status of Corsican bumblebees : Implications for conservation An integrative taxonomic approach to assess the status of Corsican bumblebees : implications for conservation. *Animal Conservation* 18: 236–248.

Leemans R, Serneels S. 2004. Understanding land-use change to reconstruct, describe or predict changes in land cover. *GeoJournal* 61: 305–307.

Lesica P, Allendorf FW. 1995. When Are Peripheral Populations Valuable for Conservation? When Are Peripheral Populations Valuable for Conservation? *Conservation Biology* 9: 753–760.

Loarie SR, Duffy PB, Hamilton H, Asner GP, Field CB, Ackerly DD. 2009. The velocity of climate change. *Nature* 462: 1052–1055.

Lobo JM, Jiménez-valverde A, Real R. 2008. AUC: A misleading measure of the performance of predictive distribution models. *Global Ecology and Biogeography* 17: 145–151.

Loveridge A. 1940. Revision of the African snakes of the genera Dromophis and Psammophis. Harvard College, Museum of Comparative Zoology.

Luo SJ, Kim JH, Johnson WE, Van Der Walt J, Martenson J, Yuhki N, Miquelle DG, Uphyrkina O, Goodrich JM, Quigley HB, Tilson R, Brady G, Martelli P, Subramaniam V,

McDougal C, Hean S, Huang SQ, Pan W, Karanth UK, Sunquist M, Smith JLD, O'Brien SJ. 2004. Phylogeography and genetic ancestry of tigers (Panthera tigris). *PLoS Biology* 2.

Mace GM. 2004. The role of taxonomy in species conservation. *Philosophical transactions of the Royal Society of London. Series B, Biological Sciences* 359: 711–719.

Mace GM, Balmford A, Boitani L, Cowlishaw G, Dobson AP, Faith DP, Gaston KG, Humphries CJ, Vane-Wright RI, Williams PH, Lawton JH, Margules CR, May RM, Nicholls AO, Possingham HP, Rahbek C, van Jaarsveld AS. 2000. It's time to work together and stop duplicating conservation efforts. *Nature* 405: 393–394.

Mantyka-pringle CS, Martin TG, Rhodes JR. 2012. Interactions between climate and habitat loss effects on biodiversity: A systematic review and meta-analysis. *Global Change Biology* 18: 1239–1252.

Marcer A, Sáez L, Molowny-horas R, Pons X, Pino J. 2013. Using species distribution modelling to disentangle realised versus potential distributions for rare species conservation. *Biological Conservation* 166: 221–230.

Maritz B. 2014. Psammophis Boie, 1825. In: Bates MF, Branch WR, Bauer AM, Burger M, Marais J, Alexander GJ, De Villiers MS (eds), *Atlas and Red List of the Reptiles of South Africa, Lesotho, and Swaziland*. South African National Biodiversity Institute, Pretoria: Suricata 1. pp. 374–380.

May RM. 1990. Taxonomy as destiny. *Nature* 347: 129–130.

McComb B, Zuckerberg B, Vesely D, Jordan C. 2010. *Monitoring animal populations and their habitats: a practitioner's guide*. Baton Rouge, Florida: CRC Press.

McDonald I, Midgley G. 1996. Impacts and implications for nature conservation. In: *Global Climate Change and South Africa*. Cleveland, South Africa: Environmental Scientific Association. pp. 83–86.

McNeely JA. 2002. Nature Conservation The role of taxonomy in conserving biodiversity. *Journal for Nature Conservation*: 145–153.

Mcpherson JM, Jetz W. 2007. Effects of Species' ecology on the accuracy of distribution models. *Ecography* 30: 135–151.

Meffe G, Carroll C. 1994. *Principles of conservation biology*. Sunderland, Massachusetts: Sinauer Associates, Inc.

Meier R, Shiyang K, Vaidya G, Ng PKL. 2006. DNA Barcoding and Taxonomy in Diptera : A Tale of High Intraspecific Variability and Low Identification Success. *Society of Systematic Biologists* 55: 715–728.

Meier R, Zhang G, Ali F, Zamudio K. 2008. The Use of Mean Instead of Smallest Interspecific

Distances Exaggerates the Size of the 'Barcoding Gap' and Leads to Misidentification. *Systematic Biology* 57: 809–813.

Meine C, Soulé M, Noss RF. 2006. 'A mission-driven discipline': The growth of conservation biology. *Conservation Biology* 20: 631–651.

Merow C, Smith MJ, Silander JA. 2013. A practical guide to MaxEnt for modeling species' distributions: What it does, and why inputs and settings matter. *Ecography* 36: 1058–1069.

Meyer C, Maree G. 2013. *State of Environment Outlook Report for the Western Cape Province*. Cape Town.

Meyer CP, Paulay G. 2005. DNA barcoding: Error rates based on comprehensive sampling. *PLoS Biology* 3: 1–10.

Michonneau F. 2015. Cryptic and not-so-cryptic species in the complex (Echinodermata : Holothuroidea : Holothuriidae). *bioRxiv*.

Mishler BD. 1985. The Morphological , Developmental , and Phylogenetic Basis of Species Concepts in Bryophytes Author (s): Brent D . Mishler Reviewed work (s): Published by : American Bryological and Lichenological Society Stable URL : http://www.jstor.org/stable/32430. *The Bryologist* 88: 207–214.

Monserud RA, Leemans R. 1992. Comparing Global Vegetation Maps with the Kappa-Statistic. *Ecological Modelling* 62: 275–293.

Morales NS, Fernández IC, Baca-gonzález V. 2017. MaxEnt ' s parameter configuration and small samples : are we paying attention to recommendations ? A systematic review: 1–16.

Morrison WR, Lohr JL, Duchen P, Wilches R, Trujillo D, Mair M, Renner SS. 2009. The impact of taxonomic change on conservation: Does it kill, can it save, or is it just irrelevant? *Biological Conservation* 142: 3201–3206.

Murphy RW, Crawford AJ, Bauer AM, Che J, Donnellan SC, Fritz U, Haddad CFB, Nagy ZT, Poyarkov NA, Vences M, Wang W zhi, Zhang Y ping. 2013. Cold Code: The global initiative to DNA barcode amphibians and nonavian reptiles. *Molecular Ecology Resources* 13: 161–167.

Nagy ZT, Glaw F, Andreone F, Wink M, Vences M. 2007. Species boundaries in Malagasy snakes of the genus Madagascarophis (Serpentes: Colubridae sensu lato) assessed by nuclear and mitochondrial markers. *Organisms Diversity and Evolution* 7: 241–251.

Naidoo R, Balmford a, Costanza R, Fisher B, Green RE, Lehner B, Malcolm TR, Ricketts TH. 2008. Global mapping of ecosystem services and conservation priorities. *Proceedings of the National Academy of Sciences of the United States of America* 105: 9495–9500.

Nelson G, Platnick NI. 1981. *Systematics and biogeography*. New York: Columbia City Press.

Nielsen R, Matz M. 2006. Statistical Approaches for DNA Barcoding. *Systematic Biology* 55: 162–169.

Nielsen R, Wakeley J. 2001. Distinguishing migration from isolation: A Markov chain Monte Carlo approach. *Genetics* 158: 885–896.

Nixon KC, Wheeler QD. 1990. an Amplification of the Phylogenetic Species Concept. *Cladistics* 6: 211–223.

Nowak EM, Theimer TC, Schuett GW. 2008. Functional and numerical responses of predators: Where do vipers fit in the traditional paradigms? *Biological Reviews* 83: 601–620.

Nowak RM. 2002. The original status of Wolves in eastern North America. *Southeastern Naturalist* 1: 95–130.

Nowak RM. 2003. Wolf evolution and taxonomy. In: *Wolves: Behaviour, ecology and conservation*. Chicago, Illinois: University of Chicago Press. pp. 239–258.

NPAES. 2009. *National Protected Area Expansion Strategy for South Africa*.

Ornelas JF, González C. 2014. Interglacial genetic diversification of Moussonia deppeana (Gesneriaceae), a hummingbird-pollinated, cloud forest shrub in northern Mesoamerica. *Molecular Ecology* 23: 4119–4136.

Ornelas JF, de León SG, González C, Licona-Vera Y, Ortiz-Rodriguez AE, Rodríguez-Gómez F. 2015. Comparative palaeodistribution of eight hummingbird species reveal a link between genetic diversity and quaternary habitat and climate stability in Mexico. *Folia Zoologica* 64: 248–258.

Ostfeld RS, Holt RD. 2004. Are predators good for your health? Evaluating evidence for top-down regulations of zoonotic disease reservoirs. *Front Ecol Environ* 2: 13–20.

Parusnath S, Little IT, Cunningham MJ, Jansen R, Alexander GJ. 2017. The desolation of Smaug: The human-driven decline of the Sungazer lizard (Smaug giganteus). *Journal for Nature Conservation* 36: 48–57.

Paz A, Crawford AJ. 2012. Molecular-based rapid inventories of sympatric diversity: A comparison of DNA barcode clustering methods applied to geography-based vs clade-based sampling of amphibians. *Journal of Biosciences* 37: 887–896.

Pearson RG, Raxworthy CJ, Nakamura M, Townsend Peterson A. 2007. Predicting species distributions from small numbers of occurrence records: A test case using cryptic geckos in Madagascar. *Journal of Biogeography* 34: 102–117.

Petit RJ, Excoffier L. 2009. Gene flow and species delimitation. *Trends in Ecology and Evolution* 24: 386–393.

Phillimore AB, Owens IP. 2006. Are subspecies

useful in evolutionary and conservation biology? *Proceedings of the Royal Society B: Biological Sciences* 273: 1049–1053.

Phillips SJ, Anderson RP, Schapire RE. 2006. Maximum entropy modeling of species geographic distributions. *Ecological Modelling* 190: 231–259.

Phillips SJ, Dudík M. 2008. Modeling of species distribution with Maxent: new extensions and a comprehensive evalutation. *Ecograpy* 31: 161–175.

Pinho C, Harris DJ, Ferrand N. 2007. Comparing patterns of nuclear and mitochondrial divergence in a cryptic species complex the case of Iberian and North African wall lizards (Podarcis , Lacertidae). *Biological Journal of the Linnean Society* 97: 121–133.

Pons J, Barraclough TG, Gomez-Zurita J, Cardoso A, Duran DP, Hazell S, Kamoun S, Sumlin WD, Vogler AP. 2006. Sequence-Based Species Delimitation for the DNA Taxonomy of Undescribed Insects. *Systematic Biology* 55: 595–609.

Posada D. 2008. jModelTest: Phylogenetic model averaging. *Molecular Biology and Evolution* 25: 1253–1256.

Possingham HP, Andelman SJ, Burgman MA, Medellín RA, Master LL, Keith DA. 2002. Limits to the use of threatened species lists. *Trends in Ecology and Evolution* 17: 503–507.

Price MR, Hayes WiK. 2009. Conservation Taxonomy of the Greater Antillean Oriole (Icterus Dominicensis): Diagnosable Plumage Variation Among Allopatric Populations Supports Species Status 22: 19–25.

Purvis A, Gittleman JL, Cowlishaw G, Mace GM. 2000. Predicting extinction risk in declining species. *Proceedings of the Royal Society of London Series B-Biological Sciences* 267: 1947–1952.

Van Der Putten WH, Macel M, Visser ME. 2010. Predicting species distribution and abundance responses to climate change: why it is essential to include biotic interactions across trophic levels. *Philosophical Transactions of the Royal Society B: Biological Sciences* 365: 2025–2034.

De Queiroz K De. 2007. Species concepts and species delimitation. *Systematic Botany* 56: 879–886.

Radosavljevic A, Anderson RP. 2014. Making better M AXENT models of species distributions : complexity , overfitting and evaluation: 629–643.

Rambaut A, Suchard MA, Xie D, Drummond AJ. 2015. Tracer v1.6.

Ratnasingham S, Hebert PDN. 2007. BOLD: The Barcode of Life Data System: Barcoding. *Molecular Ecology Notes* 7: 355–364.

Razgour O, Rebelo H, Puechmaille SJ, Juste J, Ibanez C, Kiefer A, Burke T, Dawsone DA, Jones G. 2014. Scale-dependent effects of landscape variables on gene flow and population structure in bats. *Diversity and Distributions* 20: 1173–1185.

Reading CJ, Luiselli LM, Akani GC, Bonnet X, Amori G, Ballouard JM, Filippi E, Naulleau G, Pearson D, Rugiero L. 2010. Are snake populations in widespread decline? *Biology Letters* 6: 777–780.

Redford KH, Amato G, Baillie J, Beldomenico P, Bennett EL, Clum N, Cook R, Fonseca G, Hedges S, Launay F, Lieberman S, Mace GM, Murayama A, Putnam A, Robinson JG, Rosenbaum H, Sanderson EW, Stuart SN, Thomas P, Thorbjarnarson J. 2011. What Does It Mean to Successfully Conserve a (Vertebrate) Species? *BioScience* 61: 39–48.

Reid NM, Carstens BC. 2012. Phylogenetic estimation error can decrease the accuracy of species delimitation : a Bayesian implementation of the general mixed Yule-coalescent model. *BMC Evolutionary Biology* 12: 196.

Rodda GH, Savidge J a. 2007. Biology and Impacts of Pacific Island Invasive Species. 2. Boiga irregularis, the Brown Tree Snake (Reptilia: Colubridae)1. *Pacific Science* 61: 307–324.

Rödder D, Lötters S. 2010. Potential Distribution of the Alien Invasive Brown Tree Snake , Boiga irregularis. *Pacific Science* 64: 11–22.

Rodrigues ASL, Pilgrim JD, Lamoreux JF, Hoffmann M, Brooks TM. 2006. The value of the IUCN Red List for conservation. *Trends in Ecology and Evolution* 21: 71–76.

Rogell B, Thörngren H, Laurila A, Höglund J. 2010. Fitness costs associated with low genetic variation are reduced in a harsher environment in amphibian island populations. *Conservation Genetics* 11: 489–496.

Rojas M. 1992. The species problem and conservation: what are we protecting? *Biological Conservation* 6: 170–178.

Rondinini C, Wilson KA, Boitani L, Grantham H, Possingham HP. 2006. Tradeoffs of different types of species occurrence data for use in systematic conservation planning. *Ecology Letters* 9: 1136–1145.

Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.

Rorabaugh JC. 2002. *Endangered and Threatened Wildlife and Plants: Listing of the Chiricahua Leopard Frog (Rana chiricahuensis).* Washington DC: United States Fish and Wildlife Service, Federal Register.

Rosen DE. 1979. Fishes from the uplands and intermontane basins of Guatemala: Revisionary studies and comparative geography. *Natural History* 162: 267–376.

Rouget M, Richardson DM, Cowling RM, Lloyd JW, Lombard AT. 2003. Current patterns of habitat

transformation and future threats to biodiversity in terrestrial ecosystems of the Cape Floristic Region, South Africa. *Biological Conservation* 112: 63–85.

Rowe G, Harris DJ, Beebee TJC. 2006. Lusitania revisited: A phylogeographic analysis of the natterjack toad Bufo calamita across its entire biogeographical range. *Molecular Phylogenetics and Evolution* 39: 335–346.

Rubinoff D, Holland BS. 2005. Between two extremes: mitochondrial DNA is neither the panacea nor the nemesis of phylogenetic and taxonomic inference. *Systematic biology* 54: 952–961.

Russello M a, Glaberman S, Gibbs JP, Marquez C, Powell JR, Caccone A. 2005. A cryptic taxon of Galápagos tortoise in conservation peril. *Biology letters* 1: 287–90.

Rutherford MC, Powrie LW, Schulze RE. 1999. Climate change in conservation areas of South Africa and its potential impact on floristic composition : a first assessment. *Diversity and Distributions* 5: 253–262.

Rylands AB, Mittermeier RA. 2014. Primate taxonomy: Species and conservation. *Evolutionary Anthropology* 23: 8–10.

Samper C. 2004. Taxonomy and environmental policy. *Philosophical transactions of the Royal Society of London* 359: 721–728.

Sangermano F, Eastman JR. 2012. A GIS framework for the refinement of species geographic ranges. *International Journal of Geographical Information Science* 26: 39–55.

Savidge JA. 1987. Extinction of an island forest avifauna by an introduced snake. *The Ecological Society of America* 68: 660–668.

Schorr G, Holstein N, Pearman PB, Guisan A, Kadereit JW. 2012. Integrating species distribution models (SDMs) and phylogeography for two species of alpine primula. *Ecology and Evolution* 2: 1260–1277.

Seigel RA, Mullin SJ. 2009. *Snake Conservation, Present and Future*.

Sergio F, Caro T, Brown D, Clucas B, Hunter J, Ketchum J, McHugh K, Hiraldo F. 2008. Top Predators as Conservation Tools: Ecological Rationale, Assumptions, and Efficacy. *Annual Review of Ecology, Evolution, and Systematics* 39: 1–19.

Sewell D, Guillera-Arroita G, Griffiths RA, Beebee TJC. 2012. When is a species declining? optimizing survey effort to detect population changes in reptiles. *PLoS ONE* 7.

Sexton JP, Mcintyre PJ, Angert AL, Rice KJ. 2009. Evolution and ecology of species range limits. *Annual Review of Ecology & Systematics* 40: 415–436.

da Silva JM, Tolley KA. 2017. Diversification through ecological opportunity in dwarf chameleons. *Journal of Biogeography* 44: 834–

847.

Sinclair SJ, White MD, Newell GR. 2010. How useful are species distribution models for managing biodiversity under future climates? *Ecology and Society* 15.

Sinervo B, Mendez-de-la-Cruz F, Miles DB, Heulin B, Bastiaans E, Villagran-Santa Cruz M, Lara-Resendiz R, Martinez-Mendez N, Calderon-Espinosa ML, Meza-Lazaro RN, Gadsden H, Avila LJ, Morando M, De la Riva IJ, Sepulveda P V., Rocha CFD, Ibarguengoytia N, Puntriano CA, Massot M, Lepetz V, Oksanen TA, Chapple DG, Bauer AM, Branch WR, Clobert J, Sites JW. 2010. Erosion of Lizard Diversity by Climate Change and Altered Thermal Niches. *Science* 328: 894–899.

Slatkin M. 1987. Gene flow and the geographic structure of natural populations. *Nature* 236: 787–792.

Small E. 2012. The new Noah's Ark : beautiful and useful species only . Part 1 . Biodiversity conservation issues and priorities. *Biodiversity* 12: 232–247.

SPAN. 2010. *State of Protected Areas in Namibia*. Ministry of Environment and Tourism, Windhoek.

Stamatakis A. 2014. RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312–1313.

Steffen W, Burbidge A a, Hughes L, Kitching R, Musgrave W, Smith MS, Werner P a. 2009. *Australia' s Biodiversity and Climate Change*.

Stümpel N, Rajabizadeh M, Avci A, Wüster W, Joger U. 2016. Phylogeny and diversification of mountain vipers (Montivipera, Nilson et al., 2001) triggered by multiple Plio-Pleistocene refugia and high-mountain topography in the Near and Middle East. *Molecular Phylogenetics and Evolution* 101: 336–351.

Sun L, Shine R, Debi Z, Zhengren T. 2002. Low costs, high output: reproduction in an insular pit-viper (Gloydius shedaoensis, Viperidae) from north-eastern China. *Journal of Zoology* 256: 511–521.

Sutherst RW, Maywald GF, Bourne AS. 2007. Including species interactions in risk assessments for global change. *Global Change Biology* 13: 1843–1859.

Syfert MM, Joppa L, Smith MJ, Coomes DA, Bachman SP, Brummitt NA. 2014. Using species distribution models to inform IUCN Red List assessments. *Biological Conservation* 177: 174–184.

Talavera G, Dincă V, Vila R. 2013. Factors affecting species delimitations with the GMYC model: Insights from a butterfly survey. *Methods in Ecology and Evolution* 4: 1101–1110.

Talbot SL, Shields GF. 1996. A phylogeny of the bears (Ursidae) inferred from complete sequences of three mitochondrial genes.

Molecular phylogenetics and evolution 5: 567–75.

Tattersall I, DeSalle R. 2007. Madagascar's lemurs: Cryptic diversity or taxonomic inflation? *Evolutionary Anthropology* 16: 12–23.

Taylor EB, Darveau CA, Schulte PM. 2013. Setting conservation priorities in a widespread species: Phylogeographic and physiological variation in the lake chub, Couesius plumbeus (pisces: Cyprinidae). *Diversity* 5: 149–165.

Taylor KE, Stouffer RJ, Meehl GA. 2012. An overview of CMIP5 and the experimental design. *American Meteoroligcal Society* 3: 485–498.

Taylor SA, Larson EL, Harrison RG. 2015. Hybrid zones: Windows on climate change. *Trends in Ecology and Evolution* 30: 398–406.

Thomas CD, Cameron A, Green RE, Bakkenes M, Beaumont LJ, Collingham YC, Erasmus BFN, Ferreira de Siquiera M, Grainger A, Hannah L, Hughes L, Huntly B, van Jaarsveld AS, Midgley GF, Miles L, Ortega-Huerta MA, Peterson AT, Phillips OL, Williams SE. 2004. Extinction risk from climate change. *Nature* 427: 145–148.

Thomas CD, Franco AMA, Hill JK. 2006. Range retractions and extinction in the face of climate warming. *Trends in Ecology and Evolution* 21: 415–416.

Thuiller W, Lavorel S, Araujo MB, Sykes MT, Prentice IC. 2005. Climate change threats to plant diversity in Europe. *Proceedings of the National Academy of Sciences* 102: 8245–8250.

Tolley KA, Alexander GJ, Branch WR, Bowles P, Maritz B. 2016. Conservation status and threats for African reptiles. *Biological Conservation* 204: 63–71.

Trombulak SC, Omland KS, Robinson JA, Lusk JJ, Fleischner TL, Brown G, Domroese M. 2004. Principles of conservation biology: Recommended guidelines for conservation literacy from the education committee of the society for conservation biology. *Conservation Biology* 18: 1180–1190.

Turner AA. 2012. *Western Cape Province State of Biodiversity 2012*. CapeNature Scientific Services. Stellenbosch.

van Valen L. 1976. Ecological species, multispecies, and oaks. *Taxon* 25: 233–239.

Vieites DR, Wollenberg KC, Andreone F, Köhler J, Glaw F, Vences M. 2009. Vast underestimation of Madagascar's biodiversity evidenced by an integrative amphibian inventory. *Proceedings of the National Academy of Sciences of the United States of America* 106: 8267–8272.

Vucetich JA, Waite TA. 2003. Spatial patterns of demography and genetic processes across the species' range: Null hypotheses for landscape conservation genetics. *Conservation Genetics* 4: 639–645.

Wake DB, Vredenburg VT. 2008. Are we in the midst of the sixth mass extinction? A view from the world of amphibians. *Proceedings of the National Academy of Sciences of the United States of America* 105: 11466–11473.

Waltari E, Hijmans RJ, Peterson AT, Nyári ÁS, Perkins SL, Guralnick RP. 2007. Locating pleistocene refugia: Comparing phylogeographic and ecological niche model predictions. *PLoS ONE* 2.

Walther F, Neiber MT, Hausdorf B. 2016. Species complex or complex species? Integrative taxonomy of the land snail genus Rossmaessleria (Gastropoda , Helicidae) from Morocco and Gibraltar. *Systematics and Biodiversity* 14: 394–416.

Walther G, Post E, Convey P, Menzel A, Parmesan C, Beebee TJC, Fromentin J, I OH, Bairlein F, Hoegh-Guldberg O, Bairlein F. 2002. Ecological responses to recent climate change. *Nature* 416: 389–395.

Warren DL, Glor RE, Turelli M. 2010. ENMTools: A toolbox for comparative studies of environmental niche models. *Ecography* 33: 607–611.

Wheeler QD. 2004. Taxonomy: Impediment or Expedient? *Science* 303: 285–285.

Whittaker RJ, Fernandez-Palacios JM. 2007. *Island Biogeography: Ecology, Evolution, and Conservation* (1st edn). New York: Oxford University Press Inc.

Wilcox TP, Zwickl DJ, Heath TA, Hillis DM. 2002. Phylogenetic relationships of the dwarf boas and a comparison of Bayesian and bootstrap measures of phylogenetic support. *Molecular Phylogenetics and Evolution* 25: 361–371.

Williams SE, Moritz C, Shoo LP, Isaac JL, Hoffmann A a, Langham G. 2008. Towards an Integrated Framework for Assessing the Vulnerability of Species to Climate Change. *PLoS Biol* 6: e325.

Wilson EO. 2004. Taxonomy as a fundamental discipline. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* 359: 739.

Wüster W, Duarte MR, da Graça Salomão M. 2005. Morphological correlates of incipient arboreality and ornithophagy in island pitvipers, and the phylogenetic position of Bothrops insularis. *Journal of Zoology* 266: 1–10.

Yang Z, Rannala B. 2010. Bayesian species delimitation using multilocus sequence data. *... of the National Academy of Sciences* 107: 1–6.

Zachos F. 2013. Species splitting puts conservation at risk. *Nature* 494: 35.

Zimmermann NE, Edwards TC, Graham CH, Pearman PB, Svenning JC. 2010. New trends in species distribution modelling. *Ecography* 33: 985–989.

51

SUPPLEMENTARY MATERIAL

Table S1: Sequence sources for the analyses presented in this study. All additional sequences are denoted with an 'x'.

Classification		Geographic origin					Gene region and GenBank accession number		
Genus	Species (subspecies)	Locality	Country	Lat (decimal degrees)	Long (decimal degrees)	Specimen #	Cyt-b	ND4 and tRNA	c-mos
Dipsina	*multimaculata*	—	Namibia	—	—	CMRK62	DQ486370	DQ486209	—
Dipsina	*multimaculata*	Namaqualand	South Africa	-29.02	18.09	TM84514	DQ486357	DQ486332	DQ486181
Dromophis	*lineatus*	Bujumbura	Burundi	-3.35	29.29	CMRK379	DQ486426	DQ486263	—
Dromophis	*lineatus*	Bujumbura	Burundi	-3.21	29.29	CMRK383	DQ486428	DQ486265	—
Dromophis	*lineatus*	Bello Tonga	Benin	12.05	3.21	Chir02	—	EU526861	—
Dromophis	*praeornatus*	Nienie	Benin	11.36	2.21	Chir01	—	EU526860	—
Hemirhagerrhis	*hildebrandtii*	Arusha Region	Tanzania	—	—	CMRK319	DQ486418	DQ486255	—
Hemirhagerrhis	*kelleri*	Kingori	Tanzania	-3.28	36.98	CMRK77	DQ486335	DQ486311	DQ486159
Hemirhagerrhis	*kelleri*	Kingori	Tanzania	-3.28	36.98	CMRK400	DQ486436	DQ486272	—
Hemirhagerrhis	*viperina*	Okangwati	Namibia	-17.07	13.27	CAS(AMB5989	DQ486453	DQ486289	—
Malpolon	*moilensis*	—	Tunisia	—	—	HLMD27	DQ486333	DQ486309	DQ486157
Malpolon	*monspessulanus*	Malp_05	—	—	—	—	AY058965	AY058989c	AY058936
Mimophis	*mahafalensis* (*mahafalensis*)	Mont. des Francais	Madagascar	—	—	—	DQ486363	DQ486202	—
Mimophis	*mahafalensis* (*madagascariensis*)	Mount Ibity	Madagascar	—	—	—	DQ486440	DQ486276	—
Mimophis	*mahafalensis* (?)	Tulear District	Madagascar	—	—	PEM2	DQ486461	DQ486297	—
Mimophis	*mahafalensis* (*madagascariensis*)	Mim_mad	—	—	—	—	AY188031	—	—
Mimophis	*mahafalensis* (*mahafalensis*)	Mim_mah	—	—	—	—	AY188032	AF544662c	AY187993
Psammophis	*angolensis*	Kazungula	Botswana	-17.84	25.23	CMRK263	DQ486410	DQ486248	DQ486189
Psammophis	*angolensis*	Kabwe	Zambia	-14.58	28.26	CMRK283	DQ486416	DQ486254	—
Psammophis	*angolensis*	Usa River	Tanzania	-3.37	36.85	CMRK392	DQ486433	DQ486270	—
Psammophis	*angolensis*	Kasane	Botswana	-17.82	24.86	CMRK447	DQ486439	DQ486275	—
Psammophis	*biseriatus*	Arusha	Tanzania	-3.37	36.68	CMRK169	DQ486389	DQ486228	—
Psammophis	*biseriatus*	Watamu	Kenya	-3.35	40.07	BK10724	DQ486448	DQ486284	—
Psammophis	*brevirostris*	Marondera	Zimbabwe	-18.18	31.51	CMRK186	DQ486395	DQ486234	—
Psammophis	*brevirostris*	Hole in the Wall	South Africa	-32.03	29.11	CMRK237	DQ486402	DQ486241	—
Psammophis	*brevirostris*	Hole in the Wall	South Africa	-32.03	29.11	CMRK238	DQ486403	DQ486242	—
Psammophis	*brevirostris*	Naboomspruit	South Africa	-24.36	28.83	CMRK277	DQ486412	DQ486250	—
Psammophis	*brevirostris*	Cullinan Mine	South Africa	-25.68	28.52	TM83922	DQ486470	DQ486306	—
Psammophis	cf.	Kwekwe Derba	Ethiopia	9.4	38.67		DQ486449	DQ486285	—
Psammophis	cf. (*sibilans*)	Keriyo Hamlet	Ethiopia	9.4	38.65	CMRK352	DQ486419	DQ486256	—
Psammophis	cf. (*sibilans*)	Keriyo Hamlet	Ethiopia	9.4	38.65	CMRK358	DQ486420	DQ486257	—
Psammophis	cf. (*sibilans*)	Keriyo Hamlet	Ethiopia	9.4	38.65	CMRK364	DQ486422	DQ486259	—
Psammophis	cf. *philipsii* (*occidentalis*)	Serenje	Zambia	-12.76	30.93	CMRK71	DQ486373	DQ486210	—
Psammophis	cf. *philipsii* (*occidentalis*)	Kizuka	Burundi	-3.9	29.39	CMRK377	DQ486424	DQ486261	—
Psammophis	cf. *philipsii* (*occidentalis*)	Bubanza	Burundi	-3.06	29.43	CMRK381	DQ486427	DQ486264	—
Psammophis	cf. *rukwae*	—	Senegal	13.98	-14.57	MNHN	DQ486452	DQ486288	—
Psammophis	*crucifer*	Somerset East	South Africa	-32.72	25.58	CMRK19	DQ486360	DQ486199	—
Psammophis	*crucifer*	Jeffrey's Bay	South Africa	-33.94	24.99	CMRK70	DQ486363	DQ486310	—
Psammophis	*crucifer*	Nyanga	Zimbabwe	-18.24	32.77	CMRK203	DQ486397	DQ486236	DQ486188
Psammophis	*crucifer*	DeHoop Nat. Res.	South Africa	-34.43	20.48	S3	DQ486466	DQ486302	—
Psammophis	*elegans*	La Tapoa	Niger	12.05	2.26	Chir03	—	EU526862	—
Psammophis	*elegans*	—	—	—	—	—	—	EF128027	—
Psammophis	*jallae*	Kazungula	Botswana	-17.95	25.23	CMRK256	DQ486409	DQ486247	—
Psammophis	*leightoni*	Piketberg	South Africa	-32.90	18.77	TM83620	DQ486467	DQ486303	DQ486197
Psammophis	*leightoni*	West Coast Nat. Pk.	South Africa	-33.17	18.14	AC1046	x	x	x
Psammophis	*leightoni*	West Coast Nat. Pk.	South Africa	-33.16	18.04	ARD000025	x	x	x
Psammophis	*leightoni*	Draaihoek	South Africa	-32.61	18.33	ATDHKPL1	x	x	x
Psammophis	*leightoni*	Tweekuilen	South Africa	—	—	ATTKPL1	x	x	x
Psammophis	*leightoni*	Redelinghuys	South Africa	-32.44	18.58	KTH05-03	x	x	x
Psammophis	*leightoni*	West Coast Nat. Pk.	South Africa	-32.45	18.62	KTH05-04	x	x	x
Psammophis	*leightoni*	Koeberg Nat. Res.	South Africa	-33.65	18.44	WP1601JM	x	x	x
Psammophis	*leightoni*	Koeberg Nat. Res.	South Africa	-33.63	18.44	WP1602JM	x	x	x
Psammophis	*leightoni*	Koeberg Nat. Res.	South Africa	-33.64	18.42	WP1603JM	x	x	x
Psammophis	*leightoni*	Koeberg Nat. Res.	South Africa	-33.65	18.44	WP1701JM	—	x	x
Psammophis	*leightoni*	West Coast Nat. Pk.	South Africa	-33.12	18.07	WW2798	x	x	x
Psammophis	*leopardinus*	Opuwo	Namibia	-18.47	13.8	CAS214763	DQ486456	DQ486292	—
Psammophis	*leopardinus*	Grootberg Pass	Namibia	-19.87	14.07	CAS214727	DQ486462	DQ486298	—
Psammophis	*lineolatus*	Nephtezavodsk	Turkmenistan	39.25	63.18	CAS179682	DQ486450	DQ486286	DQ486195
Psammophis	*mossambicus*	Tanga	Tanzania	-5.16	38.98	PEMR5679	DQ486363	DQ486198	—
Psammophis	*mossambicus*	Kingori	Tanzania	-3.28	36.98	CMRK81	DQ486373	DQ486212	—
Psammophis	*mossambicus*	Kingori	Tanzania	-3.28	36.98	CMRK82	DQ486374	DQ486213	—
Psammophis	*mossambicus*	Nyagatare	Rwanda	-1.36	30.35	CMRK125	DQ486383	DQ486222	DQ486185
Psammophis	*mossambicus*	Nyagatare	Rwanda	-1.29	30.23	CMRK126	DQ486384	DQ486223	—
Psammophis	*mossambicus*	Nyagatare	Rwanda	-1.29	30.23	CMRK127	DQ486385	DQ486224	—
Psammophis	*mossambicus*	Mikumi Nat. Pk.	Tanzania	-7.35	37.08	CMRK175	DQ486392	DQ486231	—
Psammophis	*mossambicus*	Sodwana Bay	South Africa	-27.69	32.37	CMRK231	DQ486400	DQ486239	—
Psammophis	*mossambicus*	Pandamatenga	Botswana	-18.64	25.63	CMRK268	DQ486411	DQ486249	—
Psammophis	*mossambicus*	Butare	Rwanda	-2.69	29.71	CMRK376	DQ486423	DQ486260	—
Psammophis	*mossambicus*	Maun	Botswana	-19.98	23.42	Bills	DQ486442	DQ486278	—
Psammophis	*mossambicus*	Makuyu	Kenya	-0.9	37.18	BK10357	DQ486447	DQ486283	—
Psammophis	*mossambicus*	Moebase	Mozambique	-17.06	38.69	PEMR13258	DQ486457	DQ486293	—
Psammophis	*mossambicus*	Namagure	Mozambique	-17.06	38.69	PEMR13217	DQ486460	DQ486296	—
Psammophis	*mossambicus*	Palaborwa	South Africa	-23.95	31.12	TM83688	DQ486468	DQ486304	—

Classification		Geographic origin					Gene region and GenBank accession number		
Genus	Species (subspecies)	Locality	Country	Lat (decimal degrees)	Long (decimal degrees)	Specimen #	Cyt-b	ND4 and tRNA	c-mos
Psammophis	*namibensis*	Port Nolloth	South Africa	-29.25	16.87	PEMR15811	DQ486455	DQ486291	—
Psammophis	*namibensis*	Port Nolloth	South Africa	-29.28	16.97	TGET569	x	x	x
Psammophis	*namibensis*	Port Nolloth	South Africa	—	—	1819	x	x	x
Psammophis	*namibensis*	Langer Heinrich Mine	Namibia	-22.81	15.37	LHU01	x	x	x
Psammophis	*notostictus*	Grahamstown	South Africa	-33.3	26.51	PEMR5660	DQ486362	DQ486201	—
Psammophis	*notostictus*	Mtn. Zebra Nat. Pk.	South Africa	-32.17	25.27	PEMR5682	DQ486366	DQ486205	DQ486182
Psammophis	*notostictus*	Mtn. Zebra Nat. Pk.	South Africa	-32.17	25.27	PEMR5669	DQ486367	DQ486206	—
Psammophis	*notostictus*	Port Nolloth	South Africa	-29.25	16.87	PEM	DQ486463	DQ486299	—
Psammophis	*notostictus*	Beaufort-West	South Africa	-32.68	22.68	EI_0291	x	x	x
Psammophis	*notostictus*	Beaufort-West	South Africa	-32.36	23.06	EI_0308	x	x	x
Psammophis	*notostictus*	Hardap Region	Namibia	-24.18	15.98	EI_0351	x	x	x
Psammophis	*notostictus*	Northern Cape	South Africa	-31.22	20.52	FP020	x	x	x
Psammophis	*notostictus*	Northern Cape	South Africa	-31.34	22.29	FP031	x	x	x
Psammophis	*notostictus*	Northern Cape	South Africa	-31.34	22.29	FP037	x	x	x
Psammophis	*notostictus*	Northern Cape	South Africa	-31.35	22.29	FP043	x	x	x
Psammophis	*notostictus*	Northern Cape	South Africa	-31.92	22.88	FP058	x	x	x
Psammophis	*notostictus*	Northern Cape	South Africa	-32.31	21.94	FP094	x	x	x
Psammophis	*notostictus*	Springbok	South Africa	-29.66	17.89	NCP16-70	x	x	x
Psammophis	*notostictus*	West Coast Nat. Pk.	South Africa	—	—	UWC001	x	—	—
Psammophis	*notostictus*	Koeberg Nat. Res.	South Africa	-33.65	18.44	WP1604JM	x	x	x
Psammophis	*notostictus*	Koeberg Nat. Res.	South Africa	-33.63	18.44	WP1605JM	x	x	x
Psammophis	*notostictus*	West Coast Nat. Pk.	South Africa	-33.15	18.02	WW2562	x	x	x
Psammophis	*notostictus*	West Coast Nat. Pk.	South Africa	-32.45	18.62	WP1606JM	x	x	x
Psammophis	*orientalis*	Handeni	Tanzania	-5.43	38.02	CMRK89	DQ486380	DQ486219	—
Psammophis	*orientalis*	Gilgil	Kenya	-0.5	36.32	NMKO/3597	DQ486386	DQ486225	—
Psammophis	*orientalis*	Nguru Mtns	Tanzania	-5.43	37.45	CMRK171	DQ486390	DQ486229	—
Psammophis	*orientalis*	Nguru Mtns	Tanzania	-5.43	37.45	CMRK172	DQ486391	DQ486230	—
Psammophis	*orientalis*	Udzungwa Nat. Pk.	Tanzania	-7.58	36.36	CMRK177	DQ486393	DQ486232	—
Psammophis	*orientalis*	Gorongosa	Mozambique	-18.18	34.11	CMRK187	DQ486396	DQ486235	—
Psammophis	*orientalis*	Moma	Mozambique	-16.76	39.22	PEMR15622	DQ486459	DQ486295	—
Psammophis	*phillipsii* (*philipsii*)	Loango Nat. Pk.	Gabon	-2.36	9.64	PEMR5451	DQ486454	DQ486290	—
Psammophis	*punctulatus* (*trivirgatus*)	Lolkisale	Tanzania	-3.77	36.42	CMRK167	DQ486387	DQ486226	DQ486186
Psammophis	*punctulatus* (*trivirgatus*)	Arusha Region	Tanzania	—	—	CMRK391	DQ486432	DQ486269	—
Psammophis	*punctulatus* (*trivirgatus*)	Watamu	Kenya	-3.35	40.02	BK10476	DQ486445	DQ486281	—
Psammophis	*rukwae*	Kondoa Region	Tanzania	-4.9	35.78	CMRK83	DQ486375	DQ486214	—
Psammophis	*rukwae*	Kondoa Region	Tanzania	-4.9	35.78	CMRK85	DQ486376	DQ486215	—
Psammophis	*rukwae*	Lake Baringo	Kenya	0.47	35.97	BK10358	DQ486443	DQ486279	—
Psammophis	*rukwae*	Kakuyuni	Kenya	-3.22	40	BK10620	DQ486446	DQ486282	—
Psammophis	*schokari*	Tantan	Morocco	—	—	—	DQ486365	DQ486204	—
Psammophis	*schokari*	Hazoua	Tunisia	—	—	—	DQ486364	DQ486203	—
Psammophis	*schokari* (*sibilans*)	Bou Hedma	Tunisia	—	—	—	DQ486441	DQ486277	DQ486194
Psammophis	*subtaeniatus*	Kazungula	Zimbabwe	-18.92	29.82	NMZB4	DQ486358	—	—
Psammophis	*subtaeniatus*	Kariba	Botswana	-17.96	25.23	CMRK249	DQ486408	—	—
Psammophis	*subtaeniatus*	Kingori	Zimbabwe	-16.52	28.8	CMRK282	DQ486415	DQ486253	—
Psammophis	*sudanensis*	Loitokitok	Tanzania	-3.28	36.98	CMRK91	DQ486382	DQ486221	DQ486184
Psammophis	*sudanensis*	Athi River	Kenya	-2.84	37.52	CMRK334	—	DQ486307	—
Psammophis	*sudanensis*	Athi River	Kenya	-1.45	36.98	CMRK385	DQ486429	DQ486266	—
Psammophis	*sudanensis*	Namanga	Kenya	-1.45	36.98	CMRK386	DQ486430	DQ486267	—
Psammophis	*sudanensis*	Tsavo Nat. Pk.	Tanzania	-2.87	36.72	CMRK390	DQ486431	DQ486268	—
Psammophis	*sudanensis*	Dodoma Region	Kenya	-2.98	38.47	BK10603	DQ486444	DQ486280	—
Psammophis	*tanganicus*	Dodoma Region	Tanzania	—	—	CMRM86	DQ486377	DQ486216	—
Psammophis	*tanganicus*	Dodoma Region	Tanzania	—	—	CMRK87	DQ486378	DQ486217	DQ486183
Psammophis	*tanganicus*	Arusha Region	Tanzania	—	—	CMRK88	DQ486379	DQ486218	—
Psammophis	*tanganicus*	Sesfontein	Tanzania	—	—	CMRK90	DQ486381	DQ486220	—
Psammophis	*trigrammus*	Brandberg	Namibia	-19.17	13.57	CAS214751	DQ486458	DQ486294	DQ486196
Psammophis	*trigrammus*	Doroba	Namibia	-21.13	14.58	TM83873	DQ486469	DQ486305	—
Psammophis	*trinasalis*	Goegap Nat. Pk.	South Africa	-29.70	17.93	GNR 002	x	x	x
Psammophis	*trinasalis*	Northern Cape	South Africa	-28.83	19.51	ARD00028	x	x	x
Psammophis	*trinasalis*	Tsawisis	Namibia	-26.18	18.16	EI_0028	—	x	x
Psammophis	*trinasalis*	Northern Cape	South Africa	-30.08	18.31	KTH583	x	x	x
Psammophis	*trinasalis*	Northern Cape	South Africa	-28.81	22.54	MB20914	x	x	x
Psammophis	*trinasalis*	Northern Cape	South Africa	-28.57	24.2	RSP144	x	x	x
Psammophis	*trinasalis*	Northern Cape	South Africa	—	—	RSP230	x	x	x
Psammophis	*trinasalis*	Karoo Nat. Pk.	South Africa	-32.25	22.19	WC3012	x	x	x
Psammophis	*trinasalis*	Beaufort-West	South Africa	—	—	WC3795	x	x	x
Psammophis	*trinasalis*	Central District	Botswana	-21.29	25.15	WC-DNA-991	x	x	x
Psammophylax	*multisquamis*	Grahamstown	Ethiopia	9.33	38.92	CMRK361	DQ486421	DQ486258	—
Psammophylax	*rhombeatus*	Mvuma	South Africa	-33.3	26.51	CMRK234	DQ486342	DQ486318	DQ486166
Psammophylax	*tritaeniatus*		Zimbabwe	-19.53	30.73	CMRK279	DQ486414	DQ486252	DQ486190
Psammophylax	*variabilis* (*variabilis*)	Udzungwa Mtns	Tanzania	-8.28	35.9	CMRK413	EU526863	EU526859	—
Rhamphiophis	*acutus* (*acutus*)	Gitaba	Burundi	-3.85	29.98	CMRK378	DQ486425	DQ486262	DQ486192
Rhamphiophis	*acutus* (*acutus*)	Luzamba	Angola	-9.12	18.06	PEMR13485	DQ486464	DQ486300	—
Rhamphiophis	*rostratus*	Dodoma Region	Tanzania	—	—	CMRK80	DQ486336	DQ486312	—
Rhamphiophis	*rostratus*	Bubye River	Zimbabwe	-21.7	30.51	CMRK185	DQ486394	DQ486233	DQ486187
Rhamphiophis	*rubropunctatus*	Kilimanjaro Airport	Tanzania	-3.43	37.07	CMRK303	DQ486417	—	—

Table S1 continued:

Outgroups									
Classification		Geographic origin					Gene region and GenBank accession number		
Genus	Species (subspecies)	Locality	Country	Lat (decimal degrees)	Long (decimal degrees)	Specimen #	Cyt-b	ND4 and tRNA	c-mos
Aspidelaps	*scutatus*	—	—	—	—	LSUMZ56251	AF217828	AY058969c	AY058923
Atractaspis	*bibronii*	—	—	—	—	—	AY188008	U49314c	AY187969
Buhomab	*procterae*	Udzungwa Nat. Pk.	Tanzania	-8.37	35.97	ZMUCR631315	DQ486353	DQ486328	DQ486177
Duberria	*lutrix*	Limuru	Kenya	-1.11	36.61	NMKO/3578	DQ486337	DQ486313	DQ486161
Lamprophis	*guttatus*	Ngotshe District	South Africa	-27.85	31.33	TM84363	DQ486355	DQ486330	DQ486179
Pseudaspis	*cana*	Nata	Botswana	-19.92	26.15	CMRK246	DQ486343	DQ486319	DQ486167

UNIVERSITY *of the*
WESTERN CAPE