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Re-assessment of the threat status of three Red Data List plant species: *Brachystelma gerrardii, Senecio triodontiphyllus* and *Streptocarpus fasciatus.*

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A dissertation submitted to the Faculty of Science, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Master of Science.

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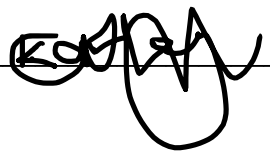
Declaration

This dissertation is submitted, in accordance with the regulations of the University of the Witwatersrand, Johannesburg in fulfilment of the requirements for the degree of Master of Science. The work described in this dissertation was carried out by me, except where otherwise acknowledged, and has not been submitted for any degree or examination to any other university or institution.

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10 August 2022

Abstract

Plants provide fundamental support systems and resources for life on earth, they serve as the foundation of ecosystems. However, many plant species are rapidly going extinct and will soon disappear. The threat assessments of plants are important as they inform which species are most at risk and call for immediate management attention and conservation. The first step to initiate conservation actions for endangered organisms is identifying species that are in decline or face extinction. A previous study for the management of Pullen Nature Reserve found that there are threatened plant species that occur on the property and in the conservancy. These plants are: (1) *Brachystelma gerrardii* Harv., (2) *Senecio triodontiphyllus* C. Jeffrey, and *Streptocarpus fasciatus* T. J Edwards & Kunhardt. *Brachystelma gerrardii* has a distribution from KwaZulu-Natal to the Waterberg in Limpopo, and is currently classified as Endangered (EN) according to the IUCN Categories and Criteria,. *Senecio triodontiphyllus* has a distribution that is restricted to the Mpumalanga province, from Barberton to Kaapmuiden. This species is currently classified as Vulnerable (VU). *Streptocarpus fasciatus* is endemic to South Africa and occurs in Mpumalanga. Its distribution ranges from Nelspruit to Kaapmuiden. This species has a current threat status of VU.

Brachystelma gerrardii, S. triodontiphyllus and *S. fasciatus* were chosen for this study because they are rare, endangered and have restricted distributional ranges. A re-assessment of their threat status is important as the previous assessments are out of date. Thus, the aim of this project is to re-assess the threat status of three plant species *B. gerrardii*, *S. fasciatus* and *S. triodontiphyllus*; to assess the potential effect of climate change on current and future distributions of these plant species; as well as to investigate aspects of the reproductive ecology of *S. fasciatus.*

The IUCN Red List Categories and Criteria were used to perform the assessments; these criteria are objective, quantifiable and have been used over many years. This project was carried out in three stages, (1) an analysis of herbarium specimens as well as fieldwork to collect data on species habitats, range sizes, population sizes, as well as Area of occupancy (AOO) and Extent of occurrence (EOO) among other information to determine the current threat status of the species; (2) an analysis of current and future climate data to determine the impact of climate change on plant species distributions; and lastly, (3) investigating the breeding strategy used by *S. fasciatus*, to determine whether pollination and germination have implications for the threat status.

Results from herbarium records and fieldwork revealed eleven, five and six sub-populations for *B. gerrardi, S. fasciatus* and *S. triodontiphyllus* respectively. According to the MaxEnt models for *B. gerrardii* and *S. triodontiphyllus*, areas that are currently suitable for the growth of these plants are predicted in the eastern parts of South Africa from East London through KwaZulu-Natal and Mpumalanga, towards the northern parts of Limpopo. Current suitable areas for *S. fasciatus* are predicted as patches along the coast in Western cape, along the border of Lesotho in KwaZulu-Natal, in parts of Gauteng as well as the eastern parts of Mpumalanga. Future

predictions for 2050 using the A1B and A2 emission scenarios showed a decrease in suitable habitats for *B. gerrardi* and *S. triodontiphyllus*, but a significant increase in the suitable habitat for *S. fasciatus*.

Field studies on the pollination of *S. fasciatus* show that this plant may be pollinated by a nemestrinid fly belonging to the genus *Stenobasipteron*, and the results also indicate the presence of a mixed breeding system involving self-pollination and cross-pollination. A low seed set was observed in open pollinated flowers, indicating that the species may be pollinator limited, however, this observation may also be as a result of the species' very specific habitat requirements. This may contribute to its low population size and thus its threatened status. According to the results of the IUCN threat assessment, *S. fasciatus* was found to be vulnerable (VU) under Criterion D (subcriteria D1 and D2), and *S. triodontiphyllus* was found to be endangered (EN) under subcriteria D1. *Brachystelma gerrardii* remains classified as EN as per the threat assessment by Styles and von Staden in 2007. Based on the results of this study further investigations are required with regards to the breeding system of *S. fasciatus*, and all three of these plant species are in need of immediate conservation actions.

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Chapter 1

General Introduction

1.1. Background

Recent years have seen a huge decrease in the amount of biodiversity, and a significant increase in the number of species threatened with extinction. The global analysis of extinction risk shows that about 1-in-5 plant species is at risk of going extinct (Ibrahim *et al.,* 2013). Species extinction threatens ecosystems through a reduction in species richness and diversity. Studies have shown that species diversity enhances ecosystem function since biological communities regulate a lot of the ecological processes such as water availability, soil fertility and its stability (Cardinale *et al.,* 2000; Cook-Patton *et al.,* 2011; Stevens & Carson, 2001;). Species extinctions are likely to affect the composition and functional traits of ecosystems (Chapin *et al.,* 2000; Gallagher *et al.,* 2013). This will affect ecosystem resilience and will result in them being vulnerable to invasions and also impact the provision of ecosystem services (Chapin *et al.,* 2000). Many species are driven to extinction by anthropogenic impacts such as overexploitation, pollution, land-use change, introduction of alien invasive plants as well as anthropogenic climate change.

Climate change is a global threat that has negative impacts on natural ecosystems (Sintayehu, 2018). Its effect on threatened and endemic plant species is of particular concern since these species have small population sizes and specific habitat requirements (Jones *et al.,* 2013). As a result, climate change may reduce their adaptive capabilities. A characteristic of global climate change is an increase in carbon dioxide (CO_2) concentration in the atmosphere due to carbon emissions (Hulme *et al.,* 2001; Karl & Trenberth, 2003). This increase in CO_2 concentration has brought about changes in global temperature (Scholes *et al.,* 2015). Temperature records in southern Africa show that this region has been warming at an exponential rate, with minimum temperatures rising faster than maximum temperature (Kusangaya *et al.,* 2014). This increase in temperature has a cascading effect on the planet's system and will lead to changes in patterns of rainfall, as well as the distribution of water runoff, groundwater reserves and soil moisture (Scholes *et al.,* 2015; Suárez *et al.,* 2002; Trenberth, 2011). All these changes will directly impact the rate and efficiency of photosynthesis, respiration, plant growth and productivity as well as other ecological processes (Hughes, 2000; Suárez *et al.,* 2002). For this reason, climate change is regarded as a major factor that controls vegetation structure, species composition and distribution in landscapes.

The earth has experienced different types of environmental change throughout its history, and these changes have been associated with the ability for species to shift their location in response to climatic events (Hughes, 2000; Kupika *et al.,* 2017; Pecl *et al.,* 2017). This shift occurs in order to stay in preferred environmental conditions (Gomez-Ruiz & Lacher, 2019). The

only problem is that rapid climate change may change the position and location of bioclimatic envelopes for species with narrow habitat tolerances (Abolmaali *et al.,* 2018; Vitt *et al.,* 2010). Most plant species can only grow and reproduce successfully within specific climate ranges. This is because for plants to survive in an environment they need suitable ecological conditions that promote reproduction and long-term genetic fitness.

Research suggests that pollinator abundance and diversity are in rapid decline due to anthropogenic factors such as habitat loss and climate change (Kearns *et al.,* 1998; Ricketts *et al.,* 2008; Wilcock & Neiland, 2002; Winfree *et al.,* 2009). This loss of pollinators as well as a mismatch of temporal and spatial co-occurrence of flowers and pollinators can affect their relationship. For this reason, climate change has been a growing concern for many years because increasing temperatures alter the flowering times of plants and pollinator behaviour (Hickling *et al.,* 2006; Sparks *et al.,* 2000). Pollination is important for the life cycle of many flowering plants as it allows for the recombination of genes and formation of genetically distinct seeds (Abrol, 2012; Pićo *et al.,* 2008). This process promotes genetic variability and increases fitness in plant offspring (Wilmer, 2011). Pollination is associated with plant speciation and evolution by natural selection and is therefore essential for the survival of plants in changing environments (Schowalter, 2006; Wilmer, 2011). Studies of pollination and plant reproduction help us identify factors affecting the reproduction of plants and maintenance of plant populations. This is especially important when it comes to endangered plant species as they are vulnerable to changing environmental conditions and their survival depends on effective reproductive biology.

Endangered and threatened plants are valuable to ecosystems and some of them have economic and medicinal value or potential which makes their management important worldwide (Wang *et al.,* 2015). Before conservation actions can be initiated, threatened species need to be identified and assessed using objective, quantifiable and consistent criteria (Tali *et al.,* 2014). The IUCN Red List Categories and Criteria are widely used to evaluate the threat status of species, which helps determine their conservation status (Nicholson *et al.,* 2009). These categories and criteria improve consistency and understanding among users (IUCN, 2019). Taxa can be assigned to nine categories, these are: Extinct (EX), Extinct in the Wild (EW), Critically Endangered (CR), Endangered (EN), Vulnerable (VU), Near Threatened (NT), Least Concern (LC) and lastly Data Deficient (DD). The categories CR, EN and VU are allocated to taxa depending on their risk of going extinct, and together, these categories are known as the 'threatened' categories (IUCN, 2001; 2019). The IUCN Criteria (A to E) on the other hand, are based on number of populations and number of individuals in populations (A & B), number of mature individuals (A, B, C & D), population reduction (A), continuing decline (B & C), fluctuations (B & C), fragmentation (B), Extent of Occurrence (EOO) (A & B), Area of Occupancy (AOO) (A, B & D), generation (A, C & E), location (B & D) and qualitative analysis (IUCN, 2019).

Assessment of threat status using the IUCN Categories and Criteria requires field-based knowledge of population size, rate of population decrease, population trend, habitat, size of range, the area that is occupied by the taxon (EOO and AOO) and current conservation actions for each species in order to assign threat status (Alam & Ali, 2010; Jetz & Freckleton, 2015; Rodrigues *et al.,* 2006; Tali *et al.,* 2014). Species assessments are a major part of conservation planning as they enable policymakers and conservation managers to identify threats as well as their severity to species and species distributions (Bleher *et al.,* 2006). Such assessments provide evidence-based, actionable and quantitative data which can help inform, improve and optimize management strategies (Collen *et al.,* 2016). Using the information obtained from species assessments, assessors can document the conservation actions that have been undertaken and propose further actions that would improve the status of each species (Hayward, 2011). Even though we know the importance of plants and the threats they face globally, there are still many plants whose extinction risk and threat status have not been assessed. Furthermore, for those species which have been assessed, the assessments were done a long time ago and their threat status may no longer apply.

A previous field- and herbarium-based study for the management plan of Pullen Nature Reserve (NR), found that there are rare and threatened plants that occur on the property, and in the conservancy (Makgopo, 2019). Pullen NR is situated in the Croc River Mountain Conservancy which is located in the Mpumalanga province approximately 30 km east of Nelspruit. The reserve is owned by the University of the Witwatersrand and managed by the School of Animal, Plant and Environmental Sciences. It is approximately 250 ha in size and is fenced with Bonsberg Game Estate which together have a determined size of 989.2 ha (Grosel, 2012). The threatened plant species that were found at Pullen are *Brachystelma gerrardii* Harv. and *Senecio triodontiphyllus* C. Jeffrey, whereas *Streptocarpus fasciatus* T. J. Edwards & Kunhardt was found to occur on Moederlief and Stonehaven, which are farms adjacent to Pullen NR. *Brachystelma gerrardii, S. triodontiphyllus* and *S. fasciatus* were chosen for this study because they are rare, threatened plant species which have a restricted distributional range. Although they have been previously assessed using desktop studies, their reassessment is vital as the previous assessments are out of date (IUCN, 2019).

Brachystelma gerrardii is a herb from the family Apocynaceae. It is characterized by its annual stems which are produced from perennial underground swollen rootstocks. This species has a scattered distribution in southern Africa (Dyer, 1983). Its distribution is from KwaZulu-Natal, through Swaziland to the Waterberg in Limpopo (Raimondo *et al.* 2009). *Brachystelma gerrardii* occupies open grassland areas at altitudes of between 400–1800 m above sea level (Lötter *et al*., 2006; Raimondo *et al.* 2009). This plant species flowers between November and April in South Africa (Dyer, 1975; K. Balkwill et al. 12487 (J); BRAHMS Online). It is one of the taller species of *Brachystelma*, and has solitary flowers on slender stalks (Dyer, 1983). In the south of

KwaZulu-Natal in southern Africa, the flowers of this plant are bright metallic green and to the north of this province the flowers are dark green, approaching black with a velvety look above (Dyer, 1983). This plant species has been recorded from 20 locations and has been classified as endangered (EN) according to the IUCN/SANBI Red List of threatened species (Styles and Von Staden, 2007). Although this species is widespread, it is rare, and its subpopulations usually consist of less than 10 mature individuals (Dyer, 1983; Raimondo *et al*., 2009). Threats to this plant species include urban and housing development, overgrazing by livestock and invasion by alien plants (Styles and Von Staden, 2007). Consequently, it is severely threatened and declining.

Senecio triodontiphyllus is a perennial herb from the family Asteraceae. This species is confined to the Mpumalanga province, from Barberton to Kaapmuiden where it occupies steep slopes and grassland habitats (Edwards & Raimondo, 2007; Raimondo *et al.,* 2009). The plant grows up to 360 mm and produces orange-yellow flowers between October and June (Raimondo *et al.,* 2009). *Senecio triodontiphyllus* is currently classified as vulnerable (VU) according to the IUCN/SANBI Red List of threatened plants (Lötter *et al.,* 2006) and its threats include invasive alien species as well as expanding forestry plantations (Raimondo *et al.,* 2009).

Lastly, *Streptocarpus fasciatus* is a rosulate perennial herb from the family Gesneriaceae. This species is endemic to South Africa and occurs in the Mpumalanga province (Lötter *et al.,* 2006). It ranges from Nelspruit to Kaapmuiden and has only been recorded from an enclave of woodland in the Crocodile River Gorge (Edwards *et al.,* 1992; Lötter *et al.,* 2006). This species occurs in Malelane Mountain Bushveld, in the scarp forest in shady woodland areas and among granite boulders (Lötter *et al.,* 2006). *Streptocarpus fasciatus* is currently classified as vulnerable (VU), with its main threats being alien plant invasions and habitat degradation due to deforestation (Lötter *et al.,* 2006). Plants of this species flower from late summer into autumn, between March and April and produce flowers that have well-defined longitudinal stripes which mark the corolla lobes (Edwards *et al.,* 1992). *Streptocarpus fasciatus* flowers have nectar, and vary in shape, size and colours, all features associated with pollination (Harrison *et al.,* 1999). Due to their floral display, flowers that are produced by *Streptocarpus* species may attract pollinator visitations and may be cross pollinated (Hughes *et al.,* 2006). However, confirmation in terms of pollinator observations is lacking. The movements of pollinators and seed dispersal ranges are of special interest in the *Streptocarpus* genus since its species exist as discrete populations that are separated by unfavourable habitats (Hughes *et al.,* 2007). Understanding how populations of this genus are connected and how they are affected by pollination and habitat variables can help us understand its evolution and biodiversity (Hughes *et al.,* 2007).

1.2. Research aims and objectives

The main aims of this study were to conduct field assessments to re-assess the threat status of *B. gerrardii, S. fasciatus* and *S. triodontiphyllus*, to determine whether the assigned status still applies; to assess the potential effect of climate change on current and future distributions of these plant species; as well as to investigate aspects of the reproductive ecology of *S. fasciatus.*

Aim 1: Re-assess the threat status of *B. gerrardii*, *S. fasciatus* and *S. triodontiphyllus*, to determine whether the assigned status still applies.

Objective (i): Determine the number of localities where the plant species occur using herbarium records, iNaturalist and personal observations.

Objective (ii): Assess land-use and threats at those localities and predict whether the species are likely to persist.

Aim 2: Determine the potential impact of climate change on threatened plants using distribution data and predictions of climate change.

Objective (i): Use current climate data to investigate current suitable habitats for the species and determine whether the known plant species distributions correspond with these predicted suitable habitats.

Objective (ii): Determine whether suitable habitats will grow or shrink under projected climate change.

Aim 3: Investigate the breeding system and pollination of *S. fasciatus* and determine whether these have implications for its threat status.

Objective (i): Determine the pollinator guild of *S. fasciatus* flowers.

Objective (ii): Determine the breeding system of *S. fasciatus*.

1.3. Structure of the dissertation

This dissertation comprises five chapters. Chapter 1 is a general overview of topics included in the dissertation and the rationale and aims of the dissertation. Chapter 2 covers the threat assessments of the three plant species using IUCN Categories and Criteria. Chapter 3 focuses on climate change, to determine impacts of this phenomenon on plant populations and predict change of future distributions. Chapter 4 focuses on the pollination and breeding biology of *S. fasciatus,* to determine if its reproductive biology contributes to its threatened status. Chapter

5 is the overall conclusion chapter, which highlights the important results of the previous chapters. The three data chapters (Chapters 2, 3 and 4) of this dissertation are written as independent papers in scientific format, although there is only a single reference list at the end.

Chapter 2

Assessment of the threat status of three endemic plant species using IUCN Categories and Criteria.

Abstract

Species threat assessments are an important tool that is used to identify species that are in decline and faced with extinction and play an important role in biological conservation. The IUCN Red List assessments provide important knowledge about the state of biodiversity and priority species for conservation. During this study, the threat statuses *of Brachystelma gerrardii* Harv.*, Streptocarpus fasciatus* T. J. Edwards & Kunhardt and *Senecio triodontiphyllus* C. Jeffrey were assessed using the International Union for Conservation of Nature (IUCN) Categories and Criteria 2010 version 8.1, and following Guidelines for application of IUCN Red List at Regional Levels 2003 version 3.0 because they are rare and under threat of extinction. Data from herbarium records revealed eleven, five and six sub-populations for *B. gerrardii*, *S. fasciatus* and *S. triodontiphyllus* respectively. *Streptocarpus fasciatus* and *S. triodontiphyllus* were each found in three localities in the Mpumalanga province where these species seem to be confined. Threats associated with these species are mainly alien invasive plants, especially *Lantana camara* L. and *Psidium guajava* L. as well as habitat loss associated with urban development. According to the results of the IUCN threat assessment, S. *fasciatus* and *S. triodontiphyllus* were found to be Vulnerable (VU) and Endangered (EN) under criterion D respectively. *Brachystelma gerrardii* remains classified as EN according to the threat assessment by Styles and von Staden (2007). There is an immediate need for conservation intervention for removal of alien species that are encroaching on suitable habitat of the species.

2.1. Introduction

Biodiversity is the variety of life on earth and includes species, genes, ecosystems and the processes that occur within them (Lister, 1997; Lötter *et al.,* 2014; Magurran, 2004). It provides the foundation for ecosystem services and is therefore linked to human well-being (Pereira *et al.,* 2012). Studies have shown that species diversity enhances ecosystem function, and this is because biological communities within these ecosystems regulate ecological processes (Cardinale *et al*., 2000; Cook-Patton *et al.,* 2011; Stevens & Carson, 2001). This means that ecosystems depend on the species within them and thus function better when they contain more species (Gascon *et al.,* 2015; Turnbull *et al.,* 2016). For instance, plant species regulate important ecosystem functions such as water availability, soil fertility as well as ecosystem stability (Eviner & Chapin, 2001). Species richness and diversity within ecosystems allow ecosystems to be better adapted to disturbances and serve as insurance for future functionality of those ecosystems (Leveque & Mounolou, 2004). Systems which are more diverse can be more productive, stable and resilient compared to less diverse systems (Cardinale *et al.,* 2007; Tilman & Downing, 1994; Tilman *et al.,* 1996). This is because communities with more species

contain a larger range of species traits that may be beneficial in the face of disturbances (Petchey, 2000).

Human beings depend on biodiversity in many ways, but also threaten it with anthropogenic activities. Threats to the diversity of plant species include land-use change and habitat degradation, fragmentation and habitat loss, alien invasive species, overexploitation, pollution and climate change. Modifications of landscape for development or agriculture result in habitat loss for many species (Kerr & Deguise, 2004). Plant diversity is highly threatened by habitat loss and fragmentation (Corlett, 2016). This is because many species are restricted to specialized habitats and loss of these areas would be detrimental to the survival of the plants. Fragmentation and loss of natural habitats due to human activities is detrimental to ecosystems and is the driving force behind biodiversity loss (Aguilar *et al.,* 2006). Fragmentation is defined as a process during which habitats are transformed into smaller patches that are isolated from each other by a matrix of habitats that are not similar to the original (Fahrig, 2003; Wilcove *et al.,* 1986). For this reason, fragmentation has been associated with loss of habitat because it results in a reduction of habitat amount (Fahrig, 2003). Additionally, fragmentation divides species populations into isolated small populations, making them susceptible to demographic effects such as loss of genetic variation as a result of inbreeding (Purvis *et al.,* 2000; Shao *et al.,* 2008). These small and isolated subpopulations will be at a greater risk of extinction (Bradke *et al.,* 2018). Habitat loss affects many aspects of biodiversity, including species richness, distribution and population size (Fischer & Lindenmayer, 2007; Sala *et al.,* 2000). Habitat degradation on the other hand, is the gradual deterioration of habitat quality. In degraded habitats, species may decline, be unable to reproduce or occur in lower densities (Fischer & Lindenmayer, 2007).

Invasive plant species threaten native plant diversity as well as ecosystems and their integrity (van Wilgen *et al.,* 2012). These plant species alter ecosystem structure and processes and impact how these ecosystems function and their provision of services (Mooney, 2005; van Wilgen *et al.,* 2012). Invasive alien plants have massive local impacts on habitat and water availability, as well as nutrient cycling because they use excessive amounts of water and nutrients resulting in landscapes being unable to support as many species as previously possible. This results in the erosion of natural capital and ecosystem instability (Richardson & van Wilgen, 2004). Alien plants are an increasing threat to South African natural ecosystems, and according to Nel *et al.* (2004), about 10 million hectares of land have been invaded. Due to all these threats resulting from human activities, ecosystems are exposed to species extinctions at a higher rate compared to previous times (Brose *et al.,* 2017; Ellison *et al.,* 2005; Ridley *et al.,* 2020). Species extinction will ultimately lead to decreased biodiversity of these ecosystems (Pereira *et al.,* 2010).

Studies by Chapin *et al.* (1998) as well as Brummitt *et al.* (2008) suggest that the earth is currently going through the sixth mass extinction event, and this event is mainly as a result of human impact on the planet. Extinctions are predicted to have an effect on the composition

and functional characteristics of ecosystems thereby altering ecosystem processes as well as affecting their resilience (Chapin *et al.,* 2000; Gallagher *et al.,* 2013). This will result in ecosystems being vulnerable to invasions and will have profound impacts on the provision of ecosystem services (Chapin *et al.,* 2000). The first global analysis of extinction risk found that almost 20 000 species were threatened with extinction, and many of the species that were driven to extinction by human impact were rare and endemic to small or isolated habitats (Chapin *et al.,* 1998; Ibrahim *et al.,* 2013). Currently, many species are considered as endangered and are at high risk of going extinct (Gundu & Adia, 2014; Wang *et al.,* 2015). These species are classified as endangered according to the IUCN Red List because they have small geographical range sizes as well as low population density, which make them vulnerable to extinction (Gundu & Adia, 2014; Purvis *et al.,* 2000).

Even though we know the importance of plants and the threats they face globally, there are still many plants whose extinction risk and threat status have not been assessed. However, the precise assessment of the threat status of a taxon is important in preventing its extinction (Alam and Ali, 2010; Vischi *et al.,* 2004). Species threat assessments have been established as a tool to identify taxa that are in decline or faced with extinction (Tali *et al.,* 2014). These are evidence-based and not only use objective, quantifiable and consistent criteria to analyse the threat status of species, but also identify threats to species populations (Mounce *et al.,* 2018; Possingham *et al.,* 2002; Tali *et al.,* 2014;). Classifying species under the IUCN Categories is important for prioritizing conservation programmes (Barik *et al.,* 2018). For that reason, the results of species threat assessments are often linked to decision-making processes and inform conservation actions where needed (Mounce *et al.,* 2018; Rodrigues *et al.,* 2006).

Species threat assessments have been established as an important tool for biodiversity conservation (Collen *et al.,* 2016; Currey *et al.,* 2009; Jetz and Freckleton, 2015). Prioritizing species conservation based on their threat status would help to improve their population size and ensure their long-term survival (Barik *et al.,* 2018). In many cases conservation efforts are established as a result of biodiversity loss, so identifying threats to species and assessing their risk status forms a big part of conservation planning. Threat assessments are not the only method in prioritising conservation efforts, but form either a primary or secondary criterion that is used alongside other considerations such as species characteristics, logistics and cost of implementation. They reveal the severity of threats to species as well as the levels of impact, which in turn enable policy makers and conservation managers to make informed decisions regarding conservation actions (Bleher *et al.,* 2006). Using information obtained from species threat assessments, assessors can document conservation actions that have been undertaken and propose further actions that would improve the status of species (Hayward, 2011).

The IUCN Red List is a powerful tool for conservation planning and highlights species that are at greatest risk of extinction (Rodrigues *et al.,* 2006). The Red List contributes to research by providing information about the decline of a species, as well as current knowledge about their conservation status and threats (Brummitt *et al.,* 2008). The IUCN Red List Categories and

Criteria are a globally accepted set of criteria used to assess the threat status of species. They are comprehensive, quantitative and a widely used method of assessing extinction risk (Brummitt *et al.,* 2008; Rodrigues *et al.,* 2006). The IUCN Categories and Criteria were developed so that threat assessments would be performed in an objective manner, as well as to promote and improve consistency among users (IUCN, 2019, pg. 5). Using these categories and criteria, species are classified on a qualitative scale into one of seven major categories that reflect different risks of extinction (Mace *et al.,* 2008; Mounce *et al.,* 2018; Nicholson *et al.,* 2009; Pereira *et al.,* 2012).

In the past, the IUCN Red List has been criticized for its biased view of extinction risk and rigidity in its criteria and protocols (Brummitt *et al.,* 2015; Webb, 2008). This is because in previous years a lot of conservation effort was focused on the species that scientists expected to be threatened and which they thought required the most attention, while some less charismatic, but possibly more threatened, taxa were neglected (Brummitt *et al.,* 2015). Due to this situation, it was argued that the Red List overestimates the overall proportion of plants that are threatened (Webb, 2008). The IUCN Red List Criteria have since been reviewed, and the indices of extinction have been limited to criteria and processes that are standardized across different species (Miller *et al.,* 2007). This review and development of the Red List Criteria also made it easier for many people to perform species assessments, and not have this task fall solely on experts (Rodrigues *et al.,* 2006). However, these assessments still need to be conducted in consultation with experts and later submitted to the IUCN assessment committee for review before publication (Callmander *et al.,* 2005; Rodrigues *et al.,* 2006).

The IUCN Red List assessments provide key knowledge about the state of biodiversity and help us identify species and regions that need immediate conservation (Jetz and Freckleton, 2015). The assessment relies on field-based knowledge of population size, rate of population decline, population trend, habitat, range size, the area that is occupied by the taxon (Extent of Occurrence (AOO) and Area of Occupancy (AOO) as well as conservation actions for each species in order to assign threat status (Alam & Ali, 2010; Jetz & Freckleton, 2015; Rodrigues *et al.,* 2006; Tali *et al.,* 2014). There are nine categories into which taxa can be classified. The first two categories are Extinct (EX) and Extinct in the Wild (EW). A taxon is classified as Extinct if there is no reasonable doubt that the last individual has died, and if it is extinct in its natural habitat but still exists in cultivation, it is classified as Extinct in the Wild (IUCN, 2001; 2019). The next three categories are Critically Endangered (CR), Endangered (EN) and Vulnerable (VU). These categories are assigned to taxa depending on varying degrees of threat of extinction, and together they are known as the 'threatened' categories.

The Near Threatened category (NT) applies to taxa that do not yet qualify as threatened but may be close to qualifying, and those that do not meet the criteria to be classified as threatened but are likely to if ongoing conservation efforts stop (IUCN, 2001; 2019). The next category is Least Concern (LC) and is applied to widespread and abundant taxa which do not

qualify as threatened or near threatened. This category means the species are of less concern compared to others (IUCN, 2019). The last two categories, Data Deficient (DD) and Not Evaluated (NE), do not reflect the threat status of taxa. The DD category calls attention to taxa for which there is insufficient data for a thorough assessment, and NE applies to taxa that have not been evaluated (IUCN, 2001; 2019). In order for a species to be added to the IUCN Red List, it needs to be evaluated against quantitative thresholds of the following measures: (a) decline in population size, (b) Small geographic range and (c) quantitative analysis. For these evaluations, IUCN has a list of Criteria ranging from A to E, which are used to classify species. These criteria are based on population size, number of mature individuals, population reduction, continuing decline, fluctuations, fragmentation, EOO, AOO, generation, location and qualitative analysis (IUCN, 2019).

A lot of progress has been made with the IUCN Red List assessments for plants on both regional and national scales (Brummitt *et al.,* 2015). For example, South Africa is one of the first mega diverse countries to assess the threat status of all its plants, however, this has only been done with desktop studies for most species, which means that field assessments still need to be done. The aim of the IUCN Red List was to identify species that are at high risk of extinction and to keep the assessments updated. However, many species have not been assessed and for some there is inadequate information with which to make an assessment (Brummitt *et al.,* 2008). Furthermore, for those species which have been assessed, the assessments were done a long time ago and their threat status may no longer apply. With the current rate of human population growth and climate change which result in biodiversity loss, it is imperative that threat assessments and re-assessments be done to ensure protection of threatened species before they are lost to extinction.

2.1.1. Aims and objectives

The aim of this study is to re-assess the threat status of *Brachystelma gerrardii*, *Streptocarpus fasciatus* and *Senecio triodontiphyllus* to determine whether the currently assigned status still applies. The first objective is to determine the number of localities where the plant species occur using herbarium records, iNaturalist and personal observations. The second objective is to assess land-use and threats at those localities and predict whether the species are likely to persist.

2.2. Methods

2.2.1. Eco-geographic survey

Species occurrence data for S*. fasciatus, S. triodontiphyllus and B. gerrardii* across their entire range were obtained by accessing distributions reported in literature (Dyer, 1983; Edwards *et al.,* 1992; Lötter *et al.,* 2006a, 2006b; Raimondo *et al.,* 2009), the Global Biodiversity Information Facility (GBIF) (https://www.gbif.org/) and major herbaria in the country (National Herbarium (PRE), Natal University Bews Herbarium (NU), KwaZulu-Natal Herbarium (NH), Buffelskloof Nature Reserve Herbarium (BNRH), Mpumalanga Tourism and Parks Agency Herbarium (LYD)). Additional records were obtained from Mountainlands Nature Reserve in South Africa, from iNaturalist as well as from the BRAHMS Online Index (http://newposa.sanbi.org/sanbi/Explore). QGIS (ver. 3.14) and Google Earth (2020) were used to geo-reference herbarium records.

2.2.2. Field exploration

Extensive field explorations were carried out in the Mpumalanga province throughout the distribution of *S. fasciatus* and *S. triodontiphyllus*. Unfortunately, no fieldwork was carried out for *B. gerrardii* due to its scattered distribution and Covid restrictions in 2020. Herbarium records, the Global Biodiversity Information Facility (GBIF), as well as BRAHMS Online Index were used to collect data relating to *B. gerradii.* The other two plant species were surveyed in three areas each. *Streptocarpus fasciatus* was surveyed in two areas within the Croc River Mountain Conservancy (Moederlief farm and Stonehaven) and also in an area north of the N4 (locality 3) (Figure 1), whilst *Senecio triodontiphyllus* was surveyed at Mountainlands, Pullen NR and Bonsberg Game Estate (Figure 2). A site near Kaapmuiden where a *Senecio triodontiphyllus* plant was once observed was also investigated. The plant was observed in 1925 by H. Munro, but unfortunately, the locality record was vague and no *S. triodontiphyllus* plants were found in the area during this study. The geographical coordinates of these sites were recorded using a GPS and camera. During the field surveys we recorded the habit, habitat and number of mature individuals whenever we located a population. Mature individuals were counted as those which bear flowers or fruits (IUCN, 2010). Direct observations were made to determine the potential and actual threats to the population of selected plant species in a given area.



Figure 1: Google Earth view of the locations of Moederlief farm and Stonehaven located in the Croc River Mountain conservancy, and locality 3 which is north of the N4 (between Mpakeni and Matsulu) in Mpumalanga, South Africa.

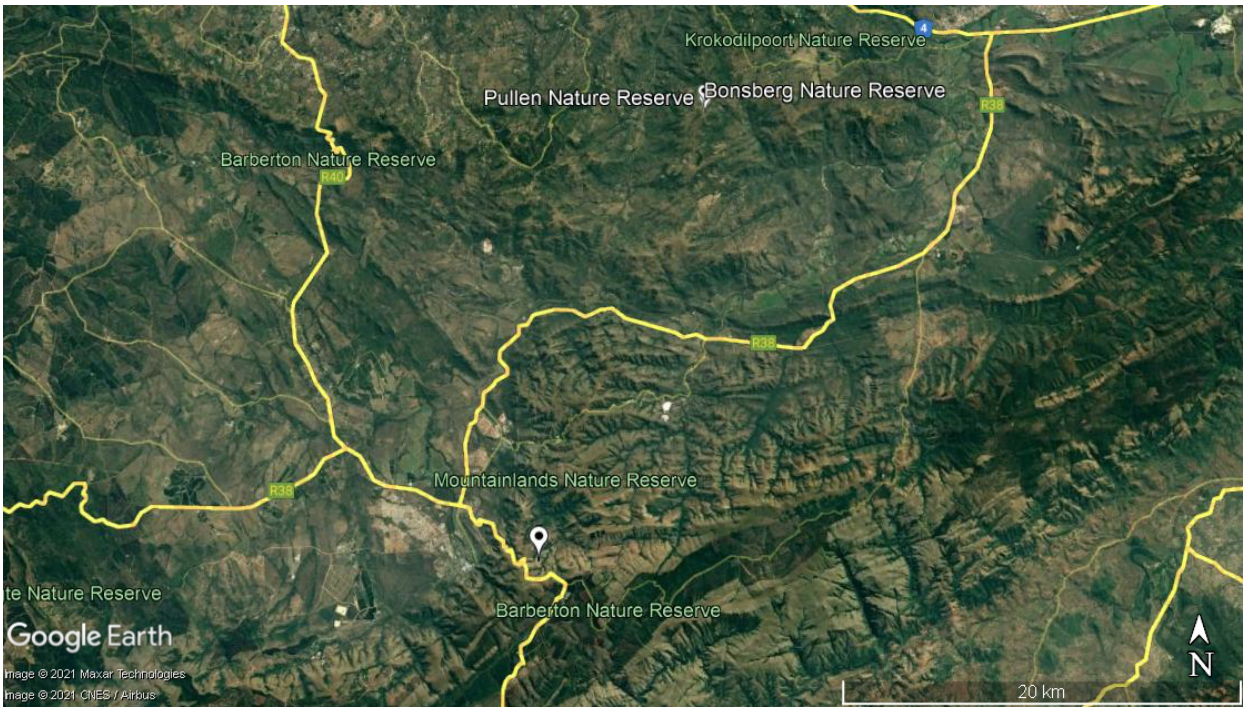


Figure 2: Google Earth view of relative positions of Pullen, Bonsberg and Mountainlands Nature Reserves in Mpumalanga, South Africa.

2.2.3. Data evaluation

The collected data were used to calculate the Area of Occupancy (AOO) and Extent of Occurrence (EOO). The Geospatial Conservation Assessment Tool (GeoCAT) was used to calculate EOO and AOO (Bachman *et al.,* 2011). This platform is consistent and performs automated EOO and AOO analyses (Bachman *et al.,* 2011). The plant species were evaluated using the IUCN Red List categories and Criteria 2010 version 8.1 and following Guidelines for application of IUCN Red List Criteria at Regional Levels 2003 version 3.0. Herbarium records for *Brachystelma gerrardii* show that this plant has been found in 11 localities which span from KwaZulu-Natal to Limpopo. In the records, 12 individual plants were found in Piet Retief (KZN) as indicated on the label of the herbarium specimen, three plants were observed in Vryheid (KZN), two plants were found in Luneburg (Mpumalanga), one plant was found in Wolkberg on the farm Mimosa 218 KS (Limpopo), another plant was found in Songimvelo Game Reserve on the farm Diepgezet (Mpumalanga), and finally one plant was found at Pullen Nature Reserve (Mpumalanga). This plant species is rare and occurs occasionally in known localities. Threats that have been previously observed include burning and plantations. Three of the plant localities recorded in KZN occur in nature reserves. However, in this present study *B. gerrardii* could not be evaluated due to incomplete data. Furthermore, Criterion A (declining population - past, present and/or projected), Criterion B (restricted geographic range size and

fragmentation, decline or fluctuations), and Criterion C (small population size and decline) could not be applied *to S. fasciatus* and *S. triodontiphyllus* because population decline and fluctuation data were not available. Criterion E (probability of extinction estimate) was also not applied because quantitative data on the extinction risk was not available. For these reasons, *S. fasciatus* and *S. triodontiphyllus* were evaluated under Criterion D (See Table 1).

Table 1: IUCN Criteria including conditions of applications and thresholds.

Criterion A: Designed to highlight taxa that have undergone major declines in the near past, or are likely to decline in the near future.			
Subcriterion A1	**Subcriterion A2**	**Subcriterion A3**	**Subcriterion A4**
Is used for a reduction [observed, estimated, inferred or suspected] that took place over the past 10 years or 3 generations, where the causes of the reduction are understood AND have now ceased AND reduction is reversible	Is used for a reduction [observed, estimated, inferred or suspected] that took place in the past 10 years or 3 generations, where the causes of the reduction are ongoing, OR are not understood, OR the reduction is not reversible	Is used for a future reduction [projected or suspected] that is expected to occur over the next 10 years or over the next 3 generations (up to a max of 100 years)	Is used for a reduction [observed, estimated, inferred or suspected] over a period including some time in the past and time in the future (up to 100 years)
Population reduction thresholds			
CR >/= 90%	CR >/= 80%		
EN >/= 70%	EN >/= 50%		
VU >/= 50%	VU >/= 30%		
Criterion B: Designed to identify populations with restricted distributions that are also severely fragmented, undergoing a form of continuing decline, and /or exhibiting extreme fluctuations.			
Criterion B1 - Estimated extent of occurrence (EOO) thresholds		**Criterion B2 - Estimated area of occupancy (AOO) thresholds**	
CR <100 km^2		CR <10 km^2	
EN <5,000 km^2		EN <500 km^2	
VU < 20,000 km^2		VU <2,000 km^2	
Subcriterion a	**Subcriterion b**	**Subcriterion c**	
To qualify for subcriterion (a), the taxon must be severy fragmented or is known to exist in only a few locations	To qualify for subcriterion (b), there must be an observed, inferred or projected continuing decline in any of the following:	To qualify for subcriterion (c), there must be evidence of extreme fluctuations in any of the following:	
Severely fragmented, OR number of locations	(i) Extent of occurrence	(i) Extent of occurrence	
CR =1	(ii) Area of accupancy	(ii) Area of occupancy	
EN </= 5	(iii) Area, extent and/or quality of habitat	(iii) Number of locations or subpopulations	
VU </= 10	(iv) Number of locations or subpopulations	(iv) Number of mature individuals	
	(v) Number of mature individuals		
Criterion C: evaluates taxa with small population sizes that are currently declining or may decline in the near future.			
To apply Criterion C, the taxon's population size must be less than 10 000 mature individuals before any of the threatened categories can be considered under this criterion [population size must be estimated]			
Population size thresholds: Number of mature individuals			
CR <250			
EN <2,500			
VU <10,000			
Subcriterion C1		**Subcriterion C2**	
For subcriterion C1, there must be continuing decline in the number of mature individuals and there must be some data available for the Assessor to be able to estimate the rate of this decline		For subcriterion C2 an observed, estimated, projected or inferred continuing decline in population size is required	

Table 1 (Cont.): IUCN Criteria including conditions of applications and thresholds.

Subcriterion C1	**Subcriterion C2**	
For subcriterion C1, there must be continuing decline in the number of mature individuals and there must be some data available for the Assessor to be able to estimate the rate of this decline	For subcriterion C2 an observed, estimated, projected or inferred continuing decline in population size is required	
C1 Thresholds	**C2a Thresholds**	**C2b**
CR - 25% in 3 years or 1 generation	*(i) Number of mature individuals in each subpopulation*	Focuses on taxa that have a higher extinction risk because they have a small population size with continuing decline and there are extreme fluctuations in the number of mature individuals
EN - 20% in 5 years or 2 generations	CR ≤ 50	
VU - 10% in 10 years or 3 generations	EN ≤250	
	VU ≤ 1,000	
	(ii) % of mature individuals in one subpopulation	
	CR : 90 - 100%	
	EN : 95 - 100%	
	VU : 100%	

Criterion D: Evaluates taxa with very small or restricted populations	
D1 - Number of mature individuals (Very small population size)	**D2 - Very restricted population and plausible threat** (VU only)
CR <50	VU (D2) typically: AOO < 20km^2 or number of locations ≤ 5
EN <250	
VU (D1) <1,000	

Criterion E: Uses a quantitative analysis to determine the probability of a taxon becoming extinct in the wild within a specific time period		
Thresholds		
CR	**EN**	**VU**
A quantitative analysis must show that there is at least 50% probability of the taxon becoming extinct in the wild within the next 10 years or 3 generations	A quantitative analysis must show that there is at least a 20% probability of the taxon becoming extinct in the wild within 20 years or 5 generations	The taxon must have at least a 10% probability of becoming extinct in the wild within 100 years

2.3. Results

2.3.1. Distribution

A total of 22 records of *Brachystelma gerrardii*, 22 records of *Senecio triodontiphyllus* and 17 records of *Streptocarpus fasciatus* were collected and used to create a preliminary distribution map (Figure 3).

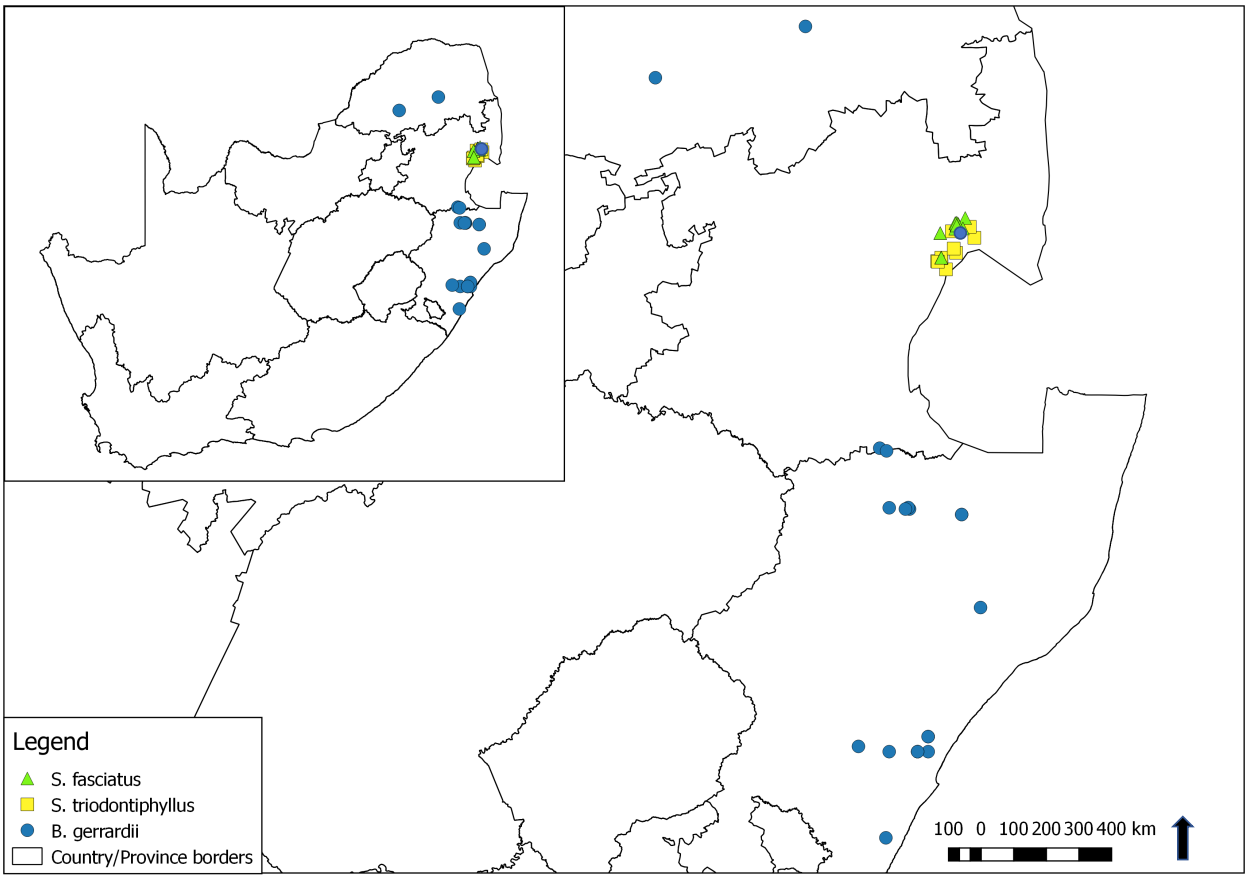


Figure 3: Map showing the preliminary distribution of *Brachystelma gerrardii, Streptocarpus fasciatus* and *Senecio triodontiphyllus* in Limpopo, Mpumalanga and KwaZulu-Natal provinces in South Africa.

2.3.2. Field exploration

A total of 11 sub-populations were recorded for *B. gerrardii*, and the calculated EOO and AOO of the species were 95 456.43 km^2 and 64 km^2 respectively. Threats associated with *B. gerrardii* are habitat destruction as a result of urban and rural and development, overgrazing and poor fire regimes (Styles and von Staden, 2007). During the present study, sub-populations of *S. fasciatus* and *S. triodontiphyllus* were surveyed in all previously recorded localities. The localities, altitudinal range and numbers of plants counted are recorded in Table 2. *Streptocarpus fasciatus* was found growing in damp areas under the shade where habitats are cool, and the plants are not exposed to direct sunlight. In Moederlief farm, *Streptocarpus* plants were primarily found on the mountain, growing in cracks in rocks and at the base of aloe plants. These areas were always damp and sometimes there was water seeping from under the rocks. The plants were always shaded from the sun by either trees, shrubs, or rock overhangs.

In Stonehaven the plants were found growing in areas along a river that passes through this property. These areas had similar characteristics to those found in Moederlief farm, however, there were large areas covered with invasive plant species. These invasive species were *Psidium guajava* L. (Myrtaceae), *Lantana camara* L. (Verbenaceae), and they covered large parts of the river that are suitable for *S. fasciatus* growth. In the locality north of the N4, the plants were found under rock overhangs, in lees of granite boulders and at the top of cliffs. This subpopulation is threatened by unofficial and illegal expansion of settlements in the Mpakeni village. In all localities, *Senecio triodontiphyllus* was found growing in grassland areas among other trees and shrubs. These plants were found growing in open areas in direct sunlight on rocky, gentle slopes.

Table 2: Names of localities, altitudinal range and number of plants surveyed of the selected plant species.

Species	Localities	Altitudinal range (m)	Number of plants
Streptocarpus fasciatus	Moederlief farm	930 – 1099,5	310
	Stonehaven	718,5 – 843,7	475
	North of the N4	425,8 – 531,3	81
Total number of plants			866
Senecio triodontiphyllus	Bonsberg Game Estate	978 – 1007,6	198
	Pullen Nature Reserve		
	Mountainlands Nature Reserve	984,1 – 1191,2	70
Total number of plants			268

Six and five sub-populations were recorded for *S. triodontiphyllus* and *S. fasciatus* respectively The total number of mature individuals for *S. fasciatus* was 497 and 172 for *S. triodontiphyllus,* and the calculated EOO and AOO of the species are recorded in Table 3.

Table 3: EOO and AOO of *Brachystelma gerrardii, Streptocarpus fasciatus* and *Senecio triodontiphyllus* in km^2 calculated using GeoCAT.

	B. gerrardii	*S. fasciatus*	*S. triodontiphyllus*
EOO	95546,43 km^2	3040,13 km^2	2332,27 km^2
AOO	64 km^2	64 km^2	48 km^2

2.3.3. IUCN threat assessment

Criteria A and E were not applied to the plant species because decline data (Criterion A) and quantitative data on extinction risk (Criterion E) were not available. To evaluate a taxon under criterion B, it must meet the thresholds for B1 and/or B2, and secondly it must meet the conditions for at least two of subcriteria a, b and c (Table 1, IUCN, 2019). *Streptocarpus fasciatus* and *S. triodontiphyllus* did not meet the last condition (observed population decline) and therefore do not qualify as threatened under criterion B (Table 4). The two plant species also don't qualify as threatened under criterion C since they don't meet conditions of subcriteria C1 or C2 (population decline data not available) (Table 1, 4), but under subcriterion C2a *S. triodontiphyllus* may be classified as EN since it has less than 250 mature individuals. *Streptocarpus fasciatus* on the other hand may be classified as VU under subcriterion C2a for having less than 1000 mature individuals. Both *S. fasciatus* and *S. triodontiphyllus* qualified for and were evaluated under criterion D. *Streptocarpus fasciatus* was found to be VU under subcriteria D1 and D2 because of its very small population size with less than 1000 mature individuals, as well as having a restricted number of subpopulations with less than 5 known localities. *Senecio triodontiphyllus* was found to be EN under D1 and VU under D2 because of its small population size (<250 mature individuals) and very restricted geographic range (<5 localities) (Table 4).

Table 4: Threat categories assigned to *Brachystelma gerrardii, Streptocarpus fascatus* and *Senecio triodontiphyllus* in each IUCN Criterion based on data collected.

			Criterion B		
Species Name	Criterion B1	Criterion B2	Subcriterion a	Subcriterion b	Subcriterion c
Brachystelma gerrardii	EN	EN	-	-	-
Streptocarpus fasciatus	EN	EN	EN	-	-
Senecio triodontiphyllus	EN	EN	EN	-	-
			Criterion C		
	Mature Individuals			Subcriterion C1	Subcriterion C2
Brachystelma gerrardii	-			-	-
Streptocarpus fasciatus	EN			-	-
Senecio triodontiphyllus	EN			-	-
			Criterion D		
	Subcriterion D1			Subcriterion D2	
Brachystelma gerrardii	-			-	
Streptocarpus fasciatus	VU			VU	
Senecio triodontiphyllus	EN			VU	

2.4. Discussion

Results from this study indicate that there are 11 subpopulations of *B. gerrardii*, five subpopulations of *S. fasciatus* and six subpopulations of *S. triodontiphyllus*. *Streptocarpus fasciatus* and *S. triodontiphyllus* were found in three localities each (Table 2), and this number of localities is below the threshold of acceptable number of locations according to IUCN standards (Table 1). For example, a taxon that occurs in less than five localities meets the conditions for an Endangered (EN) classification under subcriterion B1 (IUCN, 2019). This study also found that the number of mature individuals in each population falls below threshold. It was found that *S. fasciatus* and *S. triodontiphyllus* have restricted distributions and small population sizes as evidenced by the small EOO (Table 3). Small population sizes are a threat to plant populations because small populations tend to lose genetic diversity faster than large populations. This is because species with small population sizes suffer from phenomena such as

inbreeding and genetic drift (Ellstrand & Elam, 1993). Inbreeding and genetic drift cause a reduction in species genetic diversity as well as reduced species fitness. Genetic diversity underpins population resilience and has been identified as an important factor that influences the long-term survival of species as well as adaptation (Bouzat, 2010; Frankham, 2005; Furlan *et al.,* 2012). Thus, small and isolated populations will not only have greater sensitivity to demographic stochasticity but will also be susceptible to increased rates of extinction (Bradke *et al.,* 2018; Markert *et al.,* 2010; Mills, 2012).

The selected plant species are subject to different natural and anthropogenic threats in the region. The habitats of these species (especially *S. fasciatus* and *S. triodontiphyllus*) fall within very specific areas of the Mpumalanga province which have certain geomorphological characteristics and thereby restrict their distribution. The *S. fasciatus* subpopulation in Stonehaven is mostly affected by alien invasive plants such as *Lantana camara* and *Psidium guajava.* These alien plants were found growing in and taking over suitable habitat that would be occupied by *S. fasciatus*. This is a serious threat to the plant subpopulation because invasive plants alter ecosystem structure and have a huge impact on ecological processes such as light, water and nutrient availability (Mooney, 2005; van Wilgen *et al.,* 2012). Both *S. fasciatus* and *S. triodontiphyllus* are affected by climate change and its resulting impacts, such as increasing temperature and variable rainfall which alter plant flowering times (Chapter 3). Area of Occupancy is inversely related to extinction risk, and according to the results (Table 3) all the species have AOOs that are below threshold (IUCN, 2001). This is an indication that the plant species are exposed to high risk because there is a significant chance that one of the threats will affect most of the distribution within a given time frame (IUCN, 2019).

Species threat assessments could not be undertaken for *B. gerrardii* because data were not available, but *S. fasciatus* was found to be vulnerable (VU) under Criterion D (sub-criteria D1 and D2), while *S. triodontiphyllus* was found to be endangered (EN) under Criterion D, sub-criterion D1 (<250 mature individuals) and vulnerable (VU) under sub-criterion D2 (<5 localities) (Table 1 & 4). In accordance with the IUCN Red List Categories and Criteria, the highest threat must be taken when assessing species, therefore, *S. triodontiphyllus* is classified as endangered (IUCN, 2001; 2019). The threat status assigned to these species were mainly based on their restricted geographic ranges and population sizes which are an indication of high extinction risk (IUCN, 2019). The assigned status of *S. fasciatus* is similar to the previous one assigned by Lötter *et al.* (2006b), however, the threat status of *S. triodontiphyllus* in this study was found to be in a higher category (EN) compared to the previous assessment (VU) (Lötter *et al.,* 2006a). Only one of the *S. triodontiphyllus* localities is found in an area which is a formally proclaimed nature reserve (Mountainlands Nature Reserve, Table 2). For *S. fasciatus,* none of the localities are found in areas that are formally proclaimed nature reserves. However, two of the areas; Pullen Nature Reserve and Bonsberg Game Estate, are located within the Croc River Mountain Conservancy.

The conservation of a species greatly relies on their assessment and allocation of an appropriate threat category. The results show that effective conservation of protected areas is needed for the survival of these species in their natural habitats. The following are recommended: (a) immediate removal of all invasive plants encroaching on species habitats; (b) annual population counts to determine trends over time; and (c) seed collection for seed banking.

2.5. Conclusion

The re-assessment of the threat status of three endemic plants was carried out following the IUCN guidelines. This study presents the threat status of these species and the factors that threaten them. Conservation recommendations involve the immediate removal of invasive plants in threatened species habitats and the development of appropriate conservation strategies, as well as strong measures to reduce the general impact of current threats.

Chapter 3

Effects of climate change on future distributions of threatened plant species

Abstract

Climate change is a major threat to natural ecosystems and biodiversity on a global scale. It is of particular concern to threatened and endemic plant species that have small population sizes and specific habitat requirements. It not only affects global temperature, but also impacts rainfall patterns as well as water availability. Most species can only grow and reproduce successfully within specific climate ranges, therefore a shift in climatic conditions due to increased carbon dioxide concentrations in the atmosphere will significantly affect these species and put them at a high risk of extinction. Studies indicate that the geographic domains of certain species would shrink under climate change warming projections, and species would disappear from certain sites that were previously suitable. For this reason, our capacity to predict the potential distributions of taxa under anticipated future climate conditions is very important for their conservation. In this present study, the likely impact of climate change on selected threatened plants was determined using distribution data and predictions of climate change. Based on Maxent models for climate suitability using current climatic data (1970 – 2000), current suitable areas for *Brachystelma gerrardii* Harv. And *Senecio triodontiphyllus* C. Jeffrey are predicted in the eastern parts of South Africa from East London through KwaZulu-Natal and Mpumalanga, towards the northern parts of Limpopo. However, future predictions using A1B and A2 emission scenarios show a decrease in suitable habitat for these species. Current suitable areas for *Streptocarpus fasciatus* T. J. Edwards & Kunhardt on the other hand were predicted as patches along the coast in Western Cape, along the border of Lesotho in KwaZulu-Natal, in areas of Gauteng and eastern Mpumalanga. These suitable habitats were predicted to significantly increase with increasing climate change, but this may have been an overestimation by MaxEnt since the distribution of this species relates to the Mpakeni (Mpageni) pluton which forms part of the Nelspruit suite. These geological formations have resulted in a unique and highly specific habitat for *S. fasciatus,* therefore, likelihood that this species would migrate to new disjunct areas is low.

3.1. Introduction

Climate change is a global threat that has major negative impacts on natural ecosystems and biodiversity (Sintayehu, 2018). For this reason, we need to have an understanding of the connection between climate change, biodiversity, and ecosystem services. The effect of climate change on threatened and endemic plant species is of particular concern because these species have small population sizes and specific habitat requirements that may reduce their adaptive capabilities in response to climate change (Jones *et al.,* 2013). Currently, knowledge and data regarding the effects of climate change on dispersal and life history characteristics of certain

threatened plants is still lacking. This study provides important knowledge on the impacts of climate change on environmental conditions and will lead to a deeper understanding of the distribution patterns of threatened species as well as how these distribution ranges are affected by changing climatic conditions (Hu *et al*., 2015).

Climate change and effects on temperature and rainfall

Climate change is a phenomenon that is caused by higher atmospheric levels of greenhouse gases, and results in changes in global or regional climate patterns (Dawson *et al.,* 2011; Scholes *et al.,* 2015; Sintayehu, 2018). Greenhouse gas emissions are intensified by human activities such as the production and use of energy, urbanization and land-use change (Karl & Trenberth, 2003; Matata & Adan, 2018). In 2014, human activities (E.g. burning of fossil fuels) released 36 billion tonnes of carbon dioxide into the atmosphere, and the rate continues to increase per year, increasing the accumulation of greenhouse gases in the atmosphere and thus regional concentrations (Hulme *et al.,* 2001; Scholes *et al.,* 2015). The anthropogenic changes in concentration and composition of gases in the atmosphere are the main source of climate change (Hulme *et al.,* 2001; Karl & Trenberth, 2003). Climate change in recent times is mainly due to human activities which exceed natural variability (Karl & Trenberth, 2003).

The earth has had a natural cycle of warming and cooling in the past because of changes in climate, ocean circulation and major geological events (Scholes *et al.,* 2015). However, due to human activities, the amount of CO_2 in the atmosphere is rising at an exponential rate and causing a rise in temperature. Southern Africa has been warming at an exponential rate over the last few decades and records show that minimum temperatures are increasing more quickly than maximum temperatures thus resulting in a trend of higher temperatures overall (Kusangaya *et al.,* 2014). An increase in temperature has a cascading effect on the planet's systems. Increasing global temperature is likely to lead to changes in patterns of rainfall, as well as how and where runoff occurs, soil moisture patterns and amounts of groundwater because of changes in atmospheric circulation and the water-holding capacity throughout the atmosphere (Scholes *et al.,* 2015; Suárez *et al.,* 2002; Trenberth, 2011).

The water cycle is an amplifier of climate change as it is sensitive to changes in both precipitation and evaporation (Scholes *et al*., 2015). Changes in precipitation mean variability in rainfall which will cause a decrease in flow of rivers, thus resulting in additional pressures on water availability (Jarvis *et al*., 2008). Rainfall is variable in southern Africa and shows no significant trend, however, it is suggested that rainfall will decrease due to climate change (Kusangaya *et al.,* 2014). Southern Africa is most impacted by climate change due to variability in rainfall, scarce water resources and by the region's low adaptive capacity (Kusangaya *et al.,* 2014). Climate change, through an increase in temperature and impacts on water, will have an

effect on the biophysical environment such as ecosystems and biodiversity (Kusangaya *et al.,* 2014; Suárez *et al.,* 2002; Thomas *et al*., 2004).

Effects on species and ecosystems

Climate directly affects the rate and efficiency of photosynthesis, respiration, plant growth, productivity and other ecosystem processes (Hughes, 2000; Suárez *et al.,* 2002). Consequently, climate is regarded as one of the major factors controlling vegetation structure, species composition as well as species distributions in landscapes. Most species can only grow and reproduce successfully within specific climatic ranges, therefore, shifts in climatic conditions brought about by changes in concentrations of carbon dioxide in the atmosphere determine the impacts of climate change on ecosystems (Suárez *et al.,* 2002). Thus, climate warming in addition to other factors such as land-use change, represent a major challenge for species (Maggini *et al.,* 2014).

Earth has gone through different types of environmental change over its history (Pecl *et al.,* 2017). These environmental changes are associated with the potential for species to shift their distribution in response to either tectonic, oceanographic or climatic events (Hughes, 2000; Kupika *et al.,* 2017; Pecl *et al.,* 2017). Species may respond to changing climate by expanding or contracting their distribution in order to remain within environmental conditions they most prefer (Gomez-Ruiz & Lacher, 2019; Pecl *et al.,* 2017). The geographic distributions of species depend on whether they are able to: (a) tolerate their environment; (b) disperse without any constraints; and (c) their biological interactions with other species in the same habitats. Therefore as climate changes, species must either tolerate the changes in their environment, shift their distribution to more suitable environments, adapt to the new climatic conditions or face extinction (Pecl *et al.,* 2017).

The rate at which climate changes can potentially alter the distributions of bioclimatic envelopes for the world's flora will have significant impacts on species distributions and especially on species with narrow habitat tolerances (Abolmaali *et al.,* 2018; Vitt *et al.,* 2010). Human activities have changed the distribution and reduced the abundance of most species. Previously this was mainly due to habitat loss or alteration, but recently studies have shown that shifts in distribution may be related to climate trends (Hughes, 2000). Climate change will increase extinction risks and affect biotic interactions, thus failure of species to adapt to their changing environment will lead to their permanent loss (Blaustein *et al.,* 2010; Gomez-Ruiz & Lacher, 2019).

The distribution of the Earth's species is changing at an accelerating rate because of human induced climate change. The climate-driven redistributions alter the composition of ecological

communities and therefore affect how ecosystems function, and the dynamics of climate change itself (Gomez-Ruiz & Lacher, 2019; Hu *et al.,* 2015; Pecl *et al.,* 2017). Species range shifts in turn affect climate change, health of ecosystems and well-being of humans (Pecl *et al.,* 2017). However, species are capable of responding to pressures at different rates which may disrupt their interactions or lead to the development of new ones. The change in species interactions can result in rapid changes to the functioning of ecosystems and may also have unexpected consequences that impact biological and human communities (Pecl *et al.,* 2017).

At the primary level of biodiversity, climate change may result in directional selection and rapid migration of species populations, which will ultimately decrease their genetic diversity (Botkin *et al.,* 2007). Studies show that species diversity influences ecosystem functioning, and thus changes in diversity may affect the stability and condition of ecosystems (Sintayehu, 2018). Changes in physiology, phenology and species distribution will affect competition and other interactions between species, which will result in changes in local abundance and geographical ranges. These processes lead to change in the phenological profile of an ecosystem as species adapt to the changing climate conditions (Workie & Dabella, 2018).

Climate projections

Recent biodiversity loss has been driven by degradation and fragmentation of habitats, overexploitation of natural resources, and invasive species. However, climate change is currently projected as a major driver of species loss in the 21st century, both directly and in conjunction with other stressors (Beaumont *et al.,* 2011; Trull *et al.,* 2018). Average annual global temperatures have risen by 0.7 ˚C between 1900 and 2000 and the Intergovernmental Panel on Climate Change (IPCC) predicts that temperatures will continue to rise at an exponential rate (Blaustein *et al.,* 2010; Sintayehu, 2018). Temperature throughout the world is anticipated to increase by 1.5–6 ˚C by 2050, while average precipitation is projected to increase by 15–20% (Jarvis *et al.,* 2008). However, changes in rainfall wil be very uneven with some areas receiving substantially less rainfall than they currently do (Jarvis *et al.,* 2008). Various researchers point out that Africa is the most vulnerable continent in the world because temperatures are rising at a far higher rate in this area than anywhere else in the world (Collier *et al.,* 2008; Lawal *et al.,* 2019; Meadows, 2006).

Precipitation patterns are more variable in Africa than anywhere else, and climate change in this area is linked to extreme weather events such as El Nino and la Nina events and El Nino southern oscillation (Collier *et al.,* 2008). Studies show that southern Africa is a potential hotspot of change, with significant increases in temperatures and drying, while highlands of East Africa are projected to have an increase in rainfall (Jarvis *et al.,* 2008). Projections show an increase in rainfall further from the equator in both hemispheres, however, in the mid-southern

latitudes – specifically southern Africa, projections predict a decrease in total annual precipitation (Blaustein *et al.,* 2010). The rate at which the climate changes affects the persistence of species in intact landscapes, so when climate change is relatively slow, species can occupy certain patches for longer during the period of change (Travis, 2003). Karl and Trenberth (2003) argue that the projected rate and magnitude of climate change will most likely be too much for many plants to survive and adapt to new environments and conditions since human-induced change is faster than change induced by natural processes. Plant species are thus more likely to go extinct than adapt to this new normal.

Shifting distributions

The vegetation of southern Africa has been found to be vulnerable to increasing temperatures due to climate change, however, the severity of the impacts under different warming levels is yet to be investigated (Lawal *et al.,* 2019). Climate change presents a significant challenge for plant species with narrow habitat tolerances, as it affects their persistence and survival (Abolmaali *et al.,* 2018). Malcom *et al.* (2002) suggest that the current global warming will result in higher mean global temperatures compared to the past several million years, and as a result organisms are expected to shift their geographical distributions.

Studies indicate that the geographic domains of certain species would shrink under climate change warming projections, and species would disappear in certain sites that were previously suitable because the climate niche becomes inappropriate for regeneration (Abolmaali *et al.,* 2018). Evidence suggests that climate change may reduce biodiversity by promoting the selection of mobile and opportunistic species which have faster migration rates than those seen during post glacial times (Malcom *et al.,* 2020). Species with large suitable habitats may benefit from climate conditions in the future while those with small distributional ranges are more threatened because because the species with narrow distributional ranges are likely to have unique, less plastic phenological or physiological characteristics (Abolmaali *et al.,* 2018; Khanum *et al.,* 2013; Westoby & Burgman, 2006). Understanding how species shift their geographical distribution and factors that affect habitat suitability are important aspects in the assessment of climate change and its potential effects on ecosystems (Martinez-Meyer, 2005).

Study approach

Conservation of plant species heavily relies on our capacity to predict how climate change will affect patterns of species distribution. Essentially this provides a greater understanding of the stability of ecological systems as well as how genetic variation is distributed within species (Hu *et al.,* 2015). Species distribution modelling (SDM) is important for ecological, biogeographical, and evolutionary studies to predict the loss of biodiversity based on future climate scenarios

(Hu *et al.,* 2015; Elith *et al.,* 2006; Peterson, 2006). SDMs analyse the relationship between environmental variables and species occurrence and use that data to predict species distributions across the landscape (Abolmaali *et al.* 2018; Guisan *et al.,* 2002).

SDMs have become an important tool for determining the potential effect of climate change on species distributions (Remya *et al.,* 2015). "Bioclimatic models define the current distribution of a species as a function of current climate and then project future potential ranges based on projected future climate data" (Blaustein *et al.,* 2010, pp. 285). Therefore, assessments of the impacts of climate change on biodiversity measure changes in the size and position of species bioclimatic envelopes since these envelopes represent their potential distributions (Araujo *et al.,* 2011).

Various modelling programs (E.g. Maxent, GARP and BIOCLIM) have been developed to help scientists estimate the extent to which climate change will affect species distributions. However, the criteria they use, and input parameters are different (Garcia *et al.,* 2013; Remya *et al.,* 2015). SDMs are established on the notion that we can determine how a pattern is correlated with factors that are assumed to control it (Anderson *et al.,* 2003). Most species distribution models use both presence and absence data of a species for modelling, but Maxent is able to operate with just presence data, such as those of species' distribution records (Hu *et al.,* 2015; Phillips *et al.,* 2006; Yang *et al.,* 2013). The Maxent model is based on statistical mechanics and uses environmental data to predict the potential distribution of species (Jaynes, 1957; Phillips *et al.,* 2004; Yang *et al.,* 2013).

This model has successfully predicted the distributions of many floral and faunal species (Baldwin, 2009; Hu *et al.,* 2015; Khanum *et al.,* 2013; Trisurat *et al.,* 2011). Furthermore, Maxent has been found to be ideal for predicting distributions of rare species since it possesses a good predictive ability even with small population sizes (Garcia *et al.* 2013; Hernandez *et al.,* 2006). Many scientists prefer working with this model since it can run with only presence data, and because absence data is rarely available and difficult to collect (Elith *et al.,* 2011; Phillips *et al.,* 2006). Occurrence data and predictor variables (climate, soil, biogeography and topography) are needed to predict the location of suitable habitats for a species (Hu *et al.,* 2015; Phillips *et al.,* 2006; Remya *et al.,* 2015; Yang *et al.,* 2013).

Using Maxent for species distribution modelling has made a remarkable contribution to the prediction of biodiversity loss as a result of future climate change, as well as to the reasonable use of environmental resources (Hu *et al.,* 2015). For conservation efforts to be successful, we need to understand the potential impacts of climate change under different climate scenarios (Remya *et al.,* 2015). In this study we use current distributions of *Brachystelma gerrardii, Senecio triodontiphyllus* and *Streptocarpus fasciatus,* and selected scenarios of climate change to predict whether their potential habitat will grow or shrink under anticipated future climate scenarios.

3.1.1. Aim and objectives

This chapter aims to determine the impact of climate change on the selected plant species using distribution data and predictions of climate change. The first objective is to use current climate data to investigate current suitable habitats for the species and determine whether the known plant distributions fall within the predicted suitable habitats. The second objective is to determine whether suitable habitats will grow or shrink under projected climate change.

3.2. Methods

3.2.1. Occurrence data collection

Preliminary distribution maps of the species were drawn using the data collected for Chapter 2, and these were used to determine sample sites.

3.2.2. Climate data

Climate data were downloaded from WorldClim database (http://www.worldclim.org.org/). The data are obtained from measurements of altitude, temperature and rainfall from various weather stations. Current climatic data were for the period 1970 – 2000 and future climatic data were for the period 2021 – 2040. Seventeen bioclimatic variables with 30 seconds (ca. 1 km) spatial resolution (Table 5) from the WorldClim dataset were used to assess current climatic conditions. The WorldClim data, unlike other datasets are freely available at a high spatial resolution (30 arc-second in the WGS84 reference system and approximately 1 km at the equator) and allow the environmental variability that can be lost at lower resolutions to be captured (Hijmans *et al.,* 2005; Marchi *et al.,* 2019). This dataset has a global coverage and availability and can be applied to ecological studies (Marchi *et al.,* 2019). The WorldClim bioclimatic variables are used to model the distributions of species (E.g. Khanum *et al.,* 2013; Sanchez *et al.,* 2011) and capture annual ranges of temperature and rainfall, seasonality, as well as monthly and quarterly temperature and precipitation extremes (Hijmans *et al.,* 2005).

Future climate data for 2050 (A1B and A2 emission scenarios) were obtained from Climond (https://www.climond.org/Default.aspx). These projections are based on IPCC 4th assessment report modelling (AR4). The data are based on two Global Climate Models (GCMs), CSIRO Mk3.0 and the Miroc-H models in order to create estimates of future climate. The A1B scenario shows an emphasis on a balance across all sources of energy (for example, not relying too heavily on one energy source), whereas the A2 emission scenario describes a world characterized by an ever increasing population, high emissions and regional economic development among other things (Nakicenovic *et al.,* 2000).

3.2.3. Predictive modelling

The Maxent model (Maxent version 3.4.0; Phillips *et al.,* 2006) was used because it functions better with smaller samples relative to other models (Elith *et al.,* 2006; Phillips *et al.,* 2006; Khanum *et al.,* 2013). Maxent uses presence-only data and the theory of maximum entropy to predict species distributions (Phillips *et al.,* 2006). The aim of this model is to estimate a probability distribution of species occurrence that is near constant even when affected by environmental constraints (Elith *et al.,* 2011). Maxent estimates habitat suitability for a species on a scale from 0 (lowest suitability) to 1 (highest suitability).

The bioclimatic variables for the study areas were extracted and converted to ASCII files using ArcGIS (ArcMaps 10.5.1). One replicate was run for each species. The jackknife procedure, as well as percentage contributions were used to evaluate the influence of various bioclimatic variables. The Area under the receiver operating characteristic curve (AUC) was used to assess the performance of the model (Fielding & Bell, 1997). The AUC values range from 0 to 1, where a value of <1 shows that the model did not perform better than random whereas a value of 1.0 indicates perfect discrimination (Swets, 1988). The current 19 bioclimatic variables were examined for cross-correlations (Graham, 2003). A Pearson correlation coefficient of $r \leq \pm 0.80$ was used as a threshold to determine the exclusion of highly correlated variables (Khanum *et al.,* 2013)

3.3. Results

The Maxent models performed much better than random with test AUC values ranging from 0.968 to 0.998. The distributions of *B. gerrardii, S. triodontiphyllus* and *S. fasciatus* are mainly affected by the precipitation variables with contributions of 90.7%, 91.5% and 70.1% respectively (Table 5).

Table 5: Percent contributions of the bioclimatic variables in the Maxent models for *Brachystelma gerrardii, Streptocarpus fasciatus* and *Senecio triodontiphyllus*. Variables without any values (indicated by —) were removed because of high cross-correlations.

	B. gerrardii	*S. triodontiphyllus*	*S. fasciatus*
Annual precipitation (Bio12)	—	—	—
Precipitation of wettest quarter (Bio16)	69.8	77	39.7
Minimum temperature of coldest month (Bio6)	6.6	6.8	15.7
Precipitation of driest quarter (Bio17)	20.9	14.5	30.4
Mean diurnal range in temperature (Bio2)	—	—	—
Temperature seasonality (Bio4)	—	—	—
Precipitation of wettest month (Bio13)	—	—	—
Max temperature of warmest month (Bio5)	—	—	—
Temperature annual range (Bio7)	0	0	3.1
Precipitation of driest month (Bio14)	—	—	—
Mean temperature of coldest quarter (Bio11)	—	—	—
Isothermality (Bio3)	0.2	0	4.9
Mean temperature of driest quarter (Bio9)	—	—	—
Mean temperature of wettest quarter (Bio8)	2.6	1.7	6.1
Precipitation seasonality (Bio15)	—	—	—
Mean temperature of warmest quarter (Bio10)	0	0	0
Annual mean temperature (Bio1)	—	—	—
Precipitation of warmest quarter (Bio18)	—	—	—
Precipitation of coldest quarter (Bio19)	—	—	—

The model for *B. gerrardii* performed much better than random with an AUC value of 0.968. Current suitable habitats for *B. gerrardii* were predicted in the eastern parts of South Africa from East London in the Eastern Cape, through KwaZulu-Natal and Mpumalanga (passing through Eswatini), towards the northern parts in Limpopo (Figure 4). There is also a patch of suitable habitat in the Western Cape. This result is unusual because the Western Cape has winter rainfall and summer drought, therefore, the area cannot be suitable since rainfall is in the wrong season. Maxent primarily looks at rainfall records and not the seasons in which they were recorded. Therefore, it might be interpreting this area in the Western Cape as receiving enough rainfall to make it a suitable habitat despite the difference in rainfall season to the rest of South Africa. This 'suitable patch' in the Western Cape is also too far removed from where the plant currently oc'urs, and therefore it is unlikely that propagules will be moved from current localities to those in the Western Cape . Precipitation of wettest quarter (Bio16) and precipitation of driest quarter (Bio17) provided the most useful and unique information for *B. gerrardii* (Figure 5). These variables were the top two predictors in the Maxent model with contributions of 69.8% and 20.9% respectively (Table 5). The suitability of habitat for *B. gerrardii* showed a sharp increase with increases in both these variables (Figure 13a, b). The predicted suitable habitats contain plant localities, except for the northern parts of Limpopo which are predicted to be highly suitable, and parts of the Eastern Cape (Figure 4). The plant localities are localised in areas with a high probability of habitat suitability except for one locality in Limpopo in the Waterberg district. This locality is found in a mountainous area with elevation ranging from 1400 to 1600 m, which is an appropriate altitude for this plant species, and may have a suitable microclimate.

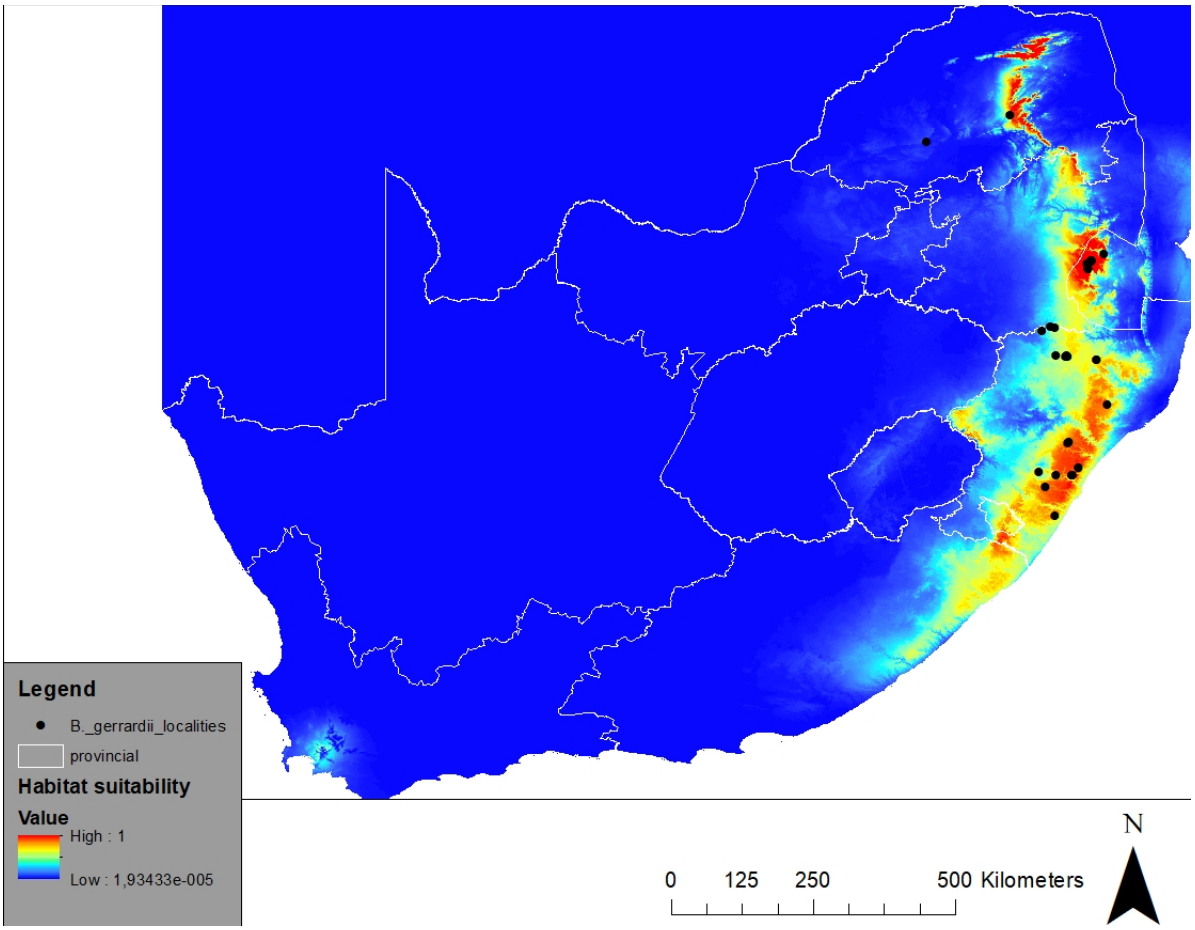


Figure 4: Predicted current (suitable and unsuitable) habitat for *Brachystelma gerrardii* based on near current (1970–2000) climatic conditions in South Africa.

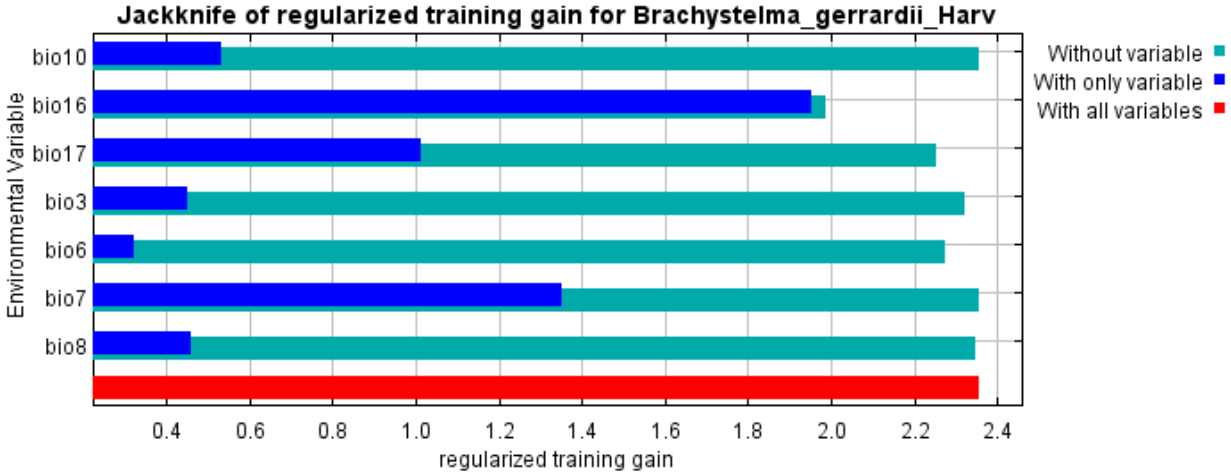


Figure 5: Relative predictive power of different bioclimatic variables based on the jack-knife of regularized training gain in Maxent models for *Brachystelma gerrardii.*

Future predictions from two climatic models for 2050 (A1B and A2 emission scenarios) showed a decrease in suitable habitat for *B. gerrardii* in the northern and eastern parts of the country (Figure 6).

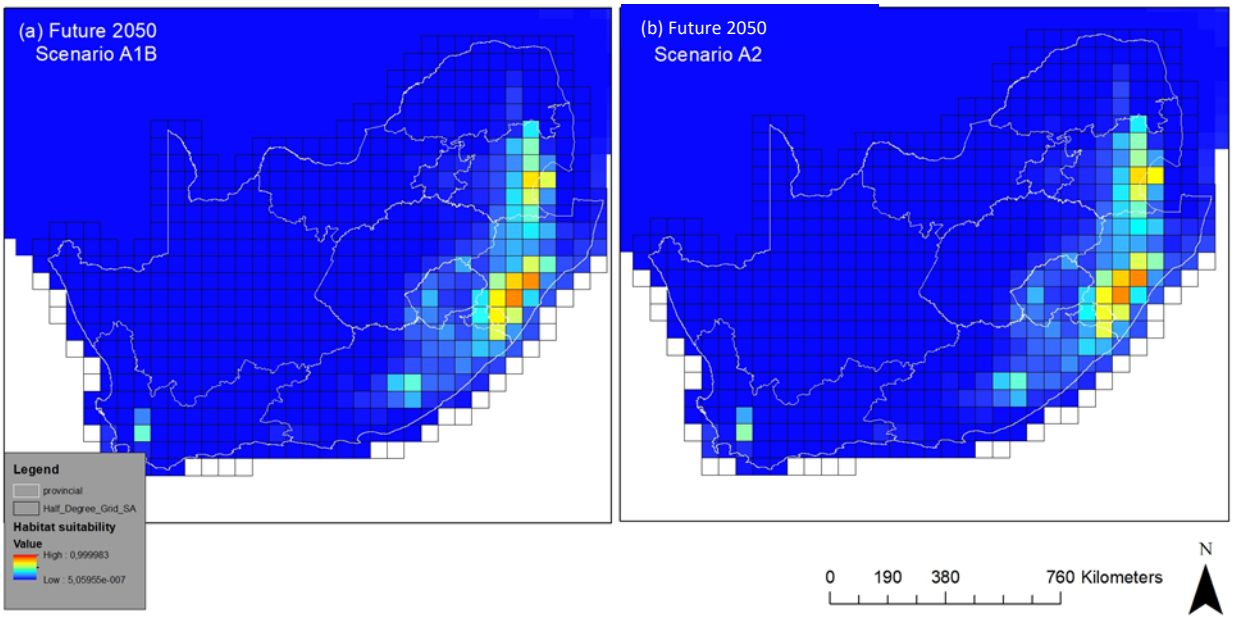


Figure 6: Predicted future (suitable and unsuitable) habitat for *Brachystelma gerrardii* in South Africa. Future predictions are based on data from two Global Climate Models for 2050, A1B (a) and A2 (b) emission scenarios.

The model for *S. triodontiphyllus* had an AUC value of 0.997. Current suitable habitats for *S. triodontiphyllus* were predicted in the eastern to northern parts of South Africa; in KwaZulu-Natal along the Lesotho border, towards Mpumalanga (passing through Eswatini) to Limpopo province (Figure 7). However, plant locality data indicate that the plant species is restricted to the Mpumalanga province and is confined to the Barberton to Kaapmuiden area. Precipitation of wettest quarter (Bio16) and precipitation of driest quarter (Bio17) were the most important in the model predicting suitable habitat of *S. triodontiphyllus* relative to other bioclimatic variables (Figure 8). Precipitation of wettest quarter (Bio16) had the top contribution of 77%, while precipitation of driest quarter (Bio17) had 14.5% contribution (Table 5).

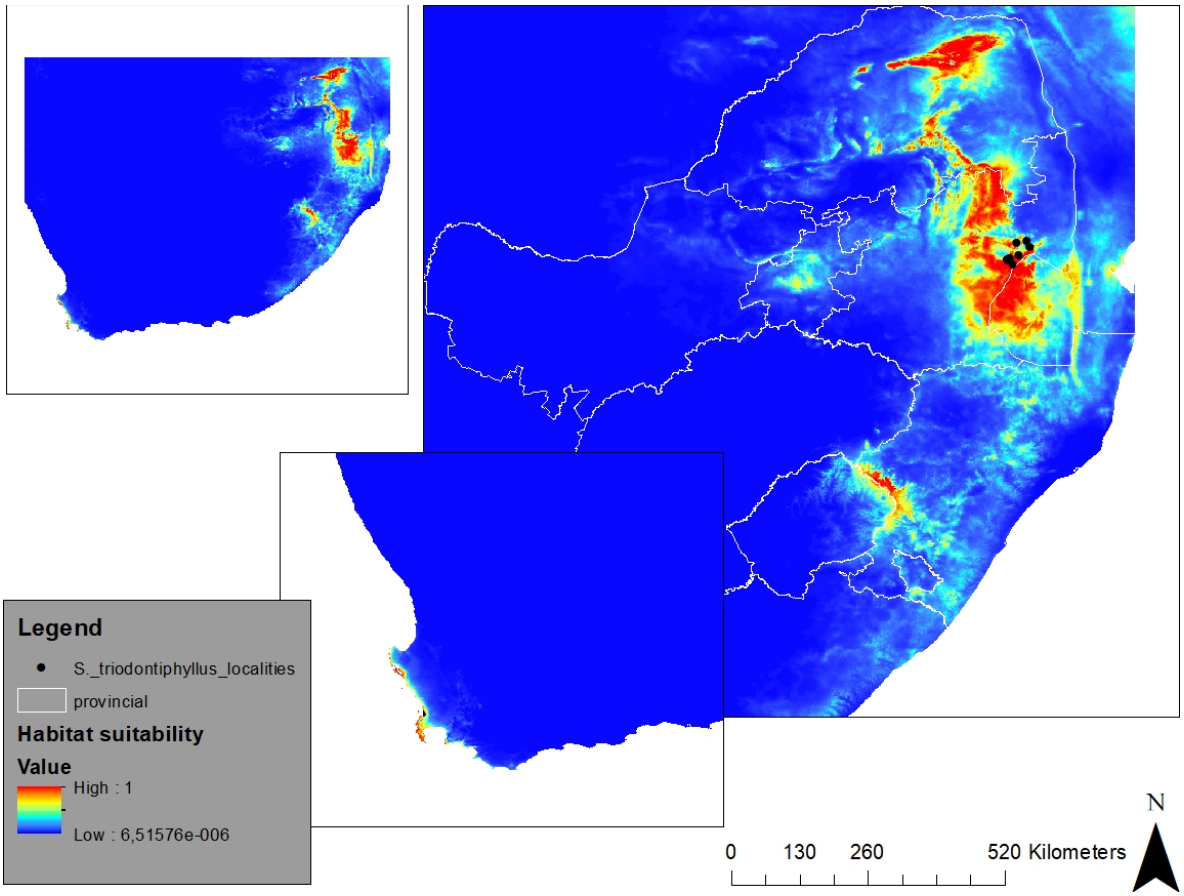


Figure 7: Predicted current (suitable and unsuitable) habitat for *Senecio triodontiphyllus* based on near current (1970–2000) climatic conditions in South Africa.

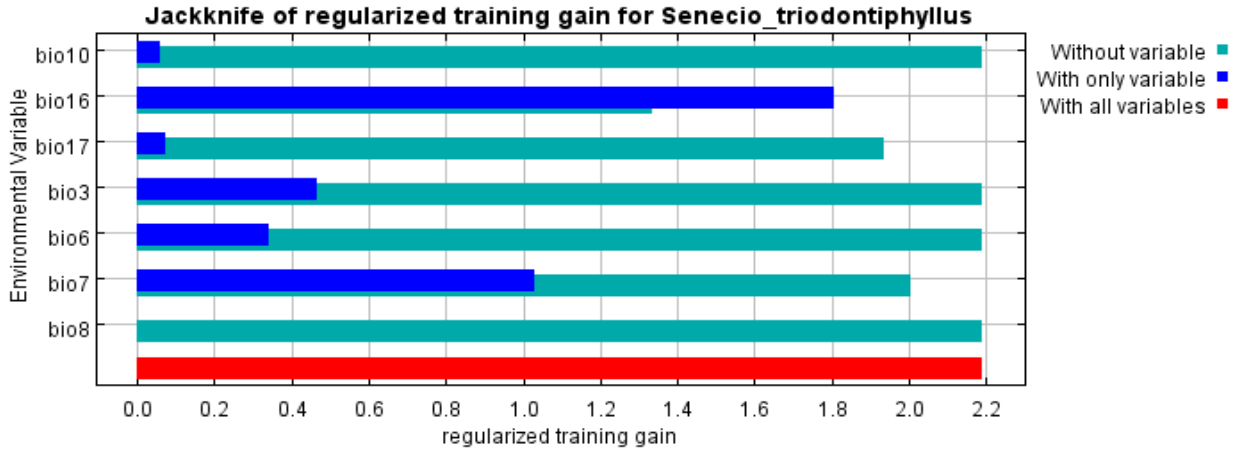


Figure 8: Relative predictive power of different bioclimatic variables based on the jackknife of regularized training gain in Maxent models for *Senecio triodontiphyllus*.

Two climatic models for 2050 based on the A1B and A2 emission scenarios predicted a loss of suitable habitat for *S. triodontiphyllus* in the eastern parts of the country and a loss in the northern parts of Limpopo as well as the Gauteng province (Figure 9). The probability of presence of *S. triodontiphyllus* increased with an increase in precipitation of wettest quarter but decreased sharply with increasing precipitation of driest quarter (Figure 13e, f).

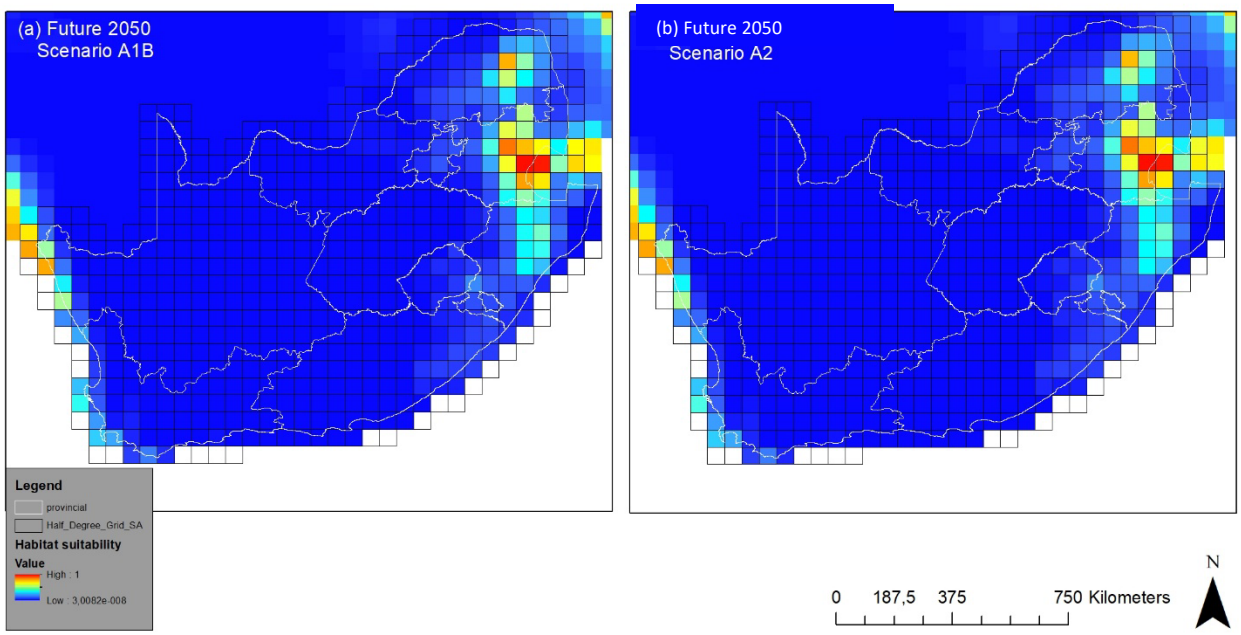


Figure 9: Predicted future (suitable and unsuitable) habitat for *Senecio triodontiphyllus* in South Africa. Future predictions are based on data from two Global Climate Models for 2050, A1B (a) and A2 (b) emission scenarios.

The performance of the Maxent model for *S. fasciatus* was much better than random with an AUC value of 0.998. Current suitable habitats for *S. fasciatus* were predicted as patches along the coast in Western Cape, along the border of Lesotho in KwaZulu-Natal, in Gauteng and on the eastern parts of Mpumalanga going towards the northern parts of the Limpopo province (Figure 10). Plant locality data indicate that this plant species is localised in the Mpumalanga province, in the Malelane and Kaapmuiden areas. Precipitation of wettest quarter (Bio16) and precipitation of driest quarter (Bio17) had higher training gain and AUC values than other bioclimatic variables for *S. fasciatus* (Figure 11). Precipitation of wettest quarter (Bio16) contributed 39.7% and precipitation of driest quarter (Bio17) contributed 30.4% to the Maxent model for *S. fasciatus* (Table 5).

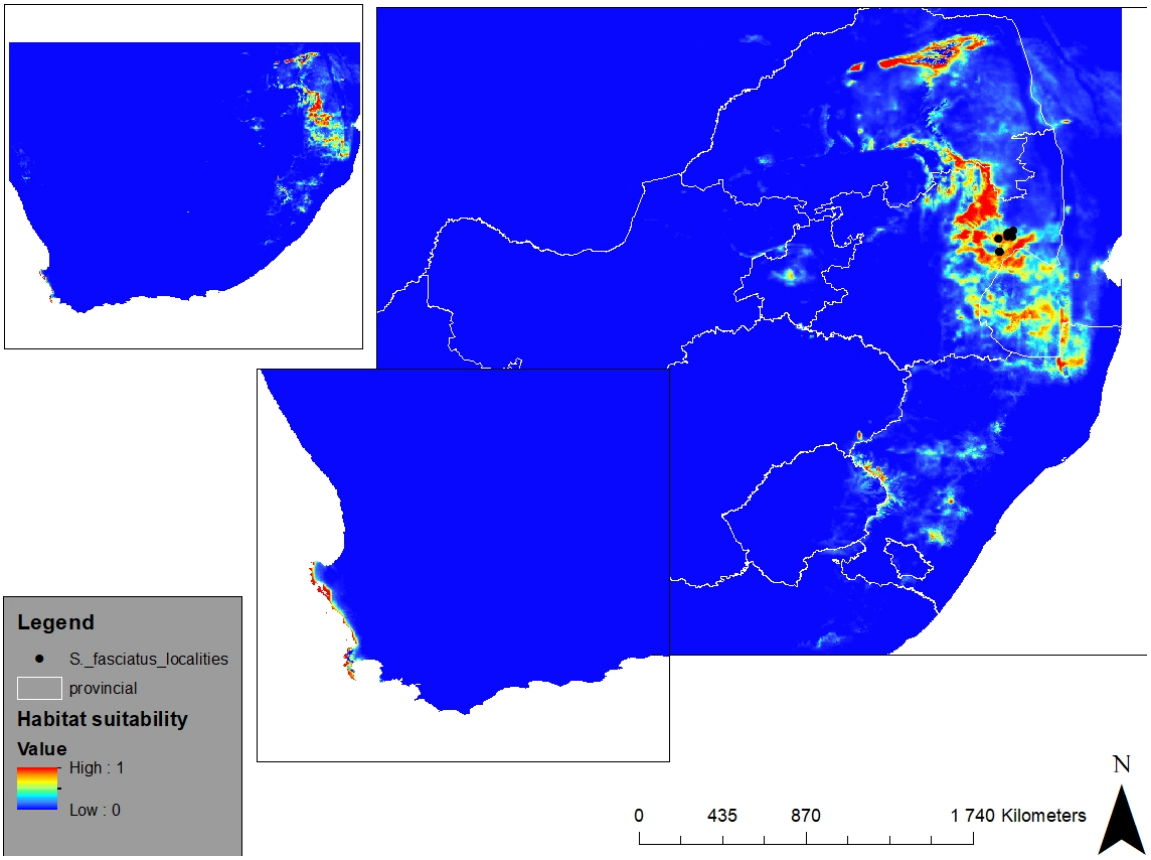


Figure 10: Predicted current (suitable and unsuitable) habitat for *Streptocarpus fasciatus* based on near current (1970–2000) climatic conditions in South Africa.

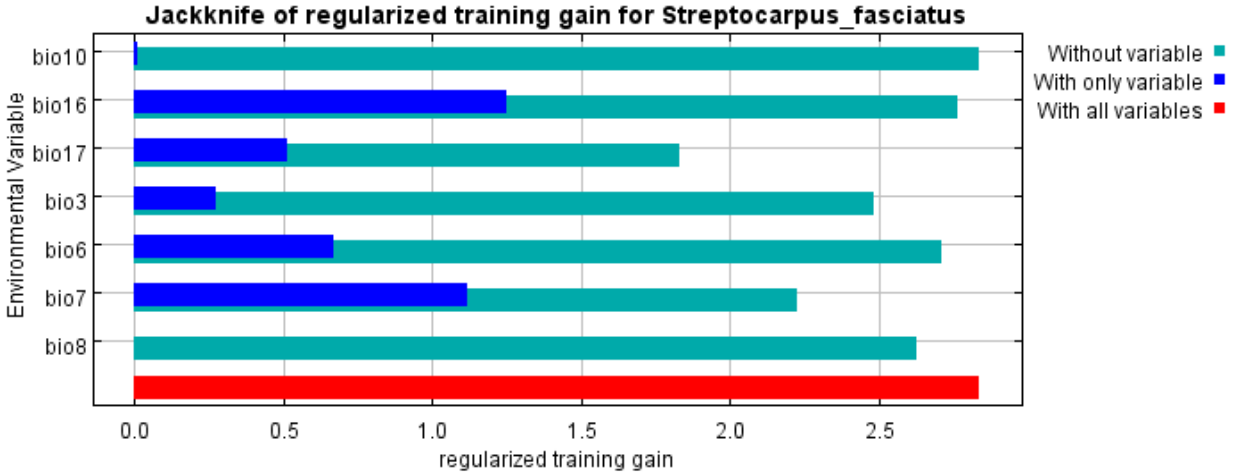


Figure 11: Relative predictive power of different bioclimatic variables based on the jackknife of regularized training gain in Maxent models for *Streptocarpus fasciatus*.

Future predictions for 2050 (A1B and A2 emission scenarios) showed a significant increase in suitable habitat for *S. fasciatus* in the north and towards the eastern parts of the country (Figure 12). The habitat suitability of *S. fasciatus* increased relatively slowly with increasing precipitation of wettest quarter, but it showed a sharp decrease with increasing precipitation of driest quarter (Figure 13c, d).

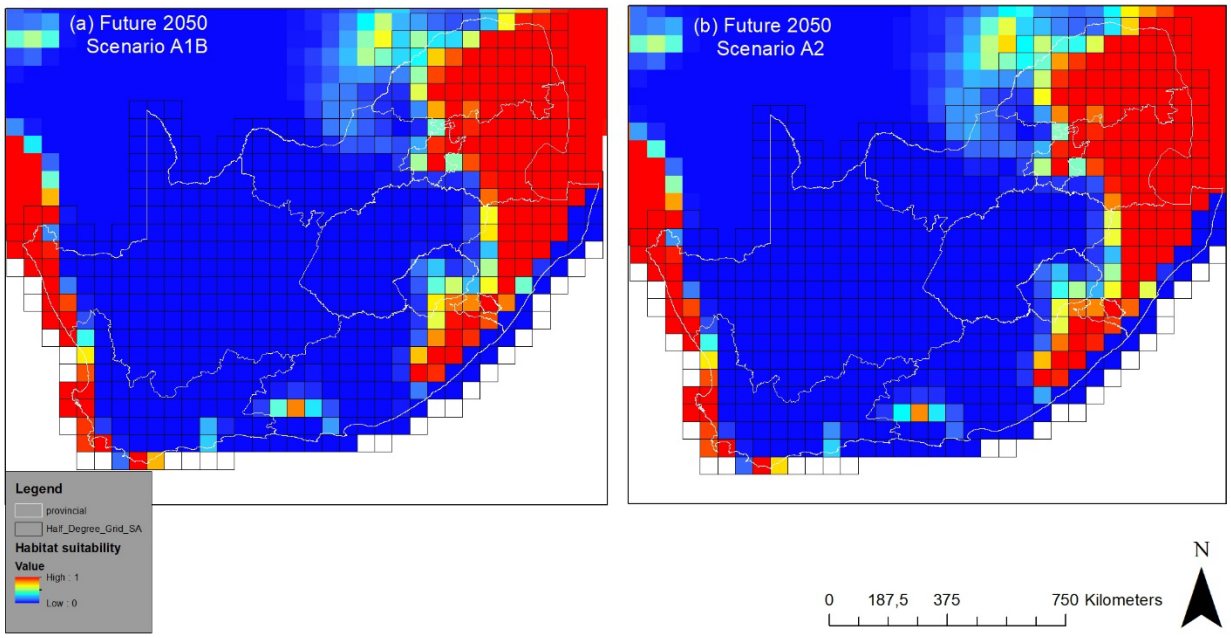


Figure 12: Predicted future (suitable and unsuitable) for *Streptocarpus fasciatus* in South Africa. Future predictions are based on data from two Global Climate Models for 2050, A1B (a) and A2 (b) emission scenarios.

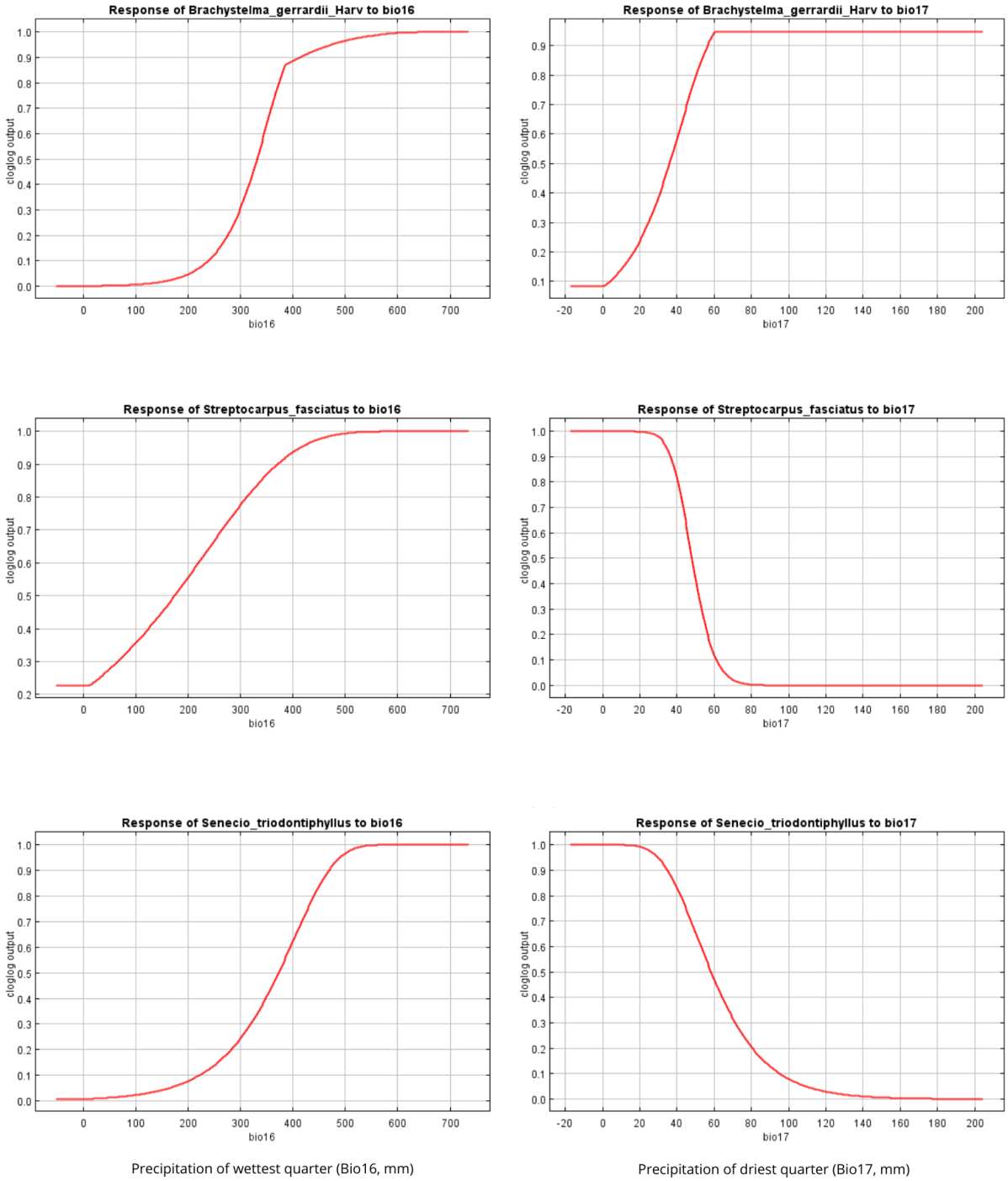


Figure 13: Response curves showing the relationships between the probability of presence of a species and two top bioclimatic predictors of *Brachystelma gerrardii, Streptocarpus fasciatus* and *Senecio triodontiphyllus* (bio16 = Precipitation of wettest quarter, bio17 = precipitation of driest quarter).

3.4. Discussion

3.4.1. Current climate

Brachystelma gerrardii and *Senecio triodontiphyllus* occupy the grassland biome which is characterized by summer rainfall and winter drought, whereas *Streptocarpus fasciatus* occurs in areas characterised by summer rainfall with dry winters and a MAP of about 600–1100mm, increasing with altitude (Lötter *et al.,* 2006a; Mucina & Rutherford, 2006; Raimondo *et al.,* 2009). Areas where *B. gerrardii* has been located fall within the predicted suitable habitat. However, one locality of this plant species which occurs in the Limpopo province falls outside of the predicted suitable habitat (Figure 5). It was not possible to verify this specimen, but upon closer inspection it was found that the locality is in a mountainous area with elevation ranging from 1400 to 1600m, which is an appropriate altitude for this plant species as it may have a suitable microclimate. This locality was recorded in 1936, a period before our current climate data were recorded, which is why the area may be predicted as having a low probability of habitat suitability. It is also important to note that due to time the locality was recorded, it is possible that the plant no longer occurs in this area.

Although Maxent predicts a large suitable habitat for *S. triodontiphyllus,* plant localities indicate that it is restricted to Mpumalanga province and confined to the Barberton and Kaapmuiden area (Figure 7). This is because even though environmental conditions may be suitable, there are some factors that may prevent the species from growing in areas outside Mpumalanga. For example, biophysical variables such as aspect, elevation, landcover, soil type and geology. This plant occurs in an area characterised by ferrallitic soils and a geology of granite, volcanic rocks and porphyritic granodiorite (Chesworth, 2007).

Streptocarpus fasciatus is restricted to the Mpumalanga province, even though Maxent predicts a much larger suitable habitat. *Streptocarpus fasciatus* is localised in Malelane Mountain Bushveld, in the high-lying area of Malelane and Kaapmuiden (Edwards *et al.,* 1992; Lötter *et al.,* 2006b; Mucina & Rutherford, 2006). Plants of the genus *Streptocarpus* grow where rock outcrops fulfil their ecological requirements, for example, some species of this genus frequent coarse granites and conglomerates (Hilliard & Burtt, 1971). The distribution of this species relates to the Mpakeni (Mpageni) pluton, which is found about 20km east from Nelspruit and forms part of the Nelspruit suite. The Mpageni pluton consists of a pinkish, coarse grained, potassic granite.

3.4.2. Future climate

Predictions from two climatic models for 2050 (A1B and A2 emission scenarios) show a decrease in suitable habitat for *B. gerrardii* (Figure 6) and *S. triodontiphyllus* (Figure 9), and an expansion of the suitable habitat for *S. fasciatus* (Figure 12). This decrease in suitable habitats for *B. gerrardii* and *S. triodontiphyllus* is primarily in the northern and eastern parts of the country, whereas habitat suitability for *S. fasciatus* is predicted to expand in the northern and

towards the eastern parts of the country, as well as on the coast of the Western and Northern Cape.

It is important to note that there are numerous uncertainties surrounding the projection of future rainfall patterns over the African continent (Engelbrecht *et al.,* 2015). This is because GCMs tend to be unreliable estimators in regions where convection is important (Davis *et al.,* 2017). As a result, there is a much greater confidence in the magnitude of changes in temperature than in the magnitude of changes in rainfall. Most GCMs project rainfall decreases for southern Africa and increases for east Africa, however, this pattern is variable across different models (Archer *et al.,* 2018; Davis *et al.,* 2017; Engelbrecht *et al.,* 2015). General drying is projected across southern Africa as a result of the drastic increases in temperature that are projected (Engelbrecht *et al.,* 2011; 2015; Malherbe *et al.,* 2013). Under the A1B and A2 scenarios the mean annual temperatures are expected to exceed 2°C over southern Africa (Davis *et al.,* 2017). Rainfall is projected to decrease in summer, the main rainfall season of the southern region (Davis *et al.,* 2017).

According to Maxent, Bio16 and Bio17 are the top two predictors in the models (Table 5), meaning they had the highest contributions in predicting the suitable habitats. Bio16 and 17 represent the wettest and driest quarters of the year respectively (WorldClim). Results show that suitable habitat for *B. gerrardii* increased sharply with an increase in both these variables (Figure 10a, b), whilst suitable habitats for *S. triodontiphyllus* and *S. fasciatus* increased with an increase in bio16 and decreased with an increase in bio17 (Figure 13c, d, e, f). These results indicate that the three plant species in this study are highly dependent on local climate, and a significant decrease in the amount of precipitation especially in the growing season, will affect the suitable habitats and thus growth of *B. Gerrardii* and *S. triodontiphyllus,* and most likely will lead to their extinction. *Streptocarpus fasciatus,* which has a restricted distribution and highly specific habitat, surprisingly shows an expansion of suitable habitat with both climate scenarios. However, the geological formations that occur along the distributional range of this species have resulted in a unique and highly specific habitat for *S. fasciatus.* Therefore, likelihood that this species would migrate to new disjunct areas is low. Our results are similar to the results from a study by Yang *et al.* (2013) as they support the statement that in most cases the predicted suitable area by Maxent appears as an over-estimation in comparison to the realised niche of the species. This is because a Maxent model considers only niche-based presence data and maps the fundamental niche of the species instead of the realised niche (Abolmaali *et al.,* 2018; Kumar & Stohlgren, 2009). Only bioclimatic variables were used as predictors in this study, however, the distribution of plant species is also affected by other factors such as dispersal ability, biotic factors, human disturbances, competition etc. Therefore, not incorporating these factors may limit the accuracy of our results (Kaky *et al.,* 2020; Urban *et al.,* 2016).

3.5. Conclusion

Climate modelling is a powerful tool for conservation, and in this study Maxent was used to model current and future suitable habitats for *B. gerrardii, S. triodontiphyllus* and *S. fasciatus.* The results of this study show that under future climate change the suitable habitats for *B. gerrardii* and *S. triodontiphyllus* would shrink, while the suitable habitat for *S. fasciatus* would expand. However, given its restricted distribution and specific habitat requirements, this expansion seems unlikely and may be an over-estimation by Maxent. Given these results and the current rate of climate change, it is likely that these plant species will go extinct unless conservation action is taken. Conservation actions for these species could be: (1) conservation of areas with suitable habitats, that is, areas that serve as climate refugia and experience lower rates of climate velocity; minimising non-climate threats such as land degradation and invasive plants in order to increase species' future resilience to climate change; (2) translocation of plant species to newly suitable habitats when their current habitats shrink; and (3) collection of seeds and banking actions to provide a safety net (Braverman, 2014; Fenu *et al.,* 2019).

Chapter 4

Pollination biology and breeding system of the endangered plant *Streptocarpus fasciatus:* implications for threat status

Abstract

The survival of plants depends on their ability to cope with ecological conditions as well as effective reproduction. Plants depend on pollination for reproductive success, and effective mating strategies to promote genetic diversity and thus increase fitness. Different breeding systems result in different reproductive outputs and therefore influence genetic structure and gene flow in plant populations. Studies of pollination and plant breeding systems help us identify factors affecting the reproduction of plants and maintenance of plant populations. This study investigated the breeding system and pollination of *Streptocarpus fasciatus* T. J. Edwards & Kunhardt in order to determine if these processes have implications for its threat status. *Streptocarpus fasciatus* was found to be visited by a nemestrinid fly of the genus *Stenobasipteron* Lichtwardt. This insect is a hovering fly that has a long proboscis which helps it obtain nectar from the long corolla tube of the flower. It is hypothesized that this fly is the most likely pollinator given it's morphological fit and two observed visits in the field. Pollination treatments were performed to test for autogamy, geitonogamy, cross pollination and emasculation. The plant species was found to have a mixed breeding system involving both self- and cross-pollination. Seeds produced by autogamous pollination had a high percentage germination, followed by geitonogamy and open pollination (97,7%, 91% and 85.5% respectively). On average, a lower seed set was observed in open pollinated flowers (112.6±5,6 average seeds per treatment) compared to hand cross pollinated (316.8±15.8) and self-pollinated flowers (140.8±7.04), indicating the possibility of pollination limitation. This disadvantage may contribute to the species' low population size and ultimately its threat status. Further investigations of the insect pollinator are needed, as well as further tests of the breeding strategy.

4.1. Introduction

The survival of plants in an environment depends on their ability to cope with ecological conditions, and whether they are able to reproduce and have long-term genetic fitness. Pollination guarantees reproductive success and genetic fitness, and thus increases the chances of species survival. Pollination is the transfer of pollen grains from stamens to a flower's stigma, which is the receptive surface of the female reproductive organ (Abrol, 2012; Inouye, 2013; Walker, 2020). Pollination usually occurs between flowers of the same species and is important for sexual reproduction in plants (Abrol, 2012; Gebner *et al.,* 2013). The transfer of pollen from the male to the female part of the flower is a movement of genetic material and results in fertilization and the production of fruits and seeds (Brauman & Daily, 2014; Potts *et al.,* 2014).

This process is thus important in the life cycle of many flowering plants as it allows for genetic recombination and formation of genetically distinct seeds (Abrol, 2012; Píco *et al.,* 2008). Pollination thus promotes genetic variability and increases fitness in plant offspring. In this sense, pollination is associated with plant speciation and evolution by natural selection and is essential for plants to survive in changing environments (Schowalter, 2006; Wilmer, 2011). Pollination highlights the mutualistic relationship between the plant and animal kingdom, where plants gain reproductive success while animals receive a food reward usually in the form of nectar (Wilmer, 2011).

As with other natural processes, there are limitations associated with pollination that may cause it to fail in plants. Pollination failure can occur at different stages during the release, transport and deposition of pollen on the stigma (Wilcock & Neiland, 2002). This failure can be as a result of: (a) pollen limitation, (b) pollination limitation, and (c) pollinator limitation. Pollen limitation occurs when pollen that is transported from the stamens to the stigma is of inadequate quality or quantity in order to result in maximum fruit set (Wilcock & Neiland, 2002). Pollen can either be sterile as a result of environmental influences during development, or it can lose viability after removal due to an extended time between transfer and deposition at the stigma (Owens & Morris, 1998; Pacini *et al.,* 1997). Pollinator and pollination limitation are related as they refer to the unavailability or lack of pollinators as well as the absence of pollen deposition on the stigma, respectively (Wilcock & Neiland, 2002). Both of these processes are related to pollen dispersal. Ultimately, without vectors, pollen transfer would not occur and thus pollination and fertilization would fail.

Research suggests that the decline in pollinator abundance and diversity is a result of anthropogenic factors such as habitat loss and fragmentation as well as the intensification of agricultural practices (Kearns *et al.,* 1998; Ricketts *et al.,* 2008; Wilcock & Neiland, 2002; Winfree *et al.,* 2009). A study by Brown & Paxton (2009) found that habitat fragmentation is one of the most important factors driving bee declines. Steffan-Dewenter *et al.* (2001) as well as Tscharnke *et al.* (2002) found that fragmentation produces declines in species richness and abundance in bees and butterflies respectively, especially those that are solitary and have narrow ranges of pollen hosts. This is because habitat fragments may not be able to sustain as many flower visiting species as more connected areas (Wilcock and Neiland, 2002).

A mismatch of temporal and spatial co-occurrence of flowers and pollinators can disrupt their relationships. For this reason, climate change has been a growing concern for many years because increasing temperatures alter the flowering times of plants (Sparks *et al.,* 2000). A change in flowering times will affect pollinator behaviour, as these pollinator species must time their emergence after winter with the blooming period of their host plants (King, 2012). Hickling *et al.* (2006) found that butterfly distributions have already been affected by recent climate change, and more severe changes in the future are expected to have greater impacts (Settele *et al.,* 2008). Plant specialization to attract certain groups of pollinators is another factor that can lead to pollination failure (Faegri & van der Pijl, 1979). The plants which depend

on specific species for pollination are at a higher risk of pollination failure due to pollinator limitation (E.g. Johnson & Steiner, 2000; Rathcke, 2000). Floral characteristics such as early closure of the stigma, absence of floral rewards as well as reduction in petal size can have considerable impact on pollination success (Fetscher & Kohn, 1999; Kudoh & Whigham, 1998; Williams *et al.,* 2000). For example, these traits can limit the amount of pollen received by the stigma (early closure) and reduce pollinator visitation.

Pollination is important to the functioning of most terrestrial ecosystems. It maintains plant communities and agricultural productivity (Potts *et al.,* 2010). Pollination failure due to pollinator decline can have negative impacts on the ecology and economy of an area, as well as affect plant diversity and ecosystem stability (Potts *et al.,* 2010). Many wild plant species depend on insect pollination to bear fruit and seed, and without this service these plants will have reduced fruit and seed set (Donaldson *et al.,* 2002; Potts *et al.,* 2010). Since a plant species' population size is limited by seed production, pollination failure may pose a huge threat to species survival as well as community composition (Donaldson *et al.,* 2002; Fontaine *et al.,* 2006; Hegland & Totland, 2008). A lack of cross-pollination highly reduces genetic diversity and the chances of plants adapting to climate change and due to this, plants may become rapidly extinct as a result of temperature and precipitation changes as well as seasonal abnormalities (Christmann, 2019).

According to Potts *et al.* (2010) nearly 75% of crops that are used for human food depend on bee pollination. The honeybee has been documented to increase crop yield in 96% of animal-pollinated crops, however, in the past few years, the bee industry has been in decline (Potts *et al.,* 2010; van Engelsdorp *et al.,* 2010). As a result, the demand for pollination far outweighs the number of available bee pollinators. There is also evidence to suggest that pollinator loss is biased towards pollinators with specific traits such as narrow pollen specialization, dietary and habitat specialists such as bumblebees (Biesmeijer *et al.,* 2006; Kleijn and Raemakers, 2008). Pollination failure could thus lead to a crop production deficit and ultimately food scarcity. When pollination fails, many plants could go locally extinct, and this may reduce other ecosystem services (Christmann, 2019). The mutually beneficial relationship between plants and pollinators represents a critical ecosystem service. The functioning of ecosystems depends severely on key species such as pollinators (Dirzo *et al.,* 2014; Hassan *et al.,* 2005). For example, conservation of soil fertility and the prevention of soil erosion depends to a great extent on plants with elaborate or extensive root systems, some of which depend on pollination for survival (Christmann, 2019).

Flowering plants depend on pollination to successfully reproduce (Chai *et al.,* 2019). If pollination fails, plant reproductive output will decrease as well as the quality of fruits and seeds. To understand factors affecting species population sizes and causes of rarity, it is important to understand their reproductive ecology and biology (Cousins *et al.,* 2013; Kaye 1999). The factors that affect plant reproductive success and therefore influence distribution of species distribution and size of populations are the ability to be pollinated and produce viable

seeds as well as to effectively disperse these seeds (Steffan-Dewenter *et al.,* 2001). Flowers that are pollinated by biotic vectors have evolved, and invest in features such as flower colour, scent and structures which allow only certain individuals to forage and pollinate them. These features help plants advertise their reward, optimise pollination and essentially maximise their reproductive fitness (Button *et al.,* 2012). Pollinators are selective and mostly forage on flowers that provide the greatest reward for minimal effort and thus drive the speciation of angiosperms (Fontaine *et al.,* 2006; Gegear & Burns, 2007; Pauw, 2019). Much like pollinator behaviour, plant breeding systems are also influenced by various plant characteristics. These include floral morphology and phenology, self-incompatibility and the structure of inflorescence (Harder & Berrett, 1996; Richards, 1986; Wyatt, 1982). This means that breeding systems are a determinant of plant population structures.

Plant breeding systems are an important part of plant reproductive biology, and they answer the question of 'who mates with whom?' (Briggs & Walters, 2016; Sakai & Weller, 1999). Different breeding systems result in different outputs and influence the genetic structure and gene flow in plant populations (Barret & Harder, 2017; Castro *et al.,* 2008; Charlesworth, 2006; Whitehead *et al.,* 2018). Mating in seed plants comes from the interaction between plant characteristics and the environment in which they are found (Barrett and Harder, 2017). Most plant species are hermaphrodites, and many are self-compatible, as a result there are two major breeding system types: (a) predominantly selfing and (b) predominantly outcrossing (Abrol, 2012; Coates e*t al.,* 2007; Eckert, 2010). Self-mating involves self-pollination, whereby pollen from the stamens of a flower is deposited on the stigma of the same flower (autogamy) or the stigma of another flower on the same plant (geitonogamy) (Abrol, 2012; Robert, 1989; Sakai & Weller, 1999). Cross-mating involves the transfer of pollen from one plant to another resulting in fertilization (Abrol, 2012).

Selfing has two major advantages, transmission advantage and reproductive assurance (Barret & Harder, 2017). Transmission advantage is as a result of self-pollination and fertilization, during which each parent (male and female) contributes both allele copies to the offspring instead of just one; and reproductive assurance means that the plant is still able to mate regardless of outcrossing opportunities, that is, even when there are no pollinators available (Barret & Harder, 2017). Plants that use autogamous and geitonogamous breeding systems have an automatic selection advantage since they contribute more genes to the next generation, and thus produce offspring that are similar to the parent compared to outcrossing plant species (Briggs & Walters, 2016). However, self-fertilization has genetic costs such as increased homozygosity which allows for recessive alleles to be expressed thus resulting in inbreeding depression and offspring with decreased fitness (Charlesworth & Willis, 2009; Whitehead *et al.,* 2018; Yang *et al.,* 2017). For this reason, self-incompatibility protects plants against the genetic effects of self-fertilization. Outcrossing individuals on the other hand contribute pollen to other plants. This mating system increases gene recombination and therefore genetic diversity in plant species by producing genetically distinct offspring (Abrol, 2012; Barrett & Harder, 2017; Richards, 1996; Sakai & Weller, 1999). Since cross-mating

provides the opportunity for mating with multiple partners, it provides an advantage for increased seed production. Multiple mating can also elevate the quality of offspring and therefore parental fitness (Barrett and Harder, 2017).

Outcrossing plants are usually self-incompatible individuals with large flowers and high pollen to ovule (P/O) ratios (Goodwillie & Ness, 2005). Conversely, self-fertilizing plants may show a reduced level of separation of male and female function, flower size and attractiveness as well as low P/O ratios (Jacquemart, 2003; Runions & Geber, 2000;). This is because the ability to self-pollinate is higher in these plants as pollination can occur even in the absence of pollinators, and so plant attractiveness is not a principal requirement. In theory, species are expected to be predominantly selfing or outcrossing in order to avoid either outcrossing or inbreeding depression. However, many plant species have a facultative breeding system (Schemeske & Lande, 1985). Understanding the reproductive biology of plant species is important for predicting their survival capacity and suggesting suitable conservation strategies when they are under threat (Rodriguez-Perez, 2005; Roy *et al.,* 2021). Studies of pollination and plant breeding systems help us identify factors affecting the reproduction of plants and maintenance of plant populations (Evans, 2003; Gan *et al.,* 2013). This is particularly important when it comes to endangered plant species (Chai *et al.,* 2019; Danieli-Silva & Varassin, 2013; Rodriguez-Perez, 2005; Roy *et al.,* 2021). Threatened plant species are vulnerable to changing environments and their survival depends on effective reproductive biology (Gong *et al.,* 2015; Osunkoya, 1999). For these plants to avoid extinction, they need to maintain a viable population size through reproduction.

Streptocarpus Lindl. Is a florally diverse genus with 146 species in Africa and Madagascar and belongs to the family Gesneriaceae (Hilliard & Burtt, 1971; Hughes *et al.,* 2006). With a few exceptions, flowers of this family are cross-pollinated and animal-pollinated (Weber, 2021). Self-pollination is known and or suspected in only a few Gesneriaceae. The evolutionary diversification, speciation and evolution of flower forms in Gesneriaceae are closely related to the mode of pollination in the species (Weber, 2021). However, field studies on pollination are limited and more work remains to be done. From the floral characteristics of the plant species, it has been inferred that animals involved in pollination are insects, birds and bats. Regarding insects, bees usually play the role (Weber, 2021). The subgenus *Streptocarpus* exhibits a fascinating evolutionary pattern in which hybridization and gene flow between species play important roles (Hilliard & Burtt, 1971).

Many small-flowered species of *Streptocarpus* produce seeds without being pollinated, and this confirms that the species may be using self-pollination to reproduce, additionally their small corolla size has been linked to autonomy (Harrison *et al.,* 1999; Hughes *et al.,* 2006). In contrast, there are many taxa with small flowers on the African mainland which are chasmogamous and are possibly receptive to pollinators (Hughes *et al.,* 2006). Unfortunately, it is unknown whether these species are able to attract pollinators. The study by Hughes *et al.* (2006) found that the main breeding system for the *Streptocarpus* genus is selfing, and that its

ancestors may have used a mixed mating breeding system. Two of the plant species investigated (*S. macranthus* and *S. lanatus*) mainly use self-pollination and have inferred outcrossing rates whilst the third species (*S. ibityensis*) can maintain a higher degree of outcrossing. Although currently classified under the IUCN Threat List, we do not know much about the reproductive biology of *Streptocarpus fasciatus* Edwards & Kunhardt, a plant species that is endemic to South Africa (Edwards *et al.,* 1992; Lötter *et al.,* 2006b).

Streptocarpus fasciatus is a rosulate, perennial herb with tubular flowers and well-defined longitudinal stripes which mark the corolla lobes (Edwards *et al.,* 1992). This plant has oblong leaves and the inflorescence is up to 12-flowered. It is distributed in the Mpumalanga province, ranging from Nelspruit to Kaapmuiden (Lötter *et al.,* 2006b). Here it occupies rocky and damp habitats as well as humid gorges (Weber, 2021). *Streptocarpus fasciatus* occurs in discrete populations separated by unfavourable habitats. This plant species has a small population size and is highly threatened by alien invasive species and habitat degradation (Lötter *et al.,* 2006b). Anthropogenic disturbances that threaten plant populations, and factors such as population size and distribution may affect plant-pollinator relationships. Therefore, studies of reproductive biology in threatened and rare plants are important in understanding factors that limit population growth, dispersal and colonization (Saunders & Sipes, 2006). Understanding the pollination biology and breeding system of *S. Fasciatus* could help us understand the constraints on sexual reproduction and assist in the development of effective conservation efforts.

4.1.1. Aims and objectives

This study aims to investigate the breeding system and pollination of *S. fasciatus* and determine whether these have implications for its threat status. The first objective is to determine which insects pollinate *S. fasciatus* flowers, and the second is to determine which breeding system the plant species uses to produce viable seeds.

4.2. Methods

4.2.1. Study area

The study was conducted in the Croc River Mountain Conservancy at Moederlief farm (25° 32’ 5.10” S, 31° 12’ 45.0” E), approximately 30 km east of Nelspruit (Figure 14). The region receives about 600 – 1100 mm of rainfall per annum (Mucina and Rutherford, 2006). The vegetation in the area is classified as Malelane Mountain Bushveld consisting of tall and short trees and shrubs on mountains and higher-lying slopes (e.g., *Vachellia davyi* (=*Acacia davyi*) (N. E. Br.) Kyalangalilwa & Boatwright, *Canthium inerme* (L. f.) Kuntze) and on rocky outcrops and lower-lying areas (e.g., *Senegalia caffra* (=*Acacia caffra*) (Thunb.) P. J. H. Hurter & Mabb*, Barleria rotundifolia* Oberm. (Mucina and Rutherford, 2006).



Figure 14: Google Earth view of the location of Moederlief farm located in the Croc River Mountain Conservancy in Mpumalanga, South Africa.

4.2.2. Flower characteristics

Flower descriptions (shape and colour) were recorded in 30 plants during the flowering period (March–May) in 2021 and were based on ex-situ observations at Moederlief farm. Ten plants were sampled in each group of small, medium and large plants. The size of the plants were measured using a ruler in the field. The descriptions of plant and flower structure were based on personal observations and additional information was taken from Hilliard and Burtt (1971). The morphological details of the flowers were studied under a dissecting microscope and documented photographically. Thirty plants were selected in the field and the inflorescences of each individual were counted. Additionally, the number of flowers, flowering period and flower lifespan were recorded.

4.2.3. Floral visitors

To determine the main floral visitors of *S. fasciatus* observations were conducted during the peak flowering time. Observations of insect visitors were conducted daily for 2 weeks for approximately 30-minute periods in the morning (08h00–10h00) and midday (11h00–14h00), and camera traps were set up for night observations and recorded footage for three minutes at 15 minute intervals from 15h00–06h00 the next day. Therefore, flowers were observed for approximately 7 hours per day, for a total of 98 hours over 2 weeks. For each survey,

branchlets with open inflorescences were selected randomly and observed. When an insect visited the plant, its identity was recorded, and it was captured using an insect net (Cardel & Koptur, 2010). Captured insects were put into an insect jar and sedated using ethanol for identification at the laboratory. A reference slide of the pollen from *S. fasciatus* was made. Small pieces of solidified agar were used to remove pollen from insects. The pollen was then checked and matched to pollen from the plant species using a light microscope. Insects were photographed under a dissecting microscope, identified, mounted, and labelled. Species were identified with the help of Dr James Harrison and Dr David Barraclough as well as using Kirk-Spriggs & Sinclair (2017) and Barraclough (2006). Voucher specimens of the insect were placed in the Zoology Museum at the University of the Witwatersrand.

4.2.4. Breeding system

Hand-pollination treatments were used to determine the breeding system of *S. fasciatus*. Plants with abundant flowers were selected for four treatments (autogamy, geitonogamy, cross-pollination and emasculation) and a control. Ten flowers were chosen for each of the four treatments due to the small population size and distance between subpopulations. All flowers in the four treatments were covered with nets before and after hand-pollination and emasculation treatments to prevent insect pollination. The control consisted of 20 flowers that were marked and left to be pollinated. In the autogamy treatment flowers were hand pollinated with their own pollen using cotton buds; for the geitonogamy treatment flowers were pollinated using pollen from other flowers on the same plant; cross pollination involved the pollination of flowers using pollen from other plants of the same species; and for the emasculation treatment, stamens were removed as soon as the buds began opening using a dissecting needle and covered without pollination to test for apomixis. The pollination experiments were carried out from 24–25 March 2021 and all fruits set were collected on the 21st of May 2021. Fruit set was calculated as the percentage of flowers per treatment that produced fruit, whereas seed set was calculated as the average number of seeds produced per pollination treatment.

4.2.5. Seed germination

Seed viability and the germination success of each treatment was evaluated using seed germination tests. Germination trials were run to determine seed viability because a trial run to determine viability with tetrazolium stain did not work. For the germination tests, 200 seeds from each treatment were randomly selected and placed on Whatman No. 1 filter paper in labelled petri dishes, saturated with water daily and kept at room temperature away from direct light. A protruded radical indicated germination success, and germinated seeds were counted on the 7th, 10th and 15th day. Percentage germination was calculated using the formula: no. of germinated seeds/total seeds in a petri dish × 100. The total number of viable seeds in

each treatment was calculated using the following formula: %fruit set × no. of seeds per fruit × % germination.

4.2.6. Data analyses

All data were tested for normality using the Fligner-Killeen test. A one-way ANOVA was used to compare differences between treatments for the fruit and seed set data, while a repeated measures ANOVA was used for germination data. A Tukey HSD test was used to determine which treatments differ from each other. Analyses were conducted using R studio and diagrams drawn with MS Excel.

4.3. Results

4.3.1. Flower characteristics

Streptocarpus fasciatus flowers were blue/violet with defined longitudinal stripes which mark the corolla lobes (Figure 15a). Individual flower size ranged from approximately 4 – 5.5 cm in width, and the corolla has a narrow, bent tube. The floral structure of *Streptocarpus* plants may be described as "corolla gamopetalous, five-lobed, zygomorphic" (Hilliard & Burtt, 1971). Only the anterior stamens in the flowers are fertile, with the other two being staminodes (Figure 15b). *Streptocarpus fasciatus* plants have an average of 3.5 ± 3.3 inflorescences and 5.6 ± 5.2 flowers per plant. The development from bud to open flower takes 3–5 days and mature flowers remain on the plant for a week before the corolla falls off following pollination and fertilization. Flowers are produced throughout the flowering period and remain open during the day and night.

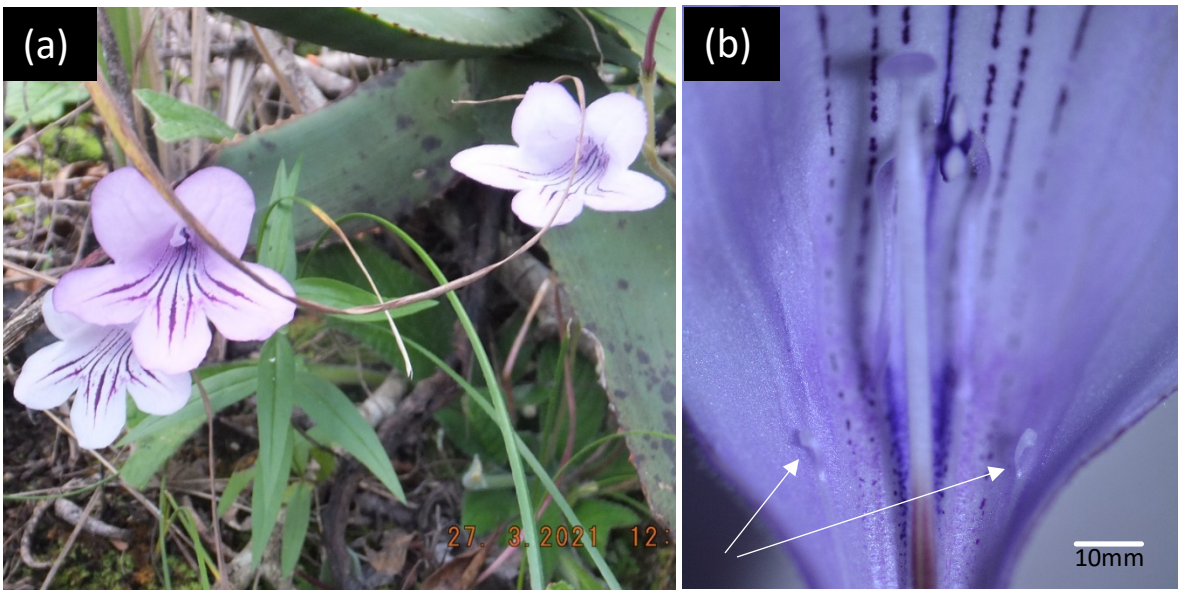


Figure 15: Photograph of *Streptocarpus fasciatus* flowers showing (a) longitudinal stripes on the corolla lobes and (b) staminodes (white arrows)

4.3.2. Floral visitors

Insect visits were only seen four times over an observation period of three days between 10h00–14h00 and on camera trap footage recorded from 15h00–06h00. An insect from the family Nemestrinidae, genus *Stenobasipteron* was the most common visitor (Figure 16). Occasional ants and flies (not identified) were found around the flowers in some subpopulations but were excluded from observations because the visits to the flowers were infrequent and they were not observed to touch the reproductive structures. The *Stenobasipteron* flies were seen visiting *S. fasciatus* plants, where they hovered above the flowers for a few seconds before inserting the proboscis and head into the corolla. However, one *Stenobasipteron* fly was seen visiting *Hypoestes aristate* var. *alba* K. Balkwill. Two specimens of this species were collected in the field. The *Stenobasipteron* fly moved between flowers on the same plant as well as on different plants of the same species. The insect carried pollen on the top left section of the thorax just above the eyes (Figure 15, white arrow) and these pollen grains matched the pollen produced by *S. fasciatus*.



Figure 16: Photograph of one of the observed floral visitors of *Streptocarpus fasciatus*. The insect is from the genus *Stenobasipteron.* The white arrow shows where pollen was carried on the insect.

4.3.3. Breeding system

Fruit set was highest with cross-pollination (90%) and lowest in the autogamy treatment (40%) (Figure 4a). Statistical analyses found a significant difference in fruit set between the different treatments ($F_{4,24}$=2.78, P<0.05). Seed set was higher with the cross-pollination treatment (316.8±15.84) and lowest with autogamy (140.8±7.04) (Figure 17b). There was a statistically significant difference in seed set between the pollination treatments (P<0.001). According to the Tukey HSD test, cross-pollination produces a significantly greater seed set than autogamy.

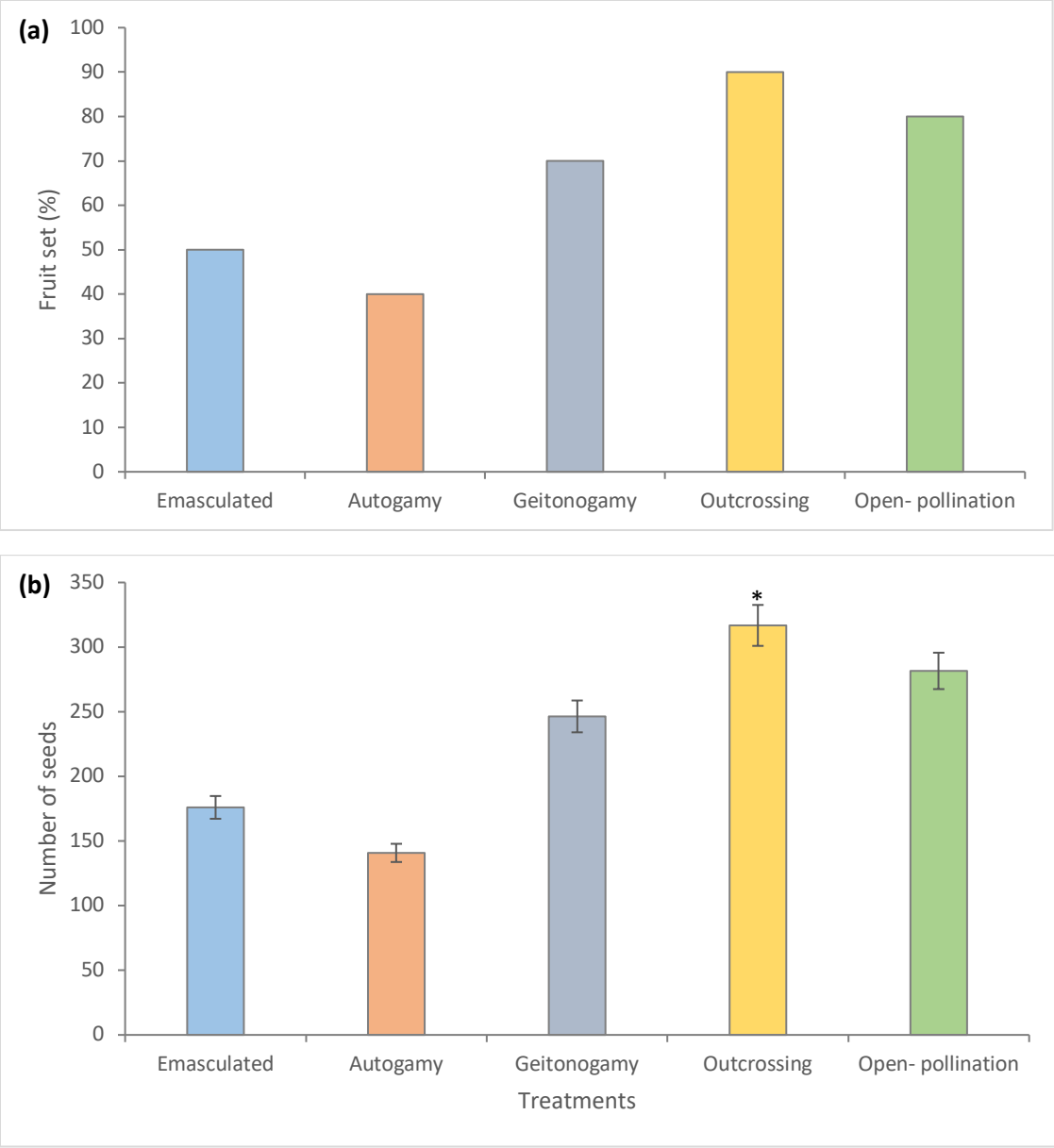


Figure 17: Results of breeding systems determined through hand-pollination treatments (n=10 for the first four treatments and n=20 for open-pollination (control)); (a) fruit set per treatment, (b) seed set treatment (mean ± SE.) ($P<0.05$). The (*) represents the level of significance.

4.3.4. Seed viability

Seed viability tests using the standard tetrazolium test were ineffective due to the small size of the seeds. Seed length ranges from 0.4–0.8 mm (Figure 18) and because of this cannot be cut to effectively expose the embryo for staining. Germination tests were conducted to test for seed viability.

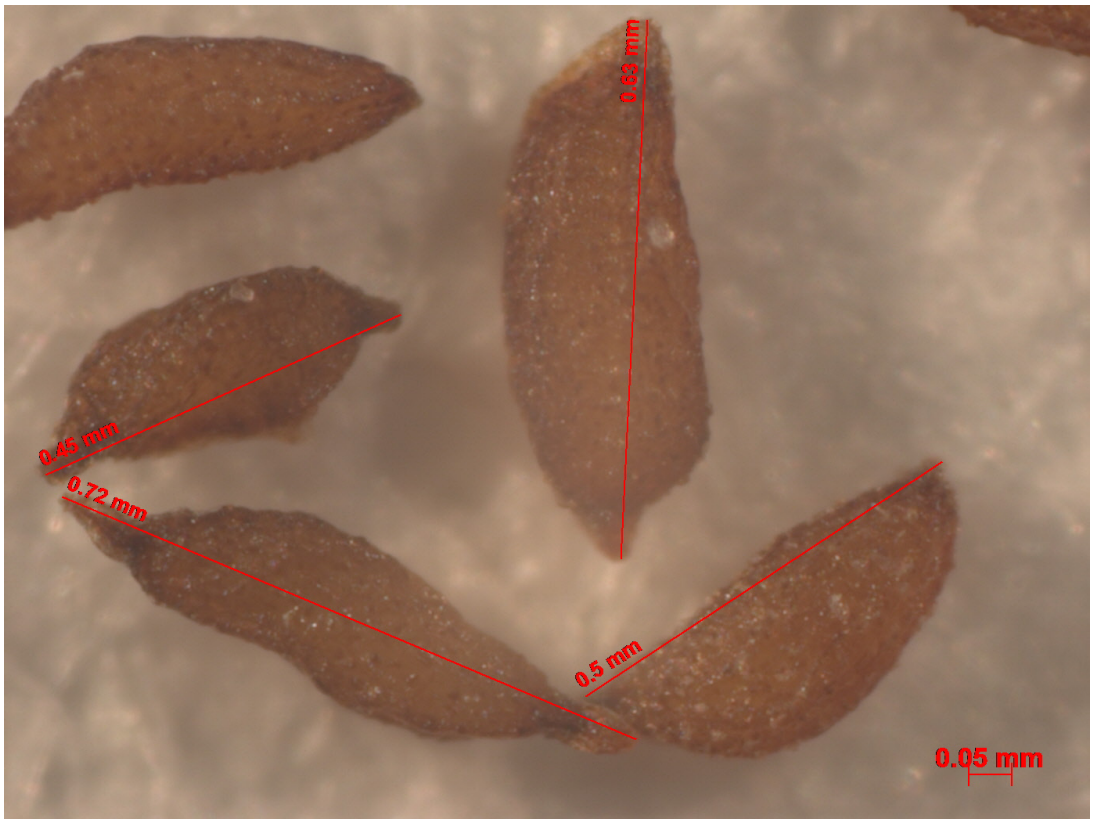


Figure 18: Seeds of *Streptocarpus fasciatus* with length measurements (mm).

4.3.5. Seed germination

The percentage of seeds that germinated within the 15-day trial period was high, ranging from 4.5–29% on day 7; 13.5–47.3% on day 10; and finally, 53.5–97.7% on day 15. Seeds from all treatments began germinating on Day 4 and 5 of the trial, and each treatment yielded the highest germination percentage by Day 15 across all treatments (Figure 19). Seeds produced by autogamous pollination had a high percentage germination, followed by geitonogamy and open pollination (97,7%, 91% and 85.5% respectively), whilst seeds produced from emasculated flowers had the lowest percentage germination (53,3%). There was a significant difference in germination success between the pollination treatments over the 15-day period ($F_{2,8}$=0.368, *P*<0.001). A Tukey HSD test revealed that seeds from the autogamy and geitonogamy treatments have a significantly greater germination success than seeds produced from the emasculation treatment.

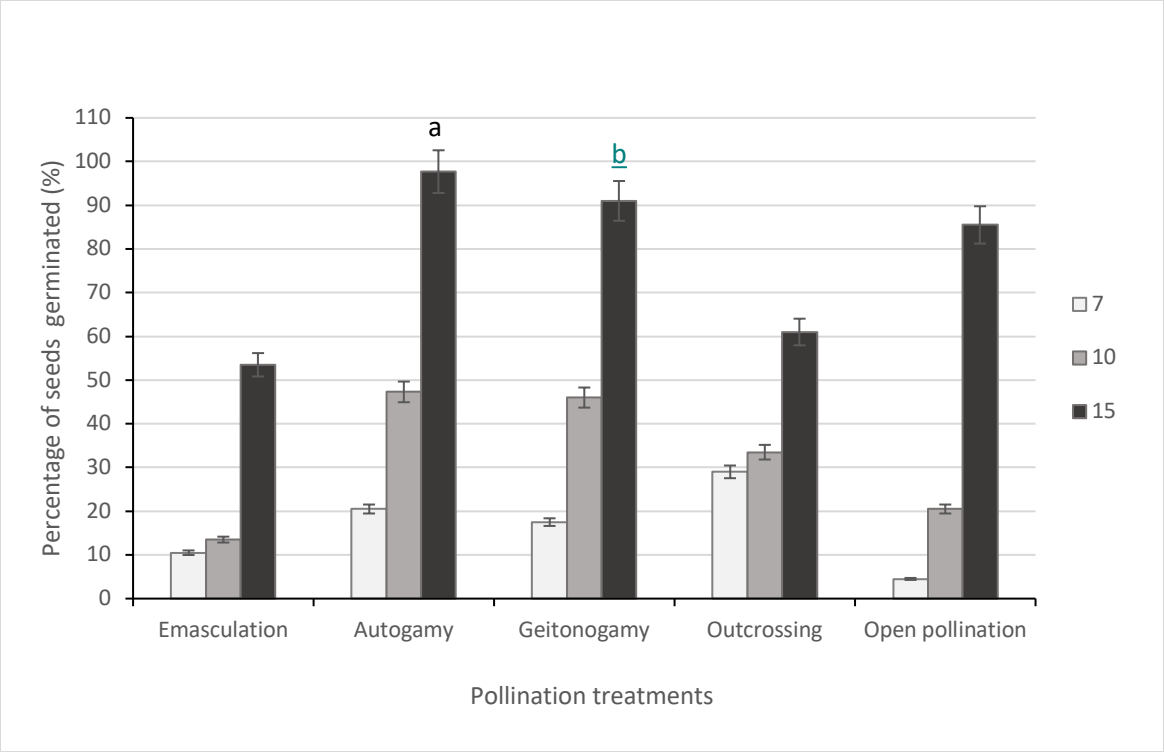


Figure 19: *Percentage of germinated seeds per pollination treatment (± SE) on days 7, 10 and 15, with a statistically significant difference between treatments (Repeated measures ANOVA, P<0.05).* The (a) and (b) above autogamy and geitonogamy represent level of significance.

According to calculations, the number of viable seeds varied between pollination treatments. The open pollination treatment had the highest number with 239 viable seeds, followed by geitonogamy with 222 viable seeds, cross-pollination with 190 viable seeds, and finally autogamy with 137 viable seeds. The emasculation pollination treatment had the lowest number of viable seeds with only 93 seeds.

4.4. Discussion

The results of this study indicate that *Streptocarpus fasciatus* is insect-pollinated, particularly by an insect species of the genus *Stenobasipteron* (Figure 16). Although there is only one formally described species of this genus in South Africa, there are several undescribed species represented in the Mpumalanga and Limpopo provinces (Barraclough, 2006; Kirk-Spriggs & Sinclair, 2017). *Stenobasipteron* species are thought to be restricted to forests (E.g. *S. wiedemanni*), but in Mpumalanga and Limpopo, species of this genus occur in grassland and savanna habitats (Barraclough, 2006; Potgieter & Edwards, 2005). Species of this genus have been recorded as pollinators for 19 plant species including Gesneriaceae, however, some

records of these pollinators in Mpumalanga refer to undescribed *Stenobasipteron* species (Barraclough, 2006).

The South African Nemestrinidae have received little to no taxonomic attention over the past years. This is highly problematic as most of the species are restricted to South Africa and are most likely the sole pollinators of more than 170 plant species (Goldblatt & Manning, 2000; Lombardi *et al.,* 2021). The long-proboscid nemestrinid species pollinate flowers with an elongate, and usually cylindrical perianth tube (Goldblatt & Manning, 2000). According to Barraclough (2006), adult nemestrinids are often found in sunlit areas where they hover near flowers, and in the eastern summer rainfall areas these insects are collected over short periods from late summer through autumn (especially in March and April). These flight periods are thought to be related to the flowering of host plants (Barraclough, 2005; 2006) and may also be related to the flowering period of *S. fasciatus.*

The results indicate that fruit and seed sets in cross-pollinated flowers were higher than in autogamous flowers (Figure 17a, b). Germination results, however, showed that seeds produced from autogamous and geitonogamous pollination had a higher germination rate than seeds produced from outcrossing (Figure 19). The fruit and seed set results indicate that this plant species is insect pollinated, and are therefore in agreement with the observed insect pollinator. These results are also in line with claims made by Hilliard and Burtt (1971) that species of *Streptocarpus* mainly use cross-pollination. Seed set in self-pollinated (autogamy and geitonogamy) flowers (Figure 17) suggests that *S. fasciatus* could have a mixed breeding system involving self-pollination and cross-pollination. A study by Otieno *et al.* (2021) found that a similar species, *Streptocarpus teitensis*, also had a mixed breeding system. Selfing has transmission advantage and provides reproductive assurance, whilst outcrossing increases genetic diversity (Abrol, 2012; Barret & Harder, 2017; Richards, 1996; Sakai & Weller, 1999). In cross-pollination plants receive two alleles from both parents, this means the species will have an increased fitness while being protected from negative effects of pollinator losses. The mixed breeding system also means that *S. fasciatus* can be self-compatible while avoiding inbreeding depression.

Since this plant species is threatened according to the IUCN Species Threat Assessment of 2006, information regarding its breeding system is useful in understanding the causes of its endangered nature (Chai *et al.,* 2019; Gan *et al.,* 2013). For example, plants of this species produced less fruits and seeds under natural conditions compared to artificial pollination (Figure 17), and this indicates that the species might be pollinator limited. This limitation may be as a result of the low frequency with which pollinators visit plants or the decline in pollinator abundance. However, the results are evidenced by only four observed insect visits and only two specimens collected in the wild. Thus another season of visitor observations is recommended. In the field, *S. fasciatus* subpopulations grew distances apart because of the unsuitable habitat between them. Chai *et al.* (2019) suggest that threatened plants in areas that are fragmented may not have access to enough pollinators because of the small number of flowers which are

unable to attract insects. Pollination limitation can lead to either reproductive failure or an increase in self-pollination. Other studies have found that pollen limitation endangers threatened species (Geerts & Pauw, 2012).

The results also show that flowers that were emasculated produce fruit and viable seeds. However, during days when it was windy or raining some of the nets covering the flowers were blown away and the plants were left uncovered for a few hours before being discovered and covered again. This happened twice in the field, and it is possible that during these times the flowers may have been visited by pollinators therefore compromising the results. Pollination tests should be performed again in a controlled environment such as a greenhouse to test if *S. fasciatus* does indeed use the mixed breeding strategy. Additional breeding system experiments should be conducted, since having pollination results over multiple flowering seasons may be beneficial for seeing if there is any variation between seasons due to different environmental factors.

In this study, only one insect species belonging to the genus *Stenobasipteron* was observed visiting and interacting with *S. fasciatus* flowers. Therefore, we can deduce that this species provides the most pollination service to these plants and thus needs to be protected. Efforts to protect this insect species would guarantee continuous delivery of pollination services to *S. fasciatus* plants. Conservation of these insects can be achieved by identifying these species down to species level and understanding their ecological needs such as the resources needed for nesting and reproduction, and by protecting these resources around *S. fasciatus* populations.

4.5. Conclusion

Streptocarpus fasciatus appears to be pollinated by a nemestrinid fly belonging to the genus *Stenobasipteron.* This insect is a hovering fly that has a long proboscis which helps it obtain nectar from the long corolla tube of the flower. Pollination studies indicate that these flower visitors are needed for an increased fruit set, but the results show that the species may have a mixed breeding system involving self-pollination and cross-pollination. The low seed set of open pollinated flowers indicates that this species might be pollinator limited. Therefore, the presence of a mixed breeding system suggests that *S. fasciatus* may be protected from negative effects of pollinator losses. Future work needs to investigate the breeding system under controlled conditions to prevent any contamination and thus obtain a better understanding of the breeding strategy.

Chapter 5

General Conclusion

5.1. Summary of major findings

In this dissertation I set out to re-assess the threat status of *Brachystelma gerrardii, Senecio triodontiphyllus* and *Streptocarpus fasciatus*. All three plant species are endemic to South Africa and have previously been classified as endangered according to IUCN Categories and Criteria. This study also involved studying the current and future impacts of climate change on plant species distributions, as well as the pollination and breeding biology of *S. fasciatus.* Before this study, little was known about the reproductive biology of *S. fasciatus* or the effects of climate change on these species. These species are threatened by habitat loss and invasion by alien plants (Styles and Von Staden, 2007; Raimondo *et al.,* 2009). I attempted to identify other factors that may influence the plant populations negatively and contribute to their extinction risk.

I assessed the threat status of the three plant species (Chapter2). In this study *B. gerrardii* was classified as Data Deficient (DD) due to certain types of data not being available. *Streptocarpus fasciatus* and *S. triodontiphyllus* were found to be vulnerable (VU) and endangered (EN) respectively. Their threat status was mainly attributed to their restricted geographic ranges and small population sizes which are an indication of high extinction risk. Using species distribution modelling (Maxent), I also identified locations with suitable environmental conditions for these plants based on current and future climate data (Chapter 3). Maxent predicted that under future climate change the suitable habitats for *B. gerrardii* and *S. triodontiphyllus* would shrink, while the suitable habitat for *S. fasciatus* would expand. The decrease in suitable habitats for *B. gerrardii* and *S. triodontiphyllus* were mainly in the northern and eastern parts of the country, while the expansion of suitable habitat for *S. fasciatus* was predicted in the north and towards the eastern parts of the country as well as on the coast of the Western and Northern Cape. These predictions are mainly attributed to changes in precipitation that are brought about by climate warming and indicate that the three plant species are highly dependent on adequate rainfall during the growing season. *Streptocarpus fasciatus* has a restricted distribution and specific habitat requirements. Due to these factors, it is likely that the expansion of suitable habitat that was predicted by Maxent may have been an over-estimation. In previous studies it was found that the predicted suitable area by Maxent almost always appears over-estimated compared to the realized niche of the species (Yang *et al.,* 2013).

Pollination experiments determined that *S. fasciatus* is pollinated by an insect of the genus *Stenobasipteron* (Chapter 4). In this study, results show that insect pollination contributed negligibly to reproductive output in *S. fasciatus* compared to hand pollination. I attributed this difference to pollination failure as a result of pollinator limitation. A vital aspect of this study which arose was the existence of a mixed breeding strategy in *S. fasciatus*. During pollination experiments, both self-pollinated and cross-pollinated flowers produced fruits and seeds and these seeds had a high germination rate. This is similar to the results found in another species,

Streptocarpus teitensis (Otieno *et al.,* 2021). A mixed breeding system suggests that this plant species may be buffered from potential negative impacts brought about by the loss of pollinators. Based on the results of studies in this dissertation and all the assessments and extent of threats in plant habitats, the survival of these species depends on effective conservation of plant populations in protected areas.

5.2. Conservation approaches

Conservation of plants relates to their maintenance whether *in situ* or *ex situ* (Li & Pritchard, 2009). *In situ* conservation is regarded as a basic conservation approach and remains the most effective and safest way of preserving endangered species in parks and nature reserves, whilst *ex situ* conservation is an important approach for the conservation of populations away from their natural habitats (Fenu *et al.,* 2019; Guerrant *et al.,* 2004; Harris *et al.,* 2014; Maunder & Byers, 2005). *In situ* conservation is the conservation of natural habitats, ecosystems and species populations in their natural habitat (Braverman, 2014). *Ex situ* conservation is a set of techniques that are used to support the conservation and recovery of threatened taxa, their genetic diversity as well as habitats (Maunder & Byers, 2005). Usually *ex situ* conservation efforts need to be made in the distribution range of the taxa, and their management needs to minimize deleterious effects to the population such as artificial selection, loss of genetic diversity and pathogen transfer (Taylor, 2007). Plant conservation success depends on baseline knowledge of the plant species and the application of this understanding, whilst considering the costs that will be involved (Li & Pritchard, 2009).

5.2.1. Ex situ conservation

There are many *ex situ* species protection methods including field genebanks (plants that are planted for gene conservation), pollen banks and *in vitro* storage (Li & Pritchard, 2009). However, since field genebanks need a lot of space and substantial financial investment, seed banking is a predominant method of *ex situ* conservation of plants (Braverman, 2014). Seed banking is an efficient and affordable conservation strategy since seeds are easy to collect and the collections capture a lot of diversity when collected from a population of individuals and over several seasons (Guerrant *et al.,* 2014; Havens *et al.,* 2006; Li & Pritchard, 2009). Seed banking uses the unique properties of plants which allows their seeds to be dried, frozen and stored for a long time, after which they can successfully germinate and grow into reproductive adults (Guerrant *et al.,* 2014). This conservation strategy is mostly used for agriculture and food supplies as it enables the conservation of many wild species of plants (Li & Pritchard, 2009). Seed banks therefore serve as an insurance against extinction.

Although seed banks offer a cost-effective way of storing large numbers of seeds or species over long periods of time, not all seeds are able to survive being desiccated and stored for long periods (Guerrant *et al.,* 2014). Furthermore, if the seeds are not collected and stored in the right conditions they can become infected and ultimately affect the quality of the collection (Liu *et al.,* 2020). Although seed banking is an excellent conservation strategy, seed collection efforts should not be a one-time occurrence because genetic characteristics of stored seeds will

change over time (Guerrant *et al.,* 2004).

5.2.2. In situ conservation

In situ conservation is regarded as a primary conservation strategy (Fenu *et al.,* 2019). Strategies for conserving plant species in their native environments include but are not limited to removal of invasive plants as well as translocation of threatened plants. Management of invasives includes strategies that involve the elimination or reduction of invasive plants (Pyšek & Richardson, 2010). Removal of invasives may range from low-impact practices such as manipulative treatments that reduce the presence, abundance or impacts of invasive species, to expensive methods that involve engineering techniques and revival of indigenous species (Pyšek & Richardson, 2010). Alien invasive plants can be controlled or removed either manually, chemically or by biocontrol. However, these methods have disadvantages, for example manual clearing can disturb soil and ultimately allow invasion by other invasive plants, whereas chemical removal may inhibit the growth of native plants in the area (Adam *et al.,* 2017). Generally, the removal of invasive species has benefits to the natural biota of the area, but observations by Courchamp *et al.* (2011) show that the benefits of this removal may vary and there may be unexpected consequences. For example, removing invasive species can favour the growth and expansion of plant species that were supressed by the alien plants (Courchamp *et al.,* 2011).

Firn *et al.* (2013) successfully used grazing as a management strategy for invasive plants in grasslands. Grazing was used as a form of biocontrol where grazing animals reduced the survival and reproduction of invasive plants by limiting growth, and as competition by increasing the growth of native species which suppressed invasive species (Firn *et al.,* 2013). Other studies have found that high intensity rotational grazing can be used as a strategy to control the growth of invasive species (De Bruijn & Bork, 2006; Olson *et al.,* 1997). However, in some instances nitrogen and phosphorus levels in plots where invasive plants were removed were not that different from control plots (Ostertag *et al.,* 2009). Removal experiments are recommended as an effective approach for conserving invaded ecosystems, these experiments can be used to examine how resident species respond to disturbances created by the act of removal (Diaz *et al.,* 2003; Ostertag *et al.,* 2009).

Conservation translocation is the placement of plants into natural or other suitable areas (Fenu *et al.,* 2019). The goal of this conservation strategy is to establish resilient and self-sustaining plant populations (Monks & Coates, 2002). This strategy contributes to the recovery of species at risk of extinction, and its positive impacts are enhanced when the strategy is part of a conservation plan. There are three types of translocations, namely: (a) augmentation; (b) introduction; and (c) re-introduction (Weeks *et al.,* 2011). Augmentation is the process of moving individuals of a species in to another population of conspecifics (Fenu *et al.,* 2019). This type of translocation can thus be used to increase the population size of threatened species (Weeks *et al.,* 2011). There are studies which show successful translocations of plants ranging from trees to herbaceous species (Fenu *et al.,* 2019; Harris *et al.,* 2014; Liu *et al.,* 2015; Monks

& Coates, 2002). These studies show that the survival of species that have been translocated strongly depends on their life-form and type of substrate used when planting (Liu *et al.,* 2015). Furthermore, using juvenile and reproductive plants has been proven to yield better results (Fenu *et al.,* 2019).

Although an effective strategy, conservation translocation is costly and time-consuming (Fenu *et al.,* 2019; Monks & Coates, 2011). For this reason, many researchers recommend using an experimental approach to translocation, as the results obtained can inform future projects by testing factors which contribute to the success of this strategy and also add to the knowledge of this science (Silcock *et al.,* 2019). The success of this conservation approach depends on the use of a large number of propagules, which is not possible when working with endangered plant species (Monks & Coates, 2002; Silcock *et al.,* 2019). In this instance, translocation might not be the best conservation action and it may be best to rely on *ex situ* conservation strategies such as seed banks, and consider *in situ* conservation methods. Certain plant species make great candidates for translocation because of their inherent traits which allow them to adapt quickly to their new environments. As a result, translocation of plant species requires that the threats to a species are known, as well as a thorough understanding of their biology and ecology (Fenu *et al.,* 2019). The process therefore needs expert-based decisions on how to select target sites, what type of outplants to use (seeds, seedlings or cuttings) and which planting methods to use (Fenu *et al.,* 2019). The success of translocation can only be determined after a long time, thus, it is recommended that translocation strategies include a monitoring protocol that is cost-effective and can detect changes in the population (Monks & Coates, 2002; Fenu *et al.,* 2019).

5.3. Recommendations

5.3.1. *Brachystelma gerrardii*

Brachystelma gerrardii is widespread; however, it is rare, and its subpopulations usually consist of less than 10 mature individuals (Dyer, 1983; Raimondo *et al.,* 2009). *B. gerrardii* is severely threatened and declining because of habitat destruction especially in KwaZulu-Natal where most subpopulations occur. Threats include urban and housing development, overgrazing by livestock as well as invasion by alien plants (Styles and Von Staden, 2007). Potential conservation strategies might include:

Seed banking

Collection of seeds for seed banking can provide an efficient form of conservation for this species since most of the plant populations fall outside of protected areas. Conservation of these populations should be prioritized as they are faced with higher levels of habitat degradation. Seeds can be gathered over the species distributional range, and this will help increase genetic diversity. However, given the small population size it might be difficult to collect large numbers of seeds without endangering the plant species. In addition, not much is

known about the seeds *of B. gerrardii* and whether they would be able to withstand desiccation and long-term storage.

Translocation of plants

Maxent predicted that suitable habitats for this plant species would shrink with increasing climate change (Chapter 3), therefore, translocation of plants to newly suitable habitats and consequent conservation of these climate refugia is highly recommended. However, given their endangered nature, it is not advisable to dig out plants and move them to another area. I recommend collecting seeds from as many populations as possible, germinating and growing the plants in a greenhouse before planting them in a suitable environment. Studies have found that it is better to use juvenile and reproductive plants since they have a higher rate of survival (Godefroid *et al.,* 2011; Liu *et al,* 2015; Menges *et al.,* 2016).

5.3.2. *Senecio triodontiphyllus*

Senecio triodontiphyllus, is confined to the Mpumalanga province and has a restricted distribution and population size (AOO=48 km^2, <250 mature individuals; Chapter 2 Results). No immediate threats were observed in the field, however, some of the localities of this species fall outside of protected areas and may be threatened by habitat destruction due to expanding forestry plantations and alien invasives. Potential conservation strategies might include:

Seed banking

Collection of seeds for seed banking may be an efficient form of conservation for this species given its restricted geographic distribution, and seeds collected from the three localities would help increase genetic diversity. However, given the low population size, collecting too many seeds might harm the species population and endanger it even further. In addition, the seeds might not have the necessary characteristics to survive desiccation and long-term storage.

Translocation of plants

Similar to *B. gerrardii,* Maxent predicted that suitable habitats for this plant species would shrink with increasing climate change. However, given the restriction in habitat for this species, it might be difficult to find other areas with the same suitable ecological, climatic and geomorphological characteristics where plants can be translocated. Furthermore, translocation requires a significant number of propagules in order to be successful (Silcock *et al.,* 2019). Translocation of this plant species would not be possible since the species has a very small population size and harvesting the plants might endanger the species even further.

5.3.3. *Streptocarpus fasciatus*

Streptocarpus fasciatus requires very specific conditions for growth, and localities of this species fall within areas of the Mpumalanga province which have certain geomorphological and climatic characteristics (Chapter 3). As a result, this species has a restricted distribution and small population size, with an AOO of 64 km^2 and less than 1000 mature individuals (Chapter 2). *Streptocarpus fasciatus* is mostly affected by alien invasive plants such as *Lantana camara* and *Psidium guajava*, as well as habitat destruction due to expansion of settlements in one of the populations. Potential conservation strategies might include:

Seed banking

Collection of seeds for seed banking may be an efficient form of conservation for this species given its restricted geographic distribution, and seeds collected from the three localities would help increase genetic diversity. Seeds from this plant species are small and produced in large numbers, which makes it easy to collect without endangering the species further and does not require a lot of storage space. The seeds are dry and able to be kept for long periods of time. Therefore, seed banking may be a suitable conservation strategy for this species.

Removal of alien invasive species

The *S. fasciatus* population and habitat in Stonehaven is being encroached by invasive plants. This population would benefit from immediate removal of alien plants in the area and this can improve population growth and dispersal. The removal of the invasive species should be followed by monitoring of the area in order to see how the species respond to alien invasive plant removal. This monitoring program could also provide important information that can be used for other plant species.

Translocation of plants

This plant species is restricted in Mpumalanga province. Translocation of the species population to other suitable areas might help increase population growth and protect it from extinction. However, this species has very specific habitat requirements which are not easy to find in most places, this will make finding other suitable areas difficult and the plant might end up not fully establishing itself in the new area and eventually dying out. When looking at this conservation strategy it might be useful to look for suitable habitats in areas that are adjacent to current localities so as to increase the chances of finding habitats with the specific requirements needed. *Streptocarpus fasciatus* plants produce a large number of very small seeds, these seeds

germinate easily in controlled environments and can be grown in a greenhouse. As a result, seedlings can be grown and later transported to a suitable habitat in the wild.

5.4. Limitations and areas of future research

Since pollination and breeding experiments for *S. fasciatus* were done in the wild, there were days when it was windy or raining and some of the nets covering the flowers were blown off (Chapter 4). The plants were left uncovered for several hours during those days, it is possible that the hand-pollinated flowers may have been visited by pollinators. It is recommended that breeding system experiments be performed again, and the breeding system studies to be conducted over multiple flowering seasons to see if there are any variations.

Given the threatened nature of the plant species in this study, it is important to know their biology so that conservation efforts can be successful. Future studies should: (a) investigate the pollination and reproductive biology of both *B. gerrardii* and *S. triodontiphyllus* as there are limited studies which focus on these topics; and (b) carry out annual population counts in order to inform conservation effort. The results of this study serve as baselines to which future data can be compared.

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