Phylogenetic relationships of *Prosopis* in South Africa: An assessment of the extent of hybridization, and the role of genome size and seed size in the invasion dynamics

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Disclaimer

The thesis/dissertation is the result of my own independent work/investigation, except where otherwise stated. Other sources are acknowledged by giving explicit references. A bibliography is appended for each of the chapters. This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree.

Signed:

Date: June, 2012.

Abstract

Invasive alien plants have had diverse ecological and social impacts on recipient ecosystems and are a major problem for land managers. Successful management demands an understanding of the ecology of invading taxa. The invasive status and impacts are documented for *Prosopis* populations in South Africa. However, unresolved taxonomic issues, the extent of hybridization, the applicability of morphology as a species identification approach, and the role that some traits plays in the invasion success have not been studied. This creates a gap that hinders implementation of effective management policies. In this thesis I use a phylogenetic approach to determine the taxonomic make-up of invasive *Prosopis* populations in South Africa (Chapter 2) and compare the results to morphological identification (Chapter 3). I also look at seedling growth rates in the context of variation in genome size and seed size (Chapter 4).

Almost all regions invaded by *Prosopis* are characterized by taxonomic uncertainty exacerbated by the ease of inter-specific hybridization. In Chapter 2 I aim to resolve taxonomic issues of invasive *Prosopis* populations in South Africa using a phylogenetic approach. In addition, I aim to unravel the extent of hybridization and the species involved in South Africa. Here, I found that *Prosopis* populations in South Africa comprise both reported and previously unreported species, indicating a need for a reassessment of the identity of invasive taxa. Hybridization is prevalent and all confirmed species are involved. These findings call for a rethink of legislation and management approaches, e.g. the selection of classical biological control agents. Overall the extent of hybridization indicates that *Prosopis* species in South Africa comprise a freely inter-breeding population typical of a syngameon.

Proper morphological identification of invasive species is crucial for ecological studies and management of invasions. In Chapter 3, I use the total evidence approach to assess whether morphological approaches for identification are adequate for identifying *Prosopis* species in South Africa. I found that *Prosopis* taxa in South Africa cannot be reliably distinguished using existing morphological keys. This is likely due mainly to the proliferation of hybrids with a diverse morphology. Therefore, molecular tools are crucial for confirming any morphological identities and for determining the presence of any unreported species.

Genome size and seed size have been reported to be associated with invasiveness in a number of plant groups, but not often in a system with multiple hybrids like *Prosopis*. In Chapter 4, I first investigate the relationship between genome size and seed size in invasive populations of *Prosopis* spp. in South Africa and secondly I investigate how genome and seed sizes influence germination and early growth. Here I found that genome size loses its distinctness, being diluted in hybridizing populations, but can still be used to assess hybridization events themselves. Large seed size seems to be important for invasiveness as it positively influences germination and early growth.

This thesis confirms the taxonomic conundrum of *Prosopis* species in invasive ranges. This coupled with inadequacy of morphological identification calls for a global study involving native and invasive range taxa to clarify the existing confusions. In view of the presence of unreported *Prosopis* species in South Africa and extensive hybridization, a rethink of the current legislation and control is needed.

Opsomming

Uitheemse indringer plante het grootskaalse ekologiese en sosiale impakte op die ekosisteme wat hulle indring en stel 'n groot uitdaging vir bestuurders van natuurlike hulpbronne. Suksesvolle bestuur en bestryding van indringer plante verg deeglike kennis oor hulle ekologie. Die indringer status en impakte van *Prosopis* populasies in Suid Afrika is reeds voorheen beskryf. Nieteenstaande, die problematiese taksonomie, die omvang van hibridisasie, die waarde van morfologiese identifikasie, en die rol wat sekere eienskappe speel in die sukses van hierdie groep is nog nie bestudeer nie. Daar is dus 'n gaping in kennis wat die effektiewe beheer van die groep in Suid Afrika belemmer. In hierdie tesis pas ek 'n filogenetiese benadering toe om die taksonomiese verwantskappe van *Prosopis* populasies in Suid Afrika te bepaal (Hoofstuk 2) en vergelyk my resultate met morfologiese identifikasie sleutels (Hoofstuk 3). Ek ondersoek ook saailing groei tempos in die konteks van variasie in genoom en saad groote in die groep (Hoofstuk 4).

Bykans alle areas in Suid Afrika waar *Prosopis* voorkom word gekenmerk deur taksonomiese onsekerheid, verder bemoeilik deur die gemak waarmee spesies vrylik hibridiseer. Ek vind dat beide bekende en voorheen-onbeskryfde *Prosopis* spesies in Suid Afrika aangetref word en beklemtoon die behoefte om die identiteit van spesies in die land te hersien. Hibridisasie kom algemeen voor tussen alle spesies teenwoordig in Suid Afrika. Hierdie bevindinge beklemtoon dat wetgewing en beheermaatreëls hersiening benodig, byvoorbeeld in die toepassing van biologiese beheer. In samevatting kom dit voor asof hibridisasie gelei het tot 'n vrytelende *Prosopis* groep in Suid Afrika, tipies van 'n singameon.

Ordentlike morfologiese identifikasie van indringer spesies is belangrik in enige ekologiese studie en die implementering van doeltreffende beheermaatreëls. In Hoofstuk 3 gebruik ek 'n 'totale bewys' benadering om vas te stel of morfologiese eienskappe alleenlik genoegsaam is om *Prosopis* spesies in Suid Afrika korrek te kan identifiseer. Ek vind dat spesies nie geloofwaardig geïdentifiseer kan word nie, heel moontlik as gevolg van wydverspreide hibridisasie tussen alle spesies teenwoordig in die land.

Genoom en saad groote is voorheen geassosieer met die indringer aard van verskeie plant groepe. In Hoofstuk 4 ondersoek ek die verwantskap tussen genoom en saad groote. Tweedens bepaal ek die invloed van genoom en saad groote op ontkieming en vroeë groei eienskappe van *Prosopis*. My bevindinge toon dat, terwyl die kenmerklikheid van genoom groote verloor word as gevolg van hibridisasie, dit steeds hibridisasie gebeurtenisse *per se* kan identifiseer. Groot sade het ook ’n positiewe invloed op die ontkieming en vroeë groei eienskappe van *Prosopis*.

Die tesis bevestig die taksonomiese onduidelikheid van indringer *Prosopis* taksa in Suid Afrika. Tesame met die onakkuraatheid van morfologiese sleutels beklemtoon my bevindinge die behoefte vir ’n dringende wêreldwye studie op indringer en inheemse populasies van *Prosopis* om taksonomiese onsekerhede op te klaar. Die identifikasie van nuwe spesies in Suid Afrika beklemtoon ook die behoefte om huidige wetgewing en beheer van die groep in die land te hersien.

Dedication

I dedicate this work to my late father for his vision to send children to school at a time when, and in a sphere where, education was largely optional. To my family for their support, and to my son, Melisizwe, and daughter Lerato, who spent most of the critical years of his early life, largely without a father around them.

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Table of Contents

List if Figures

List of Tables

List of Appendices

Preface

This thesis emanates from ideas conceptualised by my supervisors, Dave Richardson (D.M.R.), Johannes Le Roux (J.J.L.R.), John Wilson (J.W.) and myself (D.M.M.), but also Jan Suda (J.S.), an expert in genome size research (Chapter 3). Each chapter is written in a style suitable for submission to a journal, although the plan is to combine all the chapters to publish a single synthesis paper. D.M.M., J.S., and all my supervisors will co-author the publication.

Chapter 1 was entirely written by D.M.M. My supervisors D.M.R., J.J.L.R., and J.W. suggested relevant literature and edited the structural skeleton of the chapter.

Chapter 2 was conceptualised by J.J.L.R., D.M.R. and D.M.M., with input from J.W. Some reference data was obtained from a study by Bessega *et al.* (2006). Dr Rieks van Klinken provided *Prosopis* reference material from Australia. Chapter 2 was written entirely by D.M.M. with editorial input from J.J.L.R., D.M.R. and J.W.

The framework for Chapter 3 was conceptualised by D.M.M. emanating from the fascinating diversity in *Prosopis* observed during the March 2010 field trip. Critical conceptual input was provided by J.J.L.R., J.W., and D.M.R. Writing of Chapter 4 was led by DMM with editorial input from D.M.R., J.W., and J.J.L.R.

The framework for Chapter 4 was conceived by D.M.R., J.S., J.J.L.R., and D.M.M. Writing of chapter 3 was led by D.M.M. with editorial input from D.M.R., J.W., and J.J.L.R. Genome size determination was done by J.S. and data was cleaned and processed by D.M.M.

Chapter 5 was written by D.M.M., while D.M.R., J.J.L.R., and J.W provided editorial input.

Chapter 1— Literature Review

Rationale

Plant invasions have been a major concern for land managers and conservationists and there has been extensive research into understanding the underlying predictors of invasion to inform management decisions. *Prosopis* species have become invasive in most tropical and subtropical regions to which they have been introduced (Pasiecznik *et al.,* 2001). However, in most areas where *Prosopis* species have been introduced, there is uncertainty regarding species identities, partly due to the ease by which hybridization can occur among different species and a lack of knowledge on which taxa have been moved around the world and introduced. Currently it is not known which traits are important for *Prosopis* invasions (Pasiecznik *et al.,* 2001). These gaps, in part, limit success of management options currently being used against *Prosopis* invasions. This thesis aims to resolve taxonomic uncertainties in South African *Prosopis,* document the extent of hybridization throughout its invasive distribution, and investigate how traits like genome size and seed size influence life history traits of *Prosopis*.

Background

Alien plant invasions

Invasive alien plants are a major component of global environmental change, and many species have important disruptive effects on ecosystems (Theoharides & Dukes, 2007). Their impacts on the environment, economy, agriculture, water resources, and biodiversity, among others, have been widely studied (Higgins & Richardson, 1998; Lovel *et al.*, 2006; Le Maitre *et al.*, 1996; & Pimentel *et al.,* 2005). While all plant life forms can be invasive, trees have only recently been recognised as important invasive species (Richardson & Rejmánek, 2011)

Woody Invasive trees

Most woody tree species have been introduced for forestry/agroforestry and horticulture purposes (Binggeli, 2001; Richardson, 1998; Richardson & Rejmánek, 2011).

Tree species are the most widely distributed of all invasive plant species as they were introduced in comparatively higher proportions than other plant groups (Crawley *et al.,* 1996 & Petit *et al.,* 2004), and some have since become serious invaders.

From a taxonomic perspective, taxa in woody plant families are overrepresented among invaders of natural areas (Daehler, 1998), and of these the legume family Fabaceae are overrepresented among the world's most prominent invaders (Pyšek, 1998). In the southern hemisphere forestry trees from the genera *Pinus* and *Eucalyptus* are amongst the most important invasive species while invasive taxa in the Fabaceae family include the genera *Acacia*, *Leucaena, Prosopis* and *Sesbania* (Richardson, 1998).

Until recently though, alien woody trees have not been recognised as invaders of major importance with most becoming naturalised and invasive only in the last few decades (Richardson & Rejmánek 2011). This in part being due to long generation times and the delayed onset of invasion, i.e. so-called lag phases, which can take up to 130 years in trees (Petit *et al.*, 2004).

Not all alien plants become invasive. Only about 42% of all plant families contain invasive representatives (Pyšek, 1998). In terms of plants habits; aquatic grasses, nitrogen fixers, climbers, and clonal trees are considered to pose the most serious threats as invaders of natural ecosystems (Daehler, 1998). The question why some alien plants become invasive while others do not has received much attention in recent years (Scott, & Panetta, 1993; Rejmánek & Richardson, 1996; Rejmánek, 1996; Keane & Crawley, 2002; Theoharides & Dukes, 2007). To address this question studies have focussed on different aspects of the invasion process partly to inform management.

Among other objectives, studies of the introduction history aim to understand the extent to which propagule pressure contributes to invasion success (Kriványek *et al.,* 2006), and to determine which entities were introduced (problems with accurate identification of invasive taxa often hinders the implementation of effective management policies) (Richardson & Rejmánek, 2011).

General explanations, among others, of why some alien plants become invasive include release from natural enemies, the acquisition of novel mutualists (Richardson *et al.,* 2000), contemporary evolution of traits promoting spread and dispersal (Dawson *et al.,* 2011), and hybridization (Ellstrand & Schierenbeck, 2000). Results have been variable at local, regional and global scales, probably because of the diversity of approaches that have been applied (van Kleunen *et al.,* 2010).

It is generally thought that high levels of phenotypic plasticity and/or genetic re-organisation are required for alien plants to become widespread invaders (Richardson & Pyšek, 2006). Phenotypic plasticity allows introduced alien species a broader environmental tolerance that facilitates naturalisation while genetic recombination introduces a range of heritable phenotypes, some of which could survive localised selection pressures and become invasive (Ellstrand & Schierenbeck, 2000)

However, it is clear that humans have facilitated the invasion processes by non-randomly distributing 'selected' groups of plants—a scenario that helps explain the lack of taxonomic and phylogenetic patterns among invasive plants, with some taxa being markedly over-represented (Richardson & Pyšek, 2006). Overall therefore, invasive taxa have become 'natural laboratories' to study aspects of ecology and evolution. Each invading species is thus a unique assemblage for such studies and should help in the understanding of different dynamics underlying the invasion process.

Studies on *Prosopis*

Prosopis (Mimosoideae, Leguminosae) is a well-studied group, mainly because of the usefulness of species when not invasive, but also because of the invasiveness of some taxa in a number of regions. Several molecular ecology studies have been done on *Prosopis* in other regions. These have mainly focused at phylogeny and evolutionary diversification (Bessega *et al.,* 2000; Catalano *et al.,* 2008), genetic relationships, (Saidman & Vilardi, 1987; Ramírez *et al.,* 1999; Bessega *et al.,* 2005; Bessega *et al.,* 2006) and hybridization (Henziker *et al.,* 1986). To my knowledge, no study has yet confirmed the prevalence of hybridization using molecular approaches. A few studies have looked at morphology and its use in the construction of pylogenies (Burghardt, & Espert, 2007). Other attempts to resolve species identities have been confined to a few species (Pasiecznik *et al.,* 2001).

But to date, no molecular study has been done specifically to resolve taxonomic problems associated with *Prosopis* in its invasive ranges.

In the case of *Prosopis* in South Africa, there has been no detailed study on invasion dynamics of this group. *Prosopis* in this region therefore offers a good opportunity for the study of some processes associated with plant invasions as outlined above.

Research objectives

It is against this background that the proposed research is planned with four main objectives:

1. To determine which species of *Prosopis* are present in South Africa.
2. To document the incidence of hybridization, identify which parental species are involved, and map the spatial distribution of hybrids in South Africa.
3. To assess the applicability of morphological identifications of *Prosopis* species and their hybrids, with reference to molecular identification.
4. To describe the genome sizes and seed size variation in *Prosopis* and how these relates to life-history strategies, invasiveness, and environmental factors in South Africa.

Study group—the genus *Prosopis*

The genus *Prosopis* L. in the family Fabaceae comprises 44 species, (Appendix 1.1 provides a recent classification of the genus).

The genus is native to South West Asia, North Africa, and the Americas. In the Americas it is distributed across Mexico, southern U.S.A., Colombia, Ecuador, Peru, Venezuela, Paraguay, Brazil, Chile, and Argentina, adapted to arid and semiarid regions (Felker, 1990; Appendix 1.2). Globally, *Prosopis* covers most of the arid and semi-arid tropical regions, in many instances, where it has become naturalised and invasive. (Appendix 1.4 shows countries where it is present, as found in literature Appendix 1.5).

Prosopis species are generally spiny tree and shrub-like species. Leaves can be sub-aphyllous or paucifoliate but are mostly bipinnate with few to numerous leaflets per pinnae.

Flowers are small and hermaphroditic and mainly insect-pollinated (Ramirez *et al.*, 1999). The actinomorphous flowers are sessile, and can have either axillary racemes or heads. Fruits are formed in clusters of up to 12. Pods can be linear or compressed, straight, falcate, or spirally coiled. The fruit is indehiscent with sugary inter-seminal matrix covering the single-seeded segments, and a major model of dispersal is via the gut of ungulates / large herbivores. Seeds are ovoid, hard, compressed and usually brown in colour.

The taxonomy of *Prosopis* is complicated owing to intraspecific variability, and ease of inter-specific hybridization that creates inter-mediate morphological forms (Ramirez *et al.*, 1999; Pasiecznik *et al.,* 2001). The taxonomic difficulties, are particularly pronounced among species of the section Algarobia with some authors considering this section an "artificial grouping" given that it is likely not monophyletic (Bessega, *et al.,* 2006, Burghardt & Espert, 2007).

History of *Prosopis* introductions to South Africa: a taxonomic conundrum

The exact number of *Prosopis* species that have been introduced into South Africa remains unknown. The first recorded introduction of *Prosopis* to South Africa dates from the 1880s when *P. glandulosa* was introduced (Poynton, 1990). Since then a number of other species have been documented as being introduced: *P. pubescens* in 1879, *P. juliflora* in 1885, *P. velutina* around 1900, and *P. tamarugo* in 1971.(Poynton, 1990). *Prosopis cineraria* was also been introduced, but its date of introduction is unknown and reported to have shown limited establishment success in South Africa (Poynton, 1990).

Prosopis cineraria also represents the only taxon of section *Prosopis* introduced; *P. pubescens* the only representative of section Strombocarpa; while, *P. glandulosa var glandulosa, P. glandulosa var torreyana, P. velutina, P. chilensis, P laevigata,* and *P. juliflora* all belong to section Algarobia. The section Algarobia is divided into six series and all species present in South Africa belong to the series Chilensis.

Reasons for introduction

These species were introduced to be utilized as animal feed (mostly the pods), to provide shade in hot/dry environments, and for their support for a diverse array of pollinators, an important ecosystem service (Zimmermann, 1991).

Such benefits later became overshadowed as some species became invasive, and by 1988 farmers were reluctant to use it for fodder fearing invasion of their land (Zimmermann, 1991).

Status impact and current management of *Prosopis* invasions

The intentional planting of *Prosopis* was encouraged in South Africa during the 1960s but 20 years later *Prosopis* species were declared invaders under the Conservation of Natural Resources Act (Zimmermann, 1991). Currently in South Africa, only two taxa are listed: *Prosopis glandulosa* var. *torreyana* (and hybrids; *P. velutina* and hybrids. (Conservation of Agricultural Resources Act, 1983, amended 2001 D. o. Agriculture No. R. 280. Pretoria). *Prosopis* taxa have invaded more than 180,000 hectares in the Northern Cape Province alone with 200,000 hectares at potential risk of invasion (Harding & Bates, 1991). *Prosopis* invades both riparian zones and landscapes (i.e. away from rivers) and it is classified in the "very wide-spread-abundant" category of invasive plants in South Africa (Nel *et al.,* 2004; Rouget *et al.,* 2004), where its impacts have been very substantial.

Figure 1.1 Features and life cycle of *Prosopis* in South Africa. After flowering **(A)**, some *Prosopis* taxa produce copious amounts of seeds in seed pods of diverse shapes (**B)** while others do not **(C)**. Morphological variation exists in stem anatomy; some species have thorny stems **(D)** while others have no thorns. Stem bark can be rough **(E)** or smooth **(F)**. Management involves physical clearing **(G)** after which some species can resprout **(H)**, while some seeds germinate **(I)** and spread to form invasive populations, usually where water collects **(J)** and along water courses **(K).** *Photos by D.M. Mazibuko.*

The impacts of *Prosopis* invasions are many. For example, in the Nama Karoo *Prosopis* has invaded productive alluvial plains and seasonal watercourses (Richardson & van Wilgen, 2004) forming impenetrable thickets. The impenetrable thickets provide little shade and produce few of the valuable pods (Impson *et al.*, 1999). These thickets deplete large amounts of the scarce water resource with an estimated 191.94 million m^3 of rainfall annually lost to *Prosopis* in South Africa (Le Maitre *et al.,* 2000). Management efforts followed shortly after the declaration of *Prosopis* as an invader by means of biological control. These were meant to target seeds only (Zimmerman, 1991), and allow *Prosopis* to continue to be exploited for uses such as timber. In addition, South Africa's Working for Water programme is also involved in the physical clearing of *Prosopis* populations (Impson *et al.*, 1999). Successful control of *Prosopis* has been limited in part due to the fact that seedpods are consumed by animals before biocontrol agents have a chance to destroy them (Impson *et al.,* 1999). Chemical control is effective but, given the extent of invasions, is prohibitively expensive in most cases (van Klinken *et al.,* 2006 & van Klinken & Campbell, 2009). More than two decades after the introduction of biocontrol agents, dense nearly-monotypic stands of *Prosopis* are still found throughout the arid regions of South Africa (personal observation). The use of fire is not recommended as fire poses a risk to personal property and some species are fire tolerant (van Klinken *et al.,* 2006). This has led to calls for introduction of additional biocontrol agents, including species that damage leaves and young pods (Impson *et al.*, 1999; van Klinken *et al.,* 2006).

There is therefore a need to review the success and management of *Prosopis* invasions in the context of revised taxonomic information.

Study approach

This study combines a number of approaches to investigate the questions posed. Morphological approaches are used for initial comparisons of samples using the available key for identifying *Prosopis.* For genome size question, fresh leaf material (from a common garden set-up) was used for flow cytometric analysis. Common garden experiments were set up to determine growth dynamics of the different attributes to be investigated. Molecular approaches will involve amplification of a nuclear gene and a chloroplast gene which will be used to unlock the existing relationships within taxa invading South Africa. Finally desktop work will include acquisition of climatic data for correlative analyses.

Chapter overview

Chapter 2—Phylogenetic relationships of South African *Prosopis*; understanding invading taxa and extents of hybridization

Introduction histories and our current knowledge of the species present in SA indicate contradictory species assemblages.

In South Africa, it remains unclear which species of *Prosopis* are present and to what extent they hybridize. Hybridization (which can cause polyploidy and genome size variations) has been reported to promote fast growth, greater size and increased vigour (Ellstrand *et al.*, 2000; Te Beest *et al.,* 2012), acquisition of herbicide resistance (Snow *et al.,* 1999) and cold tolerance (Milne & Abbot, 2000), all attributes linked to invasiveness. Knowledge of the extent of such attributes in an invading population should therefore shed light on effective management.

Using the reference Internal Transcribed Sequence region (ITS) gene sequences of known parental species, this chapter uses a comparative approach to determine which species of *Prosopis* are invasive in South Africa. Samples were collected from the entire distribution range in an attempt to cover most of the diversity present in South Africa. Through cloning of the ITS gene, I assess the different gene copies that exist within *Prosopis* in South African populations. Being a bi-parentally inherited gene, I attempt to determine the putative parental species of any hybrids identified.

Phylogenetic relationships among South Africa's *Prosopis* species were reconstructed from nuclear ITS DNA sequence data to ascertain invasive species identities and extent of hybridization.

Chapter 3— Morphological identification of *Prosopis* in South Africa; how does it fit with molecular identification

The study of plant form and structure (i.e. morphology) has played a major role in plant science contributing to research in systematics, genetics, evolutionary biology, and ecology (Sattler & Rutishauser, 1997). Traditionally morphology is used to identify plant species.

However, in *Prosopis* populations where taxonomy of species is not clear and where hybridization is suspected, accurate morphological identification can be challenging (Whitney & Gabler, 2008).

In this chapter morphological identification is compared to molecular identification (Chapter 2) to assess whether any conflicts or congruencies exist. I attempt to provide an overview of morphological diversity and determine whether or not morphology can play a role in tentative species/hybrid identification.

Chapter 4— Relationships between genome and seed size and how they influence early growth in *Prosopis*

Genome size (the ratio of nuclear DNA content to ploidy level) has been found to affect different plants attributes (Grotkopp *et al.*, 2004), mostly life-history strategies at cellular level such as length of the cycle during cell division, and germination speed at whole plant level. Genome size has been found to directly vary with cell volume, mitotic S phase, and average cell cycle time (Grotkopp *et al.*, 2004). These in turn affect how fast plants grow (generation time) and seed size.

Since there might be a direct relationship with environmental attributes, genome size also has a bearing on the establishment success of plants and the direction of spread a population is likely to take. For example, in the genus *Pinus*, genome size was found to be an indicator of invasion success (Grotkopp *et al.*, 2004).

Using flow cytometry and fresh leaf material, I intend to determine the distribution of genome size among *Prosopis* throughout its distribution in South Africa. Genome size has been found to influence 'invasive traits' such as germination rates, growth rates and seed size. Here I will assess how these attributes influence early life in *Prosopis*.

Significance of the research

Information regarding the taxonomic identity of species that are present in South Africa will play a role in informing management policies.

Effective management of invasive aliens depends on correct taxonomic identification of species involved, considering the possibility of outdated taxonomy in native regions at the time of introduction (Le Roux & Wieczorek, 2009). Hybridization between exotic plants species is known to promote invasiveness, and to impact on biological control programmes. In case of *Prosopis*, this study is one of a few that will document the sympatric hybridization of closely related, formerly allopatric species. Since predictor traits of invasiveness have been found to vary across taxa this study provides information about how significant the two traits (genome size and seed size) are in the invasion success of *Prosopis*.

Such information feeds back into available literature and would eventually lead into formulation of viable hypotheses regarding 'suites of traits' that do predict invasiveness in plants. The potential of identifying species morphologically also provides opportunities to field ecologists. Being the first study at a molecular level on *Prosopis* from this region, it will create impetus for follow-up studies that should further improve our understanding of the reasons behind its successful invasion and what are the future risks posed by *Prosopis*.

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Chapter 2—Unraveling taxonomic identities of invasive *Prosopis* populations in South Africa and the extent of hybridization

Abstract

Aim *Prosopis* species have been introduced around the world and are considered invasive in many locations. However, it is still unclear which taxa have been introduced and which have become invasive. This is partly due to the capacity of many taxa to form inter-specific hybrids and the introduction of unidentified species. Using a phylogenetic approach, this study aims to resolve some of the taxonomic confusion that exists around the identity of introduced *Prosopis* in South Africa and to shed light on the extent of hybridization in invasive populations.

Location South Africa (with reference collections from Argentina and Australia)

Methods Nuclear ITS and chloroplast rpl32 genes were amplified, cloned, sequenced, and used to reconstruct phylogenetic relationships among *Prosopis* sampled throughout the invasive range in South Africa (n=55) in relation to reference collections from the native range in Argentina (n=17), and putatively identified invasive taxa from Australia (n=7). Phylogenetic relationships were reconstructed using Neighbour-joining, Maximum Parsimony and Bayesian approaches. Hybridization was inferred by identifying heterozygous individuals corresponding to gene copies belonging to different species clades.

Results The phylogenetic analysis corresponded poorly withmy expectations of the taxa likely to be found in South Africa based on historical records. While the presence of some taxa were confirmed largely as hybrids (e.g. *P. chilensis* hybrids 2% of samples, and *P. glandulosa* 24% of samples); other taxa were found whose presence was either debatable (*P. laevigata*, 24% of samples) or one sample never previously recorded (*P. hassleri*); taxa expected to be abundant were not found (*P. juliflora*, and *P. velutina*); and additional, as yet unidentified, taxa may present a large proportion of invasive populations (44% of samples). Moreover, hybridization appears to be common within and among invasive populations, and pure parental lineages are rare. Moreover, I found evidence of the first fertile 'inter-series' hybrid (between *P. chilensis* and *P. hassleri*).

Main conclusions The taxonomic identities of *Prosopis* populations in South Africa reported in the literature appears to be largely incorrect. This is likely due to extensive hybridization, on a scale that suggests *Prosopis* populations in South Africa are a freely inter-breeding hybrid swarm typical of a syngameon. These findings call for a reassessment of legislation and management practices, including the selection of classical biological control agents.

Key words

Biological control, biological invasions, hybridization, Internal Transcribed Spacer (ITS), phylogeny, *Prosopis*, taxonomy, tree invasions

INTRODUCTION

Invasive alien plants are a major component of global environmental change and often have important disruptive effects on ecosystems (Theoharides & Dukes, 2007). Their impacts on the environment, economy, agriculture, water resources, and biodiversity have been widely studied (e.g. see Le Maitre *et al.*, 1996; Higgins & Richardson, 1998; Pimentel *et al.,* 2005; Lovel *et al.*, 2006; and Hejda *et al.,* 2009). Much work has been undertaken in the quest to understand plant invasions and the processes underlining their success (Richardson *et al.,* 2000; van Kleunen *et al.,* 2010) and to devise strategies for management (DiTomaso, 2000; Rejmánek, 2000; Nel *et al.,* 2004). A critical first step toward understanding these aspects is a clear understanding of the taxonomic identity of the taxa involved (Pyšek *et al.,* 2004). This is even more important in cases where hybridization is suspected (Moody, 2002).

The globally invasive genus *Prosopis* (Zimmermann, 1991; Pasiecznik *et al.,* 2003; van Klinken & Campbell, 2009; Richardson & Rejmánek, 2011) represents a case in point. For example, at the time of introduction of *Prosopis* species to South Africa, *P. glandulosa* was referred to as *P. juliflora* in its native range (Nilsen *et al.,* 1986). Such mis-identifications are common in the invasive range of *Prosopis* species (Pasiecznik *et al.,* 2001)

Although the history and extent of invasion by *Prosopis* species in South Africa is reasonably well documented (Poynton, 1990; Harding & Bates, 1991; & Le Maitre *et al*., 2000) the recorded taxonomic identity of introduced and invasive taxa remains questionable (Zimmermann, 1991). Taxonomic uncertainty is exacerbated by the ease with which species in the genus hybridize (Bessega *et al.,* 2006; Catalano *et al.,* 2008).

Unless the identity of invasive *Prosopis* taxa is resolved, management will remain challenging and rigorous studies of invasions and efforts towards management strategies will be compromised (Smith *et al.,* 2008; Pyšek & Richardson, 2010). South African *Prosopis* populations emanated from seed imported on at least 23 different occasions, including from native regions like mainland USA and Mexico, secondary sources like Hawaii, and several unrecorded imports (Zimmermann, 1991). In addition to uncertainties about the introduction histories of *Prosopis* to South Africa, the effect of hybridization on accurate taxonomic identification was noted many years ago.

For example, Poynton (1990) noted hybrids between *P. glandulosa* var. *torreyana* and *P. velutina* to resemble Burkart's (1976) description of *P. juliflora*.

Poynton (1990) further speculated that pure *P. juliflora* may have only arrived in 1985 from Honduras, but these '*P. juliflora*' seed imports were later thought to represent *P. laevigata* (Poynton 1990). While Poynton (1990) assumed that six species of *Prosopis* were introduced to South Africa, Zimmermann (1991), while recognizing the problematic taxonomy of *Prosopis,* felt that the exact number of taxa in South Africa remains unknown. Introduced seed consignments arrived with a variety of names and could only be morphologically verified once plants matured (Poynton, 2009; Appendix 2.2). Farmers, who were encouraged to plant *Prosopis* seeds that they obtained from various localities in the Americas (G.B. Harding, University of Port Elizabeth, pers. comm., 2010), share such uncertainty. Given these records and the taxonomic problems outlined above, the exact number of *Prosopis* species present in South Africa remains speculative at best.

Despite taxonomic uncertainties, a biological control programme aimed at reducing the seed production and therefore spread rates of invasive *Prosopis* populations was launched in 1985 in South Africa (Zimmermann, 1991), and in Australia (van Klinken, 1999). The biological control programme in South Africa initially targeted *P. glandulosa* and *P. velutina*, but host-specificity testing found that some of the released agents did also target other *Prosopis* species (Zimmermann, 1991). Despite this, the introduction of biological control agents in South Africa has had very little impact overall (Klein, 2011; Zachariades *et al.,* 2011).

Against this background the current study aims to: 1) Use a phylogenetic approach to identify *Prosopis* species present in South Africa; and 2) Document the extent of, and the taxa involved in, hybridization.

MATERIALS AND METHODS

Study area description and Sampling

This study covers the entire invasive range of *Prosopis* in South Africa. Sites were selected between latitude -26.4156° and -32.5715 ° south and longitude 17.5391° and 25.2726° west.

These sites span the full bioclimatic range invaded by *Prosopis* in South Africa, allowing for a determination of how altitudinal, latitudinal, and climatic factors in South Africa impact on the different parameters under investigation.

While *Prosopis* is present in arid and semi-arid climates, these regions experience relatively frequent extreme rainfall events (Mason, 1999; Reason & Mulenga, 1999). Such climatic events can be strongly correlated to inter-annual variability in vegetation (e.g. Goward *et al.* 1995). This presupposes that plants growing in different climate regimes are exposed to different selection pressures and adapt variably. The heterogeneity in climate of the current study area therefore affords an opportunity to investigate how this variability has influenced the success of *Prosopis* species as invaders.

Not all populations were sampled because of limited accessibility to some farms but sampling was representative (Appendix 1.3), encompassing such variability as it exists across South Africa. Sampling was largely non-random and was done to maximise the morphological variation present in the population.

Sampling of *Prosopis* populations was done during March 2010. Five to 30 plants were sampled at each location. Initial morphological identification in the field maximised the sampling of putative species, morphological variants, and their hybrids. Leaf material was initially dried in silica gel, followed by oven-drying at 50°C for 48 hours, and then stored on fresh silica gel until further use. Where possible I also collected seedpods.

Sampling included roadside populations with deliberate efforts made to sample populations much further off from the roads, and those that covered vast areas of the landscape. Where possible, populations with old trees were also deliberately targeted, so as to sample trees that could have originated shortly after the initial introductions of *Prosopis*. Measurements of diameter at breast height (DBH) and height were taken. GPS coordinates were recorded for each collected sample. Herbarium samples were also collected from those populations that had individuals with flowers and seedpods. Appendix 3 shows the sampling distribution in context of the known distribution.

DNA extraction and Polymerase Chain Reaction (PCR) amplification

Genomic DNA was extracted from leaf tissue, following the cetyltrimethylammonium bromide (CTAB) procedure (Doyle & Doyle, 1987).

DNA quality was assessed using a nano-drop (Thermo Fisher Scientific, Wilmington, DE, U.S.A.) and high quality DNA diluted to 50ng/ µL.

Amplification of chloroplast gene *rpl32-ndhF* was done in 50 µL reaction volumes containing; 20 mM of each primer, 5 µL 10 X reaction buffer, 0.1 mM of each dNTP (AB gene; Southern Cross Biotechnologies, Cape Town, South Africa), 3 mM $MgCl_2$, 1.25 µL *Taq* polymerase (Super-Therm JMR-801; Southern Cross Biotechnologies, Cape Town), and 50 ng template DNA. The PCR cycle comprised a 4 minute denaturation step at 95 °C; 35 amplification cycles (94 °C for 30 s, 50 °C for 60 s, and 72 °C for 2 min); and a final extension step of 7 min at 72 ^{o}C. The size and quality of PCR products were visualized and assessed on 1.5% agarose gels.

For the nuclear ITS gene, ITS4 and ITS5 primers (White *et al.,* 1990, and modified by Bessega *et al.,* 2006) were used to amplify the entire ITS1, 5.8S, and ITS2 regions. Amplification was done in 50 µL reaction volumes containing; 20 mM of each primer, 5 µL 10 X reaction buffer, 0.1 mM of each dNTP (AB gene; Southern Cross Biotechnologies, Cape Town, South Africa), 2 mM $MgCl_2$, 0.5 µL *Taq* polymerase (Super-Therm JMR-801; Southern Cross Biotechnologies, Cape Town), and 50 ng template DNA. The PCR cycle comprised a 4 minute denaturation step at 95 °C; 35 amplification cycles (94 °C for 30 s, 52 °C for 60 s, and 72 °C for 2 min); and a final extension step of 7 min at 72 ^{o}C. The size and quality of PCR products were visualized and assessed on 1.5% agarose gels.

For both genes, PCR products were purified using the QIAquick PCR purification kit (Qiagen, supplied by Whitehead Scientific, Cape Town, South Africa) following the manufacturer's protocols. Due to the potential presence of heterozygotes from hybrid individuals all ITS PCR products were cloned using pGEM-TEasy Vector System (Promega, supplied by Whitehead Scientific, Cape Town, South Africa) in order to sequence both copies in putative hybrids. At least three clones were sequenced per taxon.

Sequencing was done at the Central Analytical Facility at Stellenbosch University, using the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction kit and an automated ABI PRISM 377XL DNA sequencer (PE Applied Biosystems, Foster City, CA, USA).

All cloned ITS sequences were first blasted on Genbank to determine whether they matched gene data for existing *Prosopis* taxa.

Any cloned microbial contaminants identified were discarded. All DNA sequences were edited in BioEdit (Hall, 1999), and aligned using CLUSTAL W (Thompson *et al.,* 1994) using default parameters followed by manual inspection and editing of the alignment.

Reference samples

We included all available *Prosopis* taxa from a previous systematic treatment of the group (Bessega *et al.,* 2006, Table 2.1). In addition, selected reference species of *Prosopis* were obtained from Australia, thought to represent *P. pallida*, *P. velutina*, *P. glandulosa*.

Table 2.1 Reference ITS sequences, used in this study. There are 18 reference samples out of a total of 44 species within the genus. *Prosopis pubescens* and *P. reptans* belong to the series Strombocarpa. Notation for collection areas: A, south-western USA; B, Mexico; C, Caribbean Antilles; D, Peru–Ecuador; E, central and northern Argentina; F, south-western Argentina (Patagonia) and Cuyo. Gen-bank reference numbers are in the order ITS1 and ITS 2. All data in this table is from Bessega *et al.,* (2006) study.

Species and Authority	Section, Series	Area	Collector-Voucher-Herbarium	GenBank no
Microlobius foetidus	–	–	–	AF458783
Prosopis alba Grisebach	Algarobia, Chilenses	E	BOS-JCV-0409-FCEyN-UBA-ARGENTINA	AY145692–AY145693
P. alpataco Philippi	Algarobia, Chilenses	E/F	BOS-JCV-0581-FCEyN-UBA-ARGENTINA	AY145700–AY145701
P. argentina Burkart	Monilicarpa	F	P.Villagra-0001-IADIZA-ARGENTINA	AY145708–AY145709
P. caldenia Burkart	Algarobia, Chilenses	E	BOS-JCV-0570-FCEyN-UBA-ARGENTINA	AY145686–AY145687
P. chilensis (Molina) *Stuntz* emend. Burkart	Algarobia, Chilenses	E	O. Solbrig-4215-FCEyN-UBA	DQ323141–DQ323149
P. flexuosa DC	Algarobia, Chilenses	E/F	BOS-JCV-0300-FCEyN-UBA-ARGENTINA	AY145706–AY145707
P. glandulosa Torrey	Algarobia, Chilenses	A/B	J.Evans-0005-GRS-USDA-USA	AY145696–AY145697
P. hassleri Harms	Algarobia, Ruscifoliae	E	R. Palacios 311-FCEyN-UBA	DQ323137–DQ323145
P. juliflora (Swartz) DC	Algarobia, Chilenses	C/D	J.H.Hunziker-10039-FCEyN-UBA-ARGENTINA	DQ323140–DQ323148
P. kuntzei Harms	Algarobia, Sericanthae	E	BOS-JCV-0514-FCEyN-UBA-ARGENTINA	AY145704–AY145705
P. nigra (Grisebach) Hieron	Algarobia, Chilenses	E	BOS-JCV,0428-FCEyN-UBA-ARGENTINA	AY145688–AY145689
P. pallida(Humboldt & Bonpland ex illdenow)H.B.K.	Algarobia, Pallidae		DANIDA-01622/86	DQ323139–DQ323147
P. pubescens Bentham	Strombocarpa,	A/B	J. Evans-0015-GRS-USDA-USA	DQ323142–DQ323150
P. reptans Bentham	Strombocarpa,	A/D/E	BOS-JCV-3036-FCEyN-UBA-ARGENTINA	DQ323136–DQ323144
P. ruscifolia Grisebach	Algarobia, Ruscifoliae	E	BOS-JCV-0419-FCEyN-UBA-ARGENTINA	AY145698–AY145699
P. velutina Wooton	Algarobia, Chilenses	A/B	J. Evans-0001-GRS-USDA-USA	AY145702–AY145703
P. vinalillo Stuckert	Algarobia, Ruscifoliae	E	BOS-JCV-0387-FCEyN-UBA-ARGENTINA	AY145694–AY145695
P. laevigata (Humboldt & Bonpland ex Willdenow) M.C. Johnston	Algarobia, Chilenses	B	Solbrig et Ornduff-4479-Darwinion, SI-ARGENTINA	DQ323138–DQ323146

Phylogenetic analysis

Phylogenetic analysis for the chloroplast gene was done using a Bayesian approach and General Time Reversible (GTR) using gamma + invariant sites with four gamma categories using BEAST version 1.6.2 (Drummond & Rambaut, 2007). Model-test version 3.7 (Possada & Crandall, 1998) was used to find best fitting model for the data using Akaike information criteria. *Acacia pycnantha* was used as an out-group.

For the nuclear gene (ITS) the total aligned length of the ITS1 region was 493bp with gaps (indels) ranging from five to 30 bp. Phylogenetic analysis Neighbour-joining (NJ) (Saitou & Nei, 1987) and Maximum Parsimony methods were performed in MEGA v4 (Tamura *et al.,* 2007). In the NJ analysis, evolutionary genetic distances were computed using the Kimura-2-parameter model with complete deletion of gaps in the alignment. This method was chosen because it uses base-substitution models that allow optimal estimation of evolutionary base changes in sequences with low similarity (Bessega *et al.,* 2006). Maximum parsimony analysis was done using the Close-Neighbour-Interchange algorithm with search level 3 with random addition of sequences (10 replicates) to the initial tree. For both NJ and MP analyses, 1000 bootstrap replicates were used to determine nodal support.

Only the ITS 1 region (247 bp) of the nuclear gene was used for all analyses as only this data was available for all previously published reference samples (Bessega *et al.,* 2006). I did not run Bayes analysis for the ITS gene for better comparison with the reference study (Bessega, *et al.,* 2006) which employed the methods outlined above. *Microlobius foetidus* (GenBank accession number AF458783) was used as out group due to its close relatedness to *Prosopis* (Bessega *et al.,* 2006).

RESULTS

The chloroplast *ndhF-rpl32R* gene tree showed no clear resolution for the species included, possibly due to the conserved nature of this gene region. For example, *P. pallida*, *P. velutina (*from Australia but found to be closely related to *P. laevigata* in this study*)* and *P. glandulosa* all shared 100% DNA sequence similarity (REF clade, figure 2.1).

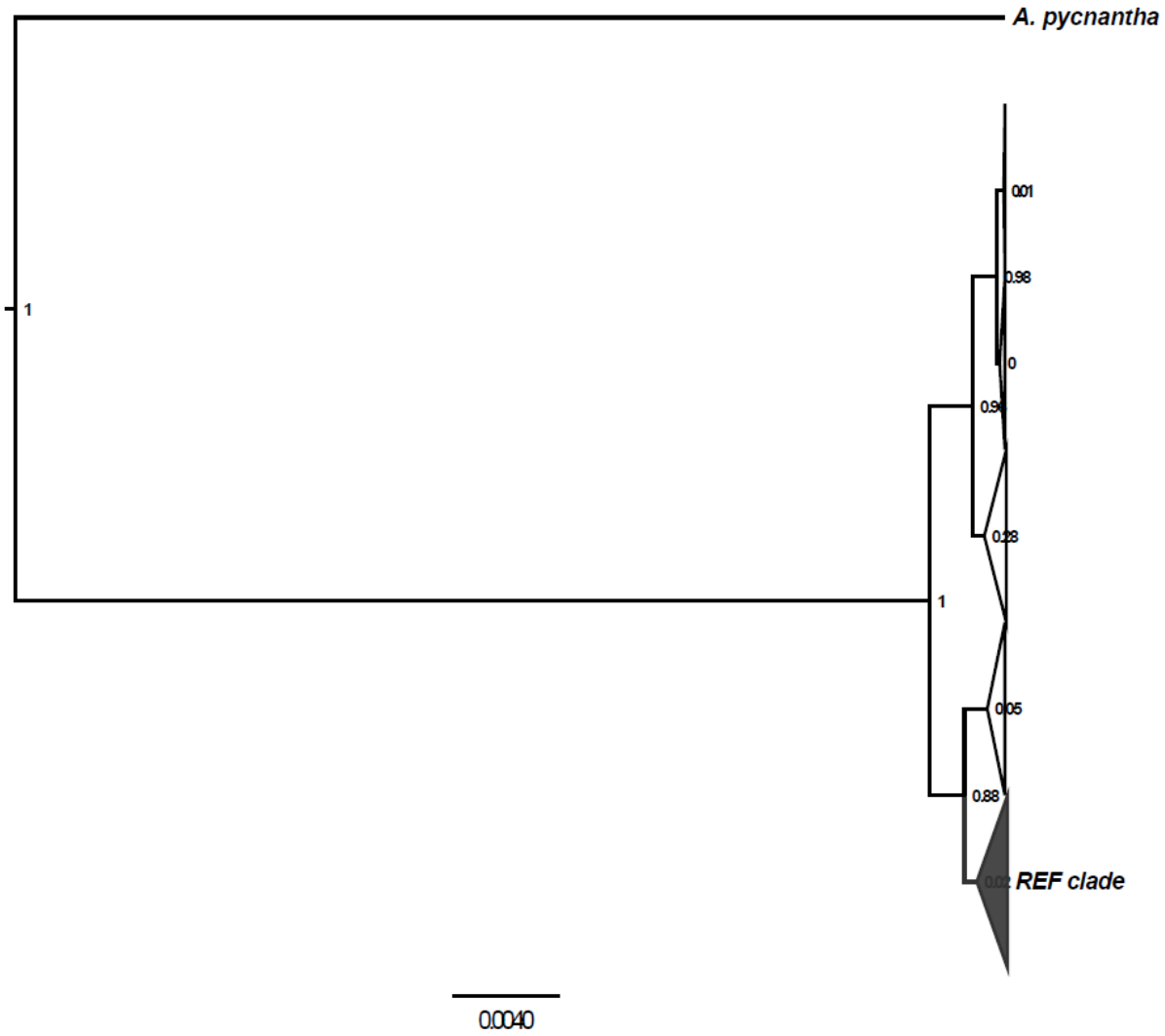


Figure 2.1 Phylogenetic tree showing relationships of South African *Prosopis* species inferred from cpDNA gene rpl32R-ndhF**.** Numbers on nodes are posterior probabilities. The four clades which had individuals that were identical are collapsed for clarity. Reference samples from Australia (*P. glandulosa, P. pallida* and *P. velutina*) are all within the REF (reference) clade. *Acacia pycnantha* is used as an out-group.

The ITS nrDNA analysis included 55 samples from South Africa and seven samples from Australia (*P. pallida* (1), *P. velutina* (3), *P. glandulosa* (1), and *Prosopis* hybrid (2)) and 17 samples as references from Bessega *et al.,* (2006). Sequencing of cloned ITS regions revealed that 37 taxa were heterozygous, i.e. having two different gene copies.

Maximum parsimony analysis from the ITS1 region yielded 110 trees (see Fig 2.2).

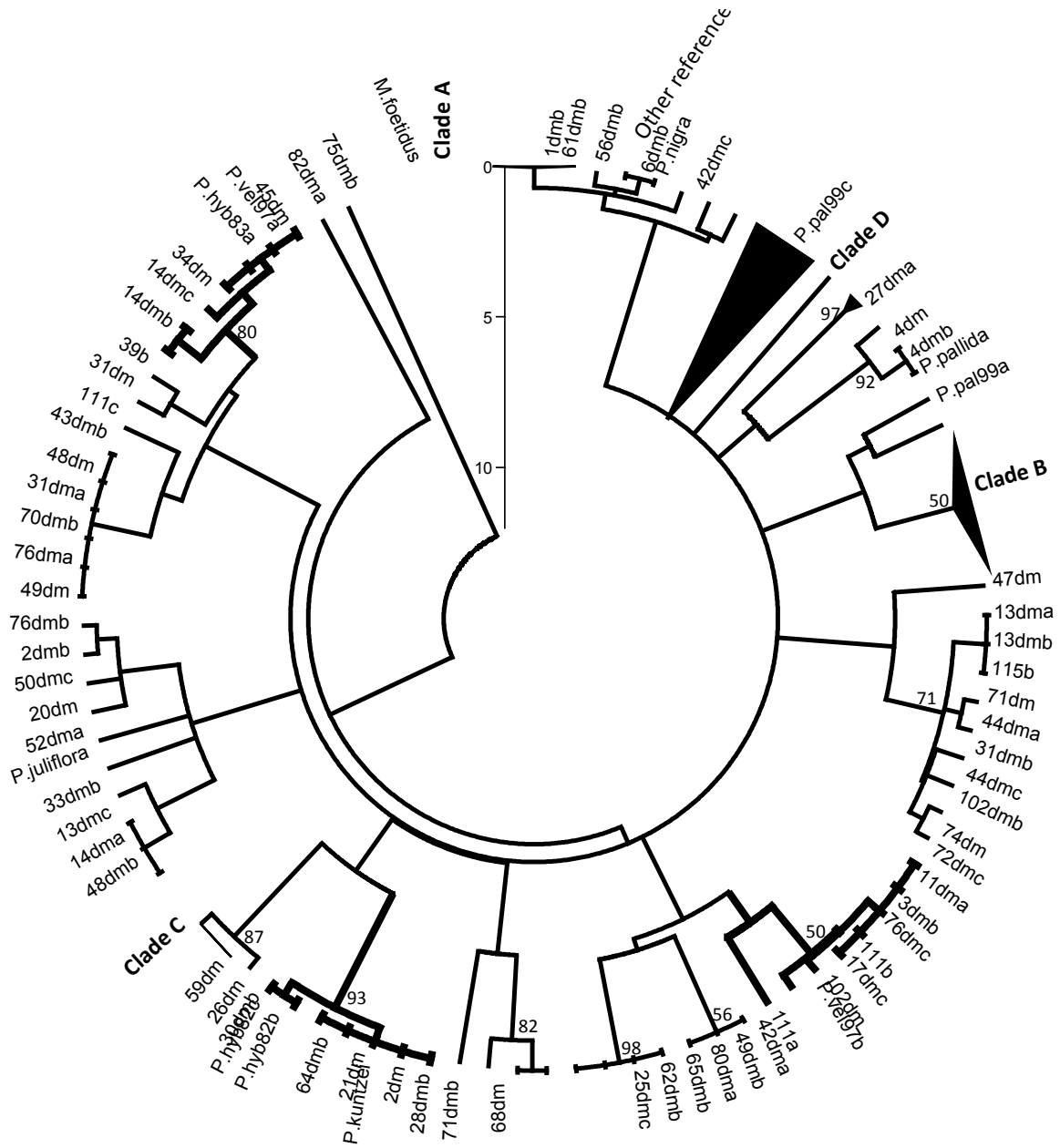


Figure 2.2 Maximum Parsimony tree showing the relationships of all South Africa *Prosopis* samples to reference samples (Bessega *et al.,* 2006), based on ITS 1 gene. Bootstrap values ≥ 50 are shown on clades. Collapsed clades **A**, **B, C,** and **D (**expanded for clarity in Fig. **2.3;** plates A-D) are those comprising South African samples and reference *P. glandulosa*, *P. laevigata*, *P. chilensis*, and *P. hassleri*, respectively. Clades containing at least one Australian accession are highlighted in bold in the figure above.

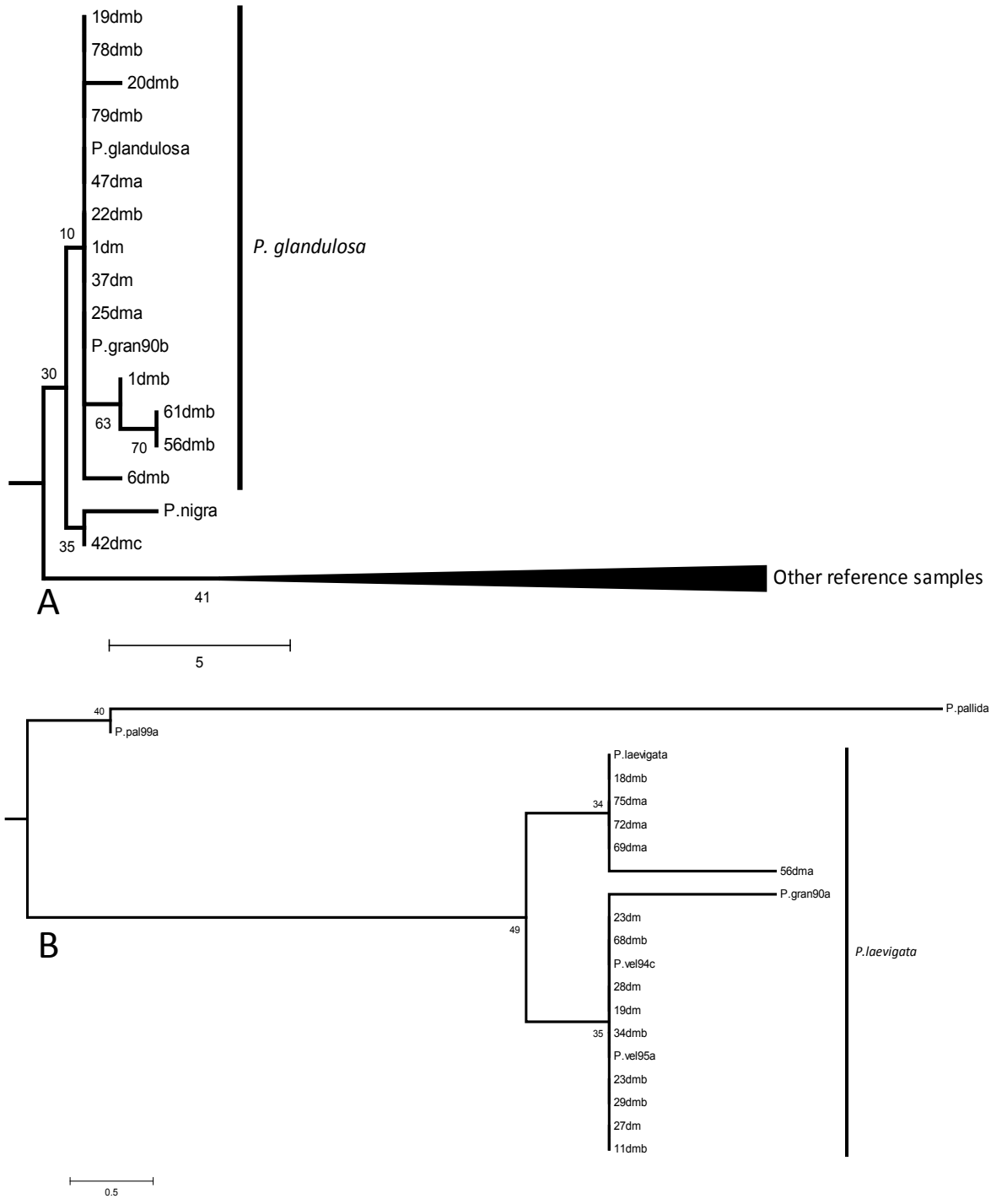
Targeted analysis

Overall there was low bootstrap support for most clades and three potential causes are suggested; 1) inclusion of unstable sequences (Sunderson & Shaffer, 2002) 2) inclusion of hybrids in phylogenetic analyses as this introduces topological changes and weakly supported cladograms, and breakdown in cladistic structure especially where hybridizing parents are distantly related (MacDade, 1992), 3) homoplasy resulting from random homogenisation of ITS copies (Nieto-Feliner *et al.,* 2001). I thus followed recommendation by Sunderson & Shaffer (2002) and "pruned" the main tree of some sequences and re-run the analysis to obtain better support for clades of interest here referred to as 'targeted analyses'.

Samples from Australia were all found to be heterozygous and some of their ITS copies did not fall within clades of the reference samples from Bessega *et al.,* (2006) included here.

For example, Australian "*P. velutina*" (95dma and 94dmc) formed a well-supported clade with *P. laevigata* with 100 % BS (in the targeted analysis Appendix 2.4), while sample 97 (also identified as *P. velutina* in Australia) was distinct from other similarly identified species (Fig 2.2 and Appendix 2.5). Australian *P. pallida* was not closely related to Argentinean *P. pallida* (Bessega *et al.,* 2006). The two ITS copies for Australian "*P. glandulosa*" were in different clades falling in both a *P. glandulosa* (reference) clade and a *P. laevigata* clade; indicating this Australian *P. glandulosa* accession may actually represent a hybrid (Fig 2.2, 2.3, and 2.4).

When the complete data set was analysed the inferred evolutionary relationships showed very weak clade support (52% for *P. chilensis* clade, 30% for *P. glandulosa* clade, and 49% for *P. laevigata* clade), except for *P. hassleri* clade (BS= 98%), see Fig 2.3 plates A-D.



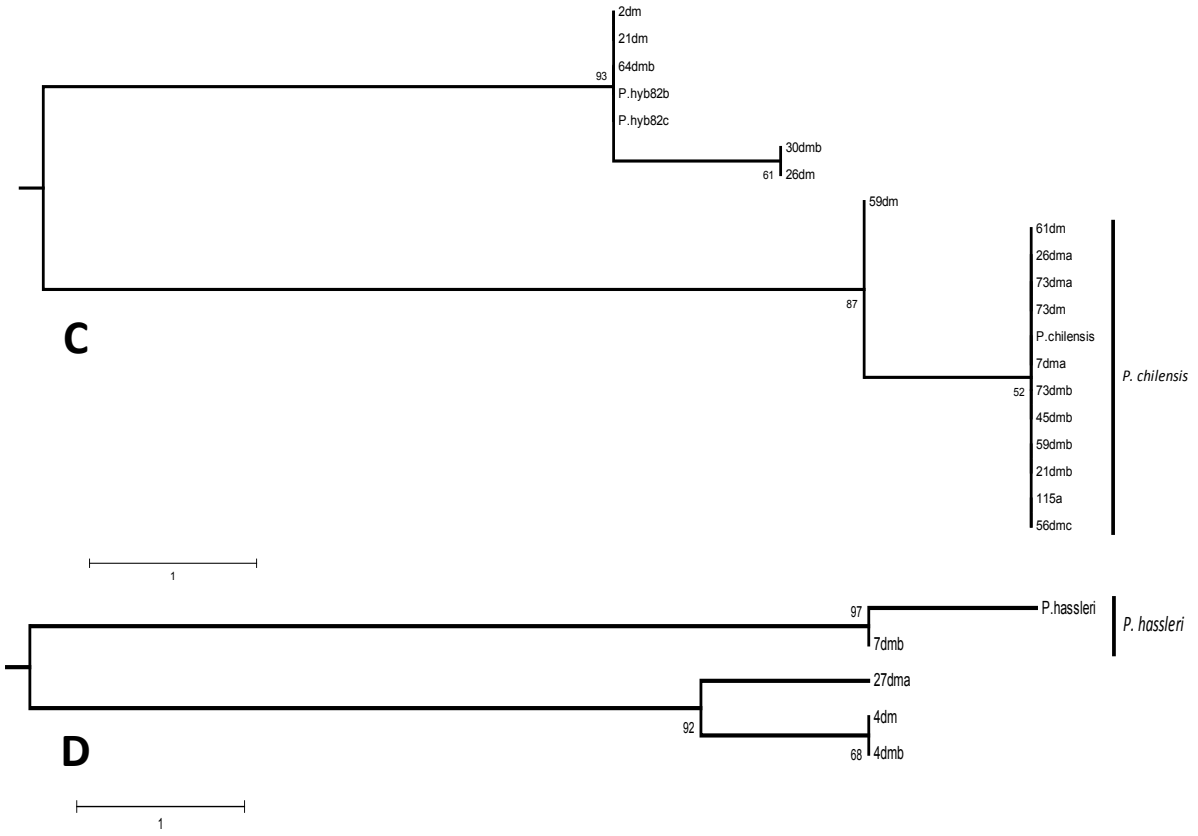


Figure 2.3 (panel A-D) Extracts of clades from Figure 2.2, showing A) putative *Prosopis glandulosa* clade; B) putative *P. laevigata* clade; C) putative *P. chilensis* clade; and D) putative *P. hassleri* clade. Samples **P. vel95a**, **P. vel94c**, **P. gran90a**, **P. hyb83a** refer to *P. velutina*, *P. glandulosa,* and a *Prosopis* hybrid (all from Australia). The reference species (from Bessega *et al.,* 2006) are given as complete species names.

When most hybrids were removed from the analysis and specific accessions targeted for analysis, South African samples formed well supported clades with some reference species (61dma with *P glandulosa* (99% BS support), 73dma with *P. chilensis* (100% BS support), *P. laevigata* and 75dma (99% BS support), *P. hassleri* and 7dmb (98% BS support) (Appendix 2.3). *Prosopis velutina* and *P. juliflora* from the Bessega *et al.,* (2006) study does not form a clade with any South African *Prosopis* taxa included here.

These evolutionary relationships are supported by the Neighbour-joining approach obtained from the Kimura 2-parameter nucleotide distances (Appendix 2.4).

Samples 1,4,14, and 73 had homozygous ITS sequences (Fig 2.2). The rest of samples had heterozygous ITS alleles corresponding to different *Prosopis* taxa, supporting the prevalence of extensive hybridization (Fig 2.2), and Appendix 2.6 is a phylogenetic relationship for some known species and their hybrids. I also observed multiple ITS copies for some samples (Fig 2.4).

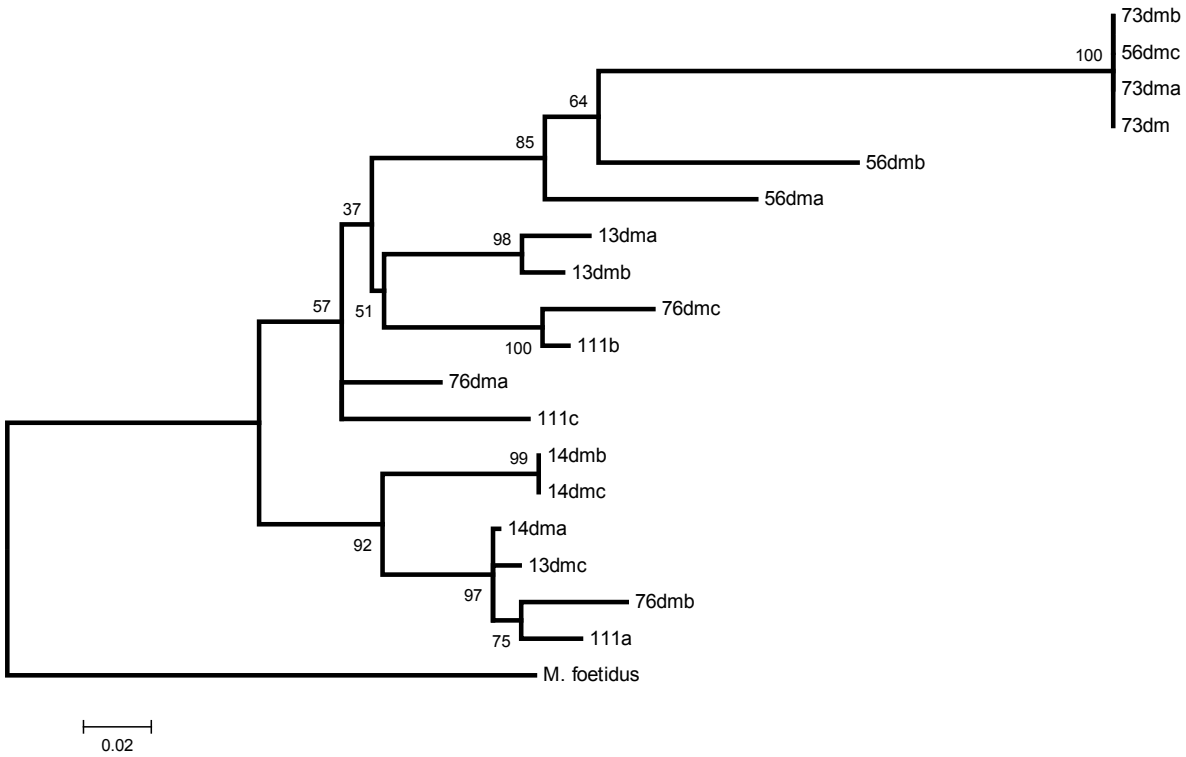


Figure 2.4 A Maximum Parsimony tree showing the relationships of multiple ITS copies of some confirmed *Prosopis* samples in this study. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. Of the six samples (each with three ITS copies), only one (sample 73) was monomorphic for ITS. Samples 14, 13, had at least two similar copies while samples 111,76, and 56 had all the three copies different.

DISCUSSION

The phylogenetic approach used here yielded numerous interesting and sometimes surprising results. First, I confirmed the presence of some suspected taxa to have been introduced to South Africa, including *P. chilensis*, *P. glandulosa*, and *P. laevigata*. Moreover, taxa thought to be present in South Africa (*P. velutina* and *P. juliflora*) could not be confirmed. This is particularly interesting since *P. velutina* was previously thought to be an abundant taxon in invasive populations in South Africa. I also identified new taxa, previously not known from South Africa, e.g. *P. hassleri* and others that could not be definitely identified to species level. Overall, it appears that most *Prosopis* taxa freely hybridize in South Africa and that invasive populations represent a hybrid swarm.

The taxonomic mystery of invasive *Prosopis*

Records indicate the importation of *P. juliflora* and *P. velutina* seeds to South Africa (Zimmermann, 1991; Poynton, 2009), yet this study found no evidence that these species are currently present in South Africa. The lack of *P. velutina* is especially surprising as it is only one of two taxa listed in current legislation (Conservation of Agricultural Resources Act (CARA) 43 of 1983), and is considered one of the most prevalent taxa in South Africa (Zimmermann, 1991; Impson *et al.,* 1999; Poynton, 2009). The apparent absence of these species could mean: 1) that they were introduced but did not survive and spread; 2) these species were misidentified at the time of introduction. The latter is a credible suggestion considering that at around the time of *Prosopis* introductions to South Africa, even in the native range, *P. glandulosa* was cited as *P. juliflora* (Nilsen *et al.,* 1986). Lastly, it is also possible that these taxa were not sampled in the current study.

More interestingly, this study has also identified some *Prosopis* species that do not fall within clades of any of the known reference species included here. I suggest four potential explanations for the existence of these 'unknown' clades.

First, the samples I collected could contain additional *Prosopis* species for which I did not have native range reference material. Out of the 44 species recognized in the genus I only included 18 in my study. My reference species contained samples of all species recorded as being introduced to South Africa as seed (6 species), but as *P. hassleri* was found, either some other species were introduced, or they were introduced under a different name.

This is feasible since some morphologically similar *Prosopis* exist sympatrically in their native ranges (Martinez, 1884). Such morphologically similar *Prosopis* taxa have been found to be very distinct genetically (Bessega *et al.,* 2006). Considering that at the time of introduction species identification was based solely on morphology, introduction of misidentified species was highly likely.

Importantly, the taxa lacking phylogenetic identities could represent unknown *Prosopis* species, not yet identified. At a morphological level, (Johnson, 1962), noted of some yet to be described species in the native region of *Prosopis laevigata*. In South Africa (Poynton, 2009) reports of some undescribed *Prosopis* species to have been under trial in Kimberly. Since most *Prosopis* introductions pre-dated the last review of the genus by Burkart, (1976), it is not clear how far this review was followed up regarding correcting previous field identifications; or whether it included descriptions of any new species as suggested by morphological observations (Johnson, 1962), prior to the review.

Thirdly, the samples that could not be assigned to a particular species clades in my phylogenetic analysis could be novel genotypes, ecotypes, strains, or even sexual species resulting from inter-specific hybridization and introgression (Abbot, 1992; Ellstrand & Schierenbeck, 2000; van Droogenbroeck *et al.,* 2006; Schierenbeck & Ellstrand, 2009). Hybridization and associated lateral gene transfer can, over time, preclude the expectation of hybrids being intermediates, at a molecular level, of associated parents (Sang & Zhong, 2000). Hybridization has long been known to be important in *Prosopis,* both in the native (Graham, 1960; Hunziker *et al.,* 1986; Vega & Hernandez, 2005) and introduced ranges (Zimmerman, 1991; van Klinken & Campbell, 2001).

Fourth, the presence of multiple ITS copies (Fig 2.4) in individual taxa, due to intra-genomic polymorphisms (IGPs), could also explain the presence of unknown taxa, since in my study I only had one ITS1 copy per reference taxa. While concerted evolution, among other processes, is expected to homogenize ITS repeats so the gene behaves as a single copy (Soltis *et al.,* 2008), incomplete concerted evolution leads to some ancestral parental repeats being obtained, after sequencing, as pseudogenes alongside functional ITS copies (Alvarez & Wendel, 2003) thus the observed multiple copies.

In *Prosopis*, hybridization is a known occurrence both in the native and introduced range as such the observed multiple copies could represent ancestral hybridization events "caught" before completion of concerted evolution (Soltis *et al.,* 2008). Such multiple copies could potentially have increased some homoplasy (Alvarez & Wendel, 2003) leading to observed low bootstrap support (Brandley *et al.,* 2009) as observed in the analysis (Figure 2.2 & Figure 2.3 A-D).

For my study, the fourth observation seems rather surprising considering that in a similar study (i.e. one where ITS was used) individuals of the same species showed < 1% ITS sequence variation (Bessega *et al.,* 2006). The high presence of heterozygosity and IGPs in ITS copies from one individual, as revealed through cloning in this study, can therefore be explained as resulting from inter-specific hybridization (and associated incomplete concerted evolution) since pure parental taxa in plants are largely known to be monomorphic for ITS (Kock *et al.,* 2009).

Samples from Australia also highlight the muddled taxonomy of *Prosopis* (Fig 2.2; Appendix 2.5). An apparent *P. glandulosa* population from Australia located at Nicholson Station near Halls Creek (18.0167°S 128.883°E) from my analysis appears to be a hybrid between *P. glandulosa* and *P. laevigata.* Similarly, what is thought to be *P. velutina* at Comongin near Quilpie (26.45°S 144.32°E) in fact comprises a hybrid between *P. laevigata* and some yet to be identified species. The population at Comongin was previously identified as *P. flexuosa* (Csurhes, 1996). Of all the samples received from Australia, none matched with molecular identification of Argentinean samples except for those which turned out to be *P. glandulosa X P. laevigata* hybrids. These examples underscore the extent of the taxonomic confusion in the invasive range of *Prosopis* species, not only in places like South Africa, but globally.

On hybrids and hybridization

Prosopis populations in South Africa comprise mainly hybrids. One critical question is whether they were introduced as hybrids or as pure parental species. For example, *P. hassleri* in South Africa is found as a hybrid with *P. chilensis,* the first known report of such a hybrid. The other species thus far confirmed in South Africa are mostly hybrids of both known and unknown parents of introduced *Prosopis* taxa.

While it can be hypothesized that some of the initial introductions included hybrids, it is also possible that the diversity of seed sources for the South African *Prosopis* populations had enabled previously allopatric species to hybridize in South Africa after being co-introduced. *Prosopis glandulosa* is native to North America whereas *P. chilensis*, *P. laevigata* and *P. hassleri* are native to South America (Pasiecznik *et al.,* 2001). It is possible that hybrids were introduced (involving the natively sympatric pairs mentioned above), but this seems unlikely for *P. glandulosa* X *P. chilensis* whose parental species are allopatric in their native ranges, suggesting that some hybridization has occurred in the introduced ranges. The apparently high levels of hybridization shown in this study confirm that species of section Algarobia do form a syngameon as previously thought (Palacios & Bravo, 1981 quoted in Catalano *et al.,* 2008).

While hybrids of some *Prosopis* species combinations are partially or completely sterile (Catalano, *et al.,* 2008) species that are in South Africa comprise the freely hybridizing ones. I found hybrids of *P. chilensis, P. glandulosa, P. hassleri,* and *P. laevigata;* producing copious amounts of seeds which germinated when planted in a greenhouse (see Chapter 4).

The report of a fertile hybrid between *P. hassleri* (Series; *Ruscifoliae)* and *P. chilensis* (Series; *Chilensis*) provides further evidence for inter-series hybridization, a scenario that led (Hensiker *et al.,* 1986) to call for a taxonomic review of the section Algarobia.

Conclusions and implications

Prosopis taxa in South Africa comprise both previously reported and unreported species. Hybridization is prevalent involves all taxa present, and pure parental lines are very rare. These findings have implications for different aspects of management especially biocontrol programmes, legislation, and autecological studies meant to inform management.

In the case of biocontrol, correct species taxonomy is particularly crucial considering that the effectiveness of control agents depends on how specific they are to a particular species, but sometimes as low as at biotypes level (Le Roux & Wieczorek, 2009).

In case of *Prosopis* in South Africa, the initial agents introduced to control *Prosopis* invasion were meant to target the species that were thought to have been introduced, mainly *P. glandulosa,* and *P. velutina* (Zimmermann, 1991). As such, the presence of unreported species (and hybrids) coupled with the high prevalence of hybridization in South Africa is worrisome; because genetic stability of both host and pest are crucial for a successful biocontrol programme (Weidemann & Tebeest, 1990), and in some hybrid plant species, hybrids are thought to limit success of biocontrol programmes (Zalucki & Day, 2007; but see Blair *et al.,* 2008). Considering that currently, biocontrol of *Prosopis* in South Africa is perceived inadequate with plans to try new agents underway (Zimmermann *et al.,* 2004 & Zachariades *et al.,* 2011); I recommend that the host-specificity of potential agents should possibly be reassessed. If there are any agents that are specific to particular taxa or varieties it is unlikely they will provide efficient control in South Africa thus more generalist enemies should be considered. Considering that hybrids can also be more susceptible biocontrol agents (Blair *et al.,* 2008), I recommend that host specificity of current natural enemies be verified for different *Prosopis* species and their hybrids to determine whether the observed inadequacy of the current agents is due to hybridization.

Legislation of poorly identified taxa does compromise management (Lacerda & Nimmo, 2010) because the true invading species stock cannot be determined. For *Prosopis* species in South Africa, there is thus a need in view of the current findings, to review its legislation to compliment management efforts.

The current legislation; Conservation of Agricultural Resources Act (CARA-43 of 83, amended 2001) recognises only *Prosopis glandulosa* (with hybrids) and *Prosopis velutina* (with hybrids) as invading *Prosopis* taxa in South Africa, a view echoed by Henderson, (2001) in her treatment of South African alien invasive plants. I thus recommend that *Prosopis* taxa in South Africa be dealt with as a genus, unless a risk assessment provides clear evidence that a particular species poses a low risk and will not hybridise.

These findings have indirect implications on autecological studies meant to inform management decisions, such as bioclimatic modelling.

Bioclimatic modelling, and habitat suitability modelling studies has been touted as being useful tools for prediction (Crossman & Bass, 2008) and also serving as an early warning before alien taxa become invasive (Thuiller *et al.,* 2005).

One of the critical assumptions of these models is that genetic and phenotypic composition of a given taxa remains constant in space and time (Jeschke & Strayer, 2008). Modelling of future distributions of *Prosopis* taxa and their hybrids will therefore remain a challenge, first because taxonomically well-defined taxa are rare hence the determination of species-specific realised niche is impossible.

Secondly the high level of hybridization and consequent introgression entails that both the genetic and phenotypic stability assumptions of the models are violated, rendering any predictions inconclusive. I thus urge caution in interpretation of any such findings regarding *Prosopis* species in South Africa considering the variability in adaptation that comes especially with hybridization.

Finally, this study highlights the need for review of the biogeography of the native range of the genus, the resolution of taxonomic huddles associated with *Prosopis* in invasive ranges can only be done in this context.

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Chapter 3—Morphological identification of *Prosopis* taxa and their hybrids in South Africa

Abstract

Aim Accurate morphological identification of invasive species is crucial for understanding their ecology and for effective management. For *Prosopis* in South Africa, historical records suggest that six species were introduced, but identifying individuals based on morphological features remains difficult. Uncertainty about which species were introduced and reported hybridization is known to complicate species identification based on morphology. This study: 1) explores whether *Prosopis* taxa throughout the South African range of the genus can be identified to species level using a 'total evidence approach' that incorporates molecular data and morphological characters; and 2) evaluates the potential for developing a field key specific for taxa present in South Africa.

Location South Africa

Methods Two approaches were used in the morphological analysis. Firstly, character matching was used to identify *Prosopis* using the identification key developed by Burkart (1976). Secondly, character coding of 22 characters was used to construct morphological relationships among *Prosopis* morpho-species. Principal Component Analysis was used to visualize and identify the presence of distinct morphological clusters. Discriminant Analysis was used to confirm the clustering of the different morpho-species identified using Burkart's (1976) key. Identified morpho-species were compared with species identified using molecular data (Chapter 2) to determine the degree of congruence between the two approaches.

Results Morphological identifications revealed the presence of *Prosopis* species previously reported from South Africa as well as species not previously reported from the region. Although morphological clustering agreed in some cases with molecular data, there were notable differences. Only one introduced species, *P. chilensis* could be identified using morphological features, but some of their putative hybrids (identified using DNA sequencing data) could not be easily distinguished from parental species.

Plants purported to be *Prosopis glandulosa* hybrids were morphologically identified as pure *P. juliflora,* indicating lack of hybrid morphological intermediacy.

The key based on morphological features identified 31% of samples as *P. juliflora*, but molecular data failed to identify this species. Some *Prosopis* individuals in South Africa could not be identified to species level using the morphological key.

Main conclusions *Prosopis* species in South Africa cannot be reliably distinguished using existing morphological keys. This is probably mainly because of the proliferation of hybrids and extensive introgression which together have diluted morphological signatures. Any identification based on morphology is likely to be erroneous which renders the development of a field key a challenge. Molecular tools are required to confirm the identity of individuals and to confirm which taxa are present in the region.

Key words

Biological invasions, identification key, morphological taxonomy, *Prosopis,* South Africa, total evidence approach

INTRODUCTION

Plant morphology, the study of plant form and structure, has played a major role in plant science and has been applied in various fields of research, including genetics, physiology, ecology, evolutionary biology, phylogeny and systematics (Sattler & Rutishauser, 1997). However the value of morphology in plant systematics has come under scrutiny. While molecular techniques and enriched phylogenetic inferences have provided alternatives, these advances have also led to some conflicts with traditional morphological approaches (Hillis, 1997). Proponents of molecular plant systematics argue that it provides a large amount of heritable data that are not affected by environmental conditions (Hillis, 1987; Jenner, 2004), whereas morphological data is sometimes less tightly linked to the underlying evolutionary relationships, often leading to homoplasy and/or polyphyletic placements in phylogenies (Thomas *et al.,* 2011). Morphological inference of taxonomic placement is further complicated by lack of consensus among taxonomists regarding morphology-based phylogenies (Packer *et al.,* 2009, and references therein), the prominence of hybridization between different plant taxa, and high levels of phenotypic plasticity in plants. Both hybridization and geographic localisation (adaptation) can produce morphologically intermediate and/or novel individuals (Albert *et al.,* 1997; Whitney & Gabler, 2008; Krishnankutty & Chandrasekaran 2008), changes that reduce the value of morphological characters as indicators of taxon identity. Therefore, some scholars argue strongly that morphology has limited value in phylogeny reconstruction (e.g. see Scotland *et al.,* 2003).

While accepting that molecular data have inherent robustness and objectivity, others contend that phylogenetic classification, when divorced from 'morphological' taxonomy, is ephemeral and erodes the accuracy and information content of the language of biology (Wheeler, 2004). Such language is crucial for field biologists who use morphology for identifying specimens and for informing many types of decisions. Proponents of the role of morphology in phylogenetics assert that if morphological evidence is ignored, the phylogeny of over 99 % of life is ignored (Jenner, 2004).

The 'total evidence approach', involving the use of both morphological and molecular data in taxonomic inference, has gained prominence (Rieseberg & Ellstrand, 1993; Fukami *et al.,* 2004; Tovar-Sánchez & Oyama, 2004).

Results have varied with both contradictions and congruence between these approaches being reported (Douzery *et al.,* 1999; Lee, 2001). I consider that both molecular and morphological approaches have a contribution to make towards identification and phylogenetic placement; and that *Prosopis* in South Africa is a group whose study can benefit from both approaches.

Identification of *Prosopis* in South Africa

Classification and identification of *Prosopis* species in South Africa and elsewhere are currently based primarily on morphology (Saidman & Vilardi, 1987; Pasiecznik *et al.,* 2001; Poynton, 2009). Such identification approaches have led to taxonomic uncertainties in part due to extensive hybridization between different taxa and phenotypic plasticity. For example, *Prosopis* species have been shown to vary in their leaves and fruits due to exposure to variable stresses (Villagra *et al.,* 2010); with seed size, shape, colour, texture, and its chemical composition being variable in geographical regions (Werker *et al.,* 1973). *Prosopis* invasions in South Africa represent a good study system for investigating how hybridization and introgression, in concert with local environmental conditions, have altered morphological diversity. More importantly, genetics results (Chapter 2) suggest that more *Prosopis* taxa are present in South Africa than previously thought. This emphasizes the need for morphological characterization of invasive populations. Furthermore, taxonomic uncertainty also exists in other parts of the world where *Prosopis* species are introduced and naturalized or invasive (Richardson & Rejmánek, 2011). The results from this work will be useful for clarifying the distribution of introduced *Prosopis* taxa and for understanding the invasion ecology in this genus.

In this study I will seek to use a total evidence approach to identify taxa of the highly invasive genus *Prosopis* in South Africa by using morphological data and including genetic data from Chapter 2. The study will first identify South African *Prosopis* species based on existing morphological key described by Burkart (1976), and construct a morphological relationship of South African *Prosopis*. Secondly I will compare species identity using morphology and molecular identification.

MATERIALS AND METHODS

Plant material: Leaves and seed pods from mature plants were collected from across the distribution range of *Prosopis* in South Africa (Appendix 2.1; see Chapter 2.). Mature leaf material was collected at a height of about 0.5 metres above the canopy base (depending of tree height). Between 10-12 leaves and between 10-50 seed pods were collected per plant. Samples were air-dried in the laboratory before measurements were taken. A total of 22 characters were analysed, (Appendix 3.2**).** These characters were chosen first because of their frequent use in morphological identification and secondly because they are used in the identification key for *Prosopis* described by Burkart, (1976) as adapted by Pasiecznik *et al.,* (2003) (Appendix 3.1).

Morphological relationships

Each sample was compared with Burkart's key descriptions for each species. Data was scored in a binary fashion, with characters corresponding to the key a scored as 1 and those disagreeing scored as 0. The total score was used to determine what species a sample is likely to represent. Each character was given equal weight and no specific combinations of characters were considered (see Table 3.1). A list of the samples analysed and their character descriptions is given in appendix **3.**2.

Table 3.1 Approach on how initial morphological identification was done. 13 characters for each sample were compared to the description in Burkart's key to determine which of the reference species it agreed with. Agreement is denoted as 1 and disagreement is denoted 0. These scores were summed to determine overall agreement. In notation xPy; x represents the sample number while y represents the population number and P is a short form for population. In this hypothetical example, the sample would be identified as *Prosopis chilensis.*

Sample identity	**Reference species**	**Character number**												
		1	2	3	4	5	6	7	8	9	10	11	12	13	**∑**
xPy	*P. juliflora*	0	1	0	0	1	0	0	1	0	1	0	0	1	**5**
xPy	*P. pallida*	1	1	0	0	0	0	0	1	1	0	1	1	1	**7**
xPy	*P. glandulosa*	0	1	1	0	0	0	0	1	0	0	1	1	0	**5**
xPy	*P. velutina*	0	1	0	1	0	0	0	1	0	0	1	0	1	**5**
xPy	*P. alba*	1	1	1	0	1	0	1	1	1	1	1	0	0	**9**
xPy	*P. chilensis*	1	1	1	1	1	1	1	1	1	1	1	1	1	**13**
xPy	*P. cineralia*	0	1	0	0	0	0	0	1	0	0	1	0	1	**4**

Measurements approach

Qualitative characters, such as thorn presence or absence and pod colour, were scored from observation. Others including pod margin and cross-section, pod shape and leaflet shape were scored using detailed descriptions by Pasiecznik *et al.,* (2003). Seeds were removed from the seed pods and counted. Seed counts were made on at least five pods per plant. For other quantitative character measurements (pod length and width, leaflet length and width, pinae length and distance between leaflets) measurements were taken to the nearest millimetre. For curved pods, a string (capturing the full length of the pod), was used. Figure 3.1 is a pictorial view of where/how some measurements were done.

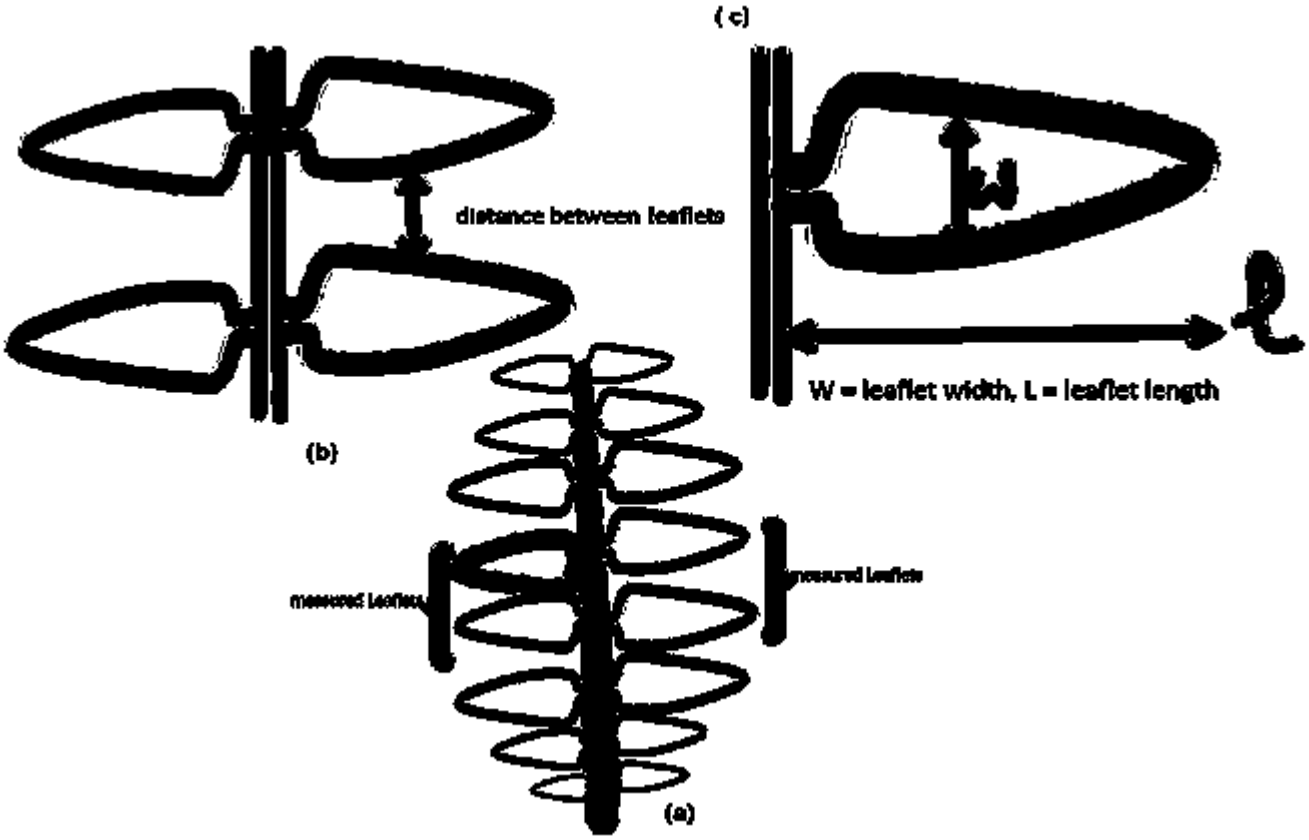


Figure 3.1 Showing positions where leaflet measurement were taken; leaflet length included leaflet-stock. Leaflets positioned midway (a) along the pinnae were used to make the measurements. Distance between leaflets, leaflet length and width were measured as shown in (b) and (c). All measurements were done on a total of five samples and the values averaged.

Data analysis

Raw character data were used in a Principal Component Analysis in Statistica 10 software (StatSoft 2010) to determine any apparent taxon groupings and to determine which characters explained most of the variation among species. A Discriminant Analysis (DA) on the morphologically identified species was done in Statistica 10 software (StatSoft 2010) to assess how the different morpho-species grouped, particularly to determine the extent of overlaps between morphological characters. The analysis included all morpho-species identified using Burkart's (1976) key.

Molecular identification and analysis

ITS sequences for samples identified morphologically (using Burkarts key, Appendix 3.1) were used to reconstruct a phylogenetic tree including sequence data from known reference taxa. Detailed methodology used for comparative phylogenetic analyses is described in Chapter 2.

RESULTS

All species reported to be in South Africa, except for *P. pubescens* and *P. tamarugo*, were identified by matching characters as set out in Burkart's (1976) key based on 68 *Prosopis* samples that were sampled in this study. However, descriptions for some individuals matched species never before reported to be in South Africa, including *P. alba* and *P. pallida* and others which could not reliably be assigned to any species and thus classified as "unknown" using morphology (Table 3.2, Fig. 3.3).

Table 3.2 Species of *Prosopis* reported to have been introduced to South Africa (Poynton, 1990 & Zimmerman, 1991; Poynton, 2009), and evidence for their presence and invasive status. Species marked with (*) were identified using morphological features but were not previously known to have been introduced. Some unknown species were found; one of these (sample 5p25 (49)) has been confirmed by both morphological and molecular approaches. Morphologically identified *P. pallida* turned out to be an unknown species based on molecular data. Those identified as *P. juliflora* morphologically turned out to be either unknown species after a molecular analysis, or hybrids involving *P. laevigata, P. chilensis, or P glandulosa.* For the notation for sample identification (column 4), refer to Table 4.2. *Prosopis* spp. refers to samples for which the identity could not be distinguished between two possible species using the identification key (the "tie" was usually between *P. juliflora* and another species). NA, refers to cases where data is not available; the symbol "?" indicates situations where no information is available

***Prosopis* species**	**Number of samples morphologically confirmed**	**Molecular confirmation of morphological identity**	**Morphological sample ID. & (DNA reference number)**	**Reported invasion status**
P. glandulosa	None	Found as a hybrid between *P. laevigata* or *P. chilensis*	5p25 (47), 4p25 (48), 7p28 (56)	Very invasive and involved in hybridization
P. velutina	8	**Not confirmed in this study**	3p34 (68), 2p37 (75)	Very invasive and involved in hybridization
P. chilensis	14	Identified as hybrids and parental	1p30 (59), 3p24 (45)	Invasive and involved in hybridization
P. juliflora	21	**Not confirmed in this study.** unknown species	4p37 (76),16p26 (52) 9p26 (50),8p36 (74)	Its presence reported but debatable.
P. pubescens	Not sampled, but presence confirmed.	Analysis not done.	NA	Present at one location
P. laevigata	Not identified	**Confirmed to be present as** hybrids with *P. chilensis* and *P. glandulosa*	No morphological comparison done *for P. laevigata*	Presence not reported, but introduction reported yet debatable.
*P. pallida**	1	"Unknown" species	5p22 (42)	?
*P. alba**	4	"Unknown" species	9p34 (70)	?
*"Unknown"**	5	1	5p25 (49)	?
Prosopis spp.*	15	-	-	?

These samples are here forth referred to as “unknown” spp. Identified morpho-species showed no clear clustering (Fig. 3.2), and the discriminant analysis of morpho-species showed overlaps in character ranges (Fig. 3.3).

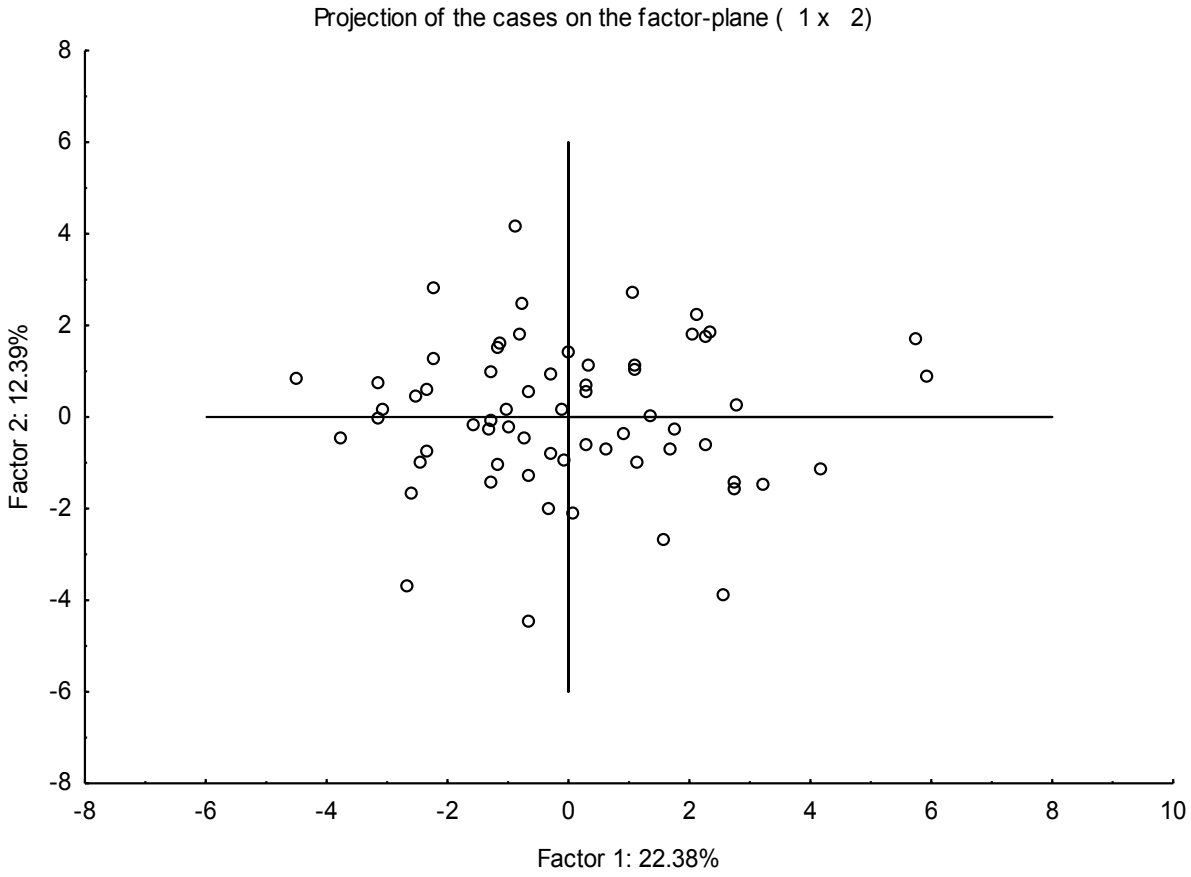


Figure 3.2 The position of *Prosopis* individuals in multivariate space using Principal Components Analysis, showing the lack of clear clustering among sampled *Prosopis* individuals in terms of morphological features.

There is some agreement between molecular and morphological characters, regarding “unknown” *Prosopis* taxa. In molecular analysis (Fig. 3.4), these form no clade with known taxa and are morphologically clustered separately as supported by the DA analysis (Fig. 3.3).

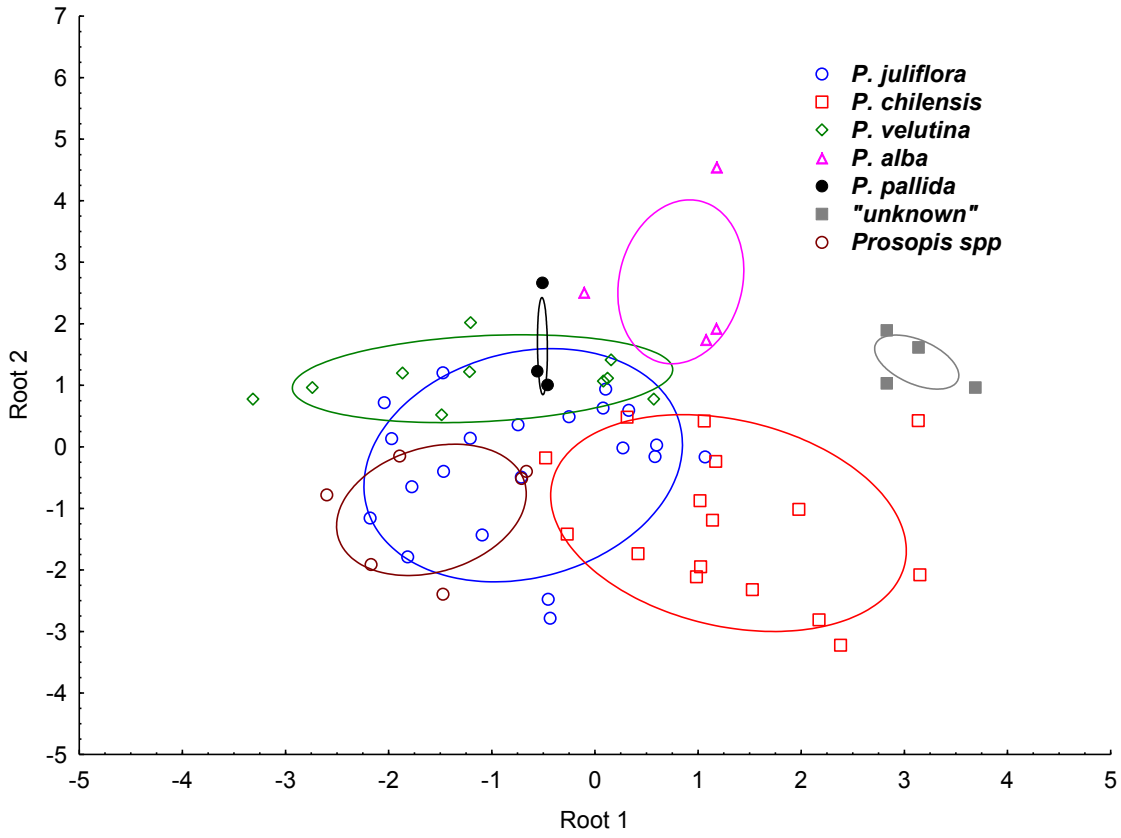
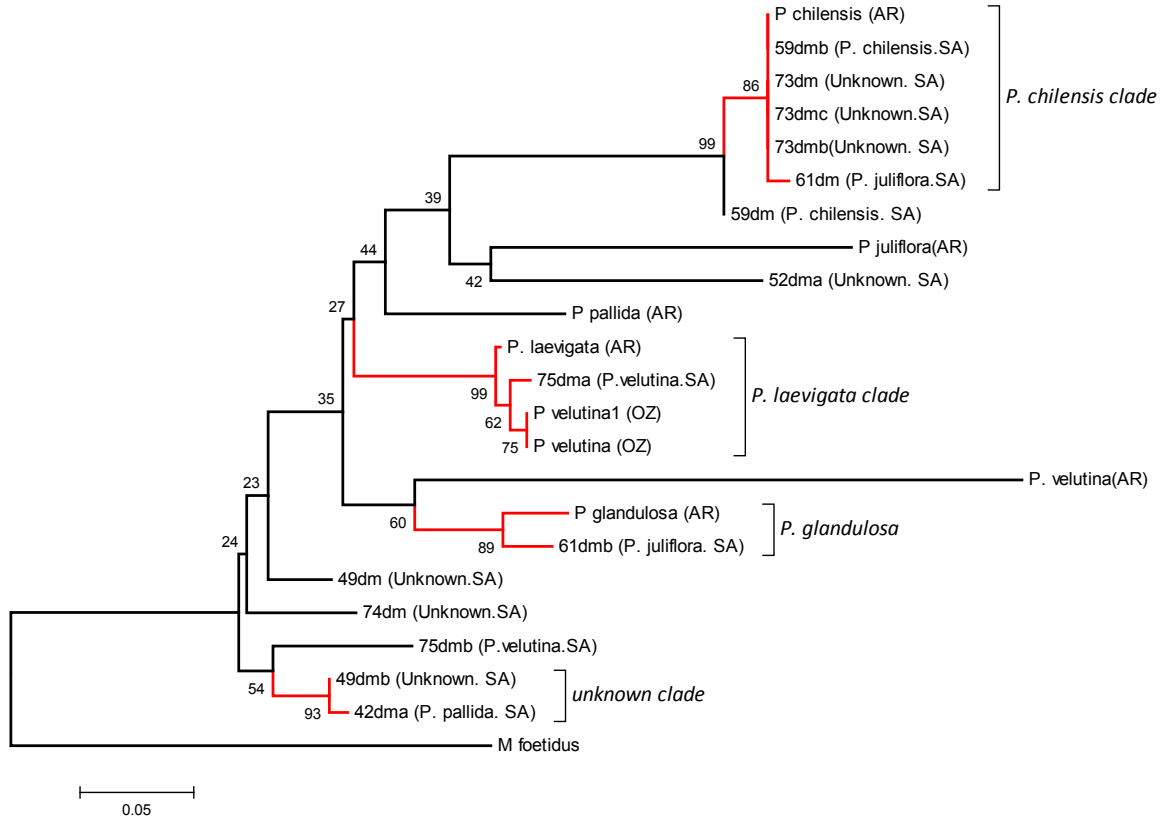


Figure 3.3 Discriminant analysis for *Prosopis* species identified using morphological features. There is overlap in morphological features between most species, but *P. chilensis* and *P. velutina* can be differentiated with some certainty. A grouping of "unknown" species also forms a distinct cluster. "*Prosopis* spp." refers to samples that could not be clearly distinguished between two species using the identification key (usually it was not possible to distinguish between *P. juliflora* and another species).

Morphological identification of *P. chilensis* matched with the identification using molecular data. One sample (number 75 in Fig. 3.4) that was morphologically keyed as *P. velutina* was actually a hybrid *P. laevigata* and an unidentified *Prosopis* taxon. Samples morphologically identified as *P. juliflora* (52 and 61, Fig. 3.4) did not match with molecular identification of the species. One of these (sample 61 in Fig. 3.4) was identified as being a hybrid between *P. chilensis* and *P. glandulosa* using DNA sequencing data. Samples 74 and 49 could not reliably be assigned to any species and thus classified as "unknown" using morphology and the molecular analysis confirmed this.



Morpho-species identity	Sample	Molecular-species identity
P. chilensis	59dm,	*P. chilensis* hybrid
P. velutina	75dm, *P. velutina* (OZ)	*P. laevigata* hybrid
P. glandulosa	none	NA
P. juliflora	61dm	*P. chilensis* X *P. glandulosa*
P. pallida	42dm	unknown
"Unknown"	49dm, 73dm, 74dm, 52dm	*P. chilensis*, and unknown

Figure 3.4 A comparison of results of morphological identification with molecular identification; morphologically identified samples end with SA (South Africa) and Australia samples end with (OZ). Reference samples end with AR (Argentina). In the analysis, two ITS copies (as obtained after cloning) of SA and OZ samples are used. Morphological species identification of SA samples is given after the sample number. The relationships were inferred using the Neighbor-Joining method (Saitou & Nei, 1987), from 1000 replicates (Felsenstein, 1985). Numbers on nodes are bootstrap test support. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method (Kimura, 1980).

The sample morphologically identified as *P. pallida* was classified as "unknown" based on the molecular analysis. Table 3.2 summarizes morphology- and molecular-based identifications. As with the genetics results (see Chapter 2), high levels of diversity were found among *Prosopis* taxa in South Africa in terms of morphological features (Fig. 3.5).

Figure 3.5 Considerable diversity in pod morphology of *Prosopis* individuals from across the range of the taxon in South Africa clearly shows the problem with species identification based on morphological features. **A**. *P. velutina* as identified using morphology but molecular identification shows it to be a hybrid between *P. laevigata* and another species. **B.** *P. chilensis* hybrid confirmed by molecular data not resembling *P. chilensis* in terms of morphology. **C.** Identified as *P. juliflora* using the morphological key, but shown to be a hybrid between *P. chilensis* and *P. glandulosa* molecular data. **D.** *P. juliflora*, as identified using morphology but shown by molecular data to be another, unknown, species. **E.** *P. chilensis* as identified using both morphological and molecular data. **F.** A *Prosopis* taxon with pods resembling those of *P. alba* but which could not be identified using either morphologically or molecular techniques. **G.** A *Prosopis* taxon that could not be identified using either morphological or molecular data. **H** *P. chilensis* X P. *hassleri* hybrid, identified using molecular data. **I-K**, are yet to be identified *Prosopis* taxa.

DISCUSSION

Combined genetic (Chapter 2) and morphological characterization of *Prosopis*, while not always congruent, did reveal that *Prosopis* invasions in South Africa are taxonomically poorly understood and that the diversity of taxa currently represented in invasive populations over a large part of South Africa has been underestimated. My identifications did not always correspond with historical records of *Prosopis* introductions to South Africa. I suggest four main reasons why the diversity of *Prosopis* species in South Africa has been underestimated: 1) unrecorded introductions; 2) lack of taxonomic expertise during introductions; 3) post-introduction hybridisation; 4) phenotypic plasticity in response to environmental variation in South Africa.

The introduction history of *Prosopis* to South Africa is not completely known, especially because unrecorded *Prosopis* accessions that were brought in by private land owners and farmers in the 1980s (Harding, 2010 pers. comm.). This has probably led to the introduction of additional species, besides those noted by Poynton, (1990). Second, some of the introductions may well have been incorrectly identified. In the native ranges, morphological identification, especially of the section Algarobia, is difficult due to similarity of species morphs which is further compounded by hybrids whose morphs defy proper placement within the genus (Saidman & Vilardi, 1987). Thirdly, hybridization could have had formative impact on morphological characters. Morphology in hybrids is assumed to be either intermediate or a blend of parental morphs (Wagner, 1969). However, in populations where multiple species are hybridizing the scenario is likely to be complicated. It has been reported that whereas first-generation hybrids are mosaics of parental intermediate characters, later generation hybrids are largely embroiled by novel characters as introgression progresses (Rieseberg & Ellstrand, 1993). Indeed, molecular markers used in this study may not fully capture the extent of hybridization based on heterozygosity. Subsequent backcrossing and even inter-hybrid cross-fertilization will greatly dilute a one-locus genetic signature of hybridization and parental contributions. There is lack of resolution of morpho-species and a noticeable overlap of morphological characters in *Prosopis* species in South Africa (Figs. 3.3 and 3.4) due to hybridization. My results have shown that for *Prosopis* in South Africa hybridization involves at least four known parental species and other unknown species.

The resulting diversity in morphology should thus be complex, and acquisition of novel characters expected.

In plants, interspecific gene flow and introgression is an important mechanism of speciation due to its immediate effects on fitness and genetic makeup (e.g. Rieseberg *et al.,* 1990; Hedge *et al.,* 2006). My results indicate that there are hardly any 'pure' parental *Prosopis* taxa in South Africa and that most populations represent a hybridization swarm of many different taxa and thus potentially evolutionary novelty (e.g. van Klinken & Campbell, 2001). The invasive range of *Prosopis* in South Africa covers a heterogeneous range of climatic and geographic habitats. In plants, morphological changes are known and are thought to be strategic adaptation to localised environment (Ellison *et al.,* 2004) which tends to vary with altitude (Meinzer *et al.,* 1985) rain fall gradients (Castro-Díez *et al.,* 1997), and temperature gradients (Boese & Hunner, 1990) among other factors. Phenotypic plasticity, could also have led to the observed morphological variants. Most of the samples identified morphologically did not much molecular identification (Table 3.2). Hence identification of *Prosopis* in South Africa based on morphology could be highly misleading. In native region of *Prosopis* geographical variation in *Prosopis* leaf morphology are reported (Graham, 1960), and have been attributed to hybridization and backcrossing, phenomena that yields intermediate morphs to parental morphs (Narajo *et al.,* 1984). These factors combined have led to *Prosopis* species being identified differently between native and introduced ranges, as reviewed in (Pasiecznik *et al.,* 2001). For Prosopis in South Africa, the direction of morphological and genetic change can clearly be determined with further studies involving all species from native range. Such a study should involve additional markers such as microsatellites which have been used to elucidate species relationships and hybridization (Queller *et al.,* 1993; Alvarez *et al.,* 2001)

CONCLUSIONS

Historical records of *Prosopis* introductions to South Africa do not reflect what is observed in the field. Morphological identification of *Prosopis* in South Africa is compromised by extensive hybridization. Despite such uncertainty, *P. chilensis* can still be identified morphologically, although it remains a challenge to determine whether individuals are pure species or hybrids. There are taxa whose morphological descriptions match those of *P. velutina* yet they are *P. laevigata* and its hybrids.

Prosopis glandulosa exists largely as a hybrid with other species and there are hardly any morphs that fit the typical parental descriptions.

Overall, *Prosopis* morphology shows great variability and plasticity, and accurate identification can only be achieved by means of molecular analyses.

There is, however, agreement between both approaches used here in indicating that there are more species of *Prosopis* in South Africa than previously thought. This lack of conclusivity of morphological identification in *Prosopis* has led to taxonomic confusions elsewhere. Future research should seek to use both molecular and morphological approaches to identify invading *Prosopis* taxa. This approach could help in implementation of effective management strategies considering that it is generally accepted that in *Prosopis*, problematical species identification is a barrier to effective management.

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Chapter 4—Genome and seed size variation in South African *Prosopis* species: Spatial extent and implications for invasiveness

Abstract

Aim Variation in genome size and seed size may influence invasiveness in a number of plant taxa. This study investigates the relationship between genome size and seed size in invasive populations of *Prosopis* spp. in South Africa and how these influence plant fitness, measured as germination and early growth. I further seek to determine how these two attributes are influenced by some geo-climatic variables

Location South Africa

Methods Seeds from 250 parental plants from throughout the distribution range of *Prosopis* in South Africa were germinated and grown under common garden conditions. Fresh leaf material was collected from the seedlings, and genome size estimated from the samples using flow cytometry. Plant height was measured and biomass harvested following three months of growth. Germination percentage for scarified and non-scarified seeds was assessed for different seed size classes

Results Genome size values for *Prosopis* taxa found in South Africa ranged from 1.17 pg to 1.26 pg. There was no significant correlation between genome size and seed size. Genome size obtained from multiple seedlings from a single parent showed up to 4.2 % variability, which suggests substantial hybridization and an open breeding system in invasive *Prosopis* populations in South Africa. Heavier seeds result in larger seedlings (plant height and biomass) three months after germination. Seed germination was much greater at higher temperatures and following scarification.

Main conclusions In invasive *Prosopis* taxa in South Africa, genome sizes represent a mosaic of variation due to extensive hybridization. Large seed size may play a role in invasiveness of *Prosopis* as it positively influences germination and early growth.

Key words

Biological invasions, biomass, common garden experiment, flow cytometry, genome size, hybridization, *Prosopis*, seed size,

INTRODUCTION

The search for factors that promote invasiveness has long dominated the literature in plant invasion ecology (Richardson & Pyšek, 2006 and references therein). Generally, propagule pressure, ecosystem invasibility and biotic characters of alien species strongly determine invasion success (Catford *et al.,* 2009). A number of studies have found support for introduction history i.e. propagule pressure (Lockwood *et al.,* 2005; Von Holle & Simberloff, 2005; Catford *et al* 2011) and residence time (Wilson *et al.,* 2007; Schimidt & Drake, 2011), as strong predictors of invasiveness. In addition, increased disturbance has long been understood to make ecosystems more vulnerable to invasion by non-native species (Baker, 1974).

Recently, studies on the role of intrinsic species traits in facilitating plant invasions has also been a focus of research (Pyšek & Richardson, 2007 and references therein) for such traits as genome size, specific leaf area, seed size, and self compatibility (van Kleunen *et al.,* 2010; Gallagher *et al.,* 2011; Buckley *et al.,* 2003 ; Baker, 1974). However no apparent generalities have emerged (Pyšek & Richardson, 2007). This is attributed to the diversity in invasive taxa and in both ecological and evolutionary responses in the variable recipient communities. In the last 30 years such studies have mostly been taxon-specific and in defined regions and ecosystems (Krivánek & Pyšek, 2006).

At least five species of *Prosopis* are known to be invasive in different parts of the world but there is considerable taxonomic uncertainty and hybridization is known to occur in many regions where multiple taxa are planted (Richardson & Rejmánek, 2011). Few studies have explored the traits associated with invasiveness in this genus (but see Archer, 1995; Brown & Archer, 1989; Bush &Van Auken, 1991; Sharma & Dakshini, 1998; Treuer, 2006). In this chapter, I focus on genome size and seed size and examine how these traits vary across the invasive range of *Prosopis* found in South Africa, as, despite *Prosopis* being invasive in a number of regions, such a study on *Prosopis* has not yet been done anywhere. For a discussion on *Prosopis* introduction and its invasiveness in South Africa see Chapters 1 and 2.

Genome and seed size in plants.

Genome size, the amount of DNA in a monoploid set of unreplicated chromosomes (Soltis *et al.,* 2003), is highly variable in plants. For example, (Bennett *et al.,* 2000), estimate an 800-fold variability in plants. Unlike the C-value which is the amount of DNA in a gamete irrespective of ploidy level, genome size as quotient of 2C-value by the ploidy level (Grotkopp *et al.,* 2004). Being fairly stable, genome size estimates have been suggested to be of some use in plant systematics (Ohri, 1998).

Seed size is one of the least variable reproductive characters in plants (Temme, 1986), with important consequences for germination, dispersal, seed-water relations, and the potential of seed emergence from different burial depths (Wulff, 1986; Buckley, 2003). In variable ecosystems seed size has been found to be phenotypically plastic (Pichancourt, & van Klinken, 2012) Variations in seed size have been attributed to nutrient levels, water availability, altitudinal, longitudal and latitudinal variability (Lee & Fenner, 1989; Baker, 1972; MacWilliam *et al.,* 1968; Rejmánek, 1996). Moreover, there appears to be a positive relationship between seed size and genome size (Bretagnolle *et al.,* 1995) and, given the arguments above, understanding these relationships may help in determining the factors that drive successful plant invasions (Figure 4.1)

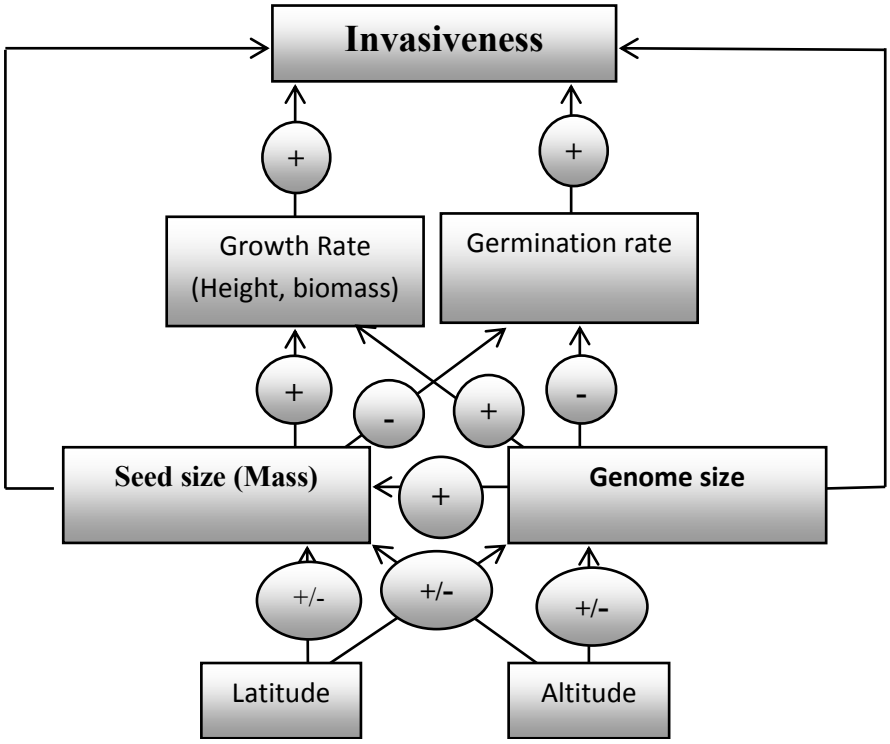


Figure 4.1 A schematic diagram showing how genome size and seed size could directly or indirectly affect plant invasiveness through their effects on growth and germination processes. Modified from Grotkopp *et al.,* (2002) and Rejmánek, (2000).

Studies on the relationship between genome size and plant growth have yielded different results for different taxa and regions. Revees *et al.,* (1987) found a negative correlation between genome size and elevation in *Dactylis glomerata*. Grotkopp, (2004) reported that tropical species have smaller genomes than their temperate counterparts. While genome size could influence adaptive plant development and growth, genome size variation due to polyploidy is thought to be deterministic (Levin & Funderburg, 1979). In *Pinus* and *Helianthus,* populations growing in higher rainfall regions tend to show smaller genomes than those from low rainfall areas (Wakamiya *et al.,* 1993; Sims & Price, 1885). Genome size was found to be positively correlated with the extent of frost resistance in British herbs (Mac Gillivray & Grime, 1995). In annual grasses, larger genomes have been found to facilitate greater CO_2 acquisition (Jasienski & Bazaaz, 1995), while in some *Acacia* species there is negative correlation between genome size and relative growth rate (Mukherjee & Sharma, 1990).

As a trait, seed size has different benefits in different situations and that there is no single strategy that could predispose a species to become invasive. Studies on the potential role of seed size and plant invasiveness have yielded variable findings. For example, evidence has been reported for larger seeds giving rise to higher rates of early development (Otto & Whitton, 2000), and that such seeds grow better under water stress owing to their rich energy reserves in cotyledons (Leishman & Westoby, 1994; Westoby, *et al.,* 2002; Zhang & Maun, 1991). In *Pinus,* small seed size was found to be correlated to invasiveness (Grotkopp, *et al.*, 2004). Small seeds have a wider dispersal advantage and fast germination, a feature that could prove advantageous under competitive environments than large seeds (Hendrix, *et al.,* 1991). Buckley *et al.,* (2003) reports evolutionary changes in seed size between native and introduced ranges, finding that seeds are heavier in introduced ranges. In a synthesis of the theory of seed plant invasiveness, Rejmánek (1996) includes small seed size, alongside short juvenile periods, and short intervals between large seeding events as factors that promote invasiveness in seed plants.

For invasive *Prosopis* no studies have been done to determine how genome size and seed size influence growth at different geographic and spatial scales. Here I used plant traits measured under common garden conditions in combination with genome and seed size estimates to answer the following questions: 1) does genome size relate to seed size in *Prosopis*; 2) does genome size does have any taxonomic value in delimiting *Prosopis* species boundaries; 3) do seed size and genome size vary with latitude, longitude and altitude; and 4) how do genome size and seed size affect fitness correlates in *Prosopis*?

MATERIALS AND METHODS

Sampling

Seed pods were collected from across the invasive range of *Prosopis* in South Africa (Appendix 1.2 Chapter 1, Appendix 2.1 Chapter 2). Pods were collected from 5-10 plants at each collection locality depending on availability and the morphological diversity of pods at each site.

Data on temperature and rainfall for collection localities were obtained from Agricultural Research Council (ARC) - Institute for Soil, Climate and Water. For this study climate data spanning the last 5-20 years (depending on availability of data) were used. Google Earth was used to determine altitudes at sampling points.

Genome size determination

Plant material

Seven to ten seeds from each collected individual were germinated, after physical scarification, in August 2011. Plants were grown in standard potting compost (AgriMark, Stellenbosch, South Africa). Plants were allowed to grow until they developed up to four bipinnate leaves. In October 2010 fresh leaf material was harvested from one seedling per parental plant and used for flow cytometric analysis. To investigate within-plant variability, 3-6 seedlings were grown from four different parental plants. Fresh leaf material was used to determine genome size. The remaining seedlings were left to grow and harvested after three months. Plants were grown randomized design and were changed every two weeks. At harvest, height, number of leaves, numbers of leaflets per leaf were measured. The plants were then oven dried at 65°C for 72 hours and dry mass measurements were made. Appendix 3.1 shows geo-climatic data obtained for this study.

Flow cytometry

For flow cytometry, fresh leaf material was homogenised in a nuclei isolation buffer. Genome sizes was determined using a Partec PA II instrument (Partec GmbH., Münster, Germany) equipped with a mercury arc lamp for UV excitation. The methodology generally follows the two-step procedure (without centrifugation) described by Suda and Trávníček (2006). Otto I buffer (0.1 M citric acid, 0.5% Tween 20) was used for nuclei isolation and Otto II buffer (0.4 M Na_2HPO_4 × 12 H_2O), supplemented with AT-selective fluorochrome DAPI (at final concentration 4 µg/mL) and ß-mercaptoethanol (2 µL/mL), was used to stain the nuclear suspension. *Bellis perennis* was used as internal standard with a known genome size of 3.84 pg. Histograms from flow cytometry were evaluated using the Partec FloMax software version 2.4d.

Seed size and germination

The germination and growth experiments were repeated twice in a fenced field in Stellenbosch; first during winter months (August to December, 2010), and then again during summer months (January to March, 2011). In August average daily temperature was 19°C with a total min-max range of -0.2°C to 27 °C and in summer average daily temperature was 28°C with a range of 20°C to 36°C.

Seed size showed a normal distribution (Fig 4.2). Based on this distribution, seed sizes were categorised as small, medium and large as follows: small = 16 mg, medium = 39 mg, large= 58 mg.

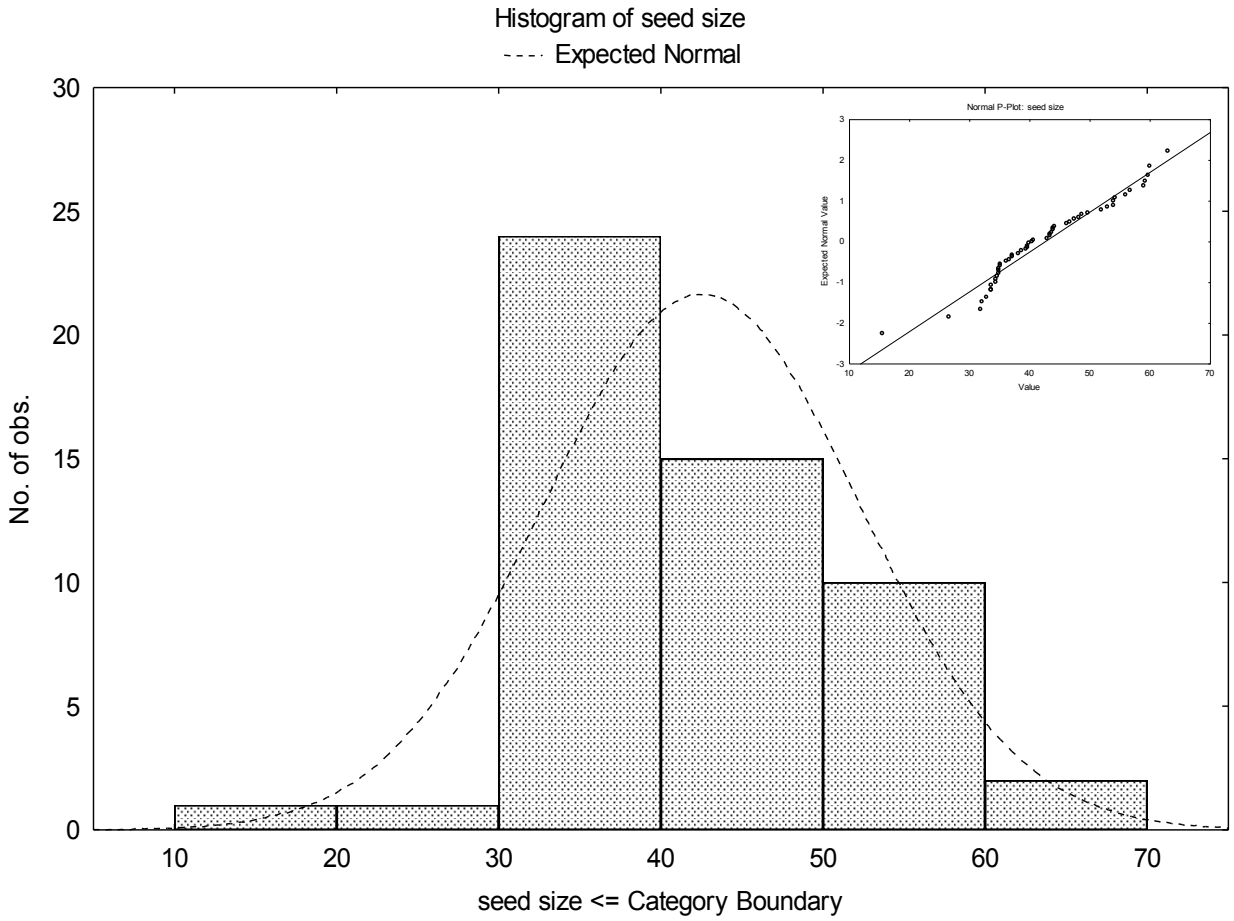


Figure 4.2 A histogram of seed size distribution in Prosopis species in South Africa. The seed size is normally distributed (Inser is a normal probability plot of seed size). Seed size categories (small and large), for germination experiments were randomly chosen at either extreme of the distribution and the medium size was the mean size.

These sizes represent extremes in the distribution i.e. smallest seed size, heaviest seed size and intermediate seeds (those with the average seed size) were classified as medium size. In the winter experiment 30 seeds were germinated for each seed size category. In the summer experiment, one hundred seeds per seed-size category. Germination was done in plastic trays 25cm by 45cm containing potting soil. Five trays per seed size class were used, each containing 20 seeds in rows of 5. Seeds were planted at a depth of about 1cm. On the day of planting the soil was watered until saturated. After which water was supplied every other morning for the duration of the experiment. Germination was noted upon the complete appearance of both cotyledons and was monitored every two days after planting.

Data analysis

For genome size, fluorescence intensity data analysis was done by comparing peak positions of 2C values on the histogram of the fluorescence intensity as follows;

$$\text{Sample 2C value} = \text{Reference 2C value}\frac{\text{sample 2C average peak position}}{\text{reference 2C average peak position}}$$

(Dolezel *et al.,* 2007).

A correlation analysis, using the Pearson correlation test was performed first between genome size and plant attributes [seed size, plant height, and biomass (root, shoot, and total)]; second between genome size and geo-climatic variables [latitude, longitude, altitude minimum rainfall maximum rainfall, minimum temperature and maximum temperature]. All correlations were performed in Statistica 10 software (StatSoft 2010).

RESULTS

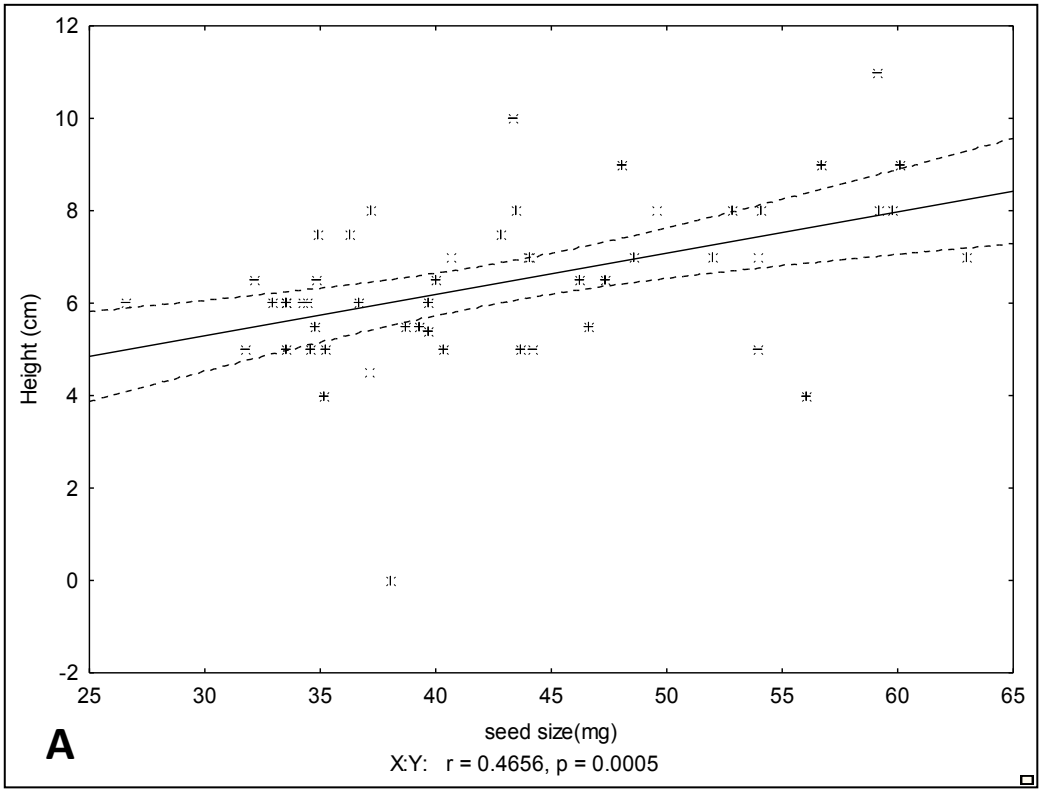
Genome size in *Prosopis* ranged between 1.167 pg and 1.263 pg. There was no clear delimitation of individual taxa present. Genome size showed up to 4.2 % variation within an individual parent plant (Table 4.1).

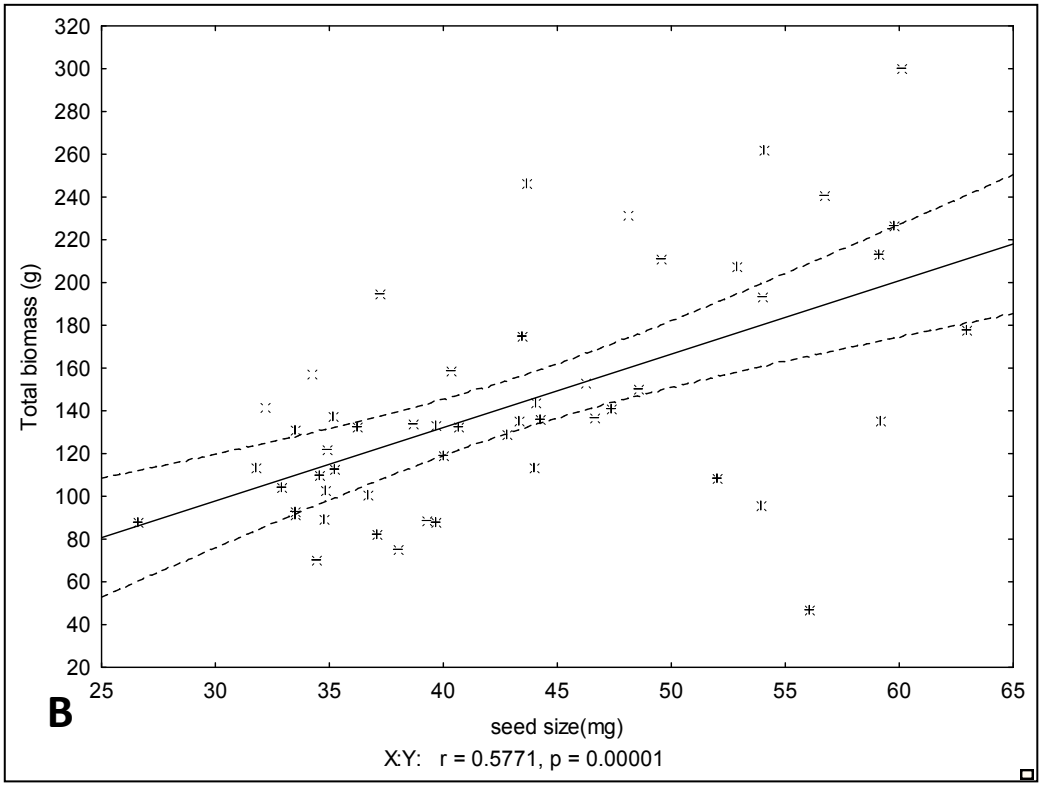
Table 4.1 Within-individual variation in genome size of for some *Prosopis* individuals in South Africa. GS= genome size, and percentage range of valued showing within individual variation. Values marked with (*) represent inter-individual genome size similarity.

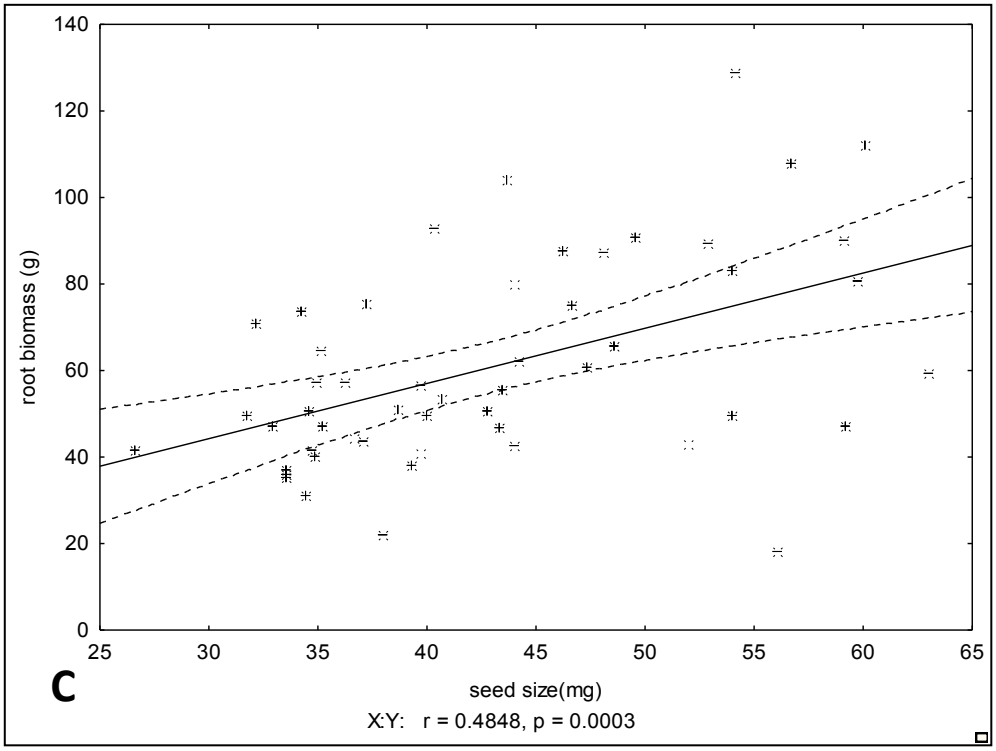
	Prosopis sample ID number			
Replicate	9p33 (2C values)	5p22 (2C values)	2p34 (2C values)	7p30 (2C values)
1	1.171	1.194	1.183	1.256
2	1.183	1.210*	1.210*	1.217*
3	1.171	1.236	1.202	1.225
4	1.190	1.233	1.206	-
5	-	1.244	-	-
6	-	1.217*	-	-
% range of values	1.6%	4.2%	2.3%	3.2%
coefficient of variation (CV)	**0.0079**	**0.0153**	**0.0099**	**0.0167**

Using the Pearson correlation test, there was no significant relationship between seed size and genome size (p=0.494, data not shown). No significant relationships were found between genome size and any of the geo-climatic variables assessed here i.e. temperature, rainfall, altitude, latitude and longitude (data not shown).

Seed size significantly influenced plant height (p = 0.0005), total biomass (p=0.0001), root biomass (p=0.0003) and shoot biomass (p=0.0001) See Fig. 4.3.







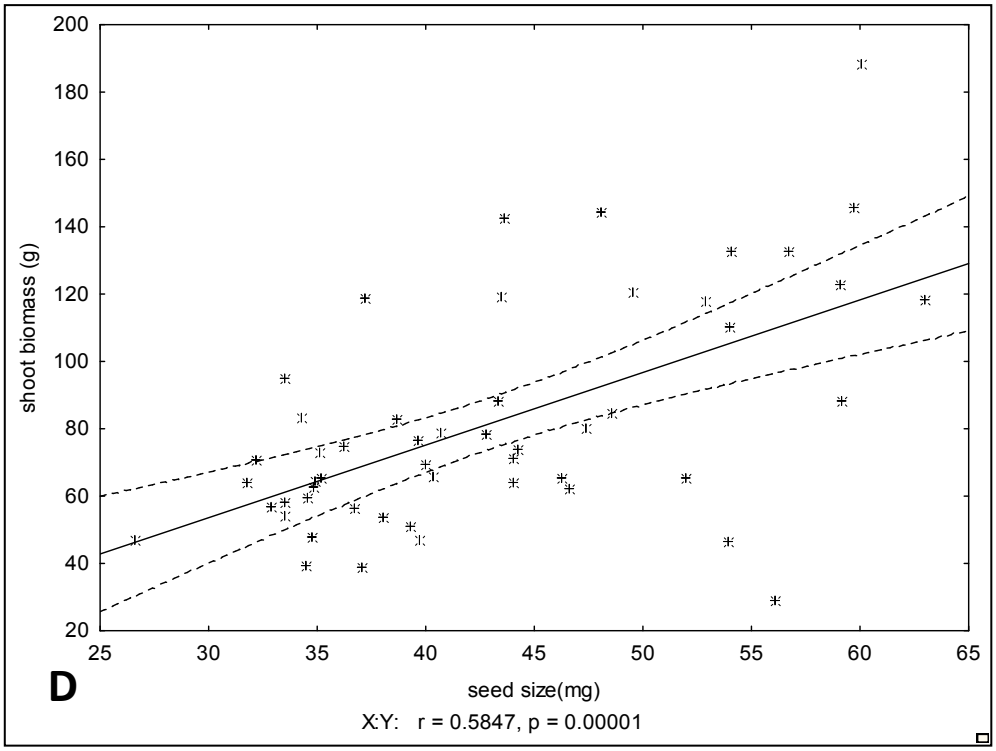


Figure 4.3 (plate A-D) Results for the correlation analysis for the trait seed size. A; seed size and plant height, B; seed size and total biomass, C; seed size and root biomass and D; seed size and shoot biomass. Correlation coefficients and associated P-values are given below each graph. The analysis was based on 52 taxa with varied seed sizes. For each taxa 3-10 seedlings were analysed and the measurements averaged. Data was analysed in Statistica 10 software (StatSoft 2010).

Germination showed variation with both scarification treatment and planting season. Seeds germinated more readily when planted in summer than in winter. On average, 98% of scarified seeds germinated in summer while 50% germinated in winter (Table 4.2), indicating that mechanical scarification promoted germination both in winter and summer. Non-scarified seeds hardly germinated at low winter temperatures while in summer; there was high germination without scarification. On average, 68% of non-scarified seeds germinated in summer where as 0.33% germinated in winter (Table 4.2). When placed on the soil surface, neither scarified nor un-scarified seeds germinated in summer or winter (data not shown).

Table 4.2 Number of seeds germinating in winter and summer with and without scarification. The fractions are for number of seed germinated divided by total number of seed planted.

	Winter		Summer	
	Scarified seeds	Non scarified	scarified	Non scarified
Small seed	10 / 30	1/30	67/70	65/100
Medium seed	17/30	0/30	66/70	50/100
Large seed	18/30	0/30	70/70	89/100

Of all the geo-climatic variables examined, i.e. temperature, rainfall, altitude, latitude and longitude, none except longitude significantly influenced any growth trait. Root biomass and plant height showed a significant negative correlation with longitude (p=0.030, and 0.029, respectively). Plant biomass, seed size, and genome size, all exhibited similar trends but were not statistically significant (p = 0.052, 0.173, 0.150, respectively).

DISCUSSION

Contrary to the general perception that genome and seed size are correlated (Beaulieu *et al.,* 2007). I found no significant relationship between these two traits in invasive *Prosopis* in South Africa. I speculate that this is because while the range is seed size is large (15mg to 67mg), there is little variation in genome sizes (1.167-1.263 pg), and as such the noise to signal ratio might be hidden. Moreover, in *Prosopis*, the genome size is generally comparatively small (Felker *et al.,* 2007), and for such plant groups it has been found that seed size ranges are higher than in plants with large genomes (Knight & Beaulieu, 2008) suggesting that in plants with smaller genomes, seed size is controlled by other factors. Genome size and seed size in *Prosopis* could still play individual roles in promoting invasiveness of *Prosopis* in South Africa by affecting other eco-physiological attributes not investigated here. There is certainly extensive hybridization, and this could partly explain the observed intra-individual variability of genome sizes (Table 4.1) which can be as high as 4.2%. The observed lack of stability in genome sizes imply that for *Prosopis* populations in South Africa, genome size can hardly be used to distinguish species but could be of use in confirming the existence of hybridization in a population.

Previous attempts to understand genome size variability in *Prosopis* were confounded by taxonomic confusions due to polyploidy and intra-specific hybridization (Bukhari, 1997).
This study has also been limited by existing hybridization events among *Prosopis* taxa represented in South Africa. It has been found (Chapter, 2) that *Prosopis* species in South Africa are virtually hybrid swarms of the species that were introduced.

It is known that hybridization can induce rapid increases and decreases in genome size (Baack *et al.,* 2005). Further, hybrid genomes are known to be variably stable depending on the parental species involved; they can be means of parental genomes, they can be significantly higher than parental genome means, or can exhibit a continuous gradation between the lowest parental genome to the highest parental genome (Rayburn, *et al*., 1993).

Results from this study, cannot be adequately compared to that of Bukhari (1987), except for *P. chilensis (*2-C value of 1.210pg) which shows some increase in genome size from the one reported by (Bukhari, 1997). If the observed slight increase in genome size (Table 4.3) is an evolutionary result of hybridization, then genome size can be suspected of aiding invasion of *Prosopis* in this region.

Table 4.3 Comparison of genome sizes for Prosopis species studied elsewhere and in South African (RSA) species and their hybrids. For *P. chilensis*, the genome size value found in this study is from a sample collected in South Africa.

***Prosopis* species**	**Genome size (2C values pg)**	
	Bukhari, (1997)	This study
*P. chilensis** (Sudan and Kenya)	1.73	1.210
P. glandulosa (Mexico)	0.827	-
P. juliflora (Senegal)	0.852	-
P. pallida (Peru)	0.836	-
P. alba (Chile)	0.840	-
P. flexuosa (Chile)	0.811	-
P. laevigata (RSA)	-	1.198
P. chilensis X *P. glandulosa* (RSA)	-	1.233, 1.206
P. chilensis X *P. laevigata* (RSA)	-	1.240
P. chilensis X *P. hassleri hybrid* (RSA)	-	1.187
P. chilensis hybrid (others) (RSA)	-	1.263, 1.206, 1.248
P. laevigata hybrids (others) (RSA)	-	1.202, 1.233

P. chilensis forms different hybrids with a lower 2-C values (1.187pg), and high 2-C values 1.263pg (Table 4.3), showing genome instability associated with hybridization. The lack of correlations between genome size and the factors investigated here should not be interpreted to imply genome size does not play a role in invasiveness of *Prosopis* but rather that hybridization has swamped the genome, resulting in individuals that do not have a signature genome size.

Seed size, germinability and invasion dynamics

Most studies that have tested factors affecting germination in *Prosopis* have involved scarification (Cony & Trione, 1996; Catalan, 1992; Catalan *et al.,* 1994; Cox *et al.,* 1993).

However, when considering the role of seed germinability in invasiveness, it is vital to determine which factors could promote germination under natural conditions, i.e. without artificial scarification. For *Prosopis*, such studies have been rare. Naturally, seed dormancy in plants and *Prosopis* in particular, it is thought to be broken due to chemical and physical process in the soil (Janzen, 1981; Ortega Baes *et al.,* 2002).

Some studies have found that passage through the digestive system of ruminants aids in promoting germination in "natural" conditions (Campos &Ojeda, 1997; but see Günster 1994; Figueiroa & Castro, 2002; Otani, 2004). The feeding of *Prosopis* to livestock is thought to facilitate germination and spread of *Prosopis* (Zimmermann, 1991). The results of this study show a natural germination average of 68% in summer and 0 % in winter, indicating that immediate germination is possible once an optimum temperature is realized as a dormancy–breaking factor. Scarification does improve germination for all seed size classes but overall more during summer than during winter.

Germination of un-scarified seed was higher and faster for larger seeds than small seeds (Table 4.2). Only 55% of 2038 scarified seeds germinated in winter, with germination starting only after 21 days, whereas 98% of scarified seeds germinated in summer within 36 hours. In a particular case, seed were noted germinating while still in their pods in the summer of March 2011.

Seed size distribution in *Prosopis* showed no altitudinal, latitudinal or altitudinal gradation in South Africa. Seed sizes were variable across the distribution range. This should predispose *Prosopis* populations to have the 'right' seed sizes to establish populations in variable bioclimatic regions.

This study has shown that seedlings from bigger seeds also accumulated significantly more shoot, root and total biomass than those from small seeds (Figure 4.3). Generally, high biomass accumulation could aid invasive plants in competing better, but specific allocation strategies depend on the particular resource being competed for (Burns & Winn, 2006). There is, however, consensus greater biomass allocation to shoots is adaptive for alien species growing under shady conditions i.e. where there is competition for light (van Kleunen *et al.,* 2011).

Actually greater investment in root biomass is one of adaptive strategies to cold hardness in plants (Linden, 2002), just as large seeds are more tolerant to winter (Erskine, 1996).Whether or not such strategies would favour bigger seeds in *Prosopis* can only be confirmed with further experiments involving resource competition.

Smaller seeds in *Prosopis* tended to stagger germination over a period whereas large seeds germinated over a shorter period, at least during summer (data not shown). Both these observations have implications for the invasiveness of *Prosopis* and on invasibility of different climatic regions of South Africa. In invasive plants, germination season and the ability to stagger germination over time are thought to increase invasive ability (Pyšek & Richardson, 2007) as it allows for germination to coincide with preferred growing conditions. In most semi-arid environments where conducive germination condition can be erratic, rapid and synchronised germination can be adaptive (Miranda *et al.,* 2011). Generally, there is thus a trade-off between fast and staggered germination.

Larger seeds have been found to germinate faster than smaller ones at the expense of dispersability in the former (Cappuccino *et al.,* 2002) whereas in invasive *Cytisus scoparius* invasive populations were found to have evolved larger seeds (Buckley *et al.,* 2003). In invasive *Rhododendron ponticum* studies have found a genetic shift towards faster germination (Erfmeier & Bruelheide, 2005).

This suggests that in *Prosopis*, large seededness is adaptive for invasiveness and that some selection for bigger seed size could be at play. All seed size classes had a shoot: root ratio of at least 1.5 meaning that there is a tendency to invest in shoot more than in root which in itself has been shown to be an adaptive strategy for invasiveness (van Kleunen *et al.,* 2011).

CONCLUSIONS

The lack of a relationship between genome size and growth traits in *Prosopis* could be due to fluidity in the genome resulting from hybridization. Genome size is of no taxonomic value in multiple hybridizing populations. This instability in genome size could be conferring a flexible platform for other invasive attributes like phenotypic plasticity, and tolerance to variable and unstable ecological conditions within the distribution range. Large seeds have adaptive potential as exhibited in high germination and biomass accumulation, features that aid invasion processes at different spatial scales.

The absence of *P. juliflora* in South Africa as observed in Chapter 2, is further confirmed by genome size data. Of all the 208 samples for which genome size was analysed there was no indication of the existence of a polyploidy genome as would be expected for the polyploid *P. juliflora* (Bennet & Leitch, 1995).

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CHAPTER 5—CONCLUSION

The taxonomic problems associated with *Prosopis* species in South Africa and other invasive ranges, are diverse and they impact on meaningful studies in invasion biology and management of invasive population. It is important therefore to understand which species are present and their inter-species interactions such as hybridization.

In this study, some previously reported species were confirmed as present, but others were not. The study also established the presence of at least species previously not known to be in in South Africa, *Prosopis hassleri*, and suggests that there are several other *Prosopis* taxa present that are yet to be identified. This alludes to inadequacy in the introduction history in determining the number of resident species, and confirms nomenclatural problems that existed not only in native ranges but also in introduced rages.

Hybridization has been confirmed, and it appears to involve most taxa recorded. This high prevalence of hybridization is likely to mean that morphological identification to a species level will be inaccurate, and, indeed, my morphological results suggest this is the case. Hybridization between *Prosopis* species, especially in the section Algarobia, is well known even from the native range. This has led to calls for the revision of this taxonomic rank (Henziker, *et al.,* 1986) that I would like to echo based on my findings here.

Due to hybridization, species traits that are thought to promote invasiveness cannot be fully investigated in hybridizing populations. For example, genome size; a trait that is expected to be species specific, has been found to show intra-individual variability. Contrary to expectation of it being of taxonomic value (Ohri, 1998), in a hybridizing species complex it is unstable. It can only thus be used to help confirm the existence of hybridization events in populations.

Significance of study

This study is the first detailed study attempting to resolve the taxonomic identity of *Prosopis* in South Africa. Previous studies have mainly focussed on management and control without knowing exactly which species were being studied. This study also provides new insights into the diversity of *Prosopis* taxa and extents of hybridization and its consequences not just on morphology based identification, but on other traits such as genome size. From an ecological perspective, these results highlight several conservation challenges: 1) studies in *Prosopis* distribution modelling are likely to face as they require species specific data which can hardly be obtained for a hybridizing species complex like *Prosopis* in South Africa. 2). From a management perspective, this study highlights the challenges associated with the choice of a control method to employ. 3) The old question of 'what is species' is all the more relevant. With the confirmed extent of hybridization species delimitation in the genus *Prosopis* need to be addressed.

Recommendations and the way forward

Due to the apparent limitation in the introduction history of *Prosopis* to South Africa, it is recommended that existing records be augmented with a survey to farm owners aimed at determining the seed source of their *Prosopis* accessions. This would provide an idea of the potential species likely to be resident in South Africa. It is recommended that *Prosopis* in South Africa be treated as a "*Prosopis* species" as any nomenclatural attempts are likely to be misleading.I further recommend a global biogeographic study of both native and invasive *Prosopis* species populations to get the extent of taxonomic mishaps and review the current taxonomic placement of some taxa where possible. Any such study should use the total evidence approach considering the likely geographic polymorphisms in morphological characters

In view of the presence of unreported *Prosopis* species in South Africa, biological control will need to consider the efficiency of agents not just on a wider range of *Prosopis* species but also on their hybrids. The acquisition of biocontrol agents from known hybrid zones in the native land could be one such step.

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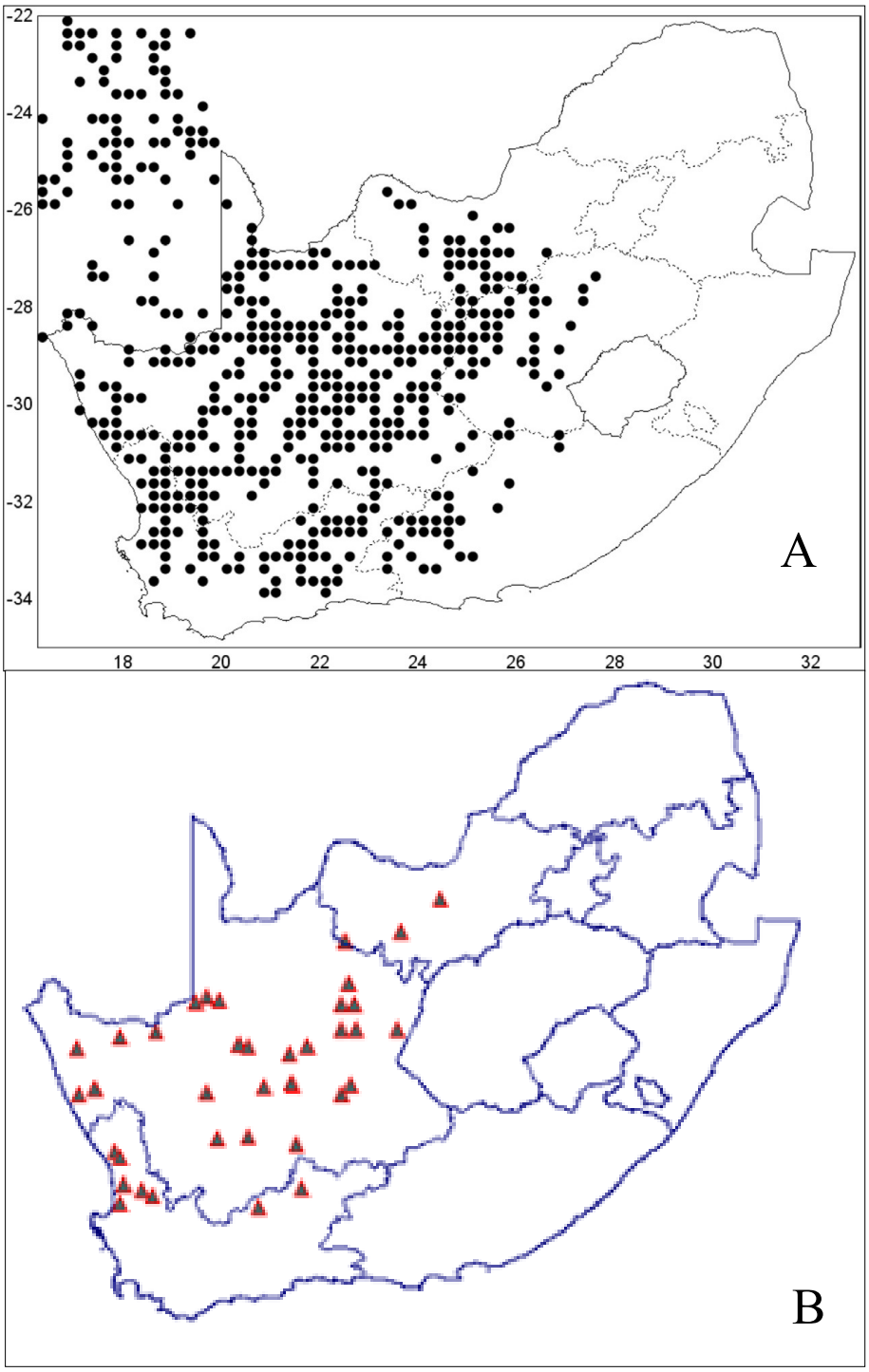
APPENDICES

Appendix 1.1 A complete classification of taxa within the genus *Prosopis* (Burkart, 1976)

SECTION	SERIES	SPECIES
Prosopis	Monotypic section	*P. cineraria, P. farcta*, *P. koelziana*
Anonychium	Monotypic section	*P. africana*
Monilicarpa	Monotypic section	*P. argentina*
Strombocarpa	*Strombocarpae*	*P. strombulifera, P. reptans* *P. abbreviate, P. torcuata* *P. burkartii, P. palmeri* and *P. pubescens*
	Cavenicarpae	*P. ferox* *P. tamarugo*
Algarobia	*Sericanthae*	*P. sericantha* and *P. kuntzei*
	Ruscifoliae	*P. ruscifolia, P. fiebrigii* *P. hassleri* *P. vinalillo*
	Denudantes	*P. denudans P. ruizleali* *P. castellanosii* *P. calingastana*
	Humilis	*P. humilis and*

SECTION	SERIES	SPECIES
		P. rojasiana
	Pallidae	*P. rubriflora, P. pallida*
		P. campestris, P. affinis
		P. tamaulipana, P. elata
		P. articulata
	Chilensis	*P. chilensis*, *P. juliflora*
		P. flexuosa. P. glandulosa, P. alba
		P. nigra, P.caldenia, P. pugionata
		P. velutina, P. alpataco,
		P. laevigata

Appendix 1.3 Maps of South Africa, showing the distribution of *Prosopis* **(A)** (Drawn by L. Henderson; data source: SAPIA database, ARC-Plant Protection Research Institute, Pretoria.) from Zachariades *et al.,* (2011), and the distribution of sampling points **(B)**



Appendix 1.4 Table of global localities where *Prosopis* taxa are known to have been introduced, with their residence status at each locality; Criteria for defining status follow those proposed by Pyšek *et al.,* (2004), but, e.g. for *P. farcta*, I used "expansive" to refer to species that considered problematic but which are native to an area. *Prosopis* has been introduced to at least 36 different countries. Of the species introduced, *P. juliflora* is predominant with reports of its invasiveness in 20 countries. *Prosopis glandulosa* is reported in at least 5 countries; *P. chilensis* in at least 4 countries; *P. velutina* in at least 4 countries; *P. pallida* in at least 4 countries and *P. alba* in at least 2 countries.

Country	***Prosopis* taxa**	**Resident status**	**Reference**
South Africa	*P glandulosa, P. chilensis, P. hassleri, P. laevigata, P. velutina,* and hybrids	Invasive	Zimmerman, (1991); (This study).
Ethiopia	*P. juliflora and P. africana*	Invasive *P. juliflora*	Schiferaw *et al.,* (2004) Weber, *et al.,* (2008)
Malawi	*P. glandulosa*	Invasive	Chikuni *et al.,* (2005)
Sudan	*P. juliflora, P. chilensis.*	Invasive	Hamsa, (2010); Rasanem *et al.,* (2001)
Europe	None	None	Iglesias *et al.,* (2007)
Mauritania	*P. juliflora and Prosopis* spp.	Not reported	Pasiecznik *et al.,* (2006)
Senegal	*P. juliflora, P. pallida, P. africana, P. cineralia*	Not reported	Pasiecznik *et al.,* (2006); Weber *et al.,* (2008), Rasanem *et al.,* (2001)
Cape Verde	*P. pallida, P. juliflora?*	Not reported	Pasiecznik *et al.,* (2006)
Morocco	*P. juliflora*	Not reported	Benata *et al.,* (2008)
Kenya	*P. juliflora*	Invasive	Mwangi & Shallow, (2005)

Country	***Prosopis* taxa**	**Resident status**	**Reference**
Niger	*P. africana, P. juliflora*	Expansive *P. cineralia,* Invasive, *P. juliflora*	Weber *et al.,* (2008); Geesing, *et al.,* (2004) Pasiecznik *et al.,* (2001)
Algeria	*P. farcta; P. juliflora*	Alien naturalised	Qasem, (2006) Mwangi & Shallow, (2005)
Somalia	*P. alba. P. juliflora, P. velutina, P. cineralia, P. glandulosa.*	Not reported	Zollner, (1986)
Tanzania	*P. chilensis*	Not reported	Jonsson *et al.,* (1988)
Tunisia	*P. farcta*	Expansive	Harzallah-Skhiri & Jannet, (2005).
Zimbabwe	*P. pallida*	Invasive	Rwegasira *et al.,* (2003)
Botswana	*P. juliflora*	Invasive	Skarpe, (1990)
Namibia	*Prosopis* spp.	Invasive	Brown *et al.,* (1985)
Eritrea	*P. juliflora*	Invasive	http://ubm.opus.hbznrw.de/volltexte/2009/2066/
Iraq	*P. farcta*	Expansive	Bazzaz, (1972)
Afghanistan	*P. cineraria*	Expansive	Malik & Kalidhar, (2007)
India	*P. cineraria, P. juliflora*	Expansive *P. cineralia,* Invasive, *P. juliflora*	Wojtusik *et al.,* (1993); Malik & Kalidhar, (2007)
Pakistan	*P. cineraria* *P. juliflora*	Expansive *P. cineralia,* Invasive, *P. juliflora*	Malik & Kalidhar, (2007); Sharma, (1998)

Country	***Prosopis* taxa**	**Resident status**	**Reference**
Iran	*P. cineraria*	Native	Malik & Kalidhar, (2007)
Saudi Arabia	*P. cineraria P. juliflora, P. alba P. chilensis P. glandulosa, P. tamarugo P. velutina, P. farcta, P. pallida*	Invasive	Al-Frayh *et al.,* (1999)
Libya	*P. juliflora*	Not reported	Dumancic & Le Houérou, (1980)
Chad	*P. juliflora*	Invasive	Geesing *et al.,* (2004); Pasiecznik *et al.,* (2001)
Egypt	*P. farcta; P. juliflora*	Expansive	Abd El-Ghani, (1999); Mwangi & Shallow, (2005)
Israel	*P. farcta P, Juliflora, P. alba, P. nigra*	Expansive *P. cineralia,* Invasive, *P. juliflora*	Zaady *et al.,* (2001)
Syria	*P. farcta*	Expansive	Al-Jassen *et al.,* 2010
Sri Lanka	*P. juliflora*	Invasive	Geesing *et al.,* (2004)
Galapagos Islands	*P. juliflora*	Invasive	Itow, (2003); Froyd *et al.,* (2010)
Dominican Republic	*P. juliflora*	Invasive	Lata *et al.,* (2001); Roth, (1999)
Colombia	*P. juliflora*	naturalised	Etter & Villa, (2000)
Australia	*P. pallida, P. velutina, P. juliflora, P. glandulosa*	Invasive	Van Klinken & Campbell, (2008)
Madagascar	*Prosopis* spp.	naturalised	Binggeli, (2003)

Country	***Prosopis* taxa**	**Resident status**	**Reference**
Puerto Rica	*P. juliflora*	Naturalised	Wunderle *et al.,* (1992)
Jamaica	*P. juliflora*	Naturalised	Wunderle *et al.,* (1992)
United Arab Emirates	*P. juliflora, P. cineraria*	Expansive *P. cineralia,* Invasive, *P. juliflora*	El-Keblawy & Al-Rawai,(2005)
Syria	*P. farcta*	Expansive	Qasem, (2006)
India	*P. farcta, P. juliflora, P. cineraria*	Expansive *P. cineralia,* Invasive, *P. juliflora*	Qasem, (2006); Love *et al.,* (2009) Robbins, (2001)
Iran	*P. farcta, P. juliflora*	Expansive	Qasem, (2006); Carillo *et al.,* (2008)
Cyprus	*P. farcta*	Expansive	Qasem, (2006)
Turkey	*P. farcta*	Expansive	Qasem, (2006)
Ukraine	*P. farcta*	Expansive	Qasem, (2006)
Jordan	*P. farcta*	Expansive	Qasem, (2006)

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Appendix 2.1 Table of DNA sample number used in this study as linked to collection points across the sampling range. The sample ID (identity) depicts two numbers; the first refers to the population number and the second refer to the actual sample number within that population. Label name is a reference number for the C•I•B Molecular plant ecology lab database. All samples for South Africa were collected in March 2010. Australian samples were received on 30 August 2010. Collectors initials: DMM= Dickson Mgangathweni Mazibuko, and NL= Nicholas Le Maitre.

DNA Sample Number.	Label	Putative Species	Collector	Sample identity	Latitude	Longitude
1	SA/PROSS/JE/02	*Prosopis* spp.	DMM and NL	2p3	-32.5715	21.42482
2	SA/PROSS/JE/18	*Prosopis* spp.	DMM and NL	2p18	-32.5715	21.42482
3	SA/PROSS/JF/01	*Prosopis* spp.	DMM and NL	3p1	-32.1953	22.34842
4	SA/PROSS/JG/02	*Prosopis* spp.	DMM and NL	4p2	-31.3355	22.21218
5	SA/PROSS/JG/08	*Prosopis* spp.	DMM and NL	4p8	-31.3355	22.21218
6	SA/PROSS/JH/01	*Prosopis* spp.	DMM and NL	5p1	-30.1553	22.14366
7	SA/PROSS/JH/03	*Prosopis* spp.	DMM and NL	5p3	-30.1553	22.14366
8	SA/PROSS/JI/06	*Prosopis* spp.	DMM and NL	6p6	-30.3374	23.18446
9	SA/PROSS/JI/08	*Prosopis* spp.	DMM and NL	6p8	-30.3374	23.18446
10	SA/PROSS/JI/12	*Prosopis* spp.	DMM and NL	6p12	-30.3374	23.18446
11	SA/PROSS/JJ/01	*Prosopis* spp.	DMM and NL	7p1	-30.1403	23.37292
12	SA/PROSS/JJ/02	*Prosopis* spp.	DMM and NL	7p2	-30.1403	23.37292
13	SA/PROSS/JJ/09	*Prosopis* spp.	DMM and NL	7p9	-30.1403	23.37292
14	SA/PROSS/JK/01	*Prosopis* spp.	DMM and NL	8p1	-29.0306	24.37088
15	SA/PROSS/JK/10	*Prosopis* spp.	DMM and NL	8p10	-29.0306	24.37088
16	SA/PROSS/JL/01	*Prosopis* spp.	DMM	9p1	-27.0978	24.44845

DNA Sample Number.	Label	Putative Species	Collector	Sample identity	Latitude	Longitude
			and NL			
17	SA/PROSS/JL/05	*Prosopis* spp.	DMM and NL	9p5	-27.0978	24.44845
18	SA/PROSS/JM/01	*Prosopis* spp.	DMM and NL	10p1	-26.4158	25.27256
19	SA/PROSS/JM/05	*Prosopis* spp.	DMM and NL	10p5	-26.4158	25.27256
20	SA/PROSS/JN/01	*Prosopis* spp.	DMM and NL	11p1	-27.2784	23.25925
21	SA/PROSS/JN/07	*Prosopis* spp.	DMM and NL	11p7	-27.2784	23.25925
22	SA/PROSS/JO/02	*Prosopis* spp.	DMM and NL	12p2	-28.1172	23.32802
23	SA/PROSS/JO/11	*Prosopis* spp.	DMM and NL	12p11	-28.1172	23.32802
24	SA/PROSS/JO/12	*Prosopis* spp.	DMM and NL	12p12	-28.1172	23.32802
25	SA/PROSS/JP/03	*Prosopis* spp.	DMM and NL	13p3	-28.5454	23.45782
26	SA/PROSS/JP/06	*Prosopis* spp.	DMM and NL	13p6	-28.5454	23.45782
27	SA/PROSS/JQ/01	*Prosopis* spp.	DMM and NL	14p1	-29.0287	23.46098
28	SA/PROSS/JQ/10	*Prosopis* spp.	DMM and NL	14p10	-29.0287	23.46098
29	SA/PROSS/JR/08	*Prosopis* spp.	DMM and NL	15p8	-28.5164	23.16031
30	SA/PROSS/JR/13	*Prosopis* spp.	DMM and NL	15p13	-28.5164	23.16031
31	SA/PROSS/JS/02	*Prosopis* spp.	DMM and NL	16p2	-29.0385	23.16031
32	SA/PROSS/JS/09	*Prosopis* spp.	DMM and NL	16p9	-29.0385	23.16031
33	SA/PROSS/JT/06	*Prosopis* spp.	DMM and NL	17p6	-29.3919	22.44748
34	SA/PROSS/JT/09	*Prosopis* spp.	DMM and NL	17p9	-29.3919	22.44748
35	SA/PROSS/JU/01	*Prosopis* spp.	DMM and NL	18p1	-30.0907	22.11701
36	SA/PROSS/JU/07	*Prosopis* spp.	DMM and NL	18p7	-30.0907	22.11701
37	SA/PROSS/JV/02	*Prosopis* spp.	DMM and NL	19p2	-31.2049	20.55066
38	SA/PROSS/JV/03	*Prosopis* spp.	DMM	19p3	-31.2049	20.55066

DNA Sample Number.	Label	Putative Species	Collector	Sample identity	Latitude	Longitude
			and NL			
39	SA/PROSS/JX/02	*Prosopis* spp.	DMM and NL	21p2	-29.5087	22.05964
40	SA/PROSS/JX/08	*Prosopis* spp.	DMM and NL	21p7	-29.5087	22.05964
41	SA/PROSS/JY/02	*Prosopis* spp.	DMM and NL	22p2	-30.1650	21.54558
42	SA/PROSS/JY/05	*Prosopis* spp.	DMM and NL	22p5	-30.1650	21.54558
43	SA/PROSS/JZ/01	*Prosopis* spp.	DMM and NL	23p1	-30.2028	21.24660
44	SA/PROSS/JZ/06	*Prosopis* spp.	DMM and NL	23p6	-30.2028	21.24660
45	SA/PROSS/K1/03	*Prosopis* spp.	DMM and NL	24p3	-30.2791	20.29330
46	SA/PROSS/K1/04	*Prosopis* spp.	DMM and NL	24p4	-30.2791	20.29330
47	SA/PROSS/K1/05	*Prosopis* spp.	DMM and NL	24p5	-30.2791	20.29330
48	SA/PROSS/K2/04	*Prosopis* spp.	DMM and NL	25p4	-29.3242	21.00013
49	SA/PROSS/K2/05	*Prosopis* spp.	DMM and NL	25p5	-29.3242	21.00013
50	SA/PROSS/K3/09	*Prosopis* spp.	DMM and NL	26p9	-29.3838	21.16872
51	SA/PROSS/K3/13	*Prosopis* spp.	DMM and NL	26p13	-29.3838	21.16872
52	SA/PROSS/K3/16	*Prosopis* spp.	DMM and NL	26p16	-29.3838	21.16872
53	SA/PROSS/K4/02	*Prosopis* spp.	DMM and NL	27p2	-28.4480	20.59255
54	SA/PROSS/K4/07	*Prosopis* spp.	DMM and NL	27p7	-28.4480	20.59255
55	SA/PROSS/K5/01	*Prosopis* spp.	DMM and NL	28p1	-28.3833	20.29975
56	SA/PROSS/K5/07	*Prosopis* spp.	DMM and NL	28p7	-28.3833	20.29975
57	SA/PROSS/K6/05	*Prosopis* spp.	DMM and NL	29p5	-28.5102	20.09085
58	SA/PROSS/K6/10	*Prosopis* spp.	DMM and NL	29p10	-28.5102	20.09085
59	SA/PROSS/K7/01	*Prosopis* spp.	DMM and NL	30p1	-29.0772	19.23889
60	SA/PROSS/K7/1	*Prosopis* spp.	DMM	30p10	-29.0772	19.23889

DNA Sample Number.	Label	Putative Species	Collector	Sample identity	Latitude	Longitude
	0		and NL			
61	SA/PROSS/K8/01	*Prosopis* spp.	DMM and NL	31p1	-29.1840	18.47596
62	SA/PROSS/K8/06	*Prosopis* spp.	DMM and NL	31p6	-29.1840	18.47596
63	SA/PROSS/K9/05	*Prosopis* spp.	DMM and NL	32p5	-29.3972	17.53909
64	SA/PROSS/K9/08	*Prosopis* spp.	DMM and NL	32p8	-29.3972	17.53909
65	SA/PROSS/KA/01	*Prosopis* spp.	DMM and NL	33p1	-30.3346	17.59509
66	SA/PROSS/KA/02	*Prosopis* spp.	DMM and NL	33p2	-30.3346	17.59509
67	SA/PROSS/KA/09	*Prosopis* spp.	DMM and NL	33p9	-30.3346	17.59509
68	SA/PROSS/KB/03	*Prosopis* spp.	DMM and NL	34p3	-32.1088	18.53602
69	SA/PROSS/KB/08	*Prosopis* spp.	DMM and NL	34p8	-32.1088	18.53602
70	SA/PROSS/KB/09	*Prosopis* spp.	DMM and NL	34p9	-32.1088	18.53602
71	SA/PROSS/KC/01	*Prosopis* spp.	DMM and NL	35p1	-31.4678	18.37618
72	SA/PROSS/KC/07	*Prosopis* spp.	DMM and NL	35p7	-31.4678	18.37618
73	SA/PROSS/KD/01	*Prosopis* spp.	DMM and NL	36p1	-31.5989	18.46265
74	SA/PROSS/KD/08	*Prosopis* spp.	DMM and NL	36p8	-31.5989	18.46265
75	SA/PROSS/KE/02	*Prosopis* spp.	DMM and NL	37p2	-32.5005	18.49226
76	SA/PROSS/KE/04	*Prosopis* spp.	DMM and NL	37p4	-32.5005	18.49226
77	SA/PROSS/KE/06	*Prosopis* spp.	DMM and NL	37p6	-32.5005	18.49226
78	SA/PROSS/KB/04	*Prosopis* spp.	DMM and NL	34p4	-32.1088	18.53602
79	SA/PROSS/KB/06	*Prosopis* spp.	DMM and NL	34p6	-32.1088	18.53602
80	SA/PROSS/KA/10	*Prosopis* spp.	DMM and NL	33p10	-30.3346	17.59509
81	SA/PROSS/K2/11	*Prosopis* spp.	DMM and NL	25p11	-29.3242	21.00013
82	OZ/PROSS/I14/0	WA hybrid	L.	41p1	-21.18333	115.96667

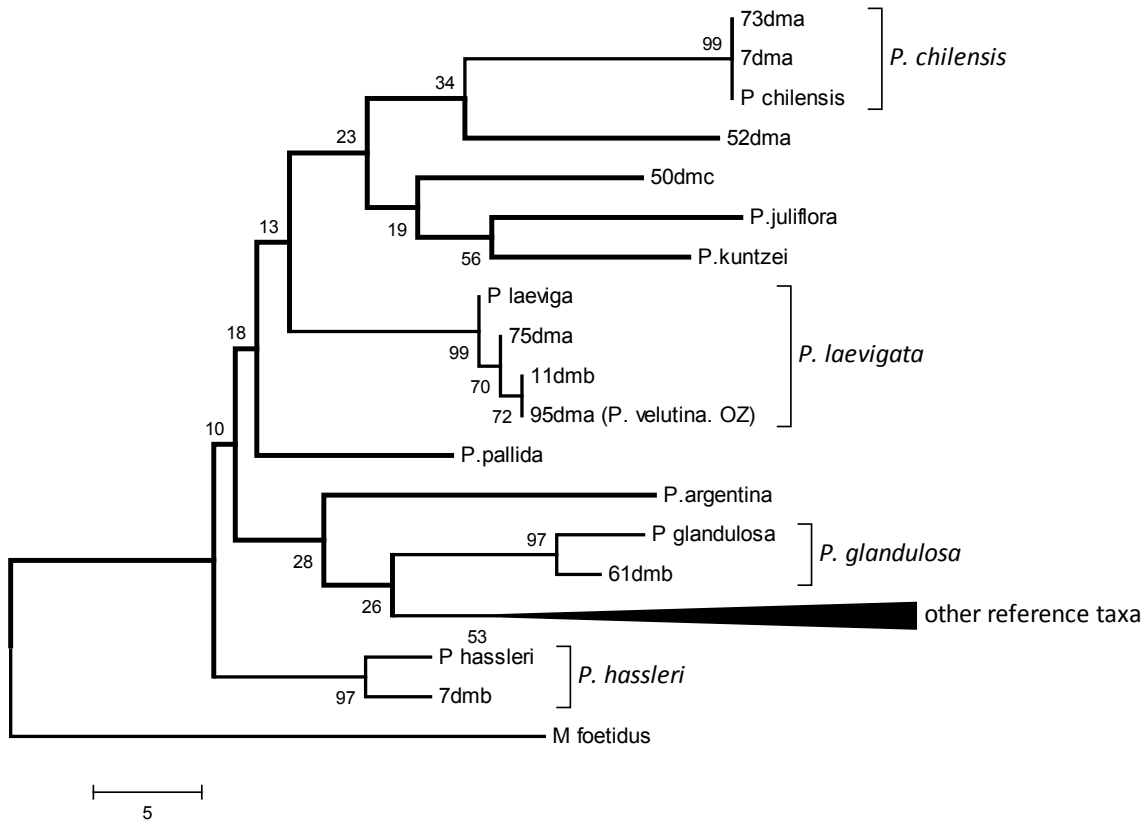
DNA Sample Number.	Label	Putative Species	Collector	Sample identity	Latitude	Longitude
	1	(*Prosopis*)	Anderson			
83	OZ/PROSS/I14/02	WA hybrid (*Prosopis*)	L. Anderson	41p2	-21.18333	115.96667
84	OZ/PROSS/I14/03	WA hybrid (*Prosopis*)	L. Anderson	41p3	-21.18333	115.96667
85	OZ/PROSS/I14/04	WA hybrid (*Prosopis*)	L. Anderson	41p4	-21.18333	115.96667
86	OZ/PROSS/I15/01	Qld hybrid(*Prosopis*)	A. White	42p1	21.4500	141.15000
87	OZ/PROSS/I15/02	Qld hybrid(*Prosopis*)	A. White	42p2	21.4500	141.15000
88	OZ/PROSS/I15/03	Qld hybrid(*Prosopis*)	A. White	42p3	21.4500	141.15000
89	OZ/PROSS/I15/04	Qld hybrid(*Prosopis*)	A. White	42p4	21.4500	141.15000
90	OZ/PROSS/I16/01	*P. glandulosa var. glandulosa*	R. van Klinken	43p1	18.0167	128.883333
91	OZ/PROSS/I16/02	*P. glandulosa var. glandulosa*	R. van Klinken	43p2	18.0167	128.883333
92	OZ/PROSS/I16/03	*P. glandulosa var. glandulosa*	R. van Klinken	43p3	18.0167	128.88333
93	OZ/PROSS/I16/04	*P. glandulosa var. glandulosa*	R. van Klinken	43p4	18.0167	128.88333
94	OZ/PROSS/I17/01	*P. velutina*	R. van Klinken	44p1	26.4500	144.31667
95	OZ/PROSS/I17/02	*P. velutina*	R. van Klinken	44p2	26.4500	144.31667
96	OZ/PROSS/I17/03	*P. velutina*	R. van Klinken	44p3	26.4500	144.31667
97	OZ/PROSS/I17/04	*P. velutina*	R. van Klinken	44p4	26.4500	144.31667

DNA Sample Number.	Label	Putative Species	Collector	Sample identity	Latitude	Longitude
98	OZ/PROSS/I18/01	*P. pallida*	A. White	45p1	20.6000	140.96667
99	OZ/PROSS/I18/02	*P. pallida*	A. White	45p2	20.6000	140.96667
100	OZ/PROSS/I18/03	*P. pallida*	A. White	45p3	20.6000	140.96667
101	OZ/PROSS/I18/04	*P. pallida*	A. White	45p4	20.6000	140.96667

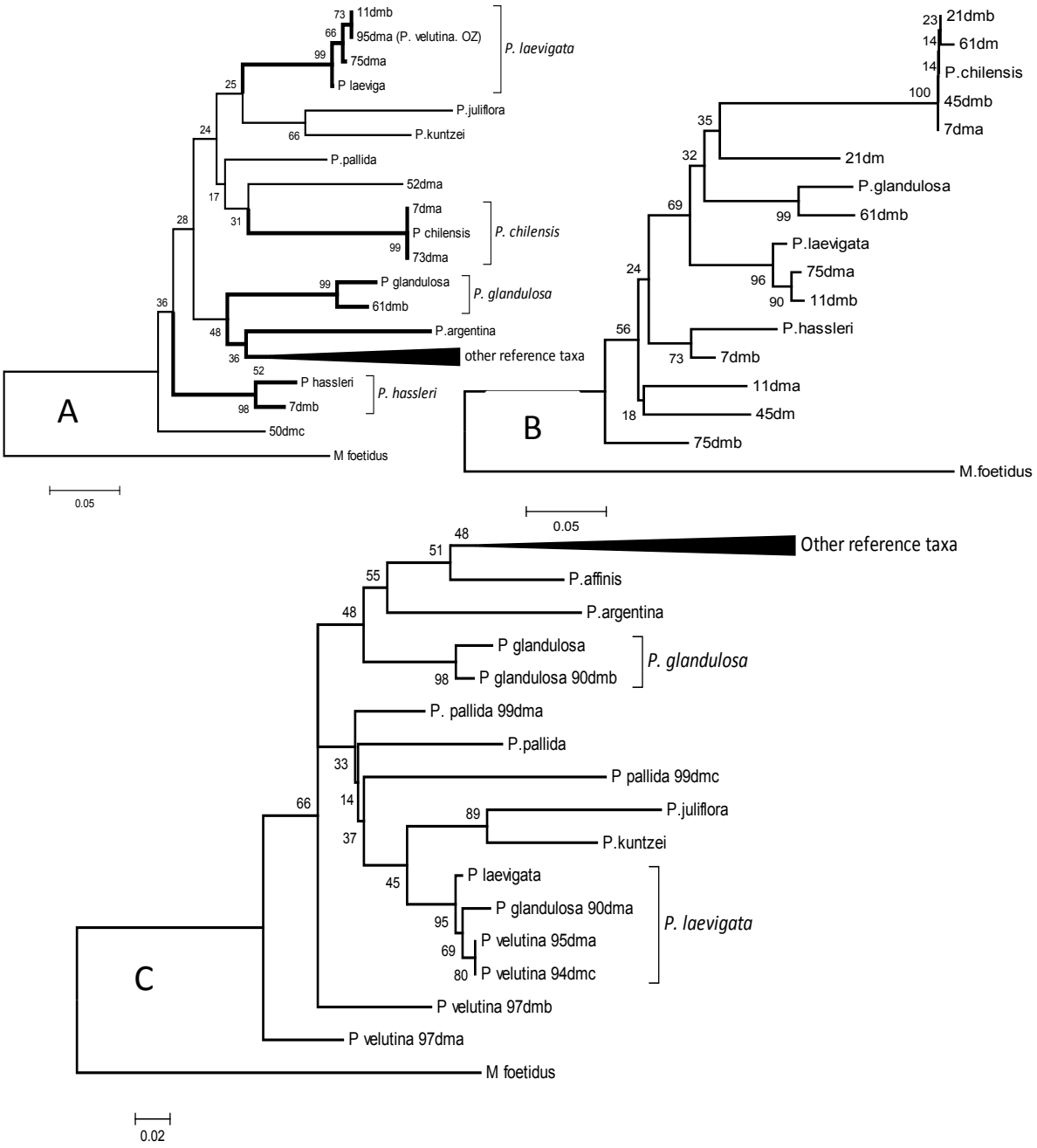
Appendix 2.2 Table showing taxonomic uncertainties at the time of *Prosopis* introduction to South Africa. There are no records for the sources of most species germplasm. Poynton, (2009) suggests local seed sources for some species. *P. dulcis* is now regarded as *P. laevigata* while *P. glandulosa* and *P. velutina* were previously considered as varieties under *P. dulcis* (Poynton, 2009*)*. Hence from the current findings, *P. laevigata* could have been introduced as *P. dulcis* in 1880, or was indeed the 1985 seed consignment from Honduras, (which was introduced under the name *P. juliflora*).

***Prosopis* species In South Africa according to Poynton**	**Identity at Introduction according to Poynton**	**Year of introduction**	**Native range**	**Seed source**
P. velutina	*P. juliflora*	1906	USA and Mexico	USA*
P. glandulosa	*P. dulcis, P. juliflora, P. velutina*	1880	USA and Mexico	unknown
P. chilensis	*P. juliflora*	unknown	South America	unknown
P. juliflora	*P. juliflora*	1985	South and Central America	Honduras
P. laevigata	*P. juliflora*	1985	Mexico	Honduras
P. tamarugo	*P. tamarugo*	1971	Chile	Chile
P. pubescens	*P. pubescens*	1879	Southwestern USA	unknown

Appendix 2.3 A Maximum Parsimony tree showing the relationships of *Prosopis* confirmed to be present in South Africa (a targeted analysis). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The tree was obtained using the Close-Neighbor-Interchange algorithm. All gaps in the analysis were treated as missing data.

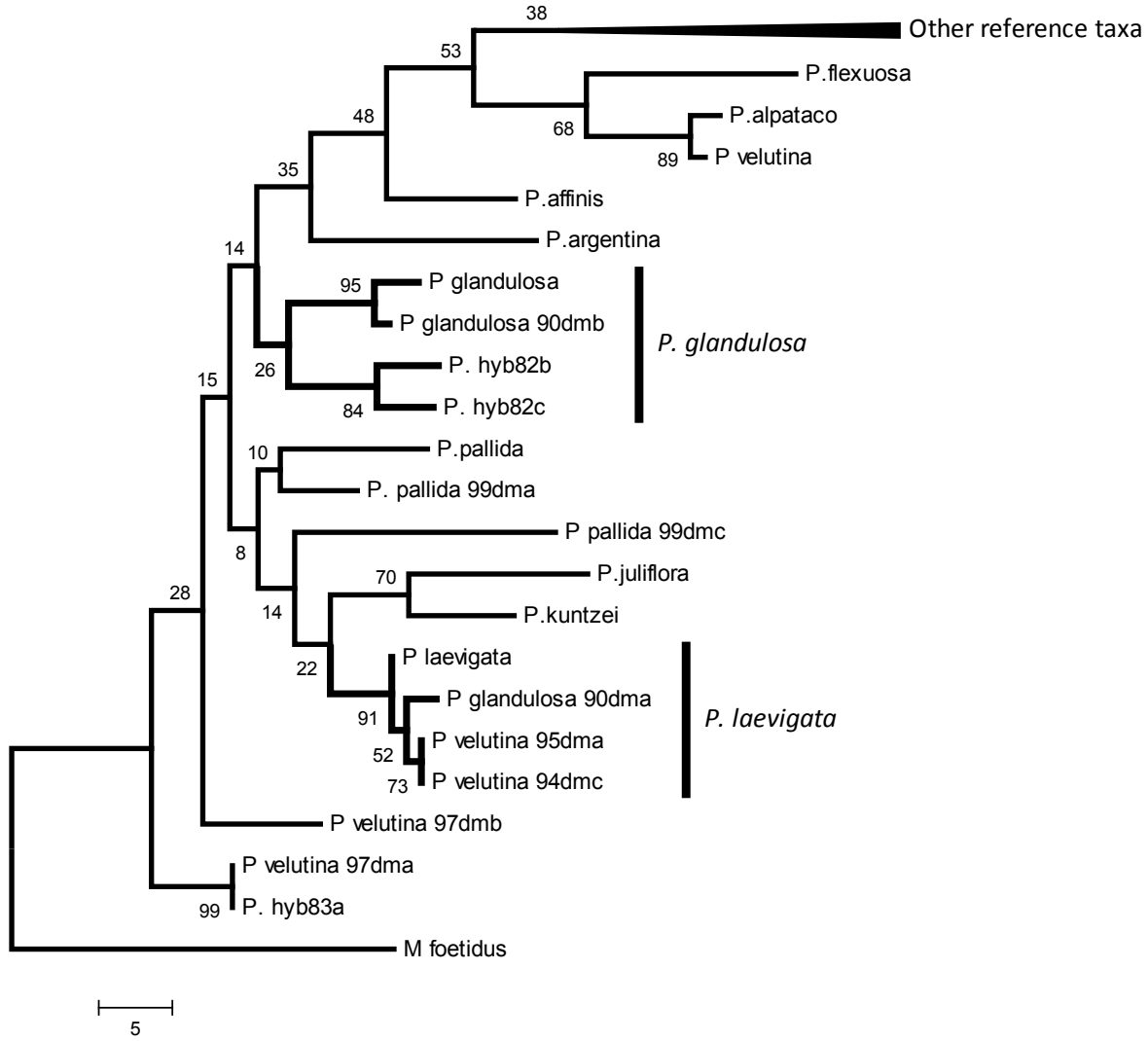


Appendix 2.4 Neighbor-joining trees showing relationships for *Prosopis* taxa in South Africa, their hybrids, and a comparison of Australian taxa and some reference taxa.



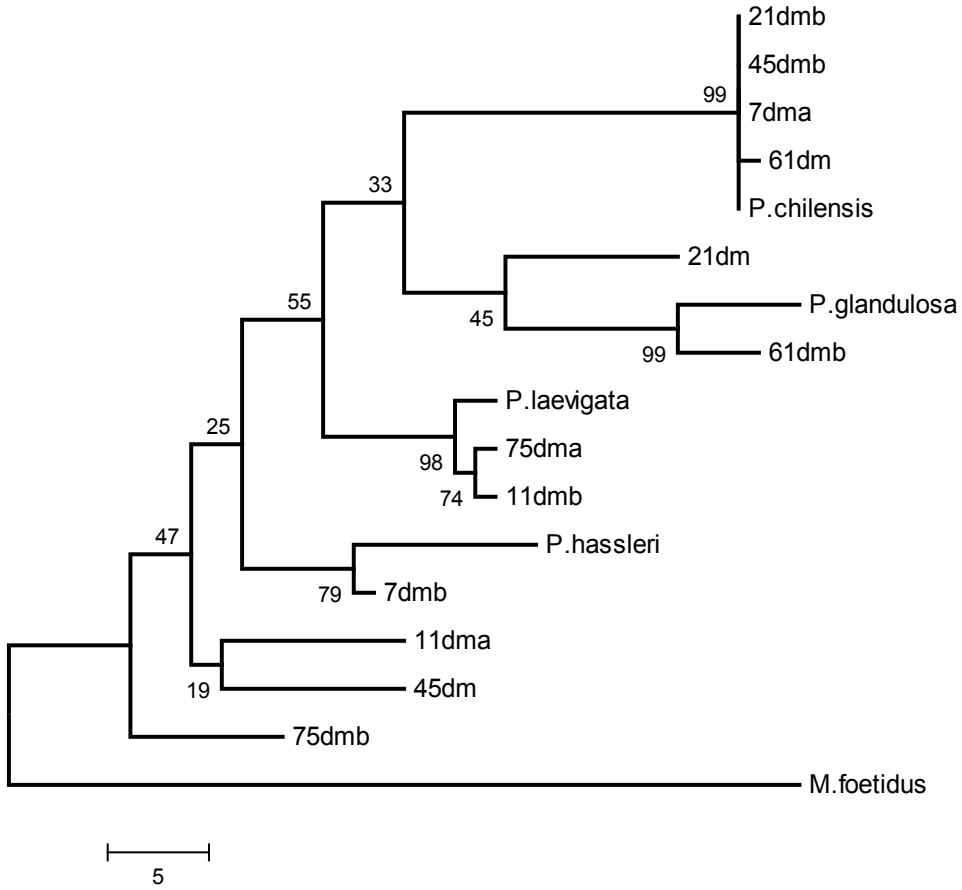
Neighbour-joining optimal trees showing evolutionary relationships of *Prosopis* taxa confirmed present in South Africa (**A**), Species involved in hybridization (**B**) and relationships of Australian reference samples as compared with those from Bessega *et al.,* (2006; (**C**)). Evolutionary distances were computed using the Kimura-2-parameter method as implemented in Mega v4 (Tamura *et al.,* 2007).

Appendix 2.5 Relationship of only Australian *Prosopis* samples in relation to reference samples form Bessega *et al.,* (2006)



A maximum parsimony analysis of Australian *Prosopis* samples in relation to reference samples form Bessega *et al.,* (2006). Samples that are referred to as *P. velutina* in Australia (here these are followed by their corresponding DNA sample number) are not closely related with reference *P. velutina*; instead, they are closely related to *P. laevigata*, except for sample 97 (*P. velutina*97) which is closely related to either. What is identified as *P. glandulosa* in Australia (*P. glandulosa* 90), is shown here as a potential hybrid between *P. glandulosa* and *P. laevigata*. Australian *P. pallida* (*P. pallida* 99) seems to be a likely hybrid between *P. pallida* and some other species (an observation confirmed in the complete dataset analysis (Figure **2.2**).

Appendix 2.6 Genetic relationships for some *Prosopis* hybrids as clarified from the targeted analysis.



A Maximum Parsimony tree showing the relationships of some confirmed *Prosopis* hybrids. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree was obtained using the Close-Neighbor-Interchange algorithm. All gaps in the analysis were treated as missing data. Hybridization occurs between *P. chilensis* and P*. glandulosa* (sample 61dm), Between *P. chilensis* and *P. hassleri* (sample 7dm), between *P. chilensis* and *Prosopis spp*. (samples 21 and 45), between *P. laevigata* and *Prosopis spp*. (sample 75dm and 11dm).

Appendix 3.1 Morphological key for *Prosopis* compiled by Burkart, (1976) as presented in Pasiecznik *et al.* (2004)

Table 1. Tree characteristics. Adapted from Burkart (1976)

Prosopis species	**Height (m)**	**Trunk**	**Branches**	**Thorn type and length**	**Foliage**	**Flower/raceme length (cm)**
P. juliflora	3-12	-	spreading, sometimes shrubby	solitary or paired 0.5-5.0 cm	glabrous, somewhat pubescent	7-15
P. pallida	8-20	to 60 cm girth	-	thornless or thorns <4.0cm	pubescent, or at least ciliate	8-15
P. glandulosa	3-9	-	-	mostly solitary, 1-4.5 cm	glabrous	5-14
P. velutina	To 15	short, to 1 m girth	drooping, rounded crown	1-2 cm	pubescent more or less on all parts	5-15
P. alba	5-15	short, to 1 m girth	rounded crown	thornless or scarce, 2-4 cm	glabrous	7-11
P. chilensis	3-10	short	rounded crown	conical, to 6 cm	glabrous rarely ciliate	7-12
P. africana	4-20	-	-	entirely thornless	glabrous, Finely pubescent	5-9
P. cineraria	To 6.5	-	-	Intermodal prickles (as in *Rosa* spp.)	glabrous or puberulous	5-15

Table 2. Leaf characteristics. Adapted from Burkart (1976)

	Pairs of pinnae	**Pinnae length (cm)**	**Leaflet pairs per pinna**	**Leaflet length and width (mm)**	**Distance apart**	**Leaflet shape**
P. juliflora	1 to 3, rarely 4	3-11	6-29	6-23 x 1.6-5.5	adjacent or leaflet width apart	emarginated or obtuse
P. pallida	2 to 4 rarely 1	1.5-6	6-15	2.5-8.3 x 1.4-4.0	adjacent but not touching, or a little distant	oblong-elliptic to ovate
P. glandulosa	1 or 2	6-17	6-17	20-63 x 1.5-4.5	distant, 7-8 mm apart	linear or oblong
P. velutina	1 or 2 sometimes 3	2-9	12-30	4-13 x 2.0-4.0	adjacent	obtuse
P. alba	1 to 3	6-14	25-50	5-17 x 1.0-2.0	adjacent	linear acute or subacute
P. chilensis	1 or 2 sometimes 3	8-24.5	10-29	11-54 x 1.1-3.0	distant, 4-12 mm apart	long-linear
P. africana	1 to 4	6-15	4-13	13-35 x 4.0-15.0	-	ovate-lanceolate
P. cineraria	1 to 3	2-7	7-14	4-15 x 2.0-4.5	-	ovate

Table 3. Pod characteristics. Adapted from Burkart (1976)

	Colour	**Pod length &width (cm)**	**Seeds per pod**	**Pod shape**	**Margins, cross-section**	**Tip shape**
P. juliflora	straw-yellow to brown	8-29 x 0.9-1.7	to 25	straight or curved	parallel, compressed	stipitate and accuminate
P. pallida	straw yellow	10-25 x 1.0-1.5	to 30	straight or curved	parallel, sub-compressed	long or short stipitate, acuminate
P. glandulosa	straw yellow or tinged violate	8-20 x 0.7-1.3	5-18	straight rarely subfalcate	compressed to subterete	short stipe and strong acumen
P. velutina	yellow sometimes reddish	8-16 x 0.6-1.0	10-17	straight or falcate	shallowly undulate, flattened	Stipe 2-10 mm, beak 2-11 mm
P. alba	straw yellow	12-25 x 1.1-2.0	12-30	falcate to ring-shaped	parallel, compressed	stipitate and accuminate
P. chilensis	straw yellow	12-18 x 1.0-1.8	20-32	nearly straight, falcate, or subfalcate	parallel, compressed	stipitate and accuminate
P. africana	brown to blackish shiny	10-20 x 1.5-3.3	many	-	subterete, ovate-compressed	-
P. cineraria	-	8-19 x 0.4-0.7	-	elongate, slender	sub-cylindrical-torulose	Stipe 8-20 mm long

Appendix 3.2 Table showing morphological attributes for preliminary identification of *Prosopis* samples collected in South Africa. Descriptions are based on Burkark's (1976) key.

Sample ID	Thorn/ pairs	Pinnae pairs	Pinnae Length (cm)	Leaflet/ Pairs/ pinnae	Leaflet Length /width (mm)	Distance between leaflets (mm)	Leaflet shape	Pod colour	Seeds/ pod	Pod margin and CS	Pod tip shape	Pod length and width (cm)	Pod shape
3P36	1	1-2	7-9	13-14	15-20 1-2	4-7	Oblong Sub-acute	yellow	4-9	Parallel, sub-compressed	Stipitate, long acumen	7-10 0.6-1.0	Straight to sub-falcate
4P36	1	1	2.5-4.5	10-18	6-10 1.5-2.0	2	Oblong to obtuse	Tinged violet	9-21	Undulating and compressed	Stipitate and short acumen	10-17 0.6-1.0	Straight to subfalcate
1P36	1	1	7-8	10-16	9-20 1	5	Oblong and subacute	yellow	10-18	Undulating, subterete to torulose	Stipitate and acuminate	7.5-17 0.7	Nearly straight
8P36	solitary	1-2	6.5-9.0	12-18	7-15 1-2	3-4	Long, linear to obtuse	Tinged violet	15-35	Parallel and compressed	Stipitate and strongly accuminate	15-28 1.5-1.7	Nearly straight
7P36	-	-	-	-	-	-	-	yellow	5-17	Parallel-undulating	Stipitate and short acumen	5-14.5 0.7-1.0	Nearly straight
6P30	thornless	1-2	7-13	20-32	14-20 1.0	4	Oblong, linear, obtuse	"Tinged violet"	9-25	Shallowly undulating and flattened	Stipitate and acuminate	8-17 0.7	Nearly straight
2P30	solitary	1-2	8-10	12-25	15-20 1.5-2.0	4	Linear, oblong, obtuse	"tinged yellow"	12-25	Parallel and compressed	Stipitate and acuminate	6-13 1.2-1.6	Nearly straight
1P30	1	1	5-8	12-15	13-15 1.1-1.5	4	Linear and obtuse	yellow	13-32	Shallowly undulating and compressed	Stipitate and acuminate	10-19 0.5-0.9	Nearly straight to Subfalcate
5P30	solitary	1	5-8.5	15-18	12-22 1.5-2.0	5	Oblong linear and subacute	Tinged yellow	7-19	Shallowly undulating to compressed.	Stipitate and acuminate	7-16 0.6-1.0	nearly straight to curved
10P30	Solitary-1 pair	1	6-9	11-19	15-25 2-2.5	5	Linear oblong obtuse	yellow	12-38	Parallel and sub-compressed	Stipitate and acuminate	9-21 1.1-1.7	Curved to curled
7P30	thornless	1-2	10-13	12-18	15-30	7	Long	Tinged		Shallowly	Stipitate and	10-18	Nearly

Sample ID	Thorn/ pairs	Pinnae pairs	Pinnae Length (cm)	Leaflet/ Pairs/ pinnae	Leaflet Length /width (mm)	Distance between leaflets (mm)	Leaflet shape	Pod colour	Seeds/ pod	Pod margin and CS	Pod tip shape	Pod length and width (cm)	Pod shape
					1.5-2		linear and acute	yellow		undulating compressed	acuminate	0.7-1.4	straight to curved
8P30	thornless	1-2	9-12	13-15	21-32 1.5-2.0	5-7	Long linear subacute	Straw yellow – tinged violet	7-27	Pararell and compressed	Stipitate & acumminate	8-20 1-1.3	Curved
3P30	thornless	1	8-10	11-20	10-16 1.5-2.0	4	Long linear subacute	Straw yellow	8-27	Parallel and compressed	Stipitate and sub-acuminate	8-19 1-1.5	Nearly straight to curved
4P30	1 pair	1	9-16	15-28	10-14 1.0-1.1	5	Linear curved and obtuse	Red blackish	8-20	Parallel to shallowly undulating to compressed	Stipitate and short acumen	9.5-15 1-1.3	Nearly straight
7P24	1 pair	1	5-8.5	20-26	8-11 1-1.5	3	Long linear subacute	brown	14-19	shallowly undulating and subcompressed	Stipitate and short acumen	8-13 0.7	Nearly straight
6P24	thornless	1	7-9	14-21	12-18 1.8-2.0	4	Linear and obtuse	Tinged violet	12-26	Shallowly undulating and compressed	Stipitate and short acumen	9-16 0.8-1.0	Mostly straight to subfalcate
5P24	1	1	4-5	12-19	4-6 1.0-1.3	3	Linear and obtuse	Tinged violet to yellow	11-26	Shallowly undulating to compressed	Stipitate and short acumen	7-14 0.8-1.0	Nearly straight to subfalcate
3P24	thornless	1	12-16	18-26	25-35 1.5-2.0	7	Long linear and subacute	Reddish	18-28	Shallowly undulating & compressed	Short Stipe & long acumen	12-23 1.0-1.2	Nearly straight to curved
2P24	1	1	8-12	14-19	15-23 1.0-1.5	6	Long linear and	Tinged violet	16-27	Parallel to shallowly	Stipitate and short acumen	10-16 1.0-1.3	Curved & falcate

Sample ID	Thorn/ pairs	Pinnae pairs	Pinnae Length (cm)	Leaflet/ Pairs/ pinnae	Leaflet Length /width (mm)	Distance between leaflets (mm)	Leaflet shape	Pod colour	Seeds/ pod	Pod margin and CS	Pod tip shape	Pod length and width (cm)	Pod shape
							acute			undulating &compressed			
4P24	Solitary and scarce	1	7-12	12-25	11-21 1.5-2.0	5	Linear and subacute	Reddish with yellow spots	11-27	Shallowly undulating & flattened	Stipitate & acumminate	13-23 ------	Nearly straight
1P24	1	1	5.5-8.5	16-22	8-13 2.0-2.5	3.5	Long linear &obtuse	Yellow with violet spots	15-28	shallowly undulating & compressed	Stipitate and short acumen	10-19 1.0-1.4	Nearly straight to subfalcate
9P37	thornless	1-2	6-7	21-27	6-10 1.5-2.0	2	Linear and sub-obtuse	Tinged violet	12-25	Shallowly undulating to compressed	Stipitate and short acumen	12-22 0.9-1.2	Nearly straight to subfalcate.
1P37	Solitary and 1 pair (2.5cm)	1	5-8.5	12-22	7-13 2.0-3.0	4	Oblong, linear &obtuse	Tinged violet	9-22	Shallowly undulating to compressed	Stipitate. And long acumen	11-19 0.7-1.0	Nearly straight
8P37	Solitary and 1 pair (2.5cm)	1-2	2.7-6	16-20	4-7 1-1.5	1.5-2.5	Linear and obtuse	Yellow to tinged violet	8-22	Shallowly undulating &compressed	Stipitate and short acumen	8-19 0.9-1.1	Nearly straight to curved.
13P37	Solitary & 1 pair	1	3-4.5	12-15	4-8 2-3	3	Short linear and obtuse	Yellow with violet spots	7-19	Shallowly undulating & compressed	Stipitate and acumminate	7-19 0.8-1.1	Nearly straight most curved
2P37	1 pair & Mostly thornless	1	3.5-5.0	13-16	6-10 2-3	3	Short linear & obtuse	yellow	8-18	Shallowly undulating & compressed	Stipitate and short acumen	8-16 0.9-1.0	Subfalcate/ slightly curved

Sample ID	Thorn/ pairs	Pinnae pairs	Pinnae Length (cm)	Leaflet/ Pairs/ pinnae	Leaflet Length /width (mm)	Distance between leaflets (mm)	Leaflet shape	Pod colour	Seeds/ pod	Pod margin and CS	Pod tip shape	Pod length and width (cm)	Pod shape
4P37	Solitary & 1 pair	1-2	3.5-6.0	12-21	7-10 1.5-2.0	3	Short linear and obtuse	Yellow with violet spots	15-28	Shallowly undulating & sub-cylindrical	Stipitate, and long acumen	12-22 0.6-0.8	Straight & subfalcate.
5P37	thornless	1-2	4.5-7.0	12-16	9-10 2-3	3	Short linear and obtuse	Tinged violet	10-17	Parallel to shallowly undulating & sub-compressed	Stipitate and short acumen	8-15 0.8-1.1	Nearly straight – subfalcate
6P37	1	1-2	3.0-4.5	12-14	6-7 2.0-2.5	2.5	Short, linear &obtuse	yellow	8-26	Shallowly undulating &compressed	Stipitate and short acumen	11-22 0.9-1.0	Nearly straight
5P22	thornless	1	3-4	10-18	5-8 1.5-2.0	2	Short linear and subacute	yellow	5-19	Undulating and sub-compressed to torulose	Stipitate and short acumen	6-13 0.5-0.7	straight to subfalcate.
10P33	1 pair	2	7-11	15-20	15-20 1.5-2.0	5	Long, linear &subacute	yellow	15-26	Parallel & compressed	Stipitate and short acumen	15-20 1.0-1.5	nearly straight to subfalcate
4P33	thornless	1	9-10	13-19	15-27 2.5-4.0	6	Long, broader at end & obtuse	yellow	9-22	Shallowly undulating &subcompressed	Stipitate and short acumen	13-22 0.9-1.8	Nearly straight to subfalcate.
9P33	1 pair	2	11-14	22-35	10-14	4	Long, linear & subacute	yellow	11-30	Parallel & compressed	Stipitate, and long strong acumen	13-24 1.0-1.5	subfalcate
10P19	1 pair strong (Conical)	1	5-9	8-16	15-20 1.5-1.9	6	Long, linear &subacute	brownish	9-26	Parallel & subcompressed	Stipitate and short acumen	10-20 0.8-1.1	Nearly straight
15P26	1 pair	1	6-7	20-22	5-10 2.0	3	Short linear & obtuse	Brown to violet	8-16	Undulating compressed and tolulose	Stipitate, and long acumen	9-18 0.6-0.9	Straight

Sample ID	Thorn/ pairs	Pinnae pairs	Pinnae Length (cm)	Leaflet/ Pairs/ pinnae	Leaflet Length /width (mm)	Distance between leaflets (mm)	Leaflet shape	Pod colour	Seeds/ pod	Pod margin and CS	Pod tip shape	Pod length and width (cm)	Pod shape
16P26	1 pair	1	5-10	15-23	11-15 2-3	3.5	Long linear & obtuse	yellow	7-21	Shallowly undulating & compressed	Stipitate and short acumen	10-26 1.0-1.3	Nearly straight &curved at the tip end
7P33	thornless	1	6-10	10-22	10-13 1.0-1.5	4	Long, linear &subacute	yellow	8-17	Parallel-shallowly undulating & compressed	Stipitate, and short conical acumen	9-16 0.8-1.1	nearly straight to subfalcate
2P33	thornless	1-2 mostly 2	9-14	18-26	16-21 1.5-2.0	6	Long, linear, subacute	Straw yellow to brownish	18-26	Shallowly undulating to subcompressed	Stipitate and short acumen	17-26 0.8-1.0	Nearly straight to curved.
4P20	1 pair	1	5-7	13-17	8-13 1.5-3.0	4	Long linear obtuse	Yellow to brown	8-25	Shallowly undulating & compressed	Stipitate, and medium long acumen	7-20 0.6-1.0	Subfalcate to nearly straight
2P20	1 pair	1	4-7	9-15	7-11 1.1-2.0	3	Long linear & obtuse	Yellowish to brown	8-28	Parallel or shallowly undulating & compressed	Stipitate, and medium long acumen	10-20 0.8-1.3	Straight to subfalcate. Curved at the tip.
1P34	1 pair	1	7-11	20-23	8-15 1.1-2.0	5	Long linear &obtuse	yellow	10-24	Shallowly undulating &subcompressed	Stipitate and very long acumen	15-26 1.0-1.4	Subfalcate to nearly straight
5P34	1	1	6-8	11-18	7-17 2-3	5	Long linear obtuse	yellow	6-15	Shallowly undulating &compressed	Stipitate, and long acumen	9-16 0.8-1.1	Nearly straight or subfalcate
6P34	solitary	1	3-7	9-15	7-11 2-3	4	Short &curved obtuse	yellow	7-15	Shallowly undulating, suterete and tolurose	Stipitate, and short thick acumen	9-16 0.5-0.7	Straight
4P34	solitary	1	4-8	7-13	8-20 1.5-2.0	6	Long, linear,	yellow	6-14	Parallel to undulating,	Stipitate and short acumen	7-13 0.8-1.2	Straight to subfalcate.

Sample ID	Thorn/ pairs	Pinnae pairs	Pinnae Length (cm)	Leaflet/ Pairs/ pinnae	Leaflet Length /width (mm)	Distance between leaflets (mm)	Leaflet shape	Pod colour	Seeds/ pod	Pod margin and CS	Pod tip shape	Pod length and width (cm)	Pod shape
							subacute.			subcompressed			
3P34	1 pair	1	7-8	20-22	10-14 1.6-2.0	4	Long, linear &obtuse	yellow	8-20	Parallel-shallowly undulating & compressed	Stipitate, and medium short acumen	11-21 1.0-1.2	Straight to subfalcate
2P34	1 pair	1	7-15	17-24	15-24 1.5-2.5	6.5	Long subacute & obtuse	yellow	11-22	Shallowly undulating to compressed	Stipitate and medium short acumen	11-24 1.0-1.4	Nearly straight to subfalcate
8P34	1 pair	1	6-12	14-20	13-18 2-3	4-7	Long, linear & obtuse	yellow	14-27	Parallel, compressed to subcompressed	Stipitate and short acumen	14-26 0.9-1.6	Heavily curved, ribbon-like.
9P34	thornless	1	8-12	12-30	12-18 1.5-2.0	5	Long, linear & subacute.	yellow	16-26	Parallel & compressed	Stipitate and short acumen	16-23 1.4-1.8	Falcate, ring shaped.
9P26	solitary	1	6-8	12-15	11-13 2.5-3.0	6	Short, linear & obtuse	yellow	15-24	Parallel to shallowly undulating & compressed	Stipitate and accuminate	16-23 1.0-1.3	Nearly straight &subfalcate.
12P26	1 pair	1	6-8	10-17	11-14 1.8-2.5	4	Short, linear & obtuse	yellow	9-24	Shallowly undulating & subcompressed	Stipitate and short acumen	10-22 0.8-1.0	falcate
11P26	1 pair	1-2	5-10	19-22	10-12 1.5-2.0	4	Long, curved and obtuse	Yellow with violet spots	9-16	Shallowly undulating and subcompressed	Stipitate and short acumen	10-18 1.0-1.3	Straight to subfalcate
3P26	1 pair	1	5-6	13-15	8-13 1.5-2.0	3.5	Linear & subacute. Long-medium	Yellow to straw yellow	9-29	Undulating and compressed	Stipitate, and long acumen	10-27 0.8-1.0	Subfalcate
10P26	1 pair	1	4-8	12-16	8-12 1.5-2.0	4.5	Long linear &	Tinged violet	10-24	Shallowly undulating &	Long stipe and acumen	12-27 0.8-1.0	Nearly straight to

Sample ID	Thorn/ pairs	Pinnae pairs	Pinnae Length (cm)	Leaflet/ Pairs/ pinnae	Leaflet Length /width (mm)	Distance between leaflets (mm)	Leaflet shape	Pod colour	Seeds/ pod	Pod margin and CS	Pod tip shape	Pod length and width (cm)	Pod shape
							obtuse			compressed			subfalcate.
13P26	1 pair	1	5-9	20-22	8-12 2-3	4	Long linear and obtuse	yellow	8-24	Parallel and compressed	Stipitate and acumminate	10-27 1.0-1.5	falcate
1P26	1 pair	1-2	5-8	13-18	10-14 2-3	5	Long, linear, obtuse to subacute	yellow	7-23	parallel to shallowly undulating, compressed	Stipitate and accuminate	9-21 0.9-1.2	Falcate to nearly straight.
2P28	1 pair	1	8-11	15-18	17-21 2-3	5	Long linear & obtuse	Tinged violet	9-21	Shallowly undulating & compressed	Stipitate and acumminate	9-18 0.7-1.1	Nearly straight to subfalcate.
1P28	1 pair	1	6-9	17-20	10-13 1.5-2.0	5	Long, linear & obtuse	Tinged violet	12-28	Parallel to shallowly undulating	Stipitate and acumminate	14-25 1.0-1.8	Falcate; s-shaped
7P28	1 pair	1	9-13	17-24	15-20 1.0-1.5	6	Long, linear & subacute	Yellow	15-21	Shallowly undulating & subcompressed	Stipitate and acuminate (sharp)	9-18 0.9-1.2	Nearly straight to subfalcate.
11P25	1 pair	1	6-9	15-20	7-12 1.5-2.0	3	Long, linear & obtuse	yellow	9-20	Shallowly undulating and compressed	Stipitate and short acumen	9-19 0.7-0.9	Nearly straight to subfalcate.
5P25	1 pair	1	4-6	10-15	8-11 1.5-2.0	3	Long, linear and obtuse	yellow	13-22	Undulating, cylindrical to subterete torulose, long and slender	Stipitate and acumminate	12-26 0.4-0.6	Falcate.

Appendix 4.1 A list of locations where *Prosopis* samples were collected in March 2010 across the invasive distribution range in South Africa, with geo-climatic data. Minimum and maximum values for both temperature (Degrees Celsius) and rainfall (millilitres) are annual averages for the different localities.

Location	Latitude	Longitude	Altitude (m)	Temperature (Min) °C	Temperature (Max) °C	Rainfall (Min) mm	Rainfall (Max) mm
Halfway between Beaufort West and Laingsburg	-32.5715	21.42482	803	10.4	25.1	10.96	50.8
Outside Beaufort West	-32.1953	22.34842	1390	11.2	27.2	3.6	47.1
Loxton	-31.3355	22.21218	1407	8.1	23.8	5.3	40.8
Between Carnavon and Vosburg	-30.1553	22.14366	996	9.7	26.4	3.6	41.7
Britstown(20Km on road to Vosburg	-30.3374	23.18446	1135	11.2	27.2	3.6	47.1
On road to Kimberly(40Km form Britstown)	-30.1403	23.37292	1048	11.2	27.2	3.6	47.1
20Km South of Kimberly	-29.0306	24.37088	1114	9.5	27	3.2	56.4
5Km out of Vryburg on road to Kimberly	-27.0978	24.44845	1308	9.1	25.79	0.74	82.09
Delareyville	-26.4158	25.27256	1342	11.3	26	2.1	114.8
Kuruman	-27.2784	23.25925	1200	11.5	26.7	3.3	66.8
Danielskuil	-28.1172	23.32802	1488	8.6	25.2	0.48	90.8
15Km North of Douglas	-28.5454	23.45782	1437	8.6	28.5	9.9	110.7
Douglas	-29.0287	23.46098	1304	8.6	28.5	9.9	110.7
Griquastad	-28.5164	23.16031	1398	8.6	25.2	0.48	90.8
30Km from Griqua on road to Prieska	-29.0385	23.16031	1267	10.16	29.3	1.55	59.6
Prieska	-29.3919	22.44748	905	10.16	29.3	1.55	59.6

Location	Latitude	Longitude	Altitude (m)	Temperature (Min) °C	Temperature (Max) °C	Rainfall (Min) mm	Rainfall (Max) mm
Williamstown	-30.0907	22.11701	1006	9.7	26.4	3.6	41.7
40Km west of Williamstown on road to Carnavon	-31.2049	20.55066	1043	9.9	25.2	7.7	39.4
VanWyksvlei	-31.153	21.16957	1214	9.9	25.2	7.7	39.4
Saaipoort Farm	-29.5087	22.05964	1074	12.4	27.8	3.7	42.5
25Km from **Van Wyksvlei** on road to Prieska	-30.165	21.54558	933	9.7	26.4	3.6	41.7
Klaas titusvlei farm	-30.2028	21.2466	901	9.7	26.4	3.6	41.7
Brandvlei	-30.2791	20.2933	929	11.4	29	0.5	33.3
Keimoes town	-28.448	20.59255	850	11.3	28.4	2.8	52.7
2Km south of Augrabies National Park	-28.3833	20.29975	876	16.9	32.9	0.3	19.7
85Km west of Pofadder	-28.5102	20.09085	639	16.9	32.9	0.3	19.7
Pofadder	-29.0772	19.23889	796	12.38	26.8	3.3	28.4
Aggeneys	-29.184	18.47596	648	7.3	24.3	1	11.8
Springbok	-29.3972	17.53909	418	11.8	24.6	4.5	29.1
Garies	-30.3346	17.59509	269	10.7	22.5	7.47	87.36
Clanwilliam	-32.1088	18.53602	189	11.3	28.2	2.5	42.6
Klawer	-31.4678	18.37618	177	13.4	27.2	3.7	35.9
Near Rondeberg Resort	-31.5989	18.46265	103	11.1	27.1	1.11	30.6
10Km north of Piketberg	-32.5005	18.49226	169	11.7	25.8	4.9	47.54
25Km south of Kenhardt along R27	-29.3242	21.00013	832	11.7	28.3	3.5	29.5

Location	Latitude	Longitude	Altitude (m)	Temperature (Min) °C	Temperature (Max) °C	Rainfall (Min) mm	Rainfall (Max) mm
40 Km south of Kenhardt on road to VanWyksvlei	-29.3838	21.16872	802	11.7	28.3	3.5	29.5

Appendix 4.2 Genome sizes for all South African samples of *Prosopis*. The symbol (*) stands for specific sizes of the corresponding identified sample. For each population, a pooled genome size value is also given and is the same for all samples in any given population. Samples are identified by notation XpY where X is a specific individual sample number and Y is the population number where the individual X was sampled.

Sample Identity	Fluorescence intensity	Genome size (pg)	Latitude	Longitude
4p2	0.309	1.18656	-32.5715	21.42482
18p2	0.312	1.19808*	-32.5715	21.42482
16p2	0.309	1.18656	-32.5715	21.42482
14p2	0.309	1.18656	-32.5715	21.42482
13p2	0.309	1.18656	-32.5715	21.42482
10p2	0.313	1.20192*	-32.5715	21.42482
8p2	0.309	1.18656	-32.5715	21.42482
7p2	0.309	1.18656	-32.5715	21.42482
3p2	0.309	1.18656	-32.5715	21.42482
5p3	0.314	1.20576*	-32.1953	22.34842
1p3	0.311	1.19424	-32.1953	22.34842
10p4	0.308	1.18272*	-31.3355	22.21218
4p4	0.307	1.17888	-31.3355	22.21218
2p4	0.307	1.17888	-31.3355	22.21218
7p4	0.307	1.17888	-31.3355	22.21218
3p4	0.308	1.18272*	-31.3355	22.21218
8p4	0.307	1.17888	-31.3355	22.21218
1p4	0.307	1.17888	-31.3355	22.21218
9p4	0.307	1.17888	-31.3355	22.21218
3p5	0.309	1.18656*	-30.1553	22.14366
6p5	0.311	1.19424	-30.1553	22.14366
7p5	0.311	1.19424	-30.1553	22.14366
13p5	0.311	1.19424	-30.1553	22.14366
8p5	0.311	1.19424	-30.1553	22.14366
6p6	0.308	1.18272*	-30.3374	23.18446
7p6	0.307	1.17888	-30.3374	23.18446
8p6	0.307	1.17888	-30.3374	23.18446
12p6	0.307	1.17888	-30.3374	23.18446
4p6	0.313	1.20192*	-30.3374	23.18446
3p6	0.307	1.17888	-30.3374	23.18446
2p6	0.307	1.17888	-30.3374	23.18446
1p6	0.307	1.17888	-30.3374	23.18446
1p7	0.321	1.23264*	-30.1403	23.37292
7p7	0.318	1.22112	-30.1403	23.37292
2p7	0.318	1.22112	-30.1403	23.37292
9p7	0.318	1.22112	-30.1403	23.37292
10p8	0.313	1.20192*	-29.0306	24.37088

Sample Identity	Fluorescence intensity	Genome size (pg)	Latitude	Longitude
9p8	0.31	1.1904	-29.0306	24.37088
4p8	0.31	1.1904	-29.0306	24.37088
3p8	0.31	1.1904	-29.0306	24.37088
2p8	0.313	1.20192*	-29.0306	24.37088
1p8	0.31	1.1904	-29.0306	24.37088
5p8	0.31	1.1904	-29.0306	24.37088
1p10	0.305	1.1712*	-26.4158	25.27256
4p10	0.309	1.18656	-26.4158	25.27256
5p10	0.309	1.18656	-26.4158	25.27256
7p11	0.314	1.20576*	-27.2784	23.25925
1p11	0.31	1.1904	-27.2784	23.25925
2p11	0.31	1.1904	-27.2784	23.25925
13p12	0.312	1.19808*	-28.1172	23.32802
14p12	0.313	1.20192	-28.1172	23.32802
12p12	0.313	1.20192	-28.1172	23.32802
11p12	0.313	1.20192	-28.1172	23.32802
2p12	0.313	1.20192	-28.1172	23.32802
6p13	0.315	1.2096*	-28.5454	23.45782
10p13	0.313	1.20192	-28.5454	23.45782
3p13	0.313	1.20192	-28.5454	23.45782
1p13	0.313	1.20192	-28.5454	23.45782
1p14	0.319	1.22496*	-29.0287	23.46098
7p14	0.315	1.2096	-29.0287	23.46098
8p14	0.315	1.2096	-29.0287	23.46098
10p14	0.315	1.2096	-29.0287	23.46098
12p15	0.311	1.19424*	-28.5164	23.16031
8p15	0.311	1.19424	-28.5164	23.16031
13p15	0.311	1.19424	-28.5164	23.16031
14p15	0.311	1.19424	-28.5164	23.16031
11p15	0.311	1.19424	-28.5164	23.16031
1p16	0.314	1.20576*	-29.0385	23.16031
2p16	0.313	1.20192	-29.0385	23.16031
8p16	0.313	1.20192	-29.0385	23.16031
10p16	0.313	1.20192	-29.0385	23.16031
9p16	0.313	1.20192	-29.0385	23.16031
6p17	0.315	1.2096*	-29.3919	22.44748
7p17	0.311	1.19424	-29.3919	22.44748
8p17	0.311	1.19424	-29.3919	22.44748
10p17	0.311	1.19424	-29.3919	22.44748
9p17	0.311	1.19424	-29.3919	22.44748
1p18	0.309	1.18656*	-30.0907	22.11701
2p18	0.309	1.18656	-30.0907	22.11701

Sample Identity	Fluorescence intensity	Genome size (pg)	Latitude	Longitude
7p18	0.309	1.18656	-30.0907	22.11701
11p18	0.309	1.18656	-30.0907	22.11701
6p18	0.309	1.18656	-30.0907	22.11701
10p19	0.323	1.24032*	-31.2049	20.55066
5p19	0.324	1.24416	-31.2049	20.55066
4p19	0.324	1.24416	-31.2049	20.55066
11p19	0.326	1.25184*	-31.2049	20.55066
2p19	0.324	1.24416	-31.2049	20.55066
1p19	0.324	1.24416	-31.2049	20.55066
1p20	0.304	1.16736*	-29.5087	22.05964
2p20	0.31	1.1904	-29.5087	22.05964
4p20	0.31	1.1904	-29.5087	22.05964
5p20	0.31	1.1904	-29.5087	22.05964
6p20	0.311	1.19424*	-29.5087	22.05964
7p20	0.31	1.1904	-29.5087	22.05964
8p20	0.31	1.1904	-29.5087	22.05964
7p21	0.315	1.2096*	-29.5087	22.05964
3p21	0.311	1.19424	-29.5087	22.05964
4p21	0.311	1.19424	-29.5087	22.05964
2p21	0.311	1.19424	-29.5087	22.05964
5p21	0.307	1.17888*	-29.5087	22.05964
6p21	0.311	1.19424	-29.5087	22.05964
10p21	0.311	1.19424	-29.5087	22.05964
1p21	0.311	1.19424	-29.5087	22.05964
5p22	0.313	1.20192	-30.165	21.54558
3p22	0.313	1.20192*	-30.165	21.54558
4p22	0.313	1.20192	-30.165	21.54558
2p22	0.313	1.20192	-30.165	21.54558
6p22	0.313	1.20192	-30.165	21.54558
4p24	0.323	1.24032*	-30.2791	20.2933
5p24	0.316	1.21344	-30.2791	20.2933
3p24	0.316	1.21344	-30.2791	20.2933
6p24	0.316	1.21344	-30.2791	20.2933
7p24	0.317	1.21728*	-30.2791	20.2933
1p24	0.316	1.21344	-30.2791	20.2933
2p24	0.316	1.21344	-30.2791	20.2933
11p25	0.306	1.17504*	-29.3242	21.00013
10p25	0.308	1.18272	-29.3242	21.00013
7p25	0.308	1.18272	-29.3242	21.00013
5p25	0.308	1.18272	-29.3242	21.00013
4p25	0.308	1.18272	-29.3242	21.00013
11p26	0.312	1.19808*	-29.3838	21.16872

Sample Identity	Fluorescence intensity	Genome size (pg)	Latitude	Longitude
12p25	0.313	1.20192	-29.3838	21.16872
10p26	0.313	1.20192	-29.3838	21.16872
1p26	0.313	1.20192	-29.3838	21.16872
16p26	0.308	1.18272*	-29.3838	21.16872
13p26	0.309	1.18656*	-29.3838	21.16872
15p26	0.313	1.20192	-29.3838	21.16872
9p26	0.313	1.20192	-29.3838	21.16872
14p26	0.313	1.20192	-29.3838	21.16872
3p26	0.313	1.20192	-29.3838	21.16872
1p27	0.316	1.21344*	-28.448	20.59255
7p27	0.314	1.20576	-28.448	20.59255
3p27	0.314	1.20576	-28.448	20.59255
6p27	0.314	1.20576	-28.448	20.59255
2p27	0.314	1.20576	-28.448	20.59255
7p28	0.314	1.20576*	-28.3833	20.29975
1p28	0.315	1.2096*	-28.3833	20.29975
3p28	0.317	1.21728	-28.3833	20.29975
2p28	0.317	1.21728	-28.3833	20.29975
9p29	0.312	1.19808	-28.5102	20.09085
10p29	0.312	1.19808*	-28.5102	20.09085
4p29	0.312	1.19808	-28.5102	20.09085
5p29	0.312	1.19808	-28.5102	20.09085
8p29	0.312	1.19808	-28.5102	20.09085
4p30	0.329	1.26336*	-29.0772	19.23889
1p30	0.324	1.24416	-29.0772	19.23889
2p30	0.324	1.24416	-29.0772	19.23889
5p30	0.324	1.24416	-29.0772	19.23889
7p30	0.324	1.24416	-29.0772	19.23889
3p30	0.325	1.248*	-29.0772	19.23889
8p30	0.324	1.24416	-29.0772	19.23889
10p30	0.324	1.24416	-29.0772	19.23889
6p30	0.324	1.24416	-29.0772	19.23889
2p31	0.321	1.23264*	-29.184	18.47596
6p31	0.319	1.22496	-29.184	18.47596
3p31	0.319	1.22496	-29.184	18.47596
1p31	0.319	1.22496	-29.184	18.47596
7p32	0.31	1.1904*	-29.3972	17.53909
1p32	0.309	1.18656	-29.3972	17.53909
2p32	0.309	1.18656	-29.3972	17.53909
3p32	0.309	1.18656	-29.3972	17.53909
5p32	0.313	1.20192*	-29.3972	17.53909
6p32	0.309	1.18656	-29.3972	17.53909

Sample Identity	Fluorescence intensity	Genome size (pg)	Latitude	Longitude
8p32	0.309	1.18656	-29.3972	17.53909
9p32	0.309	1.18656	-29.3972	17.53909
6p33	0.318	1.22112*	-30.3346	17.59509
5p33	0.319	1.22496	-30.3346	17.59509
7p33	0.319	1.22496	-30.3346	17.59509
2p33	0.319	1.22496	-30.3346	17.59509
9p33	0.319	1.22496	-30.3346	17.59509
1p33	0.319	1.22496	-30.3346	17.59509
6p34	0.316	1.21344*	-32.1088	18.53602
9p34	0.312	1.19808	-32.1088	18.53602
5p34	0.312	1.19808	-32.1088	18.53602
4p34	0.312	1.19808	-32.1088	18.53602
1p34	0.312	1.19808*	-32.1088	18.53602
1p34	0.312	1.19808	-32.1088	18.53602
2p34	0.312	1.19808	-32.1088	18.53602
3p34	0.312	1.19808	-32.1088	18.53602
3p35	0.311	1.19424*	-31.4678	18.37618
7p35	0.314	1.20576	-31.4678	18.37618
2p35	0.314	1.20576	-31.4678	18.37618
5p35	0.314	1.20576	-31.4678	18.37618
1p35	0.311	1.19424*	-31.4678	18.37618
6p35	0.314	1.20576	-31.4678	18.37618
4p35	0.314	1.20576	-31.4678	18.37618
12p35	0.314	1.20576	-31.4678	18.37618
4p36	0.315	1.2096*	-31.5989	18.46265
3p36	0.314	1.20576	-31.5989	18.46265
7p36	0.314	1.20576	-31.5989	18.46265
1p36	0.314	1.20576	-31.5989	18.46265
8p36	0.314	1.20576	-31.5989	18.46265
5p37	0.313	1.20192*	-32.5005	18.49226
5p37	0.306	1.17504	-32.5005	18.49226
6p37	0.306	1.17504	-32.5005	18.49226
13p37	0.306	1.17504	-32.5005	18.49226
8p37	0.309	1.18656*	-32.5005	18.49226
9p37	0.306	1.17504	-32.5005	18.49226
2p37	0.308	1.18272*	-32.5005	18.49226
4p37	0.306	1.17504	-32.5005	18.49226

LIST OF ACRONYMS

AR: Argentina

ARC: Agricultural research council

BS: Bootstrap value

CARA: Conservation of Agricultural Resources Act

C•I•B: DST-NRF Centre of Excellence for Invasion Biology

cpDNA: Chloroplast Deoxyribonucleic acid

CS: Cross section

CTAB: Cetyl trimethylammonium bromide

DA: Discriminant analysis

DAPI: 4'-6-Diamidino-2-phenylindole

DBH: Diameter at breast height

DNA: Deoxyribonucleic acid

dNTP: Deoxy-nucleotide-tri phosphate

GPS: Geographic positioning unit

GS: Genome size

GTR: General Time Reversible model.

ID: Identity (referring to taxa sample identity)

IGP: Inter-genomic polymorphism

ITS: Internal transcribed spacer

MP: Maximum Parsimony

NJ: Neighbour -joining

OZ: Australia

PCR: polymerase chain reaction

rpl32: Ribosomal protein L32

RSA/SA: Republic of South Africa

UV: Ultra-violet