INVESTIGATING HERBIVORY AND PLANT ORIGIN ON TALL-STATURED GRASSES IN SOUTH AFRICA

Kim Canavan

A thesis submitted for the degree of Doctor of Philosophy of
Rhodes University

Department of Zoology and Entomology
Rhodes University, South Africa

June, 2016

DECLARATION

I hereby certify that all materials in this thesis is my own work. Any quotation from, or description of the work of others is acknowledged by reference to the sources, whether published or unpublished.

Kim Canavan

ABSTRACT

South African riparian zones have been heavily degraded through anthropogenic activities such as dam construction and extraction of water for irrigation, which has resulted in a loss of ecosystem services and functioning. A consequence of such disturbances to riparian areas is in their susceptibility to invasive alien species (IAS). One such IAS is the giant reed, *Arundo donax* L. (Poaceae), introduced to South Africa in the 1700s largely for erosion control. *Arundo donax* has since greatly expanded in the country and is now one of the most abundant IAS. *Arundo donax* has been found to displace native vegetation and in South Africa this will most likely lead to the displacement of the native tall-statured grasses, *Phragmites australis* (Cav.) Trin. ex Steud. and *Phragmites mauritianus* Kunth.

This study aimed to enhance our understanding of the tall-statured grasses *A. donax, P. australis* and *P. mauritianus* to better manage them in riparian areas. For *A. donax,* biological control is seen as the most viable option to control stands in the long-term. However, before such a programme is put in place, it is important to first collect baseline data that can be used to guide the direction of the biological control project in South Africa. For the *Phragmites* spp., despite being a dominant vegetative type in riparian areas, very little is known about their status in South Africa. Furthermore, there have been increasing reports of both *Phragmites* species having an expansion of their range and abundance. In North America, there has been a similar trend of reed expansion and through molecular work it was determined that a cryptic invasion has occurred with the introduction of an invasive non-native haplotype from Europe. It is therefore unknown if *Phragmites* spp. populations are expanding due to anthropogenic activities or due to a cryptic invasion. To address these shortfalls in knowledge the study investigated the tall-statured grasses in two parts; firstly, molecular techniques are used to explore the plant origin and genetic diversity of *A. donax, P. australis* and *P. mauritianus* and secondly using the Enemy Release Hypothesis as a framework, herbivore assemblages for each reed was determined across their distribution in South Africa.

Molecular-techniques determined that both *P. australis* and *P. mauritianus* had only one haplotype – known as haplotype K and haplotype V respectively, across their distribution. For *P. australis,* haplotype K shares a close connection with populations from a Mediterranean lineage and this was further confirmed with a shared *grass-waxy* band. The direction and timing of genetic exchange between the two regions could not be ascertained and thus still remains unknown. Microsatellite analysis determined that both *Phragmites* spp. had a high genetic diversity compared to worldwide lineages. With no evidence of any cryptic invasions of haplotypes from other regions, both *Phragmites* spp. populations are likely to be native to

South Africa. For *A. donax* all populations across South Africa were determined to be haplotype M1; a cosmopolitan haplotype that has an ancient native range in Afghanistan and Pakistan (Indus Valley). Populations were found to have no genetic diversity and thus can be considered one clone.

A pre-introductory survey determined a list of herbivores associated with each tall-statured grass. For *A. donax,* a total of seven herbivores were found. Of these, one herbivore, a galling wasp, *Tetramesa romana* Walker (Hymenoptera: Eurytomidae) was found to be highly abundant and widely distributed in South Africa. *Tetramesa romana* is already a biological control agent in North America and thus is likely exerting some pressure on *A. donax* populations in South Africa. For both *Phragmites* spp. a total of ten herbivores were found, although having higher species richness compared to *A. donax,* when compared to other regions, these native species have a relatively low species richness.

Providing baseline data on plant origin, genetic diversity and herbivory on *A. donax, P. australis* and *P. mauritianus* has provided important information on managing these species in riparian ecosystems in South Africa. For the *Phragmites* spp. with no evidence of any cryptic invasions, it is recommended that reed stands continue to be managed as native species. *Phragmites* spp. are important dominant vegetative species and thus should be protected; however, if reed stands become expansive, control methods can be put in place to focus on managing spread and abundance. For *A. donax,* this study was able to provide pivotal information in guiding the biological control programme. By determining the ancient lineage of South African populations, research can be focused in this area to find potential biological control agents. Lastly, the pre-introductory survey determined that a biological control agent, *T. romana* was already established with an unknown introduction and also highlighted potential plant parts that should be targeted. In particular, no rhizome feeding herbivores were found in South Africa and therefore this highlights an important niche that should be explored in biological control agents.

ACKNOWLEDGEMENTS

The Working for Water programme (WfW) of the Department of Environmental Affairs: Natural Resource Management Programmes (DEA:NRM), and the South African Research Chairs Initiative of the Department of Science and Technology and the National Research Foundation (NRF), South Africa are thanked for their financial support throughout this PhD thesis. I would also like to thank Rhodes University for all the support throughout my academic career, with a special thanks to the Biological Control Research Unit. I am so grateful to have been part of the family and have always felt so welcomed and supported by the group. A particular thank you to Candice Owen, Julie Coetzee, Jackie Hill, Rosie Mangan, and Jeanne van der Merwe.

I am incredibly grateful to have had two supervisors, Dr. Iain Paterson and Prof. Martin Hill, who have been my mentors. To Martin, from your first lecture in 2007, you inspired me to pursue this career and I am grateful for the opportunities you have given me over the years. To Iain, you have always given me so much support and time and have continued to keep me motivated. I would also like to extend my gratitude to Dr. Carla Lambertini who has also been a source of inspiration and has taught me so much.

To my friends and family, thank you for always being there for me. Geraldine Taylor I consider myself very blessed to have you as a friend and to have been able to walk this path alongside you. To my mum, Dee thank you for always making me stop and notice the little lives around us from the tiny ants to the beautiful beetles, I owe my love of nature to you. To my dad, Sean you have always pushed me to be more and I am so thankful to have had someone believe in me like you do. To my brother and sister, Shane and Suzy, you have been my pillars of strength and support. To my husband, Sashank, and my beautiful kids, Ashwin and Kara, I love you so much and I hope I have made you proud.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

PUBLICATIONS ARISING FROM THIS THESIS

Canavan K, Paterson I, Hill MP. 2014. The herbivorous arthropods associated with the invasive alien plant, *Arundo donax*, and the native analogous plant, *Phragmites australis*, in the Free State Province, South Africa: short communications. *African Entomology* 22: 454–459.

1. CHAPTER 1: INTRODUCTION

Riparian areas can be defined as areas in and near river channels that are directly influenced by river-related processes; these habitats are unique ecosystems that are ecotones between aquatic and terrestrial ecosystems (Malanson 1993; Richardson et al. 2007). Hydrology and disturbance regimes in the surrounding landscape shape riparian ecosystems (Naiman and Decamps 1997; Holmes et al. 2005). As a result, riparian areas have naturally high species diversity, a diverse array of biological and physical processes, and a mosaic of vegetation types and structural components (Gregory et al. 1991; Malanson 1993; Naiman and Decamps 1997; Holmes et al. 2005; Richardson et al. 2007). These attributes allow riparian areas to provide a wide number of important ecological functions such as provision of food, moderation of stream water temperature, providing a buffer zone that filters sediment, controlling nutrient export, controlling against erosion, and providing refuge and corridors for the movement of biota (Gregory et al. 1991; Malanson 1993; Barling and Moore 1994; Naiman and Decamps 1997; Hood and Naiman 2000; Ewel et al. 2001). They are thus known as 'critical transition zones' for surrounding ecosystems (Ewel et al. 2001).

Despite their importance, riparian areas are amongst the world's most heavily invaded systems (Pyšek and Prach 1993; Ewel et al. 2001). Hydrology has been impacted in riparian systems worldwide through river impoundment, water management and lowering water tables as they have been the focus of human habitation and development (Hupp and Osterkamp 1996; Naiman and Decamps 1997; Washitani 2001). Such hydrologic disturbances result in these systems being pushed outside of their predictable range and thus has important consequences to ecosystem functioning (Resh et al. 1988, Jansson et al. 2000; Hooper et al. 2005; Richardson et al. 2007). In particular, disturbance is a critical determinant of the success of exotic species whereby such events result in the killing, displacement or damage to one or more individuals that ultimately results in the establishment of new individuals (Sousa 1984). For example, nutrient enrichment from stormwater and agricultural runoff will generally lead to dominance of exotic species that thrive under eutrophic conditions (Lake and Leishman 2004).

In South Africa, riparian areas have particularly high diversity, as the region is ecologically diverse with nine terrestrial biomes that have rich flora and high levels of endemism (Cowling and Hilton-Taylor 1997; Rutherford et al. 2006). Riparian areas support a wide range of species for example, the Cape clawless otter and water mongoose that can only survive in a healthy ecosystem (State-of-Rivers Report 2003). However, South African riparian zones, like elsewhere, have been heavily degraded in particular with dam construction and

extraction of water for irrigation which has reduced flows and altered riparian vegetation (Jansson et al. 2000; Shafroth et al. 2002). All of the major rivers are impounded (Hughes 2001).

The disturbance of South African riparian areas has resulted in these areas becoming susceptible to invasive alien species (IAS) (Richardson et al. 2007). Wetlands are landscape sinks for nutrients and propagules which makes them particularly vulnerable to plant invasions as they are usually downstream from most disturbances (Zedler and Kercher 2004). Many of these IAS are 'transformer' taxa that can change the character of natural riparian ecosystems (Rowntree 1991; Naiman and Decamps 1997; Tickner et al. 2001). Such transformations have major economic implications; it is estimated that IAS may be using as much as 6.7 % of the country's water runoff (estimated to be using 3300 million m^3 per year) (Versfeld et al. 1998). For example, a study in the Agulhas Plain area in South Africa estimated that the cost of water loss due to plant invasion was about US$ 163/ha and thus for this 20,000 ha area it could amount to over US$ 3.2 billion (Turpie and Heydenrych 2000).

One such IAS is the giant reed, *Arundo donax* L. (Poaceae), estimated to be the third most abundant IAS in South Africa (van Wilgen et al. 2007). The reed is an aggressive invader that forms dense monospecific stands that can rapidly degrade riparian areas (Guthrie 2007). *Arundo donax* has been found to displace native vegetation (Coffman 2007) and in South Africa this will most likely lead to the displacement of the tall-statured grasses, *Phragmites australis* (Cav.) Trin. ex Steud. and *Phragmites mauritianus* Kunth. *Phragmites* spp. are dominant plants in riparian ecosystems in temperate regions throughout the world, including South Africa (Gordon Gray and Ward 1971). It is the dominant species that are most influential in an ecosystem and as such, the greatest functional changes to an ecosystem will occur if the abundances of these species changes (Richardson et al. 2007). Often, major changes in growth form or life-history traits of the dominant species results in changes in the abiotic and biotic processes that shape the structure and composition of a riparian community (Holmes et al. 2005; Richardson et al. 2007).

Riparian areas in South Africa should be prioritised for conservation and in particular should be protected against the threat of the IAS *A. donax*. With the growing impacts of *A. donax* the potential for a biological control programme is being investigated (Guthrie 2007; Dr. Angela Bownes, ARC-PPRI, pers. comm.). However, before biological control is implemented, it is necessary to get baseline data on tall-statured grasses in South Africa. This study researches plant origin and herbivory on *A. donax, P. australis* and *P. mauritianus* to provide such information and get a better understanding of these dominant plants to help guide

future restoration and control efforts. For the native *Phragmites* spp., no prior research has been conducted on their plant genetics and also associated herbivores which is a critical part of understanding their status in riparian areas. For *A. donax,* such pre-introductory work will not only provide a clearer understanding of their populations but will also likely yield important benefits for biological control. Previous studies have shown the benefits of this retrospective approach, for example, Dudley et al. (2008) initiated a pre-introductory survey on *A. donax* in the U.S.A. and were able to determine that a biological control agent, *Tetramesa romana* Walker (Hymenoptera: Eurytomidae) was already established and available for augmentative biological control.

This chapter introduces the three tall-statured grasses *A. donax, P. australis* and *P. mauritianus* found in South Africa. The chapter provides background literature on each reed and ends with the aims and rationale of this study.

1.1 *Phragmites australis* (Cav.) Trin. ex Steud

1.1.1 Taxonomy

Phragmites australis is a tall monocot grass in the family Poaceae, tribe Arundineae (Blossey et al. 2002). A review by Clevering and Lissner (1999) lists four species in the genus *Phragmites, P. australis, P. mauritianus, P. japonicus* Steudel. and *P. karka* (Retz.) Trin. ex Steud. according to Veldkamp (1994). Since then, two additional species have been recognised, *P. frutescens* H. Scholz (Greuter and Scholz 1996), the poorly known species, *P. dioica* Hackel ex Conert (Conert 1961). The species are all morphologically similar and presumed to be closely related (Lambertini et al. 2006). Many intra-specific varieties of *P. australis* have been described however these differences are now recognised as local variants and do not have taxonomic value (Clayton 1968).

Phragmites australis is the most common species in the genus and is further considered the most widely distributed angiosperm in the world (Windham and Lathrop 1999; Chu et al. 2011; Kulmatiski et al. 2011). The other species have relatively restricted ranges with *P. mauritianus* being limited to tropical Africa, *P. japonicas* to Eastern China, Japan and the Russian Far East, *P. vallatoria* to tropical Asia but reaching Polynesia, Australia and tropical Africa, *P. frutescens* to Greece and Turkey, *P. dioica* to Uruguay and Argentina and lastly, *P. berlandieri* is found only in North America (Conert 1961; Mutlu 2002; Lambertini et al. 2006; Lee and Scholz 2007).

Ploidy level is complex in *Phragmites, P. australis* generally have a chromosome number of 12 but ploidy levels range from 3 x to 22 x (Clevering and Lissner 1999). This cytotypic plasticity has been an important factor in the evolution and diversification of the genus (Lambertini et al. 2006). The origin of *P. australis* is highly debated as it has generally been accepted that the reed is a cosmopolitan species, yet evidence points to an ancient origin in East and Central Asia (Clevering and Lissner 1999; Blossey et al. 2002).

1.1.2 Biology

Phragmites australis is classified as a perennial emergent macrophyte (Hart 1995). The reed is highly variable worldwide; this is largely a result of high plasticity and variation between genotypes (Ailstock and Center 2000; Achenbach et al. 2012; Meyerson et al. 2016). Most genotypes of *P. australis* are aggressive competitors, which is a direct result of the reed's adaptive features and biology (Ailstock and Center 2000; Saltonstall et al. 2010). *Phragmites australis* is a C3 grass species however its photosynthetic rate closely matches C4 species due to young leaves having C4-like anatomy (Engloner 2009). Furthermore, the reed's physical attributes and biology allow the plant to be highly tolerant to extreme conditions such as drought, altitude, and disturbance (Chambers et al. 1998; Saltonstall et al. 2010).

Phragmites australis is a clonal grass with woody hollow cane-like culms that can grow up to 6 m high (Blossey et al. 2002; Bonanno and Giudice 2010). Leaves are alternate with narrow-lanceolate laminae usually 20 - 70 cm long and 1 - 4 cm wide (Haslam 1972). Ligules at the base of the leaf-laminae and sheaths are 1.5 - 3 mm long; leaf bases form overlapping, smooth sheaths around the stems (Figure 1-1) (Mal and Narine 2004). Branches are smooth with scattered silky hairs (Haslam 1972). Annual culms or stems grow from extensive rhizomes 1.5 - 2.5 cm in diameter and reach a density of about 200 m^2 (Hellings and Gallagher 1992; Kiviat and Hamilton 2001; Vasquez et al. 2005). Reed stands produce new shoots annually with relative growth rate being highest in spring (Engloner 2009). In riparian areas *P. australis* stands are responsible for the bulk of energy fixation (Hart 1995).

Figure 1-1 a. *Phragmites australis*. Artist: H.W. du Toit. b. Spikelet of *P. australis* (17 x 8 mm). Artist: S.B. Chiliza. c. Spikelet of *P. australis.* Photographer: M. Koekemoer (Fish et al. 2015).

Phragmites australis' ability to persist and spread can also be attributed to the reed's reproductive strategies; reed stands can reproduce both sexually and asexually (Ailstock and Center 2000; Saltonstall et al. 2010). Sexual reproduction involves the production of flower panicles that are produced from late summer through to autumn (Engloner 2009). The terminal panicle is about 30 cm long and contains thousands of seed-containing florets, which have long

silky hairs on the rachilla to allow for wind dispersal (Chambers et al. 1998). Germination rates in *P. australis* are highly variable, with three extrinsic factors influencing seed viability; these are nutrients, disturbances and herbivory (McKee and Richards 1996; Ailstock and Center 2000; Hazelton et al. 2014). Nutrients and disturbances positively impact spread however herbivory has a negative effect through reduced seed production (Hazelton et al. 2014). Sexual reproduction in *P. australis* allows the reed to achieve mass recruitment and widespread distribution (Chambers et al. 1999; Blossey et al. 2002). When high density seedbanks occur, seeds can remain viable for at least a few years (Galatowitsch et al. 2016). Seed germination and viability have not been investigated in South Africa to date.

Vegetative reproduction is the primary mode of reproduction in *P. australis* with spread occurring from a root system known as a rhizosphere (Hellings and Gallagher 1992; Clevering and Lissner 1999). Rhizome segments are perennial and contain at least one axillary bud, which have horizontal (expand stands) and vertical (grow shoots) components (Mal and Narine 2004). New populations form when these vegetative propagules are broken up and washed downstream in flooding or earth removal events (Mal and Narine 2004). Rhizomes reach depths of one to two metres (Blossey et al. 2002; Chambers et al. 2012). In addition to growth, rhizomes play an important role in storing plant energy and uptaking nutrients (Ailstock and Center 2000; Van Rooyen et al. 2004).

Phragmites australis is highly efficient in acquiring oxygen, carbon dioxide and mineral nutrients; this is accomplished by an internal ventilation system (Armstrong and Armstrong 1988; Armstrong et al. 1996). *Phragmites australis* is the only marsh grass that can use Venturi-enhanced convective throughflow of gases to provide oxygen to the roots and also to remove accumulated toxic gases (Armstrong et al. 1992; Windham and Lathrop 1999). Convection gas flow is an important adaptation in anoxic conditions and can give a competitive advantage in deeper waters compared to species that rely solely on diffusive gas transport such as *Spartina alterniflora* Loisel (Poaceae) and *A. donax* (Brix et al. 1992). Lastly, *P. australis* is capable of tolerating high levels of salinity by excluding sodium or by adjusting cation and water loss; allowing reeds to grow in waters with salinities of up to 45 g L^{-1} (Windham & Lathrop 1999; Mal & Narine 2003; Engloner 2009; Bonanno & Giudice 2010).

In South Africa, few autecological studies have been performed on *P. australis* and therefore little is known about its biological relationship with its environment (Van Rooyen et al. 2004).

1.1.3 Utilisation

Phragmites australis lends itself to a plethora of uses due to its unique characteristics of being strong, hollow, lightweight, rapidly growing and slow-decomposing (Kiviat and Hamilton 2001). It has been a critical resource for many ancient cultures being used to serve a number of purposes including arrowshafts, smoking equipment, hats, baskets, furniture, fencing, mats, canoes, roof thatching and clothing (Kiviat and Hamilton 2001; Mal and Narine 2004; Dogan et al. 2008; Papayannis and Pritchard 2011). It was also used as a food source, to make sugars, coffee-like drinks and alcohol (Kiviat and Hamilton 2001; Mal and Narine 2004). Certain cultures also used reeds in folk medicine to treat a wide range of symptoms such as digestive problems, boils and pneumonia, arthritis, rheumatism, leukemia, cancer, typhoid, rheumatoid arthritis, anti-hemorrhagic, diabetes and gout (Kiviat and Hamilton 2001; Rahmatullah et al. 2010).

Today, *P. australis* is still being used in some countries to make crafts, however it is now more commonly being employed for maintenance of wetlands (Dogan et al. 2008; Tomimatsu et al. 2014). *Phragmites australis* acts as an ecosystem engineer in wetlands as stands impact hydraulic conditions, filter nutrients, promote the presence of microorganisms, aid in sedimentation, and control against erosion (Hart 1995; Coetzee et al. 1996; Ye et al. 1997; Koppitz 1999; Kotschy et al. 2000; Van Rooyen et al. 2004; Lee and Scholz 2007). Furthermore, reed stands can tolerate a wide range of conditions including areas with high pollution and thus can aid in phytoremediation (Ye et al. 1997; Lee and Scholz 2007; Borin et al. 2011; San Miguel et al. 2014). In addition, *P. australis* ability to uptake various pollutants and bioaccumulate metals makes it useful as a biomonitor for ecosystem health (Bonanno & Giudice 2010). *Phragmites australis* is also increasingly used for commercial purposes including production of cardboard, hardboard, paper, biofuel and as a nutrient source in agriculture (Lewandowski et al. 2003; Hansson and Fredriksson 2004; Mal and Narine 2004).

In South Africa, *P. australis* has had a long history of use and is highly revered by local tribes, for example the Zulu and Xhosa people who refer to the plant as 'emhlanga' have named many rivers and villages after the plant (Gordon Gray and Ward 1971). *Phragmites australis* is used as a non-food commodity and is still one of the most widely harvested indigenous fibers (Van Rooyen et al. 2004; Kotze and Traynor 2011). Reeds are most commonly employed for building walls, thatching, fences, huts, weaving, arrow shafts, tobacco pipestems, medicine, musical instruments and livestock enclosures (Van Rooyen et al. 2004; Kotze and Traynor 2011). *Phragmites australis* is most extensively harvested in the KwaZulu-Natal Province, with about 60 000 people involved in harvesting in the region alone (Van Rooyen et al. 2004).

The Department of Water Affairs and Forestry have also begun to use the reeds to reduce eutrophication and identify degraded catchments (Hart 1995; Coetzee et al. 1996; Kleynhans 1996).

1.1.4 Distribution and abundance

Phragmites australis has a high ecological tolerance and can therefore be found in a wide variety of ecotypes including non-tidal and tidal aquatic habitats, brackish marshes of river deltas, alkaline or saline inland wetlands, river banks, oases, springs and marshes in arid land (Haslam 1972; Chambers et al. 1998; Kiviat and Hamilton 2001; Minchinton and Bertness 2003). Reeds stands are however most abundant in warm, low-lying, moist, bare saturated soils that are eutrophic and even disturbed including drained wetlands and sewage ponds (Bonanno and Giudice 2010; Chambers et al. 2012). *Phragmites australis* is found in almost every country worldwide reaching as far south as Argentina, as far north as Greenland and as high as 3000 m in Tibet (Mal and Narine 2004).

Distribution of *P. australis* is primarily determined by salinity, elevation and temperature (Warren et al. 2001). The common reed thrives in freshwater mesohaline to polyhaline marshes, preferring less than 18 ppt salinity (Chambers et al. 1998; Warren et al. 2001). *Phragmites australis* can extend into inter-tidal estuarine areas especially if there is reduced salinity (from 0.3 % to 3.1 %) from fresh water inflow (Gordon Gray and Ward 1971). Elevation is also important as it influences hydroperiod, which at lower elevations can restrict the spread of *P. australis* (Warren et al. 2001). Lastly, temperature influences *P. australis* distribution with reed stands being most prolific in temperate regions as reeds are negatively impacted in areas with shorter growing seasons and lower temperatures (Victorin 1995; Chambers et al. 1999).

In most areas of the world *P. australis* is highly abundant and in most cases even spreading, particularly into disturbed habitats (Chambers et al. 1999; Warren et al. 2001). In North America for example, *P. australis* has been a small part of wetland communities for over 40 000 years (Hansen 1978). However, in the last 150 years the reed's distribution has greatly expanded especially along the Atlantic coast and the Mississippi delta region of the Gulf of Mexico (Chambers et al. 1999; Windham and Lathrop 1999; Kiviat and Hamilton 2001; Blossey et al. 2002; Saltonstall 2002). Genetic studies have since found evidence that the invasive nature of *P. australis* in the U.S.A. was the result of a cryptic invasion of a non-native haplotype from Europe (Saltonstall 2002). In Europe however, *P. australis'* range is declining (Van der Putten 1997; Fogli et al. 2002). A number of explanations have been proposed

including anthropogenic activities, management practices such as controlling water level, consumer demand and lack of stand regeneration (Van der Putten 1997; Koppitz 1999; Kotschy et al. 2000; Fogli et al. 2002).

Phragmites australis is widespread and abundant in most countries in Africa and is known to have been established since the Holocene era (Scott 1982; Coetzee et al. 1996; Meadows and Baxter 2001; Neumann et al. 2008; Russell and Kraaij 2008; Zhao et al. 2016). For example, studies of Pleistocene and Holocene pollen in South Africa in the 'Wonderkrater' thermal springs, Limpopo Province (Scott 1982) and Lake Sibaya, KwaZulu-Natal Province (Neumann et al. 2008) found *P. australis* species to be the dominant plants during that period. *Phragmites australis* is still highly abundant nationwide (Figure 1-2), particularly in areas with restricted drainage such as river back-waters that are usually isolated from the main stream or in standing water up to 70 cm deep to form extensive reed stands (Gordon Gray and Ward 1971; Howard-Williams and Liptrot 1980; Beckley 1984). *Phragmites australis* is also prevalent in the upper reaches of estuaries across the country including the Mzingazi River, Sundays River, St. Lucia, Kosi Bay, Sondwana Bay, and the Umlalazi estuaries (Macnae 1963; Gordon Gray and Ward 1971; Beckley 1984; Cyrus et al. 1997; Adams and Bate 1999).

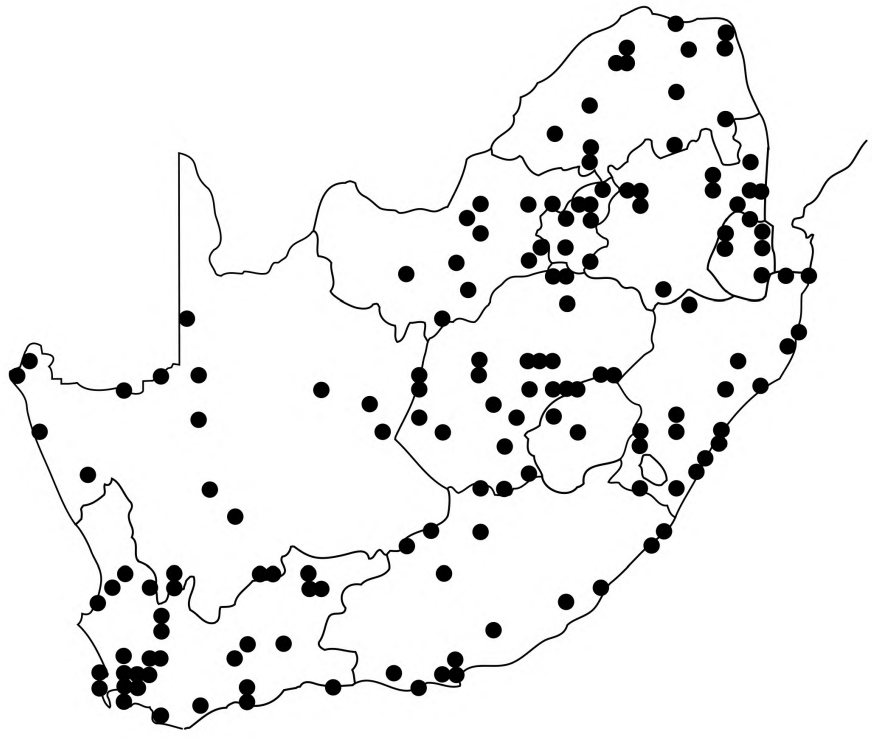


Figure 1-2 Distribution of *P. australis* in South Africa (Fish et al. 2015).

Since the 1970s, *P. australis* stands have been recorded to be expanding in their range and abundance and are encroaching into previously unoccupied habitats (Russell and Kraaij 2008; Council for Scientific and Industrial Research 2010; Quick 2014). For example, vegetative mapping in the Wilderness area, Western Cape Province, found that between 1975-97, *P. australis* distribution increased by 53% (Russell and Kraaij 2008). Similar expansion of reeds in the Verlorenvlei, Eland's Bay, Western Cape prompted the Department of Water Affairs and Forestry (DWAF) to put aside R 10 million in 2010 to develop and implement protocol for reed management (Council for Scientific and Industrial Research 2010).

It is currently not known why *P. australis* is undergoing expansion of its range and abundance in South Africa. There are two main theories that can be proposed 1) a cryptic invasion has occurred with an introduction of non-native haplotypes as has happened in the North America, 2) anthropogenic activities have altered riparian areas in favour of reed expansion. To investigate the potential of a cryptic invasion, it will be necessary to use genetic techniques to determine *P. australis* haplotypes present across South Africa (chapter 2). A number of studies have explored the anthropogenic disturbances to riparian areas and the resulting reed expansion. It has been found that changes to these systems, particularly agricultural run-off, reduced water flows and a loss of large herbivores have favoured *P. australis* growth (Council for Scientific and Industrial Research 2010; Quick 2014).

1.2 *Phragmites mauritianus* Kunth

1.2.1 Taxonomy

Phragmites mauritianus is a tall monocot grass in the family Poaceae, tribe Arundineae (Gordon Gray and Ward 1971). Like with *P. australis, P. mauritianus* is morphologically and genetically closely related to the other species in the genus (Lambertini et al. 2006). *Phragmites mauritianus* has been found to share a particularly close taxonomic relationship with both *P. frutescens* and *P. vallatoria* according to morphological and genetic characteristics sharing for example woody, perennial stems with short rigid leaves (Scholz and Böhling 2000; Lambertini et al. 2006). *Phragmites mauritianus* is restricted to a tropical African origin (Gordon Gray and Ward 1971).

1.2.2 Biology

Phragmites mauritianus is an aquatic perennial reed that grows up to 8 m with erect woody culms 2-8m high and with average diameters of 4 cm (Fanshawe 1972; Blackmore et al. 1988). Culms are cylindrical, hollow except at the nodes with walls about 2 mm thick, smooth and almost hidden by the overlapping sheathing leaf-bases arising from each node (Fanshawe 1972). Leaf blades are linear to narrow lanceolate, from 0.8-75 cm long and 6-40 mm wide, pale green, smooth above (although some have hairs when young), rough to touch beneath the leaves and on the margins, with minute bristles pointing towards the tip (Gordon Gray and Ward 1971; Launert 1971). Leaves are alternate but lie in one plane (Fanshawe 1972). At the base, leaves are constricted to form ligules, ligules are pale cream and marked by twin tufts of long white hairs, one on either side opposite the leaf blade (Fanshawe 1972).

Phragmites mauritianus spreads by both sexual and asexual reproduction and its reproductive biology is reviewed by Fanshawe (1972) below. Sexual reproduction occurs in *P. mauritianus* through the production of flower panicles. Flower panicles are greenish, purplish or pale brownish, profusely branched and are 30-70 cm long and 10-30 cm wide. Usually there is one panicle per culm except if the main culm is damaged and two or more branches form and produce panicles. Like with *P. australis,* seed viability is variable and is generally rare in natural populations with most culms producing sterile seeds. In South Africa flowering occurs from June to October (Gordon Gray and Ward 1971). In asexual reproduction, *P. mauritianus* populations spread from the rhizosphere. Buds emerge from nodes in the rhizomes and grow to long acuminate, cream coloured, cylindrical shoots and have varying diameters depending on rhizome vigour. Culms that have fallen can also act as rhizomes if they have become covered with soil and will send out roots and shoots from every node. Roots develop aerial stems, which can allow reeds to tolerate flooding. Rhizomes are very vigorous and can run for long distances over and through water, this allows reeds to encroach on streams, dams and rivers.

Phragmites mauritianus is very similar to *P. australis* morphologically and in fact differentiating between the two species can be difficult; the keys that have been created to distinguish between *Phragmites* species, including Sturgeon (1954), Chippindall (1955) and Clayton (1968), have been found to be insufficient in identifying between the species in South Africa but Gordon Gray and Ward (1971) determined a number of distinguishing characteristics that can be used to determine between the two species under natural conditions which are summarised below. The most conspicuous characteristics of *P. mauritianus* is the form of the mature inflorescence which is lax and has drooping branches. After maturing, the

reeds will also have bare internodes with exposed axillary buds that tend to turn a pinkish purplish colour. In *P. australis* only the leaf blades fall and the sheath envelopes the stem and thus does not leave the internodes bare. *Phragmites mauritianus* have well-branched flowering stems unlike *P. australis,* as after flowering culms remain photosynthetically active. The outermost protective bud sheath is hairier in *P. mauritianus* with a thicker more conspicuous fringe. *Phragmites mauritianus* leaf blades are also a different colour to *P. australis; P. mauritianus* are a yellowish green colour compared to the blueish-green of *P. australis*. Leaves are also stiffer and harder.

1.2.3 Utilisation

Phragmites mauritianus has been utilised for a wide range of purposes on the African continent (Fanshawe 1972). The structure of the reeds has made them useful as a building material; reeds have been used to make hut walls, thatching, fences, fish traps, musical pipes, arrow shafts and drinking pipes. Reeds are also used as a fibre to manufacture different kinds of mats. *Phragmites mauritianus* has also been utilised as a food source by puncturing culms to produce a sugar from the exudate. This sweet extract has also been used in the treatment of pneumonia. In Zambia, the Ila tribe use an ointment prepared from the burnt flowers to treat scabies. The rhizomes, although containing silicic acid, are edible and have been used as a diuretic and diaphoretic. In South Africa, Bemba tribes use the root infusion to wash children suffering from malaria. Bemba chiefs also transplant the reeds from the riverside to their own wells and use the spot as a shrine for their ancestors.

Phragmites mauritianus also plays an important role in ecosystem functioning of riparian areas in South Africa. *Phragmites mauritianus* is recognised as an ecosystem engineer and is therefore both protected and used as an indicator species (Coetzee et al. 1996; Kleynhans 1996). For example, in the Kruger National Park, South Africa, *P. mauritianus* is protected in order to prevent damage from flooding and maintain sedimentation (Kotschy et al. 2000). The reeds assist in fixing stream banks by causing deposition of sand and silt (Fanshawe 1972).

1.2.4 Distribution and abundance

Phragmites mauritianus is a tropical species and is restricted to the African continent with a northern limit of Ethiopia, Sudan and the Congo Republic and being found through Zambia and down to South Africa (Gordon Gray and Ward 1971; Launert 1971). It also occurs in Madagascar and the Mascarene Islands (Fanshawe 1972). The reed is replaced in North and

West Africa by its analogous species *P. vallatoria* (syn. *P. karka*) (Fanshawe 1972). In South Africa, *P. mauritianus* is restricted to the more tropical areas of country in the Mpumalanga, KwaZulu-Natal and Limpopo provinces (Figure 1-3) (Van Coller et al. 1997).

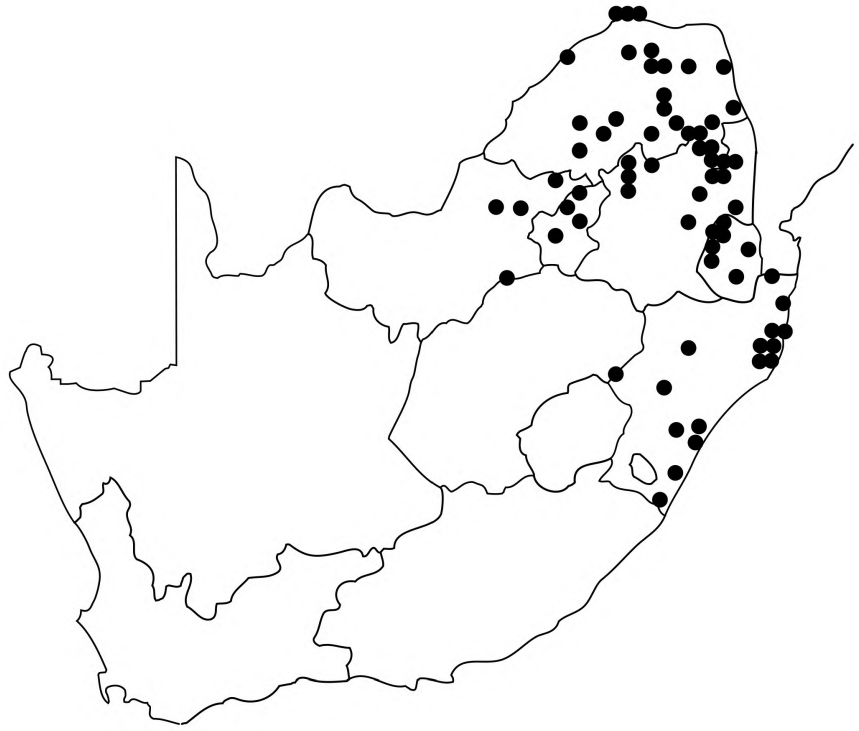


Figure 1-3 Distribution of *P. mauritianus* in South Africa (Fish et al. 2015).

Phragmites mauritianus is restricted to riparian zones where reeds are gregarious and occur on banks and shallow waters (Launert 1971; Van Coller et al. 1997). Unlike, *P. australis, P. mauritianus* prefers well-drained, often sandy soils and permanently flowing or frequently moving water, especially in areas with recent disturbance (Gordon Gray and Ward 1971). The reeds form dense stands along the river edge and in backwaters; and can even be found in areas with strong currents (>1 m sec^{-1}) (Gordon Gray and Ward 1971). The horizontal rhizomes readily colonise sandy alluvial deposits so that the reeds are often the first perennials after flood events (Gordon Gray and Ward 1971). *Phragmites mauritianus* needs better drainage and aeration of the substrate and is better able to survive drier conditions compared to *P. australis* (Gordon Gray and Ward 1971).

In South Africa, *P. mauritianus* stands are currently a protected endemic species and due to their wide array for benefits are recognised as important to riparian ecosystem functioning (Van Coller et al. 1997; Kotschy et al. 2000). However, the abundance of *P. mauritianus* has

been found to vary considerably between systems where in certain areas reed stands are expanding beyond previous limits (Van Coller et al. 1997). The increase in reed beds has been attributed to anthropogenic disturbances, with a rise in sedimentation believed to be a primary driver (Van Coller et al. 1997).

1.3 *Arundo donax* L. (Poaceae)

1.3.1 Taxonomy

Arundo donax (giant reed, bamboo reed, river cane, carrizo cane) is in the family Poaceae, tribe Arundineae (Danin 2004). The *Arundo* genus is characterised by tall perennial reed-like grasses and is made up of five species, *A. donax, A. collina* Ten., *A. plinii* Turra., *A. formosana* Hack. and *A. micrantha* Lam. (syn. *A. mediterranea* Danin) (Danin 2004; Hardion et al. 2012b; The Plant List 2013). The species within the *Arundo* genus can be differentiated by the lemma according to Danin et al. (2002). The species are otherwise morphologically similar with long culms and similar inflorescences which has often caused taxonomic confusion (Danin 2004). *Arundo donax* is the largest species in the genus (Saltonstall et al. 2010). All *Arundo* species are found in the Mediterranean except for *A. formosana* which has an origin in temperate Asia (Danin 2004; Clayton et al. 2006; Mascia et al. 2013). *Arundo donax*, like the other *Arundo* species, was believed to have a Mediterranean origin however through molecular work it has been determined that the species has an ancient origin in the Middle East and has since radiated out to have a cosmopolitan distribution (Hardion et al. 2014b).

For *A. donax,* there are two distinct lineages identified worldwide, that are classified as Asia/Middle East and the Mediterranean populations (Mariani et al. 2010). *Arundo donax* stands outside of Asia/Middle East populations generally lack genetic diversity which has been attributed to the movement of only a few selected genotypes and lack of sexual reproduction (Ahmad et al. 2008; Flores and Wood 2009; Khudamrongsawat et al. 2009; Mariani et al. 2010; Saltonstall et al. 2010). In South Africa, it is not known which lineage reed stands belong to and whether or not stands are genetically distinct from worldwide populations.

1.3.2 Biology

Arundo donax is one of the most biological productive plants worldwide (Angelini et al. 2005; Cosentino et al. 2006; Saltonstall et al. 2010). Furthermore, *A. donax* reaches heights of 6 - 8 m making it one of the tallest herbaceous grasses in the world (Perdue 1958). Under ideal

conditions it can grow up to 5 cm per day; this can lead to stands accumulating 100 tons of fresh matter per hectare annually (Rieger and Kreager 1989; Lewandowski et al. 2003; Seawright et al. 2009). High productivity is linked to the grass' ability to maintain photosynthetic rates and unsaturated photosynthetic potential similar to C4 species despite using a C3 pathway like most other grasses (Else 1996; Papazoglou et al. 2005; Cosentino et al. 2006). Furthermore, *A. donax* has evolved to be polylpoid which may have further contributed to the success of the reed (Prentis et al. 2008; Hardion et al. 2014b). Polyploids occur with greater frequency among invasive plants and this is believed to be due to increased fitness or competitive ability from chromosome doubling (Prentis et al. 2008).

Arundo donax has many-stemmed cane-like clumps made up of culms that are tough and hollow (Figure 1-4) (Pilu et al. 2012). Culms have an average diameter of 1 - 4 cm and are divided by nodes at distances of about 20 cm (Oakins 2001). Branching of culms with lateral shoots, generally only occurs in the second year of growth (Spatz et al. 1997). Culms have high mechanical stability as a result of lignification and the presence of silica in epidermal cells (Spatz et al. 1997). Leaves grow up to 70 cm long, are blue-green in colour, alternate, broad, glabrous and striate (Spencer et al. 2010). Leaves clasp the stem with an earlobed-shaped base and taper at the tip (Dudley 2000).

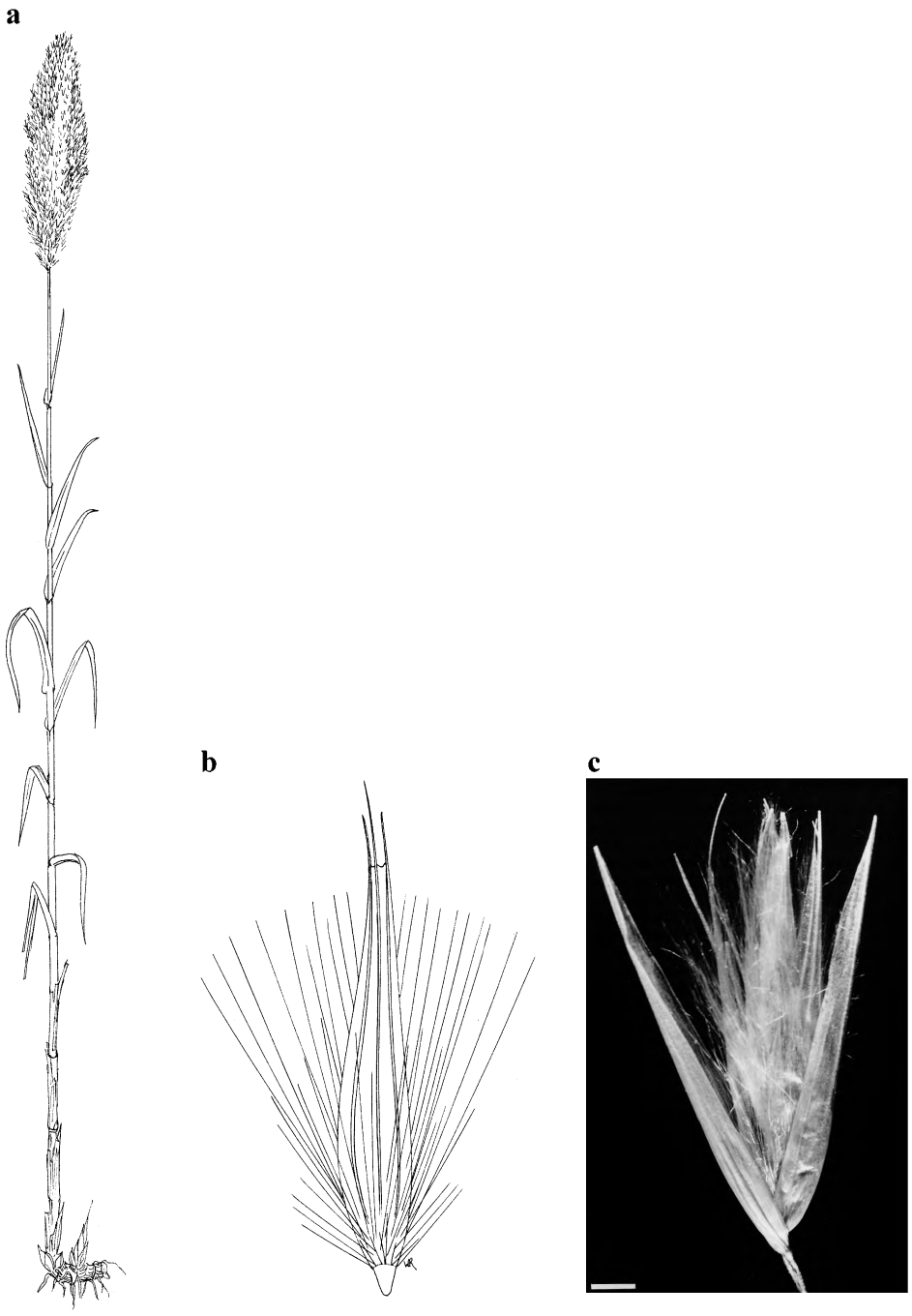


Figure 1-4 a. *Arundo donax.* Artist: H.W. du Toit. b. Lemma of *A. donax* with long hairs. Artist: W. Roux. c. Spikelet of *A. donax* (8-15mm). Photograph: M. Koekemoer (Fish et al. 2015).

Arundo donax can reproduce both sexually and asexually (Else 1996; Dudley 2000). Sexual reproduction involves the production of tall, plume-like terminal panicles at the upper tip of stems usually 20 to 45 cm in length (Oakins 2001; Johnson et al. 2006). Flowers consist of closely packed cream-coloured plumes (about 200 florets per plume) borne in early spring to early autumn (Else 1996; Dudley 2000). Seeds are dispersed by wind and by transportation

in water channels (Bell 1997; Cosentino et al. 2006). Sexual reproduction is believed to only occur in the proposed native range in Asia and even then it is irregular with evidence showing higher levels of genetic diversity compared to the adventive range (Mariani et al. 2010). In the introduced range, *A. donax* is sterile, in such cases, healthy stamens are present, however, no pollen is produced (Dudley and Lambert 2007; Saltonstall et al. 2010). Balogh et al. (2012) determined that sterility is linked to a lack of megasporocyte and microsporogenesis development in *A. donax* clones. Furthermore, it has been found that seed fertility is linked to ploidy levels with fruiting only occurring in ploidy levels of 2n = 72 (only found in Asia) (Hardion et al. 2014a).

Vegetative reproduction is thus the primary means of spread for *A. donax* in worldwide populations (Boland 2006). *Arundo donax* expands by rhizomes, fragments and layers (Boland 2006). Root systems are responsible for the production of stems and are made of fleshy, fibrous and compact rhizomes, reaching depths of up to 5 m (Rieger and Kreager 1989; Else 1996; Oakins 2001). New colonies form when rhizomes are transported downstream where they sprout new culms in moist soils (Bell 1997). Clumps can then expand from stem fragments or layering (growth from a stem still attached to parent plant) (Boland 2006).

In South Africa, *A. donax* does not produce viable seeds and therefore relies solely on vegetative growth to spread (Milton 2004; Guthrie 2007). No studies on the mechanism of seed viability have been carried out in South Africa but it is most likely that seed sterility occurs through the processes outlined in Balogh et al. (2012).

1.3.3 Utilisation

Arundo donax has intentionally been distributed worldwide as a result of its wide range of uses most notably erosion control, building material (thatching for roofs, fencing and screens), musical instruments and crafts (Bell 1997; Dudley 2000; McWilliams 2004; Cosentino et al. 2006). The oldest records of *A. donax* utilisation are from Egypt where reeds were used from 5 000 B.C. to line underground storage bins and to preserve mummies (Perdue 1958; Oakins 2001). *Arundo donax* was recorded in many Greek and Roman texts from Neolithic times, for example Sumerian tablets mention the use of bundles of reed culms being imported across Mesopotamia since 2500 BC (Faiella 2006). During the 4th Century A.D. the reed was used for medicinal purposes, including a sudorific, a diuretic, a diaphoretic, an antilactant and treatment of dropsy (also known as edema) (Perdue 1958). It was later most

commonly used for light construction work such as for thatch roofing, fencing, baskets and matting (Cheatham et al. 1995; Cortes et al. 2011).

Arundo donax has also had a long history in many recreational activities most notably fishing and music (Perdue 1958). The plant's physical attributes of strength and flexibility have made it an ideal material for the production of fishing rods, with reeds still being used for this purpose today (Pilu et al. 2012). *Arundo donax* played an important role in the development of music, dating back over 5000 years from Egypt and the Arab world to North Africa (Perdue 1958). The reed was developed as a component of a wide range of instruments including the clarinet, bagpipes, saxophones and flutes (Figure 1-5) (Perdue 1958). At present there are still no adequate substitute parts for woodwind instruments and the reed is still widely cultivated for this purpose (Perdue 1958; Spatz et al. 1997).

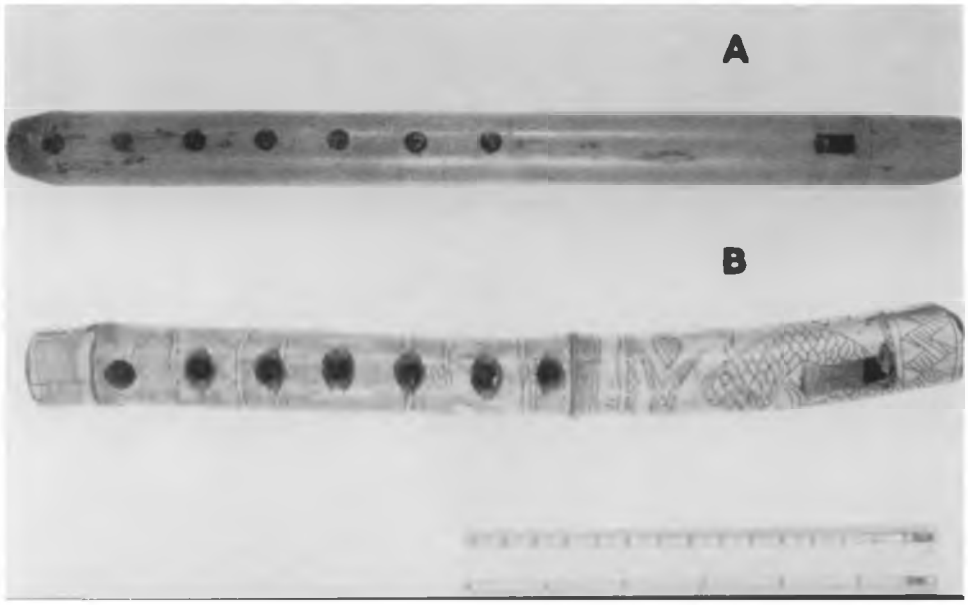


Figure 1-5 Fipple flutes formed from *A. donax* culms (Perdue 1958).

Arundo donax was first introduced outside its native range primarily for erosion control in North America, Australia and Eurasia where reed stands aided in stabilising drainage canals and dunes (Bell 1997; Oakins 2001).The reed was also then used for horticultural purposes such as for fencing, windbreaks, trellises, and as an ornamental plant (Bell 1997). Since the 1930s, *A. donax* is increasingly being adopted for agricultural and industrial purposes; *A. donax* produces lignocellulosic biomass which allows it to be applied to the manufacturing of high luminosity paper, textiles, rayon viscose, cardboard, activated carbons (ACs) and as a biofuel (Vernersson et al. 2002; Lewandowski et al. 2003; Angelini et al. 2005).

In South Africa, *A. donax* has most commonly been employed for erosion control (Guthrie 2007). Stems have also been used for building, thatching, screens and fences (Guthrie 2007). Leaves have also been adopted for craft-making including weaving baskets and mats, mainly in poorer subsistence communities (Guthrie 2007).

1.3.4 Distribution and abundance

Recent evidence has found that *A. donax* originated in India, the Arabian Peninsula and Nepal and was then introduced to the Mediterranean region thousands of years ago (Hardion et al. 2014b). *Arundo donax* has since been transported worldwide due to its plethora of uses (Bell 1997). In the areas where *A. donax* was introduced, particularly tropical and warm-temperate regions, it has naturalised and in most cases become highly invasive (Dudley 2000; Quinn and Holt 2008).

Arundo donax grows in Mediterranean-type climate zones and can tolerate a wide range of conditions such as varying soil types, salinity and drought (Tracy and DeLoach 1998; Lewandowski et al. 2003; Calheiros et al. 2012). The reed favours temperate habitats along floodplains of medium- to large-sized streams with low gradients, well-drained, coarse-grained soils with high organic content and oxygen (Rieger and Kreager 1989; Bell 1997; Oakins 2001; Angelini et al. 2005; Lambert et al. 2014). Soil composition has a strong influence on *A. donax* plant biomass with water and light resources having a lesser effect (Lambert et al. 2014). As a result, *A. donax* has been found to thrive in areas with high nutrient loading such as agricultural and urban areas (Coffman 2007; Quinn et al. 2007; Lambert et al. 2014). Lastly, latitude and elevation play an important role in *A. donax* distribution; reed stands are frost sensitive and thus struggle in higher elevations with regular freezing (Oakins 2001;Cantaluppi et al. 2016).

In introduced areas *A. donax* has in most cases become naturalised and invasive in a wide range of regions including sub-tropical U.S.A. through Mexico, the Caribbean islands, South America, Pacific Islands, and Australia (Else 1996; Dudley 2000; Haddadchi et al. 2013). The U.S.A. is considered to be one of the worst areas of *A. donax* invasion (Rieger and Kreager 1989; Dudley 2000; Herrera and Dudley 2003).

Arundo donax is widespread and abundant throughout the African continent (Milton 2004). It was deliberately introduced into South Africa in the late 1700s, primarily for erosion control (Guthrie 2007). The reed spread throughout the country as vegetative growth was facilitated by anthropogenic activities including building dams and soil stabilisation; it has since become one of the worst IAS in the country and is now present in all provinces (Figure 1-6 and Table 1-1) (Van der Merwe et al. 1990; Guthrie 2007; van Wilgen et al. 2007). The

reed has been listed as a Category 1 invasive alien species according to the National Environmental Management: Biodiversity Act (NEMBA) (Act No 10 of 2004) (Henderson 2001; van Wilgen et al. 2007). Category 1 plants are prohibited from being sold or planted and additional efforts are needed to keep the plant under control (Henderson 2001). Using climate envelope models (CEMs) a study by Rouget et al. (2004) estimated that 79% of South Africa, Lesotho, and Swaziland is potentially suitable for *A. donax* to invade. Sensitive and disturbed habitats are most at threat such as the fynbos biome where *A. donax* now occupies 50 quarter degree squares (Guthrie 2007).

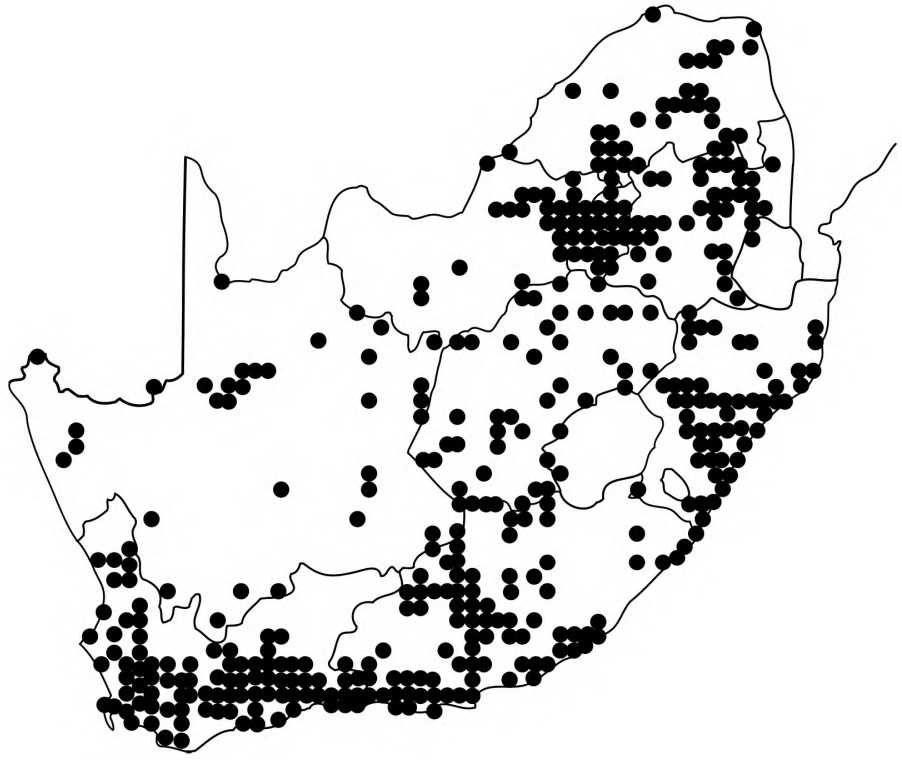


Figure 1-6 Distribution of *Arundo donax* in South Africa (Fish et al. 2015).

Table 1-1 Area of land invaded and estimated water use by *A. donax*, per province per year in South Africa (Versfeld et al. 1998).

Province	Area invaded (ha)	Water use (m^3)
Eastern Cape	15 078	214 000
Free State	695	28 000
Gauteng	Unknown	Unknown
KwaZulu-Natal	112 307	2 168 000
Northern Cape	456	2 000
Northern Province	392	9 000
North-West	47 948	1 758 000
Western Cape	11 072	170 000
South Africa	187 948	4 348 000

1.3.5 Impact

Biodiversity

One of *A. donax*'s greatest impacts is in its ability to outgrow or shade out native vegetation and thus alter native species habitats (Dudley 2000; Oakins 2001). The growth of dense monospecific reed stands, significantly reduces available sunlight, soil moisture and nutrients (Guthrie 2007). Studies in California have found that plant diversity was 57 % lower in *A. donax* infested areas compared to uninfested ones (Dudley and Lambert 2007).

Arundo donax's negative impacts on native plant biodiversity further impact animal biodiversity (Herrera and Dudley 2003). Riparian plants support a wealth of organisms most notably fish, arthropods and other invertebrates that use the vegetation for feeding, resting, refuge and reproduction (Herrera and Dudley 2003). Reed stands negatively impact the functional and physical attributes of these ecosystems by altering their composition and structure (Guthrie 2007; Moore et al. 2010). In addition, *A. donax* is not palatable to most native generalist herbivores as they are not adapted to the reed's host of chemical defenses (including triterpines, hydrozamic acid, alkaloids and sterols) (Moore et al. 2010), low nitrogen content (Going and Dudley 2008) and tough, fibrous leaves that are high in silica and nearly 65% cellulose (Neto et al. 1997). Such defenses further result in unsuitable soil litter reducing invertebrate diversity by up to 50% (Guthrie 2007). Overall, *A. donax* stands lead to native species being outcompeted by a few exotic generalist species (Oakins 2001).

In South Africa, *A. donax*-dominated communities are generally associated with living alongside other alien species in degraded ecosystems (Guthrie 2007). *Arundo donax* has been found to reduce the diversity of native species; a study by Guthrie (2007) found that stands reduced indigenous species to about 0.09 species m^{-2} compared to 0.13 species m^{-2} in native *Typha capensis* (Rohrb.) N.E.Br. (Typhaceae) dominated stands. The loss of indigenous species further leads to reduced ecosystem functioning (Guthrie 2007).

Fire

In general, riparian areas serve as fire barriers and are characterised by cooler air temperatures and higher relative humidity than adjacent land (Dwire and Kauffman 2003; Holmes et al. 2005). However, when *A. donax* invades these areas can become habitats that are fire-prone (Rieger and Kreager 1989; Bell 1997). *Arundo donax* stands can produce up to 20

tons of dense highly flammable aboveground biomass per hectare (McWilliams 2004). This increased biomass has the potential to increase the risk, intensity and frequency of fires throughout the year, especially with the occurrence of wildfires (Herrera and Dudley 2003; Guthrie 2007; Coffman et al. 2010). Furthermore, *A. donax* rhizomes respond rapidly after fire due to an underground carbohydrate reserves and actually benefit from such events, allowing them to outcompete native species that are not fire-adapted (Oakins 2001; Guthrie 2007; Lambert et al. 2010). Coffman et al. (2010) found that *A. donax* abundance increased by 25 % and comprised more than 99 % of vegetation cover after a fire event.

In South Africa, *A. donax* is altering riparian ecosystem's fire regimes (Guthrie 2007). However, the impact on such regimes is site-specific and depends on a wide range of factors including seasonal rainfall (Guthrie 2007). In most cases the reed results in an increase in unseasonal fire hazards such as during wet winter months when the reeds are at their lowest moisture content (Guthrie 2007).

Habitat Transformation

The physical attributes of *A. donax* stands, such a lack of canopy structure, alter the functioning of riparian ecosystems and can thus transform these environments (Herrera and Dudley 2003; Guthrie 2007). *Arundo donax*'s simple canopy reduces shading in riparian areas resulting in increased water temperature, increased pH, lower oxygen, lower aquatic diversity and altered water chemistry (Bell 1997). As a result, riparian areas develop poor water quality with increased algal activity and levels of toxic ammonia (Rieger and Kreager 1989; Tracy and DeLoach 1998). In addition, reed stands generally increase bare soils which reduces available resources for arthropods, rates of decomposition, water content and nutrient cycling (Herrera and Dudley 2003; Guthrie 2007).

Water

Arundo donax has the potential to greatly reduce water resources (Seawright et al. 2009; Gowda et al. 2011). Reed stands are highly efficient at trapping sediment, which can have a negative impact on groundwater levels, stream flow, navigability, and morphology which all lead to major water loss (Dudley 2000; Oakins 2001; Flores and Wood 2009). A study by Gowda et al. (2011) found that *A. donax* has an hourly evapotranspiration (ET) rate of 0.4 to 0.5 mm per hour. As much as 1100 mm of water annually can be taken up by dense stands, which is usually about three times more water than native plants (Gowda et al. 2011).

In South Africa, *A. donax* is considered a national problem primarily due to the threat it poses to water security (Milton 2004). Dense reed stands have the ability to rapidly change stream hydrology and sedimentology (Milton 2004). *Arundo donax* is already being reported to be reducing the carrying capacity of small waterways by constricting and narrowing channels (Guthrie 2007).

1.3.6 Control

The management of *A. donax* stands should always be long-term and site-specific (Oakins 2001). Control options include chemical, manual and biological control; the end goal of any control programme is to kill off rhizomes and thus prevent regrowth (Bell 1997). For the most part, traditional control methods (chemical and manual) have been ineffective at controlling *A. donax* and biological control is seen as the best long-term solution (Dudley 2000; Goolsby and Moran 2009; Silva et al. 2011). In South Africa, management of *A. donax* has been minimal mainly due to a lack of information and difficulty in eradication (Guthrie 2007). Also, in many cases *A. donax* is overlooked due to its similarity to the native *Phragmites* spp. (Guthrie 2007).

Manual

Manual control is generally only used in minor infestations with the aim of complete eradication (Dudley 2000). There are four main mechanical control methods - cut-only, root-removal, burning and soil solarisation (Oakins 2001; Guthrie 2007). The cut-only method is used in environmentally sensitive areas where chemical control is not an option (Oakins 2001). This method requires cutting the reed canes at the base of the plant during the growing season and moving them away from the site for disposal (Silva et al. 2011). The root-removal method is a method that involves digging up rhizomes and removing them from the site (Dudley 2000). However, rhizomes grow as deep as 3 m and therefore their removal is highly damaging to the system and can also increase risk of downstream spread (Dudley 2000). Burning stands is rarely used as a control method as it is counterproductive by promoting re-growth and is damaging to riparian ecosystems (Racelis et al. 2010). Lastly, soil solarisation or tarping is a method that uses plastic to trap solar radiation in order to increase soil temperature to ensure no regrowth of rhizomes (Bell 1997). However, this method is expensive in large infestations and may lead to soil erosion (Guthrie 2007).

The efficiency of manual control is highly site dependent however in most cases the results are only temporary because reeds re-sprout rapidly (Oakins 2001). In addition, in all cases of manual removal there is a large amount of biomass left over and removal of such

debris is expensive (Guthrie 2007). In South Africa, present control methods have generally been restricted to the cut-only method which has resulted in little success (Guthrie 2007).

Chemical

In almost all cases in managing *A. donax* stands, it is necessary to use chemicals (Dudley 2000). The two systematic herbicide, glyphosate and imazapyr can be applied to *A. donax* stands however glyphosate is more commonly employed (Spencer et al. 2008; Spencer et al. 2009; Puértolas et al. 2010). The type of product used depends on site conditions, for example Rodeo® has generally been employed for wetlands and Round-Up® for stands outside riparian zones (Racelis et al. 2010). There are two main methods to apply herbicides - 1) spray-only and 2) cut and spray (Oakins 2001). The spray-only method simply applies the chemicals as a foliar spray to whole stems and leaves (Oakins 2001; Spencer et al. 2008). The cut and spray method involves cutting stems at the base prior to spraying and then applying a 1.5 % by volume glyphosate or imazapyr with 0.5 % v/v non-ionic surfactant onto cut plants (Dudley 2000; Spencer et al. 2009). In both methods the timing of the chemical application is pivotal; the most effective results have been achieved when treatment is done post-flowering and pre-dormancy (Dudley 2000). Overall, the cut and spray approach has been more effective as it only targets *A. donax* plants (Dudley 2000). A study by Bautista (1998) in the Angeles National Forest, U.S.A., found that the cut and spray approach reduced stands to 1 % of vegetative cover compared to 30-80 % prior to treatment. Chemical control however is expensive, requires authorisation and can be environmentally harmful (Oakins 2001).

Chemical control of *A. donax* in South Africa is rare but has been attempted at a few sites (Guthrie 2007). It was found that herbicide initially reduced density, height and growth rates but in the long-term only stimulated plants and led to increased density (Guthrie 2007). Chemical control is unlikely to aid in controlling *A. donax* stands in South Africa (Guthrie 2007).

Biological Control

Biological control using insect specialists is seen as the best solution to addressing *A. donax* invasion in the long-term (Goolsby et al. 2009b; Moore et al. 2010; Cortes et al. 2011; Moran and Goolsby 2014). *Arundo donax* is considered a good candidate for biological control due to low genetic diversity and a relatively high number of host specific herbivores (Tracy and DeLoach 1998; Spencer et al. 2010). Surveys to find potential biological control agents began in 2001 when the University of California, Berkeley began exploration in the native range in Nepal and India (Oakins 2001). These surveys found a number of highly damaging

herbivores including stem-boring moth larva, a stem boring beetle larva, a leaf-mining moth, leaf-hoppers and other Hemiptera (Oakins 2001). The United States Department of Agriculture's Agricultural Research Service (USDA-ARS) then established a biological programme at their European Biological Control Laboratory in Montpellier, France in 2003 (Goolsby and Moran 2009).

From 2005, potential biological control agents were imported from Europe to the ARS Beneficial Insects Research Unit in Texas for further evaluation (Goolsby and Moran 2009). Four agents were imported; *Tetramesa romana* Walker (Hymenoptera: Eurytomidae), the Arundo scale, *Rhizaspidiotus donacis* Leonardi (Homoptera: Diaspididae); the Arundo fly *Cryptonevra* sp. (Diptera: Chloropidae); the Arundo Leaf sheath-miner, *Lasioptera donacis* Coutin and Faivre-Amiot (Diptera: Cecidomyiidae) (Flores and Wood 2009). Since then, the United States Department of Agriculture (USDA) and the ARS identified a further 21 herbivores and a number of *A. donax* diseases from the Mediterranean area (Goolsby and Moran 2009; Goolsby et al. 2009a; Moran and Goolsby 2009; Seawright et al. 2009).

In 2010, two biological control agents, *R. donacis* and *T. romana* were cleared for release in the U.S.A. (Goolsby et al. 2011). These herbivores were chosen as they each attack a different part of the plant (Flores and Wood 2009). *Rhizaspidiotus donacis* is a root feeder, damaging *A. donax* by puncturing mesophyll and parenchyma cells and sometimes vascular tissues in rhizomes and *T. romana* makes galls in the lateral shoots and stems (Flores and Wood 2009; Goolsby et al. 2009a; Moore et al. 2010; Cortes et al. 2011). In addition, *T. romana* populations were discovered in the U.S.A. with an unknown introduction and were thus readily available for augmentative biological control (Dudley et al. 2008; Goolsby and Moran 2009).

In South Africa, a biological control programme for *A. donax* is being investigated. The Working for Water (WfW) programme together with the Agricultural Research Council - Plant Protection Research Institute (ARC-PPRI) are currently investigating the potential of a number of biological control agents for introduction (Dr Angela Bownes, ARC-PPRI, pers.comm.).

1.4 Thesis aims and rationale

A critical part of understanding the status of a plant in a natural landscape is to have a clear understanding of its evolutionary history (Pyšek et al. 2004). In South Africa, to date there have been no studies using genetic techniques to determine the origin of the tall-statured grasses *P. australis, P. mauritianus* and *A. donax*. For the *Phragmites* spp. although considered native, it is important to consider that they have wide-ranging populations in other regions and

thus *Phragmites* genotypes can have reticulate interrelationships with indeterminable borders (Lambertini et al. 2012c). This is particularly true for the cosmopolitan *P. australis* where for example in North America, cryptic invasions have occurred with the introduction of invasive European haplotypes (Saltonstall 2002). It is therefore important to determine what haplotypes are present in South Africa and whether or not the *Phragmites* genotypes present can be considered native to the region. Such information will help guide management of *Phragmites* spp. in riparian systems. For *A. donax,* genetic techniques will provide a clearer understanding of the plant's ancestral lineage from which introductions occurred. Biological control efforts can then be directed to source populations that could help determine biological control agents that are climatically suited.

The thesis aims to provide baseline data for the tall-statured grasses *A. donax, P. australis* and *P. mauritianus* in South Africa; this will be achieved in two parts 1) investigating plant origin for each reed, *P. australis* and *P. mauritianus* (chapter 2) and *A. donax* (chapter 3) using genetic techniques, and 2) determining herbivore assemblages on all three reed species (chapter 4).

Lastly, the study considers the plant-insect interactions on all three tall-statured grasses. Using the Enemy Release Hypothesis as a framework the study aims to compare insect communities on *A. donax* (exotic), *P. australis* (native and cosmopolitan), and *P. mauritianus* (African endemic). The distribution of herbivore diversity, across plant taxa is highly variable and this is believed to be influenced by a plant's evolutionary history (Futuyma and Agrawal 2009). Herbivory pressure on tall-statured grasses should therefore have historical context and thus reflect plant origin. Herbivory is known to have a strong influence on vegetative characteristics so assessing herbivore pressure will also give an indication of the role of top-down pressure on tall-statured grasses (Butler 1995; Naiman and Rogers 1997). Lastly, the study will serve as a pre-introductory survey for the biological control programme on *A. donax,* to determine what herbivores are already present and thus guide the selection of potential biological control agents (Dudley et al. 2008).

2. CHAPTER 2: EXPLORING PLANT ORIGIN OF *PHRAGMITES AUSTRALIS* (CAV.) TRIN. EX STEUD. AND *PHRAGMITES MAURITIANUS* KUNTH IN SOUTH AFRICA

2.1 Introduction

Phragmites australis is considered an indigenous species in South Africa. It has had a long history of utilisation in the country and is abundant nationwide (Guthrie 2007). The plant is known to have occupied riparian areas for thousands of years as palynological investigation found pollen of *P. australis* present in the Bushveld savanna and Highveld grasslands in the Transvaal (now Gauteng Province) dating back to the Late Quaternary period, roughly about 35,000 B.P. (Scott 1982). Although it is considered indigenous, in the last thirty years, *P. australis* distribution and abundance has increased in certain areas, forming dense monospecific stands (Russell and Kraaij 2008). In the Orange River, South Africa for example, *P. australis* has expanded by 40,000 ha and is already impacting ecosystem services and river dynamics (World Commission on Dams 2000).

The expansion of *P. australis* in South Africa has shown similar trends to reed expansion in North America. As, in South Africa, *P. australis* has been a small component of marshes dating back several thousand years (Lynch and Saltonstall 2002; Saltonstall et al. 2010; Kulmatiski et al. 2011), but in the past 100-150 years the reed greatly expanded and began to encroach on previously unoccupied habitats (Kulmatiski et al. 2011). To understand this change in *P. australis'* ecology, a study by Saltonstall (2002) sequenced two non-coding regions of the chloroplast to determine the genetic haplotypes of *P. australis* in North America. Saltonstall (2002) showed that a cryptic invasion had occurred with the introduction of one non-native haplotype, known as Haplotype M that had been introduced from Europe (Saltonstall 2002). This introduced haplotype has been found to thrive in the adventive range in North America and can readily displace native haplotypes (Lynch and Saltonstall 2002). Since then, four additional introduced haplotypes have also been discovered in the U.S.A. and Canada known as haplotype L1, the "Greeny" haplotypes AD and AI originating in Europe and the haplotype M1 (or Med lineage) originating in the Mediterranean basin, including North Africa, the Middle East and southern Europe (Lambertini et al. 2012c; Meyerson et al. 2012; Guo et al. 2013).

Work on genetic haplotypes in North America has highlighted the role of plant genotype on *P. australis* morphology and ability to adapt. In the Gulf Coast, U.S.A., the five sympatric haplotypes of *P. australis* were found to have varying rates of photosynthesis and thus are

recognised as different ecotypes (Nguyen et al. 2013). It is believed that these differences are a result of adaptation to their original native range as the reeds now grow in close proximity (Nguyen et al. 2013). For example, the lineages believed to originate from tropical Africa known as Land-type (haplotype I), Greeny3-type (haplotype AI) and Delta-type (haplotype M1), are adapted for warmer and more arid climates having thicker leaves and higher photosynthetic capacity (A_{max}), pigment content and specific leaf area (Nguyen et al. 2013). Similarly, the invasive haplotype M in North America has been found to have more efficient photosynthetic capacity than the native *P. australis* genotypes and this ability was inherited from its native population in Europe and further improved post-introduction (Guo et al. 2014; Tho et al. 2016). Lastly, plant haplotype has also been found to be an important factor influencing herbivore load across the different genotypes (Schwarzländer and Häfliger 2000; Tewksbury et al. 2002; Häfliger et al. 2005; Blossey 2014). Such adaptations within genotypes will play a strong role in determining haplotype distribution and contribute to introduced haplotypes having a competitive advantage over native *P. australis* in North America.

An investigation into *P. australis'* present status as a native species in South Africa is required. Molecular methods are seen as the most reliable method to investigate *P. australis* diversity and discriminate between native and exotic populations that cannot otherwise be differentiated based on their morphological characteristics (Saltonstall 2003a). *Phragmites australis* phylogeography is complex and with more molecular markers being used this complexity is only beginning to unravel (Lambertini 2016), therefore approaching the question of origin is not straightforward. Although *P. australis* has long been seen as a cosmopolitan species and thus native in all areas it is found, evidence points to a region of origin in Africa or East Asia (Lambertini et al. 2006). It has become apparent that *P. australis* is a complex of cryptic species and its classification as either native or introduced is difficult to establish (Chambers et al. 1998; Saltonstall 2002) without global sampling. Currently, the best approach to address this is using chloroplast DNA to trace dispersal events at a worldwide scale (Lambertini et al. 2006, 2012c). Nuclear diversity gives insight into reproductive ability (Saltonstall et al. 2010; Hauber et al. 2011). When *P. australis* clones establish in a new environment, over time they become genetically distinct through spontaneous mutations that respond to climatic conditions and therefore in general, the greater the geographic distance the more genetically different plants will be from each other (Koppitz 1999). However, the extent of differentiation is often proportional to the time since introduction (Guo et al. 2014).

Phylogeographic studies have been under-represented in Asia and Africa (Lambertini et al. 2012c). As a result, it is uncertain whether or not cryptic invasions have occurred in South

Africa or native haplotypes are still present. In considering this, it is important to include the native, congeneric species, *P. mauritianus* in any analysis. *Phragmites mauritianus* is endemic to the African continent and so will allow for comparisons of genetic diversity levels with a known native species. Genetic variation is generally agreed to be structured in space and time (Loveless and Hamrick 1984); it has generally been found that higher levels of genetic diversity are found in plants from the source area (Lambertini et al. 2012b). However, this is not always the case, as hybridisation events with native species can increase genetic diversity and trigger rapid evolutionary change (Ellstrand and Schierenbeck 2006). Determining the genetic diversity of a plant will addresses the amount of variability among individuals of a population which has arisen from mutation and recombination in DNA over time (Brown 1983; Rao and Hodgkin 2002). Genetic diversity is the basis for plant survival and adaptability and increased diversity is linked to increase fitness (Rao and Hodgkin 2002). *Phragmites mauritianus* on the whole is a poorly studied species where at present there has been no in-depth investigation of its genetic diversity, dispersal mechanisms and haplotypes in South Africa (Van Coller et al. 1997).

In South Africa, an in-depth study of the genetic diversity of the two co-occurring reeds *P. australis* and *P. mauritianus* will yield important results for *Phragmites* spp. on a global scale. As the two species have overlapping distributions, and yet remaining distinct species and it was traditionally believed that they cannot form hybrids due to the difference in chromosome number (Lambertini et al. 2006). Yet with improved molecular markers, more evidence of hybridisation has been uncovered (Chu et al. 2011, Meyerson et al. 2012). For example, hybrids between *P. mauritianus* and *P. australis* have resulted in the Land-type in the Gulf Coast, North America (haplotype I or *P. australis* var. berlandieri) (Lambertini et al. 2012b). Furthermore, studies of the heteroplasmic diversity of chloroplastic DNA in South Africa has hinted at hybridization in haplotypes (Lambertini 2015). With such evidence of a history of *P. mauritianus* hybridisation it is hypothesized that in South Africa where the two species co-occur there will be evidence of an exchange of genetic material.

This study investigated the status of *P. australis* and *P. mauritianus* in South Africa by assessing the haplotypes present and their genetic diversity. The results from this study help link how plant origin influences the herbivore assemblages associated with *Phragmites* spp. in South Africa (chapter 4) and identifies the intercontinental phylogeographic relationships of the South African populations to address the native vs. their introduced status. Lastly, determining what haplotypes are present will guide management goals and determine if there is a need to protect native haplotype populations if they are threatened.

2.2 Materials and methods

2.1.1 Sampling and DNA extraction

Leaf tissue was collected from the young apical leaves of *P. australis* and *P. mauritianus* during the growing seasons (October - March) from 2011-2013. Samples of both *P. australis* (Figure 2-1 and Table 2-1) and *P. mauritianus* (Figure 2-2 and Table 2-2) were collected to represent their distribution in South Africa. For *P. australis,* sampling was carried out on reed stands in South Africa's nine biomes, namely the Albany thicket, grassland, savanna, Nama-Karoo, forest, fynbos, desert, Indian Ocean Coastal Belt, and thicket biomes (Rutherford et al. 2006). For *P. mauritianus,* sampling was done across the reed's range from northeastern South Africa, Swaziland and Zambia (Gordon Gray and Ward 1971). In each stand, 15 - 30 leaves from different plants were collected per population. Fresh leaves were dried in silica gel according to the protocol of Chase and Hills (1991).

DNA was isolated from samples with the Qiagen DNeasy Plant Mini Kit as described in Lambertini et al. (2006). The Qiagen protocol was modified whereby leaf tissue was crushed under liquid nitrogen before the addition of the extraction buffer.

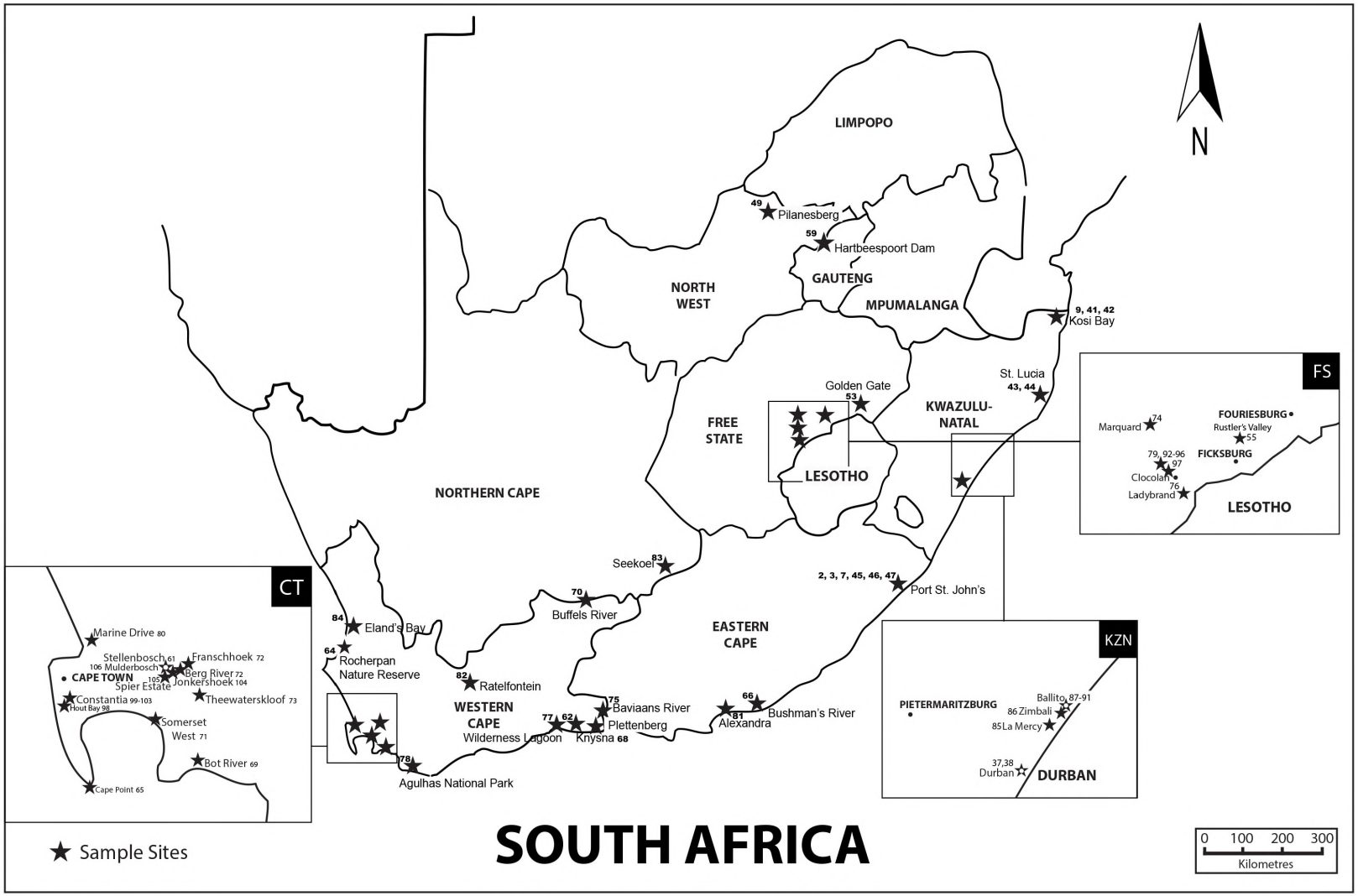


Figure 2-1 Map of South Africa showing *P. australis* sampling sites (Table 2-1).

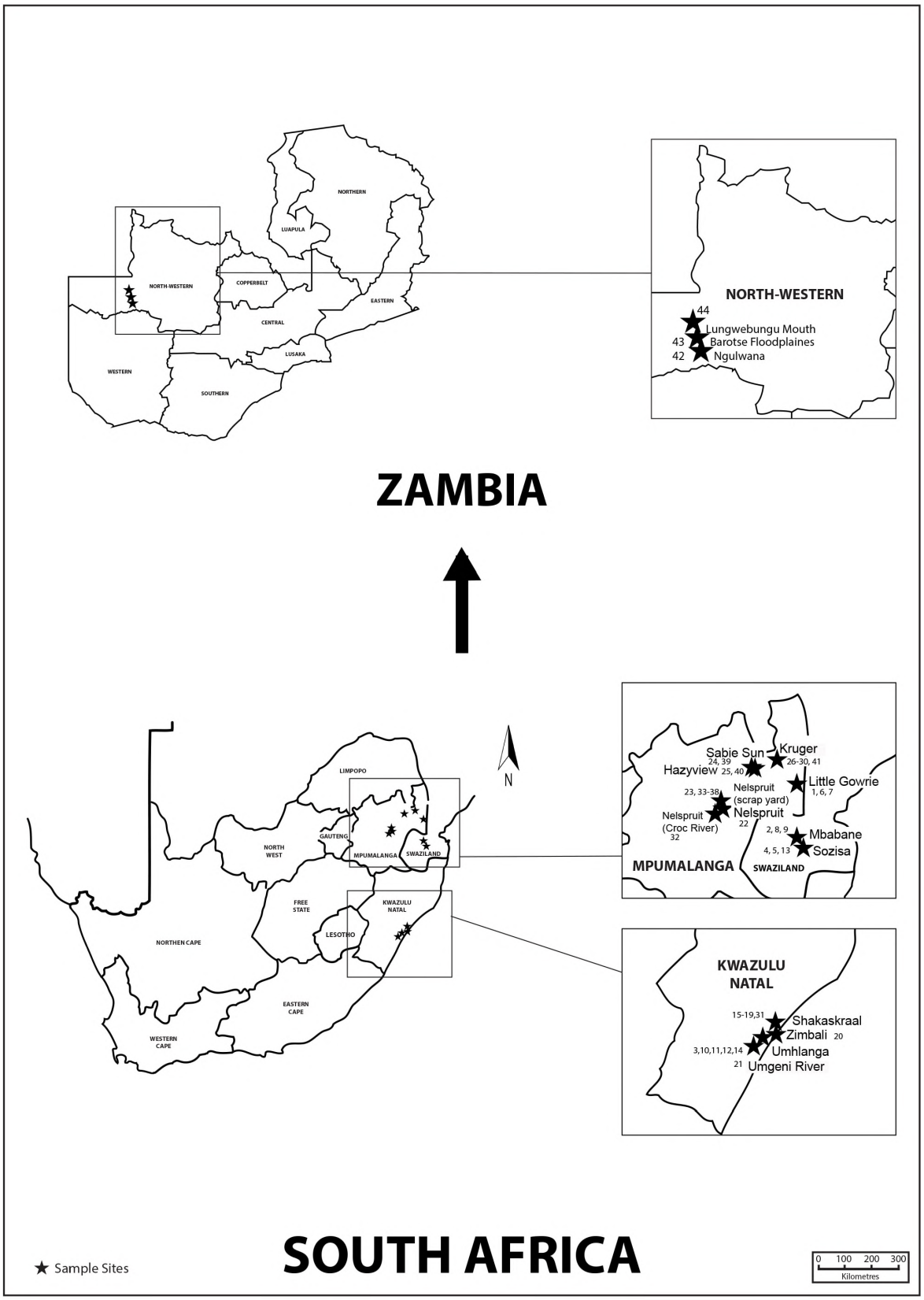


Figure 2-2 Map of South Africa, Swaziland and Zambia showing *P. mauritianus* sampling sites (Table 2-2).

Table 2-1 Sampling sites for the collection of *P. australis* genetic material.

Sample number	Population Number	Site Location	Province	GPS coordinates
1,2,3,7,45,46,47	1	Port St. John's	Eastern Cape	31°35'58.11''S, 29°31'55.51''E
9,41,42	2	Kosi Bay	KwaZulu-Natal	26°57'39.46''S, 32°49'36.23''E
37,38	3	Durban	KwaZulu-Natal	29°48'31.72''S, 31°01'05.94''E
43,44	4	St Lucia	KwaZulu-Natal	28°22'10.82''S, 32°24'35.84''E
49	5	Pilanesberg Nature Reserve	North West	25°15'36''S, 32°27'06'43''E
53	6	Golden Gate Nature Reserve	Free State	28°29'58.51''S, 32°49'09''E
55	7	Rustler's Valley	Free State	28°43'12.51''S, 28°01'33.79''E
59	8	Hartebeespoort Dam	Gauteng	25°44'22.37''S, 27°54'18.77E
61	9	Stellenbosch	Western Cape	33°58'06''S, 18°47'27''E
62	10	Knysna	Western Cape	34°02'33''S, 23°04'01''E
64	11	Rocherpan Nature Reserve	Western Cape	33°40'16.28''S,26°38'36.78''E
65	12	Cape Point	Western Cape	34°14'28''S, 18°25'28''E
66	13	Bushmans River	Eastern Cape	33°40'16.28''S,26°38'36.78''E
68	14	Plettenberg Bay	Western Cape	34°04'04''S,23°21'45''E
69	15	Bot River - Kleinemond	Western Cape	34°18'47.06''S, 19°08'54.69''E
70	16	Buffels River	Northern Cape	33°11'44.66''S, 20°51'09.60''E
71	17	Somerset West	Western Cape	34°05'25.63''S, 18°50'15.37''E
72	18	Berg River -Franshoek	Western Cape	33°52'39.84''S, 19°02'02.38''E
73	19	Theewaterskloof	Western Cape	34°04'11''S, 19°17'41''E
74	20	Marquard	Free State	28°42'05.56''S, 27°26'33.14''E
97	20	Clocolan (3P-FS-1)	Free State	28°51'30.10''S, 27°32'27.38''E
75	21	Baviaans River	Western Cape	33°58'24''S, 23°38'51''E
76	22	Ladybrand	Free State	29°10'59.60''S, 27°28'25.85''E
77	23	Wilderness Lagoon	Western Cape	29°10'59.60''S, 22°34'58.33''E

78	24	Agulhas National Park	Western Cape	34°44’33.32’’S, 19°39’25.66’’E
79	25	Clocolan (2P-FS-1)	Free State	28°51’30.10’’S, 27°32’27.38’’E
80	26	Marine Drive	Western Cape	33°52’42.96’’S, 18°29’25.56’’E
81	27	Alexandria	Eastern Cape	33°38’01’’S, 26°19’07’’E
82	28	Ratelfontein	Northern Cape	31°30’34.66’’S, 23°37’46.26’’E
83	29	Seekoei	Northern Cape	31°38’01’’S, 26°19’07’’E
84	30	Eland’s Bay	Western Cape	32°18’52.89’’S, 18°21’19.79’’E
85	31	La Mercy (2P-DB-1)	KwaZulu-Natal	29°38’47’’S; 31°07’18’’E
86	32	Zimbali (3P-DB-1)	KwaZulu-Natal	29°33’38’’S; 31°10’19’’E
87,88,89,90,91	33	Ballito (5P-DB-1-5)	KwaZulu-Natal	29°28’17’’S; 31°14’28’’E
92,93,94,95,96	34	Clocolan (2P-FS-1-5)	Free State	28°51’30.10’’S, 27°32’27.38’’E
98	35	Hout Bay (1P-CT-1)	Western Cape	34°02’05.64’’S; 18°21’13.93’’E
99,100,101,102,103	36	Constantia (2P-CT-1-5)	Western Cape	34°01’24.92’’S; 18°26’18.85’’E
104	37	Jonkershoek Nature Reserve (3P-CT-1)	Western Cape	33°58’33.98’’S; 18°56’40.24’E
105	38	Spier Wine Estate (4P-CT-1)	Western Cape	33°58’30.82’’S;18°47’07.84”E
106	39	Mulderbosch Wine Estate (5P-CT-1)	Western Cape	33°56’51.80’’S; 18°45’55.06”E

Table 2-2 Sampling sites for the collection of *P. mauritianus* genetic material.

Sample number	Population Number	Site Location	Province	GPS coordinates
1M,6M,7M	1	Little Gowrie	Mpumalanga	24°40'51''S, 31°33'17.60''E
2M,8M,9M	2	Mbabane - Swaziland	Swaziland	26°19'15.54''S,31°07'36.60''E
3M,10M,11M,12M,14M	3	Umhlanga	KwaZulu-Natal	29°42'49.80''S,31°05'31.23''E
4M, 5M, 13M	4	Sozisa - Swaziland	Swaziland	26°19'42.24''S 31°08;34.78''E
15M,16M,17M,18M,19M,31M	5	Shakaskraal	KwaZulu-Natal	29°27'28''S; 31°13'00''E
20M	6	Zimbali	KwaZulu-Natal	29°33'38''S; 31°10'19''E
21M	7	Umgeni River	KwaZulu-Natal	29°47'48''S; 30°57'57''E
22M	8	Nelspruit	Mpumalanga	25°26'03.57''S; 30°52'02.49''E
23M,33M,34M,35M,36M,37M,38M	9	Nelspruit- scrap yard	Mpumalanga	25°01'52.73''S; 31°01'25.43''E
24M,39M	10	Hazyview	Mpumalanga	25°27'17.05''S; 30°53'07.36''E
25M, 40M	11	Sabie Sun-Hazyview	Mpumalanga	25°02'10.75''S; 31°07'05.83''E
26M, 27M,28M,29M,30M,41M	12	Kruger National Park	Mpumalanga	24°58'48.04''S; 31°28'53.93''E
32M	13	Nelspruit – Hippo Lodge	Mpumalanga	25°26'06.83''S; 30°52'04.82''E
42M	14	Ngalwana Village, Zambezi	Zambia	14°59'76.90''S; 23°11'29.70''E
43M	15	Barotse Floodplains	Zambia	15°51'18,30''S; 23°13'42.00''E
44M	16	Lungwebungu Mouth	Zambia	14°30'77,30''S; 23°18'76.70''E

2.1.2 cpDNA

Two noncoding regions in the chloroplast genome were amplified according to Saltonstall (2002) (Table 2-3). These were the *trnL*b region, which is a segment of the intergenic spacer region between *trn*T (UGU) and *trn*L (UAA) and the *rbc*L region, a segment of the intergenic spacer region between *rbc*L and *psa*I. For the *trnLb* and *rbc*L regions, ten picomoles of forward and reverse primers were added to 12.5 µl of Promega MasterMix (Madison, WI, USA) (reaction concentration of 1 U of *Taq,* 1.5mM $MgCl_2$ and 0.2 µM dNTPS), 2 µl of Promega Magnesium chloride and 7 µl of template DNA per reaction. Promega nuclease-free water was added to reach a final volume of 25 µl. Amplification was run in one of the following machines: Labnet Multi Gene II or Applied Biosystems 2720 thermal cycler. For the *trnLb* region, the PCR cycling protocol was 94 °C for 2 minutes, 35 cycles of 94 °C for 1 minute, 56 °C for 1 minute, 72 °C for 2 minutes, followed by a final extension at 72°C for 5 minutes. For the *rbcL* region, the same cycling protocol was used however the annealing temperature was lowered to 45 °C.

PCR products were cleaned at Stellenbosch University, Stellenbosch, South Africa or at Inqaba Biotec™, Johannesburg, South Africa. Cycle sequencing reactions were done using BigDye Terminator ver. 3.1 Cycle Sequencing Kit (Applied Biosystems™, Foster City, CA) with the same primers used as in the PCR reactions. Cycle sequencing products were purified using ethanol-sodium acetate precipitation. Capillary electrophoresis was done using an ABI 3100® genetic analyser at Stellenbosch University or using an ABI 3500® genetic analyser at Inqaba Biotec™.

Table 2-3 Primer sequences used to obtain *P. australis* haplotypes.

Primer	Sequence	Length of obtained sequence
*trn*T(UGU)"a "[1,2]	5' CATTACAAATGCGATGCTCT	900 bp
trnL(UAA) "b"[1,2]	5' TCTACCGATTTCGCCATATC	
rbcL[2]	5' TGTACAAGCTCGTAACGAAGG	1080 bp
psaI[2]	5' CTAAGCCTACTAAAGGYACG	

[1]Taberlet et al. 1991; [2]Saltonstall 2001

2.1.3 Microsatellites

Ten microsatellite primers were developed by Saltonstall (2003b) to study North American and introduced European populations in North America. For this study, four

microsatellite primer pairs were selected - Pagt4, Pagt8, Pagt9 and Pagt22 (Table 2-4); due to their high variability (Saltonstall 2003b), also among South African samples (Lambertini et al. 2012b). The PCR reaction contained 18 µl of Promega Master Mix (Madison, WI, USA), 10 µl pmol forward and reverse primers, 3 µl of template DNA and Promega nuclease free water was added to reach a volume of 20 µl. PCR cycling protocol was 94 °C for 12 minutes, followed by 35 cycles of 94 °C for 30 s, 50-56 °C for 30 s, 72°C for 40 s and an extension of 72 °C for 5 minutes. Primers were fluorescently labelled by Applied Biosystems Inc., U.K. (Table 2-4). PCR products were diluted 20x with Promega nuclear free water and sent to either Stellenbosch University, Stellenbosch, South Africa for analysis or the Inqaba Biotec™ lab, Johannesburg, South Africa. Capillary electrophoresis of DNA fragments was done using either an ABI 3100® genetic analyser (Stellenbosch University) or an ABI 3500® genetic analyser (Inqaba Biotec™).

Microsatellite results can contain error due to allelic dropout or false allele amplification and this will influence allele frequency estimates and distinguishing genotypes (Bonin et al. 2004). To address this error, 10% of samples were duplicated. To further test the accuracy of the results, the same samples were sent to a different lab for PCR set up and fragment analysis (Inqaba Biotec™). Lastly, to avoid subjectivity in scoring of peaks, any peaks that were ambiguous and in particular with stutter peaks, such samples were scored as missing data.

Table 2-4 Primer sequences used in this study and their allelic diversity measures for four microsatellite loci of *P. australis* from Saltonstall (2003b).

Locus	Primer sequence (5'-3')	Fluorescent Dye	No. repeat units	Allele size range (bp)
PaGT4	F:TGCTCCCTGCCAGTTTCTTG R:TATCCACCCTCGAAGGCAC	6-FAM	$(CA)_9$	266-284
PaGT8	F:TCTGAACATAATCCTGGTGG R:TCTGTGTGAAGCAGTTCTGC	6-FAM	$(CA)_8$	170-193
PaGT9	F:CCATGTGTTAATGTTGTCC R:ATTGAATCCACACGTTTCCG	NED	$(CA)_{10}$	188-224
PaGT22	F: TTGAGTGCCTGGTGTATTCG R:AAGCTTCTGTCATGGAACCG	6-FAM	$(AC)_8CTT(GA)_5$	159-209

2.1.4 *Grass-waxy* analysis

For the *grass-waxy* analysis, samples were sent to Dr. Carla Lambertini at the Department of Bioscience, Aarhus University, Denmark. Primers were developed by Dr Lambertini (Table 2-5) based on sequences isolated from her previous work, and were run according to Lambertini

et al. (2012) protocol. Template DNA (1 µl) was added to 10 µl 2 x Mastermix (VWR Amplicon), 10 pmol of forward and reverse primers, and sterile water to reach a total volume of 20 µl. The cycling protocol was 94 °C for 3 min, 40 cycles of 94 °C for 30 s, 62 °C for 40 s, 72 °C for 40 s, followed by 72 °C for 7 min. Products were run on a 1.5 % agarose gel for 1 h 30 min at 125 V, 84 mA and stained with ethidium bromide.

The presence or absence of the resulting bands of 100bp for *P. mauritianus* and 200 bp for Mediterranean *P. australis*, was recorded and the PCR product was then sequenced at Inqaba Biotec™, Johannesburg, South Africa. Sequences were aligned with global samples.

Table 2-5 Primer sequences for *grass-waxy* analysis.

Primer	Sequence	Length of sequence
WaxyF[1]	5' TGCGAGCTCGACAACATCATGCG	200 bp
Waxy200(25)R[1]	5' GATCCCTCGCCATCACARCATCGCC	
Waxy100F[2]	5′- CGATAGGATGAGCAGTTAGG	100 bp
WaxyR	5′- GGCGAGCGGCGCGATCCCTCGCC	

[1]Lambertini et al. 2012c,[2]Lambertini, pers. comm.

2.1.5 Data analysis

Chloroplast DNA chromatograms were examined and contiguous sequences were assembled and manually edited in GeneStudio™ ver. 2.2.0.0. (GeneStudio, Inc.). Alignment of sequences was done in MEGA ver. 5.2.2 including all worldwide haplotypes downloaded from GenBank (Appendix - Table 0-1) using ClustalW set to default parameters (Kumar et al. 2012).

For microsatellite data, chromatogram alignment was first constructed with Geneious ver. 8.1.7 (Kearse et al. 2012). Peak size markers were all aligned to ensure amplified peaks could be aligned by fragment size. Given the polysomic nature of the sample set (more than two alleles per locus) the dataset was entered into a binary matrix (1 = presence, 0 = absence of homologous alleles) and analysed using GenAIEx ver. 6.5 (Peakall and Smouse 2012). Pairwise genetic distances were calculated based on the number of shared-alleles per locus (Euclidean distances). The output matrix of genetic distances was then used to run a Principal Component Analysis. Allelic diversity was measured as the total number of alleles per locus. Genetic diversity was compared between *P. mauritianus* and *P. australis* by calculating Nei's unbiased genetic identities (Nei 1973) and Shannon Information Index (Lewontin 1995) with

the program PopGene ver. 1.32 Population Genetic Analysis (Yeh and Boyle 1997) as done in previous studies (Lambertini et al. 2012c).

The samples were initially classified as either *P. australis* or *P. mauritianus* based on their cpDNA matrilineages (*trn*T-*trn*L and *rbc-psaI*). The nuclear ancestry of the two populations was subsequently tested based on the four microsatellite loci using a Bayesian genetic clustering algorithm implemented in STRUCTURE version 2.3.4. (Pritchard et al. 2000). An admixture model was used that assumed independent allele frequencies with 10 iterations for each run. Each run consisted of 1,000,000 MCMC steps and a burn-in period of 100,000. The number of populations (K) was tested from 1-10 and K was inferred with Harvester (following Evanno et al. (2005)). Models were also run including only *P. australis* or *P. mauritianus* samples according to the same parameters.

2.3 Results

2.1.6 cpDNA

The analysis of chloroplast sequences for *P. australis* samples found that all samples from across South Africa aligned with Saltonstall (2002) Haplotype K with *trn*T-*trn*L samples aligning with Haplotype 3 (Accession number: AY016326) and *rbc*L-psaI samples aligning with Haplotype 4 (Accession number: AY016335). No variation in the chloroplast sequences was found across all samples.

The analysis for chloroplast sequences for *P. mauritianus* found two haplotypes. All South African samples were found to be haplotype V with *trn*T-*trn*L samples aligning with Haplotype 5 (Accession number: AY016328) and *rbc*L-psaI samples aligning with Haplotype 10 (Accession number: AF457387). Two samples from Zambia (42M and 43M) had variation in the *trn*T-*trn*L region that aligned with Haplotype 12 and are therefore Haplotype AP according to Lambertini et al. (2012c).

Both *P. australis* and *P. mauritianus* were found each to have only one haplotype in South Africa, this shows a significantly lower haplotypic diversity compared to worldwide *Phragmites*. For *P. australis,* in Europe 21 haplotypes have been recorded, 17 have been found in North America, and similarly in Asia and Australia 27 and 12 haplotypes have been found respectively (Saltonstall 2002). In Africa, to date only 3 haplotypes have been recorded for *P. australis*. With only one of these haplotypes having been found to be present in South Africa. For *P. mauritianus,* only 5 haplotypes have been recorded in Africa and in this study only one haplotype was found to occur within South Africa. However, genetic variation in the African

continent is still largely unexplored.

2.1.7 Microsatellites

An overall error rate of 15.22 % was found for the microsatellite analysis based on replication of 10 % of samples. The error was a result of both false allele amplification and allelic dropout with both factors equally contributing to the error rate. The relatively high error rate is most likely also a result of the fact that the duplicated samples were run on different ABI machines.

The PCoA (Figure 2-3) separated the two species into two distinct groups, which however did not fully match with haplotype. One *P. australis* sample (based on cpDNA) with haplotype K was found to cluster within *P. mauritianus* samples (sample 49P, from Pilanesberg, North-West) (Figure 2-3). For both reeds, there was no strong geographic pattern in allelic phenotypes as no clear clustering was found either according to geographic provinces or biomes sampled (Figure 2-3).

For *P. australis,* three stands sampled in Kosi Bay (9P, 41P, 42P), St. Lucia (43P,44P), Clocolan (92P-96P) were found to have multiple allelic profiles (Figure 2-4 and Appendix-Table 0-2). For *P. mauritianus,* only two stands didn't have variation and most had different allelic profiles (Figure 2-5 and Appendix-Table 0-3).

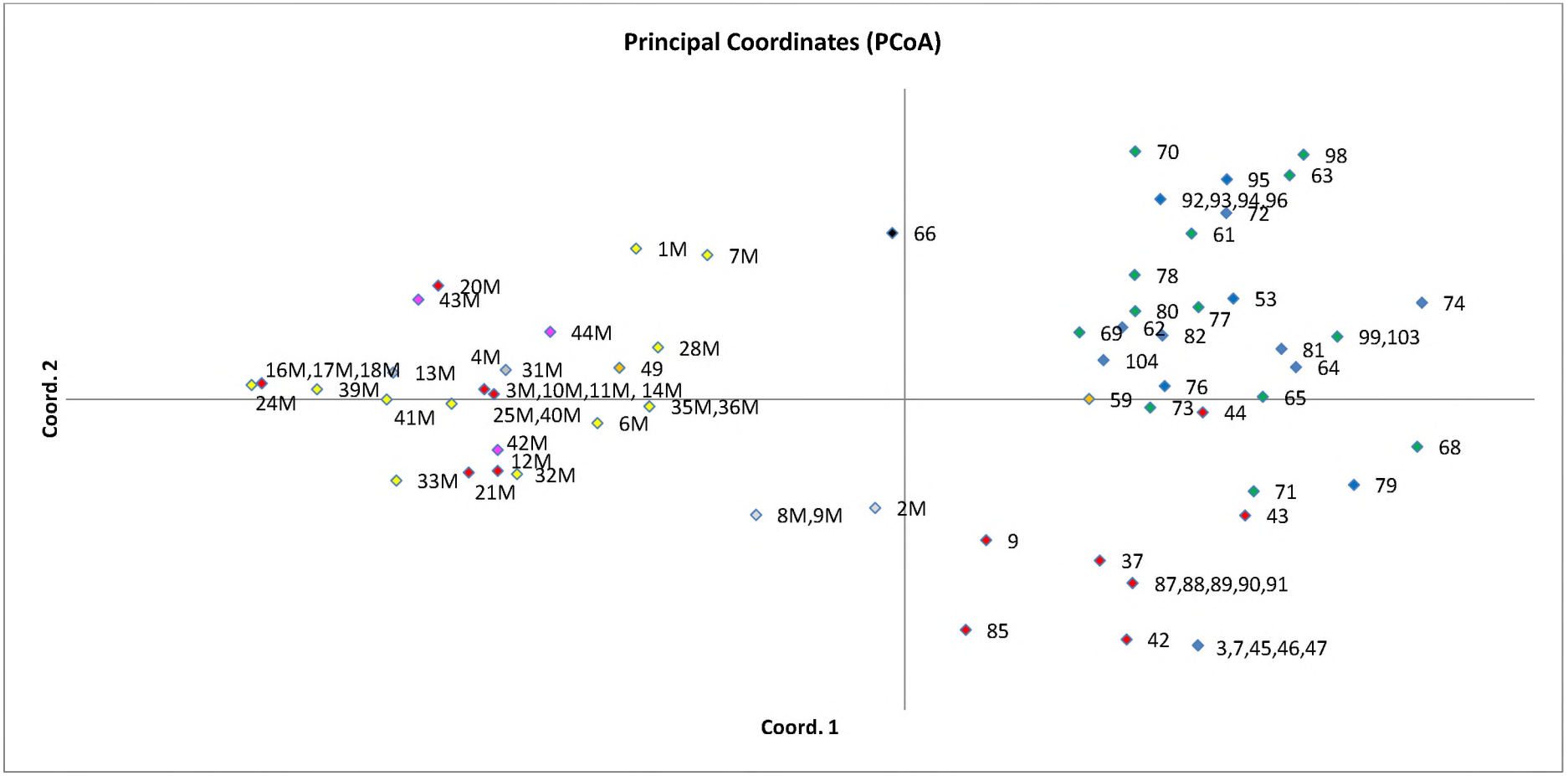


Figure 2-3 Microsatellite diversity in *P. australis* and *P. mauritianus* samples in South Africa using PaGT4, PaGT8, PaGT9 and PaGT22 primers. Samples with missing data were excluded. Coordinate 1 accounts for 22.55% and Coordinate 2 accounts for 12.25% of the variation. Colours represent geographic area defined by province in South Africa; red – KwaZulu-Natal, pink – Namibia, blue – Free State, yellow- Mpumalanga, black- Eastern Cape, green- Western Cape, orange- North-West and grey- Swaziland. *Phragmites mauritianus* samples all clustered to the left axis and *P. australis* samples clustered to the right axis. Samples with letter M following number denote *Phragmites mauritianus* samples, no letter denotes *P. australis* samples.

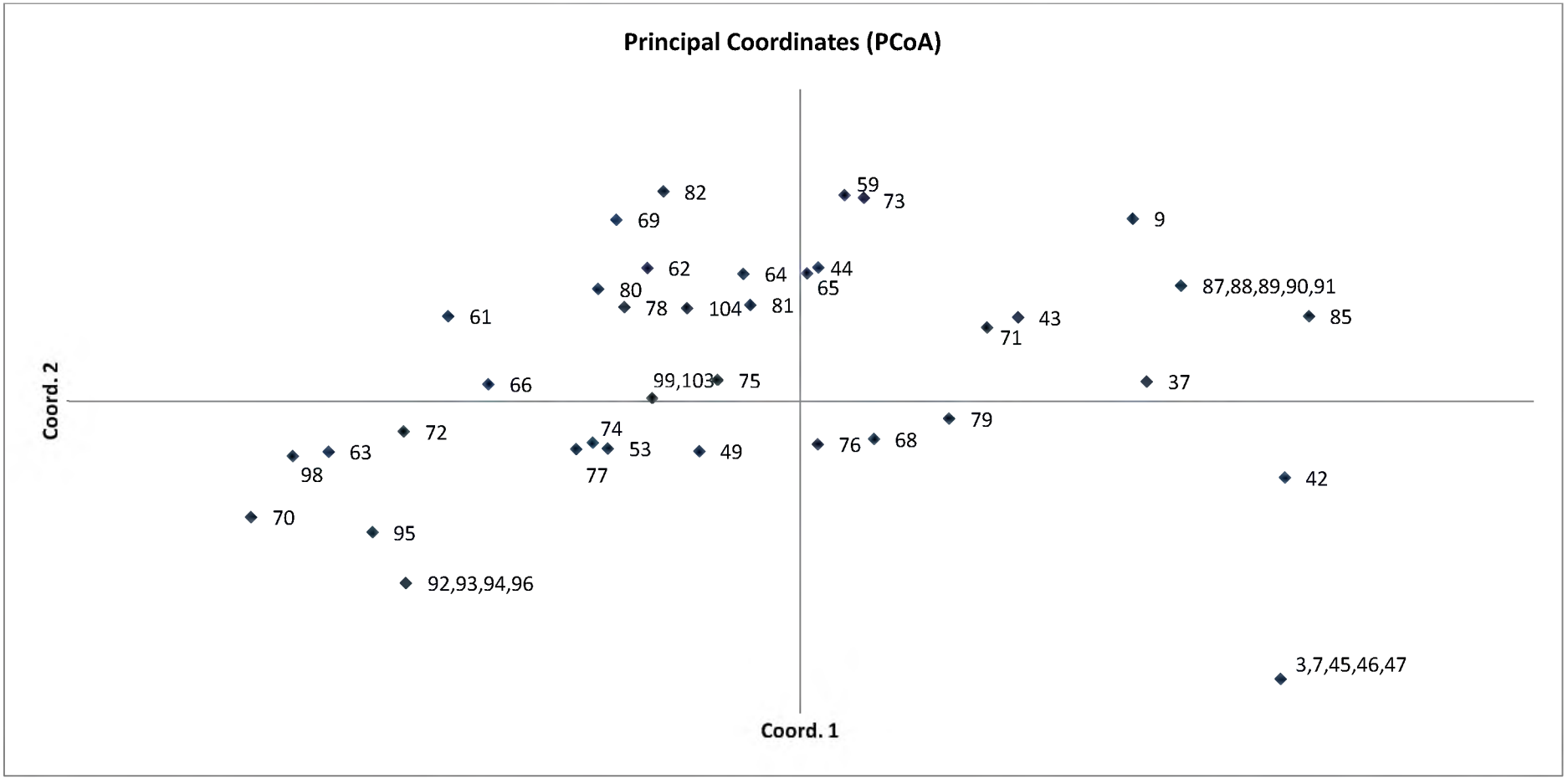


Figure 2-4 Microsatellite diversity in *P. australis* in South Africa using PaGT4, PaGT8, PaGT9 and PaGT22 primers. Graph includes only samples with no missing data. Coordinate 1 accounts for 22.79% and Coordinate 2 accounts for 13.66% of the variation.

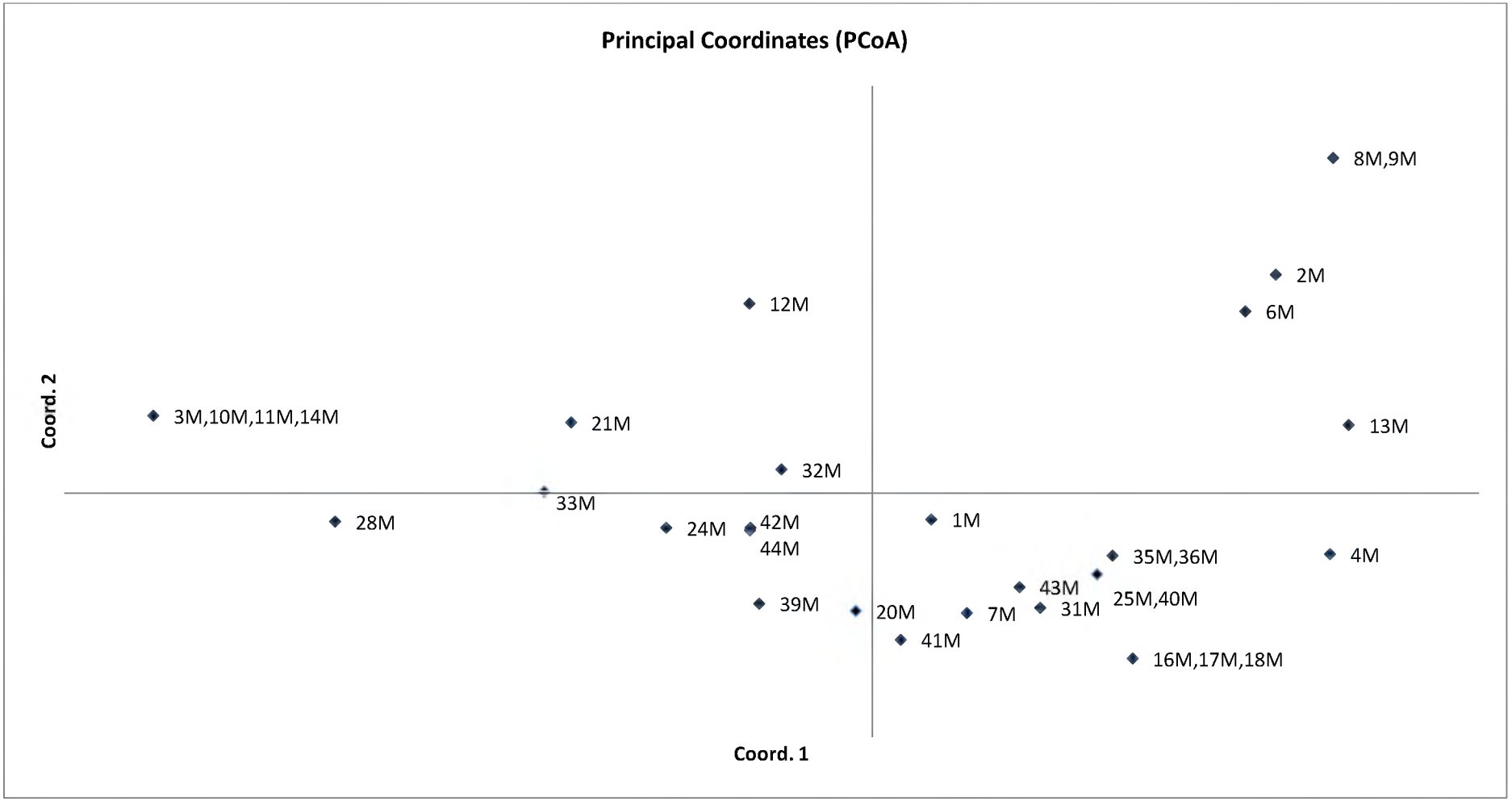


Figure 2-5 Microsatellite diversity in *P. mauritianus* in South Africa using PaGT4, PaGT8, PaGT9 and PaGT22 primers. Graph includes only samples with no missing data. Coordinate 1 accounts for 20.78% and Coordinate 2 accounts for 17% of the variation.

Phragmites mauritianus was also found to have a higher Shannon Information Index (I), number of effective alleles (Ne) and higher Nei's diversity (h) compared to *P. australis* samples (Table 2-6). However, these differences were not statistically significant. Comparing the South African samples to global populations, the two *Phragmites* spp. in South Africa have higher genetic diversity (Shannon Information Index and Nei's gene diversity) compared to all worldwide regions according to Lambertini et al. (2012a) (Table 2-6).

Table 2-6 Comparison of genetic diversity for *P. australis* (Pa) and *P. mauritianus* (Pm) in South Africa from this study (in bold) based on four microsatellite primer pairs (over populations for each loci). Sample size (N), number of populations (Npop), observed number of alleles (Na), Number of effective alleles (Ne), Shannon Information Index (I), Nei's gene diversity (h), and standard deviation (s.d.). No significant differences were found between *P. australis* and *P. mauritianus* in South Africa (p values obtained with Mann Whitney *U* test, $p < 0.050$). Worldwide distributed populations from Lambertini et al. (2012a) were included to compare the genetic diversity levels (I and h) in global samples. (The diversity values borrowed from Lambertini et al. (2012a) are based on six microsatellite loci).

	N	**Npop**	**Na (s.d.)**	**Ne (s.d.)**	**I (s.d.)**	**h (s.d.)**
Pa- SA	**61**	**39**	**2 ± 0.0**	**1.339 ± 0.358**	**0.333 ± 0.231**	**0.208 ± 0.177**
Pm- SA	**44**	**16**	**2 ± 0.0**	**1.446 ± 0.333**	**0.425 ± 0.204**	**0.273 ± 0.161**
GC-Land	55				0.044 ± 0.118	0.024 ± 0.079
GC-Delta	53				0.036 ± 0.115	0.021 ± 0.072
GC-EU	18				0.093 ± 0.199	0.060 ± 0.131
GC-Greeny1	3				0.073 ± 0.205	0.051 ± 0.143
SAM	6				0.008 ± 0.072	0.006 ± 0.050
NAMintr	33				0.151 ± 0.224	0.094 ± 0.151
NAMnat	6				0.066 ± 0.165	0.041 ± 0.104
Europe	16				0.191 ± 0.254	0.123 ± 0.173
Afr/Med	18				0.180 ± 0.230	0.111 ± 0.155
Senegal	4				0.034 ± 0.147	0.024 ± 0.105
ZA	2				0.036 ± 0.154	0.026 ± 0.111
Pm	2				0.080 ± 0.223	0.058 ± 0.161
Pk	3				0.114 ± 0.246	0.080 ± 0.172
Pj	3				0.033 ± 0.141	0.023 ± 0.099

The number of alleles amplified varied for *P. australis* and *P. mauritianus*. *Phragmites australis* phenotypes on average had more alleles amplified, with most samples having two alleles compared to only one allele for *P. mauritianus* (Table 2-7). For *P. australis*, a maximum of four alleles were found however, *P. mauritianus* had a maximum of only two alleles. The higher amplifications of alleles for *P. australis* indicates a higher ploidy level compared to *P. mauritianus*. This agrees with the finding of Gordon Gray and Ward (1971) that found that South African *P. australis* is octoploid (2n = 8x = 96) and *P. mauritianus* is a (disomic) tetraploid (2n = 4x = 48).

Table 2-7 Summary of allele amplification for *P. australis* and *P. mauritianus*.

	P. australis	***P. mauritianus***
Number of samples (n)	220	150
Mean number of alleles	2.16 ± 0.94	1.56 ± 0.71
1 allele amplified (%)	27.27%	44%
2 alleles amplified (%)	34.54%	56%
3 alleles amplified (%)	33.20%	0%
4 alleles amplified (%)	5%	0%

The resultant microsatellite phenotypes found in both *Phragmites* spp. in South Africa were each compared to *P. australis* lineages occurring in North America and Europe (Table 2-8). For *P. australis*, populations in South Africa have considerably higher number of alleles (A_o = 10.25 ± 2.06) when compared to the number of alleles of both the European and North American populations. However, *P. mauritianus* have similar number of alleles to the European introduced populations in North America and its native population in Europe with an average of 6.75 ± 1.23 alleles compared to 7 ± 4.36 and 7.67 ± 4.73 respectively. Similarly, *P. mauritianus* also has about the same level of heterozygosity as the native European lineage at 0.56 ± 0.14 compared to 0.50 ± 0.29 respectively. This is likely due to a similar ploidy level as *P. australis* has a higher ploidy level in South Africa (Table 2-8). This may explain the higher number of alleles and allelic combinations (i.e. heterozygotic phenotypes).

Table 2-8 Genetic characteristics of four microsatellite loci from Saltonstall (2003b), comparing four *P. australis* haplotype lineages to samples from South Africa including *P. australis* and *P. mauritianus*. *n*: Number of samples genotyped; A_o Number of alleles; Dominant phenotypes: dominant allele phenotypes, values in parentheses are the frequency of each phenotype; H_o: observed heterozygosity. For the total at all loci, the worldwide lineages (introduced, native, Gulf Coast and Europe) summarise from 10 microsatellite primers however for this study of *P. australis* and *P. mauritianus* in South Africa only four microsatellite primers were used.

Locus	**Haplotype Lineage**					
	Introduced	**Native**	**Gulf Coast**	**Europe**	***P. australis* South Africa**	***P. mauritianus* South Africa**
All Loci						
Mean A_0	7 ± 4.36	5 ± 3.46	2.67 ± 0.58	7.67 ± 4.73	10.25 ± 2.06	6.75 ± 1.23
Mean H_0	0.38 ± 0.18	0.15 ± 0.14	0.95 ± 0.05	0.50 ± 0.29	0.74 ± 0.19	0.56 ± 0.14
PaGT4						
n	150	125	21	57	58	43
A_0	9	3	3	6	7	7
Dominant phenotypes	274 (0.12) 276 (0.43) 274/276 (0.30)	266 (0.73) 274 (0.16)	274/276/280 (0.86)	274 (.40) 276 (0.11) 274/276 (0.19)	272/274/276 (0.26) 274/276 (0.26)	270 (0.35) 270/278 (0.18)
H_0	0.40	0.10	0.90	0.33	0.88	0.42
PaGT8						
n	131	92	22	57	56	42
A_0	2	3	2	4	7	7
Dominant phenotypes	176 (0.76) 176/178 (0.19)	178 (0.71) 180 (0.14)	176/189 (0.95)	176 (0.49) 178 (0.18) 176/178 (0.25)	173/175/180 (0.13) 175/180/182 (0.2)	177/180 (0.19) 177/184 (0.19)
H_0	0.19	0.04	0.95	0.33	0.80	0.72
PaGT9						
n	121	109	21	45	57	44
A_0	10	9	3	13	13	5

Dominant phenotypes	198 (0.34) 198/202 (0.24)	210 (0.62) 210/212 (0.20)	192/196 (0.95)	198/204 (0.09) 198/206 (0.11)	192/200/231 (0.11) 200/202/215 (0.1)	200 (0.34) 196/200 (0.16)
H_0	0.55	0.31	1	0.83	0.95	0.52
PaGT22						
n	95	25	7	36	62	35
A_0	7	7	3	11	9	8
Dominant phenotypes	181 (0.20) 197 (0.13) 181/193 (0.21) 181/197 (0.18)	185 (0.24) 191 (0.40) 193 (0.12)	175/183/197 (1.00)	183 (0.08) 195 (0.14) 199 (0.08) 195/199 (0.08)	183 (0.52) 183/202 (0.24)	183 (0.27) 188 (0.12) 175/183 (0.18)
H_0	0.58	0.12	1	0.58	0.46	0.53

The software programme, STRUCTURE, clustered *P. australis* and *P. mauritianus* into two clear groups ($k = 2$) (Figure 2-6 and Figure 2-7) based on their microsatellite phenotypes and thus was able to differentiate the two reed species. One sample, 49P (population 5; Pilanesberg, North-West Province) stood out as a hybrid as also detected by the PCoA. This sample had a signature of shared ancestry, with 96.8 % membership in the *P. mauritianus* population and only 3.2 % membership in the *P. australis* population. This shows that there has been almost complete introgression of *P. mauritianus* alleles into this *P. australis* genotype.

However, there were a number of samples both within the *P. australis* and *P. mauritianus* clusters that had a significant proportion of shared alleles with the other species. These samples are also potential hybrids however not all the alleles have been replaced yet. For *P. australis,* sample 66P (population 13; Bushman's River, Eastern Cape) has a signature of shared ancestry, with 51.8 % membership in the *P. mauritianus* group and only 48.2 % membership in the *P. australis* group. For *P. mauritianus,* samples 2M, 8M, 9M (population 41; Mbabane, Swaziland) have a mean population signature of shared ancestry, with 67.2 % membership in the *P. australis* group and only 32.8 % membership in the *P. mauritianus* group. These samples also stood out in the PCoA and clustered half way between the *P. australis* and *P. mauritianus* groups. All potential hybrids were amplified twice to ensure reproducibility of the results. All peaks remained the same and thus the microsatellite phenotypes were taken to be correct.

The separate STRUCTURE analysis of each reed species found that *P. australis* samples cluster into two groups (Figure 2-8 and Figure 2-9) and *P. mauritianus* samples cluster into three groups (Figure 2-10 and Figure 2-11). For *P. australis,* the two populations were found to correspond with groupings found in the PCoA (Figure 2-4). Almost all samples that grouped with population one were found along the coast of South Africa except for one sample (79P, from the Free State Province). The remaining samples are found across the range of *P. australis* distribution in South Africa. A number of samples from this second group, as well as sample 79P were found to have an admixed ancestry from the two populations (Appendix-Table 0-4). For *P. mauritianus,* STRUCTURE analysis determined that there are three populations in South Africa (Figure 2-10), agreeing with the PCoA analysis there was no geographical pattern among these populations (Appendix - Table 0-5).

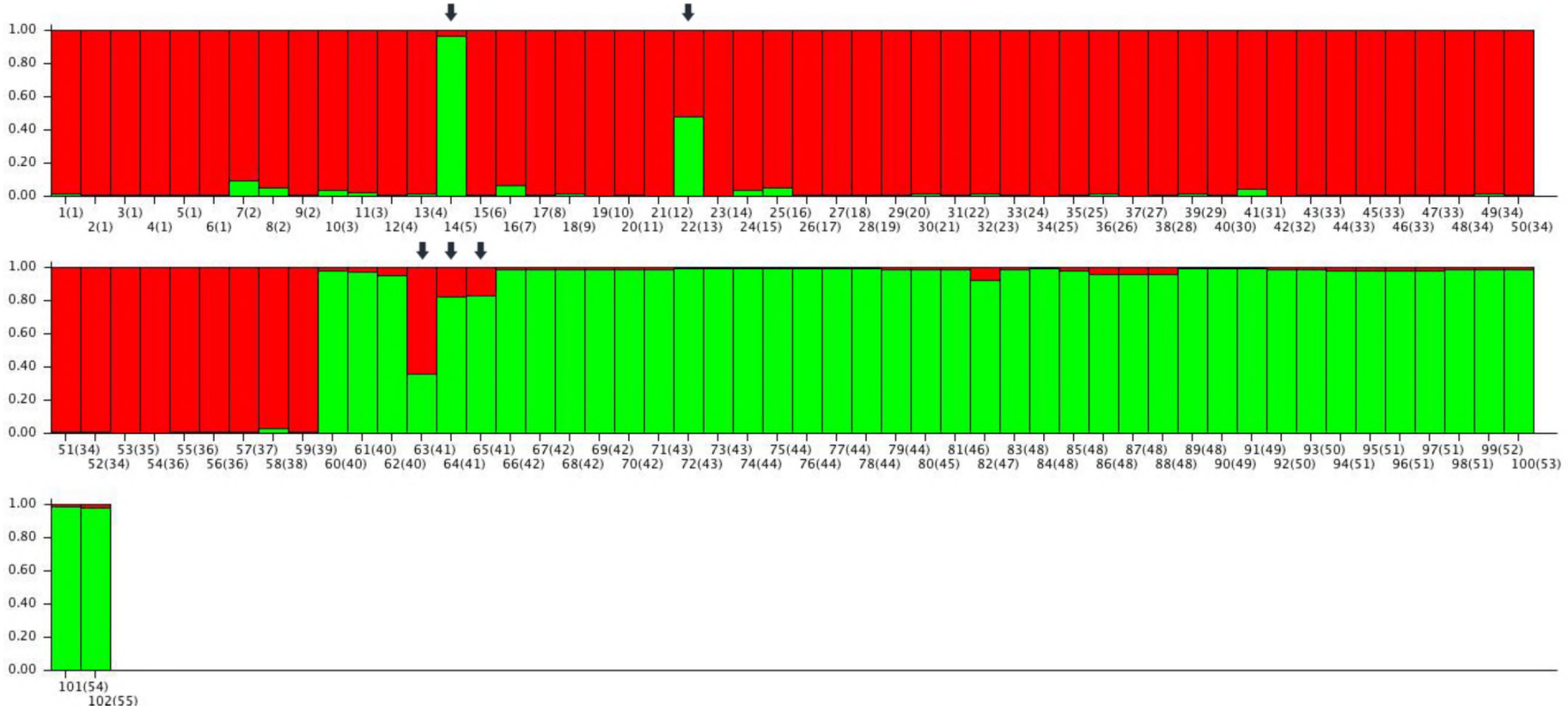


Figure 2-6 Genetic population structure of 102 individuals of *Phragmites australis* and *Phragmites mauritianus* from 55 populations in South Africa, based on Bayesian clustering analysis of 4 microsatellite loci with STRUCTURE (Pritchard et al. 2000). According to the Evanno method, two populations were inferred (Figure 2-7). Based on cpDNA matrilineage, the red cluster corresponds to *P. australis* individuals, whereas the green cluster represents *P. mauritianus*. The values in ordinate the shared ancestry according to percentage membership into *P. australis* or *P. mauritianus* populations. The downward arrows point to the potential hybrids.

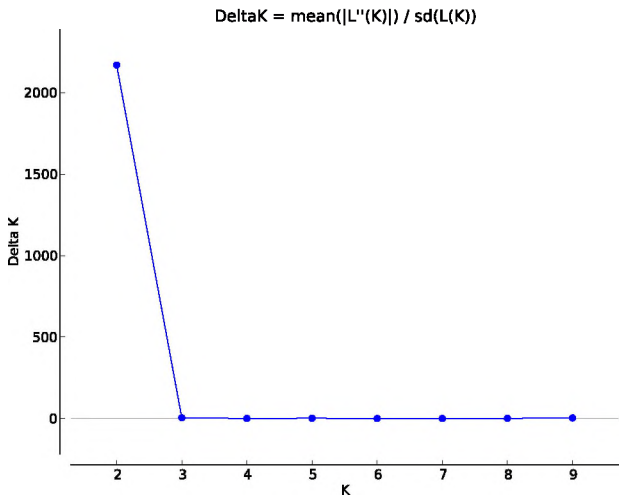


Figure 2-7 Graph of Delta K values showing the ideal number of populations as $k = 2$ based on 55 populations of *P. australis* and *P. mauritianus* in South Africa, using 4 microsatellite primer pairs and the Evanno method implemented in STRUCTURE HARVESTER program according to Earl and von Holdt (2012).

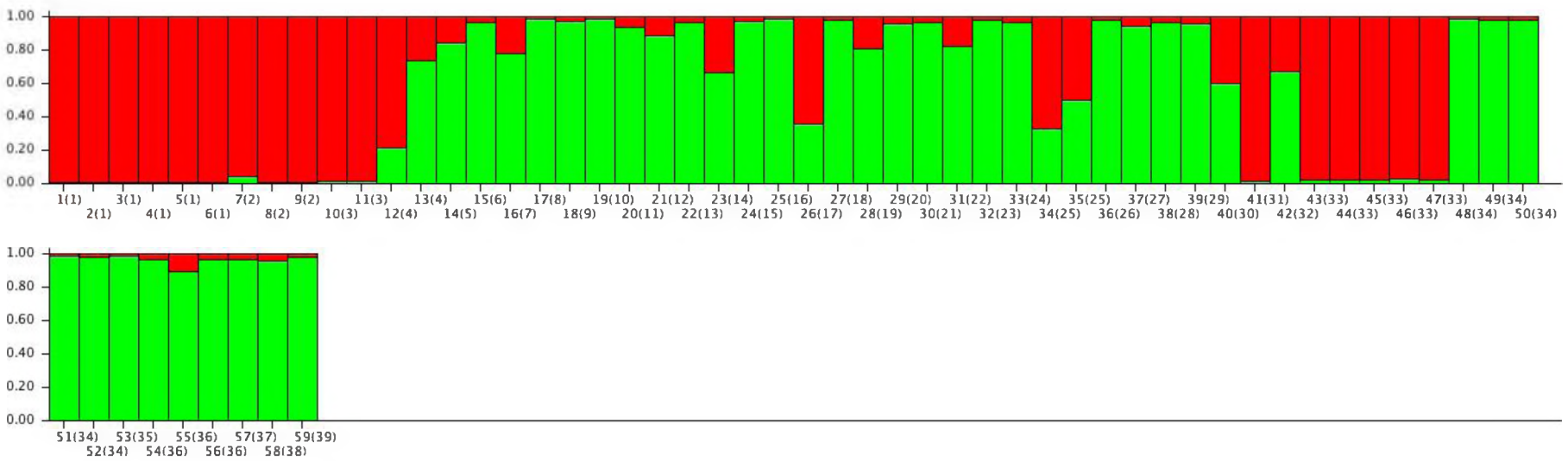


Figure 2-8 Genetic population structure of 59 individuals of *Phragmites australis* from 39 populations in South Africa, based on Bayesian clustering analysis of four microsatellite loci with STRUCTURE (Pritchard et al. 2000). According to the Evanno method, two populations were inferred (Figure 2-9) (in green and red). Based on cpDNA matrilineage all samples correspond with *P. australis* and are haplotype K, however according to their microsatellite phenotypes, they cluster into two distinct populations ($k = 2$), shared ancestry is indicated in the ordinate.

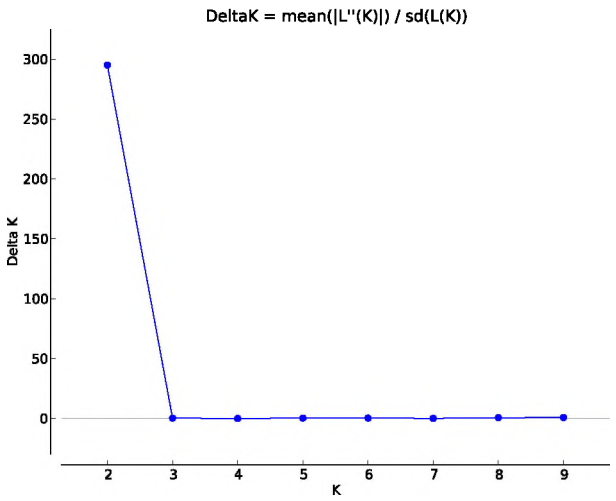


Figure 2-9 Graph of Delta K values showing the ideal number of populations as $k = 2$ based on 39 populations of *P. australis* in South Africa, using four microsatellite primer pairs and the Evanno method implemented in STRUCTURE HARVESTER program according to Earl and von Holdt (2012).

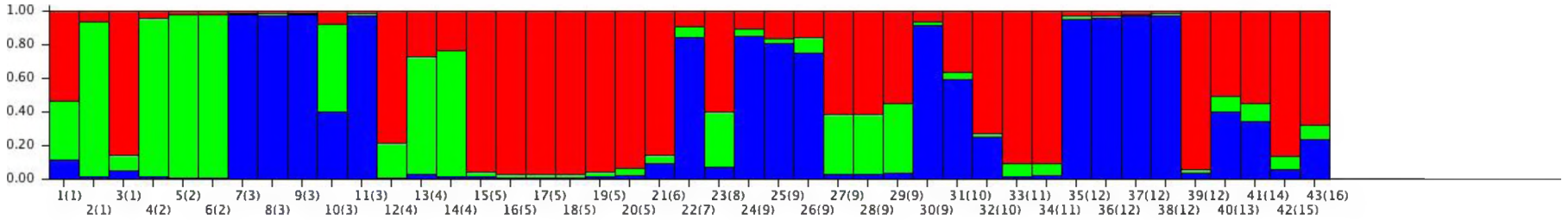


Figure 2-10 Genetic population structure of 44 individuals of *Phragmites mauritianus* from 16 populations in South Africa, based on Bayesian clustering analysis of four microsatellite loci with STRUCTURE (Pritchard et al. 2000). According to the Evanno method, three populations were inferred (Figure 2-11). Based on cpDNA matrilineage all samples correspond with *P. mauritianus* and are haplotype V, however according to their microsatellite phenotypes cluster into three distinct populations ($k = 3$) indicated by red, blue, and green clusters. The values in ordinate, indicate shared ancestry according to percentage membership in each population.

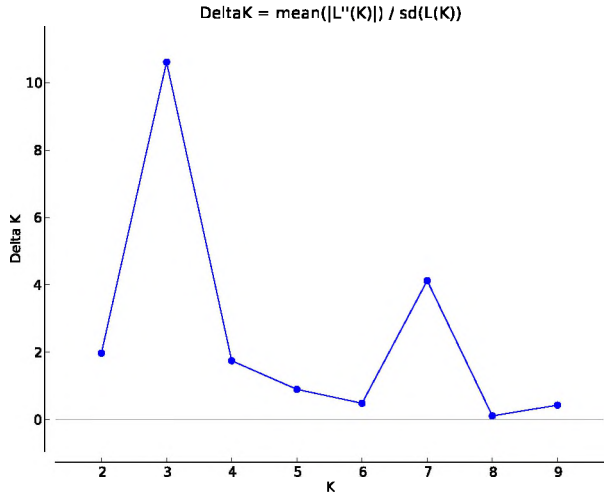


Figure 2-11 Graph of Delta K values showing the ideal number of populations as $k = 3$ based on 16 populations of *P. mauritianus* in South Africa, using four microsatellite primer pairs and the Evanno method implemented in STRUCTURE HARVESTER program according to Earl and von Holdt (2012).

2.1.8 Grass-waxy analysis

A nuclear DNA band of about 200 bp was amplified in most of the *P. australis* samples (Table 2-9). This band has previously been found to be exclusive to the Mediterranean genotypes and was used as a marker to trace genotypes of this region (Lambertini et al. 2012c). Not all *P. australis* samples were found to have this band present (38P, 49-51P, 55-57P, 62P, 70P, 71P). The shorter band of about 100 bp, previously recorded to be exclusive to *P. mauritianus, P. frutescens* and associated hybrids with *P. australis* (Lambertini et al. 2012c) was also found in a number of *P. australis* samples and in some cases was the only band present (Table 2-9). One population of *P. australis* (samples 37P and 38P) had different *waxy* genes despite having being sampled from the same reed stand. For the *P. mauritianus* samples, all reeds were found to have the 100 bp band and none amplified the 200 bp band (Table 2-10).

The DNA sequence of the 200 bp fragment was the same sequence found in the introduced *P. australis* Delta-type in the Gulf Coast of North America, North African, Mediterranean and in one Senegal sample (NCBI accession no. JF317300) (Lambertini et al. 2012b). The sequence of the 100 bp fragment aligned with *P. mauritianus,* and its hybrid population in Senegal, South America, and the Land-type in the Gulf Coast of North America (NCBI accession no. JF317301) (Lambertini et al. 2012b). However, it varied by one base pair at the end of the sequence. This substitution can be used to distinguish South African populations from these global samples.

Table 2-9 List of *P. australis* samples and the presence or absence of the waxy bands, Waxy100 and Waxy200. (+/-) presence/absence. Samples not included from Table 2-1, did not have clear amplification of a *waxy* band.

Samples	Location	Waxy 100	Waxy 200
1,2,3,7,45,46,47	Port St. Johns, Eastern Cape	+	+
9,41,42	Kosi Bay, KwaZulu-Natal	+	+
44	St. Lucia, KwaZulu-Natal	+	+
37	Bird Park, KwaZulu-Natal	+	+
38	Bird Park, KwaZulu-Natal	+	–
49,50,51	Pilanesberg, North-West	+	–

52,53,54	Golden Gate, Free State	–	+
55,56,57	Rustler's Valley, Free State	+	–
58,59	Hartebeespoort Dam, Gauteng	–	+
62	Knysna, Western Cape	+	–
63	Kars River, Western Cape	+	+
64	Rocherpan Nature Reserve, Western Cape	+	+
65	Cape Point, Western Cape	–	+
69	Bot River, Western Cape	+	+
70	Buffels River, Western Cape	+	–
71	Somerset West, Western Cape	+	–
74	Marquard, Free State	–	+
75	Baviaans River, Western Cape	–	+
76	Ladybrand, Free State	+	+
79	Clocolan, Free State	–	+
81	Alexandria, Eastern Cape	–	+
84	Eland's Bay, Western Cape	–	+
87,88,89,90,91	Umgeni River, Kwazulu-Natal	–	+
98	Hout Bay, Western Cape	+	+
104	Jonkershoek Nature Reserve, Western Cape	–	+
105	Spier, Stellenbosch	–	+

Table 2-10 List of *P. mauritianus* samples and the presence or absence of the waxy bands, Waxy100 and Waxy200. (+/-) presence/absence. Samples not included from Table 2-2, did not have clear amplification of a *waxy* band.

Samples	Location	Waxy 100	Waxy 200
1,7	Little Gowrie, Mpumalanga	+	–
2,8,9	Mbabane, Swaziland	+	–
3,10,11,12,14	Umhlanga, KwaZulu-Natal	+	–
4,5,13	Sozisa, Swaziland	+	–
15,16,17,18,19,31	Shakaskraal, KwaZulu-Natal	+	–
20	Zimbali, KwaZulu-Natal	+	–
22	Nelspruit, Mpumalanaga	+	–
23,33,34,35,36,37,38	Nelspruit (Scrap yard), Mpumalanga	+	–
24,39	Hazyview, Mpumalanga	+	–
25,40	Hazyview (Sabie Sun), Mpumalanga	+	–
26,27,28,29,30,41	Kruger National Park, Mpumalanga	+	–
32	Crocodile River, Nelspruit, Mpumalanga	+	–
42	Ngalwana Village, Zambezi	+	–
43	Barotse Floodplains	+	–
44	Lungwebungu Mouth	+	–

2.4 Discussion

The origin and status of *Phragmites* spp. in South Africa was investigated using chloroplast DNA, *grass-waxy* and microsatellite analysis. Chloroplast DNA was used as a first step to identify and classify *Phragmites* lineage according to Saltonstall (2002). For both

Phragmites species, only one haplotype each was found across South Africa; *P. australis* populations were determined to be Haplotype K and *P. mauritianus* were determined to be Haplotype V. Haplotype K is presently considered an African haplotype unique to *P. australis*, due to wide-ranging populations extending throughout South Africa, Namibia and Botswana (Lambertini et al. 2012c, Saltonstall 2002). Similarly, Haplotype V is also considered an African haplotype and is only found in *P. mauritianus* populations. Such low haplotype diversity for both *Phragmites* species was surprising considering high diversity in other geographic regions (Saltonstall 2002; Lambertini et al. 2012c), high diversity in microsatellite markers found in this study and a long period of establishment in Africa (Scott 1982). It is therefore important to assess the origin of haplotypes and their global distribution to determine if such low haplotype diversity has been a natural process or human-mediated.

For *P. australis* the occurrence of Haplotype K despite being labelled an African haplotype (Saltonstall 2002), has global reaches. Phylogeographic studies have determined that the haplotype is not restricted to the African continent and has been found in Europe and more specifically in Spain (Lambertini et al. 2012c). Furthermore, a closely related haplotype, haplotype AI, is also found in Romania and only differs by one substitution in the *rbcL-psaI* chloroplast region and is also an octoploid like South African populations (Lambertini et al. 2012c). Looking at the diversity of chloroplast haplotypes, Haplotype K has been found to cluster with European haplotypes (Lambertini et al. 2012c) and is most closely related to the European Haplotype M (Saltonstall 2002). Such close relationships between the two lineages has opened debate to the origin of Haplotype K and the direction of dispersal to or from Europe. South Africa could be the native range of haplotype K and the Mediterranean lineage carrying the 200 bp *waxy* band like many of the South African samples could have a larger native range including South Africa as suggested by Guo et al. (2013). Alternatively it is possible that haplotype K originated in Europe and upon introduction to South Africa has differentiated; this theory is supported by the fact that European K-related genotypes have different nuclear alleles from South African populations but not from the European gene pool (Lambertini et al. 2012c).

Another relationship with *P. australis* in the Mediterranean was revealed by the *waxy-gene* 200 bp band which was amplified in both populations in South Africa and in the Mediterranean/North Africa. Lambertini et al. (2012c) found that populations of *P. australis* in South Africa are closely related to the populations in the African/Mediterranean region based on six nuclear microsatellites and AFLPs. Guo et al. (2013) studied the invasion of the Mediterranean lineage in the Gulf Coast of North America and considered South Africa is within the native range of the Africa/ Mediterranean gene pool (Figure 2-12). The present study

confirms that South African *P. australis* populations are related to the Mediterranean lineage, however the direction of dispersal and at what time scale is still not possible to ascertain. Based on this present data, it is unlikely that the two populations represent different origins in South Africa.

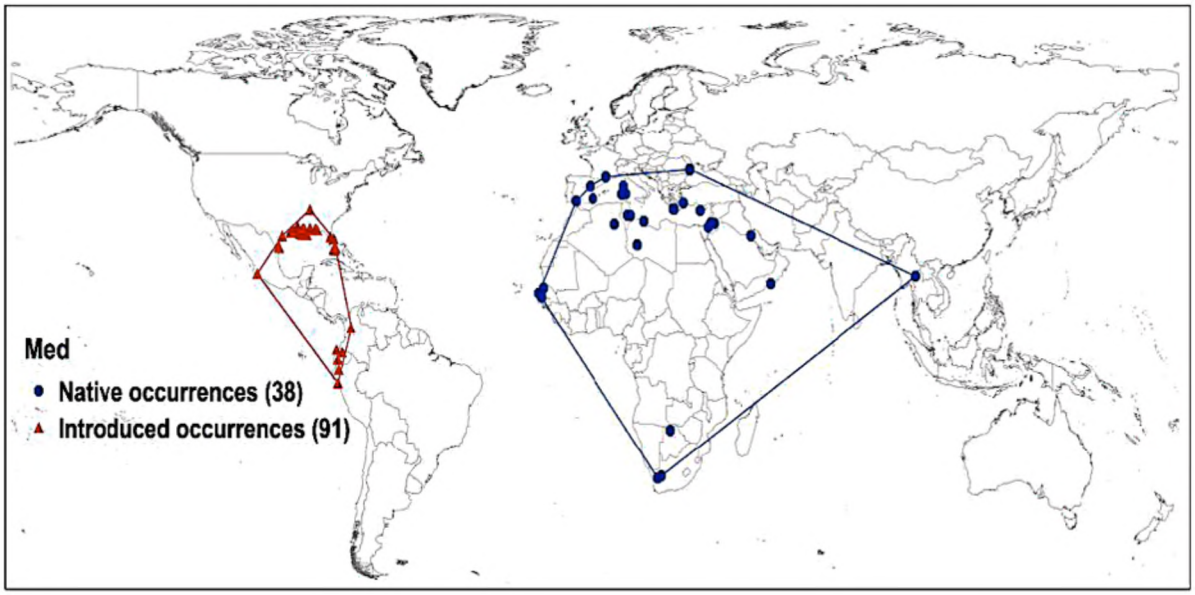


Figure 2-12 Distribution of the occurrence records from Guo et al. (2013) of the Mediterranean invasive *P. australis* genotype – Med. The red triangles indicate records of the introduced range and the blue circles are the records in the native region.

The presence of a South African *P. australis* population that is closely linked to the European/Mediterranean lineage is an important finding in highlighting the evolutionary history of South African *P. australis* and its phylogeographic relationships. It is now clear that dispersal can occur at continental scales through the movement of *Phragmites* anemochorous seeds that can travel long distances before establishing (Lambertini et al. 2012b, Lambertini et al. 2012c). For example, in the Danube Delta, Romania where related haplotypes are found; of the 350 bird species that occur there, 300 are migratory with some being found to reach as far as South Africa (Stiuca 2008). These birds are likely distributing *P. australis* seeds across the two continents.

To gain further insight into the status of *P. australis* in South Africa, the genetic diversity of populations was analysed with highly variable markers like the microsatellites. Populations from native regions are expected to have higher genetic diversity than introduced populations derived from them, which would be expected to be from a limited number of

founders (Nei et al. 1975; Barret and Kohn 1991; Dlugosch and Parker 2008; Lambertini et al. 2012b). Despite *P. australis* having low haplotype diversity in South Africa, overall stands were found to have higher genetic diversity compared to each worldwide lineage from Lambertini et al. (2012a). *Phragmites australis* had slightly lower diversity compared to *P. mauritianus* however this was not significant. These results suggest that haplotype K populations in South Africa may be native. For example, studies in the Gulf Coast of North America determined lower genetic levels compared to native European and African/Mediterranean populations; it was concluded that these populations have been introduced from Europe (Lambertini et al. 2012b).

For *P. australis,* two distinct ancestral populations were identified by STRUCTURE and such distinct populations appeared independent from the presence or absence of the *waxy-gene* 200 bp band, marking a potential introduction from the Mediterranean region, as well as from the occurrence of hybrids with *P. mauritianus*, rejecting the hypothesis of two independent evolutionary histories as could be expected in the case of a native and an introduced population, or of two independent introductions.

For these reasons, the two populations in South Africa are most likely a result of habitat differentiation rather than different phylogeographic origins. Population 1 (Figure 2-8) contained eight populations across South Africa; seven of these populations were found along the coast with six being found in the KwaZulu-Natal Province. Only one population that contained a mix of alleles from both populations was found inland in the Free State (79P, Clocolan, Free State). Population 2 on the other hand was found across the range of *P. australis* in South Africa. Although, population 1 occurs mostly in contact areas between *P. australis* and *P. mauritianus* there is no evidence that this is what differentiates this population. The one sample (49P, Pilanesberg, North-West) that was determined to be a hybrid with *P. mauritianus* was found to cluster instead with population 2. Therefore, the differentiation of population 1 could be a result of these reeds being adapted to a coastal niche and could reflect adaptation to ecological heterogeneity or different ecotypes (Turesson 1922; Wu et al. 2008). *Phragmites australis* is known to have halophytic and glycophytic populations (Waisel Y 1972; Takahashi et al. 2007; Gao et al. 2012). For example, in the Yellow River Delta, China, *P. australis* was found to have population structuring in microsatellite alleles that is believed to be a result of varying soil salinity (Gao et al. 2012). In such cases, *P. australis* adapts to different saline conditions by adjusting its plant physiology which results in genetic differentiation over time (Takahashi et al. 2007). This has also been seen in other riparian grasses, for example, creeping bentgrass, *Agrostis stolonifera* L. (Poaceae) in the Netherlands was found to have differences

in plant physiology and isozyme phenotypes between coastal and inland populations (Kik et al. 1990).

For *P. mauritianus,* this was the first study of haplotype diversity and population structure in South Africa. Similarly, to *P. australis,* low haplotype diversity was found with haplotype V being found across all populations in South Africa. Haplotype V is unique to *P. mauritianus* and therefore restricted to the African continent (Saltonstall 2003a). A southern African origin was further confirmed by Lambertini et al. (2012b) who found that haplotype V clusters with southern African haplotypes R, AP and AO (Appendix- Table 0-1). Haplotype V, R, AP and AO are distributed across *P. mauritianus* range in Africa, extending from the northern limit of Ethiopia and the Congo Republic down to South Africa (Gordon Gray and Ward 1971). The wide-range of haplotype V was confirmed in this study with the inclusion of samples from west Zambia where a population of haplotype V was found, in addition to those in South Africa and Equatorial Guinea previously identified (Lambertini et al. 2012c). In these areas in Zambia, the closely related haplotype AP (Lambertini et al. 2012c) was also found in nearby reed stands. Haplotype AP was previously found to share a diagnostic mutation in the *trnT-trnL* region of native North American haplotypes of *P. australis* ssp. *americanus,* despite its closest relatives are haplotypes P and AN, in East Asia and Australia (Lambertini 2012b; Lambertini 2016). Similarly, *P. mauritianus* populations from Uganda and Burkino Faso share the Haplotype I with *P. australis* populations known as the *Phragmites* Land-type in the Gulf Coast of North America (Lambertini et al. 2012b). These relationships show evidence of dispersal events between the African and American continents, however further research is needed to determine the direction of flow and at what time scale.

Microsatellite analysis was able to determine that *P. mauritianus* stands in South Africa, like *P. australis,* have high genetic diversity compared to worldwide populations from Lambertini et al. (2012a). High genetic diversity, haplotypic variation and an almost exclusive African distribution of the *P. mauritianus* haplotypes identified in this study suggest that South Africa is within the native range of *P. mauritianus*. Compared to *P. australis* overall *P. mauritianus* can be said to have higher diversity in populations as, although not significantly, both the Shannon Diversity Index (I) and Nei's Diversity (h) were higher. High diversity is likely to lead to a number of advantages for *P. mauritianus* populations in South Africa such as the potential for increased plant productivity and adaptability (Tomimatsu et al. 2014). Genotypic polycultures with higher diversity are more likely to contain productive genotypes that dominate communities (Loreau and Hector 2001).

The PCoA identified three ancestral populations for the sampled *P. mauritianus*

genotypes found across the range in South Africa and like with *P. australis* the genetic structure was independent from each other because of hybridization with *P. australis* or multiple phylogeographic origins. In South Africa, all three populations were found to share the same chloroplast DNA being haplotype V. Both within and among these three populations no clear geographical patterns were observed and genotypes of the three different populations could be found within the same site. Lastly, the hybrids with *P. australis* (2M,8M,9M, Mbabane, Swaziland) were found within population 3, a population including samples from Mpumalanga to KwaZulu-Natal (Appendix -Table 0-5). This suggests a high level of gene flow both within and between *P. mauritianus* sites, as well as between the populations of *P. mauritianus* and *P. australis.*

Evidence of gene flow between *P. australis* and *P. mauritianus* populations in South Africa is further supported by the *grass-waxy* gene bands. A 100 bp band that has previously been found to be unique to *P. mauritianus* populations (Lambertini et al. 2012b, 2012c) was also carried by a number of *P. australis* samples in South Africa. Interestingly, the occurrence of the 100 bp band did not affect the structure of *P. australis* populations and was not only found in the *P. australis* populations in contact zones between the two species. It is therefore likely that the high frequency of this band may highlight ancient exchanges of genetic material between the two species that have been conserved. This provides empirical evidence that hybridisation has taken place multiple times in the evolution of the genus *Phragmites* as it was previously hypothesized (Chu et al. 2011, Lambertini et al. 2012c, Lambertini et al. 2012b, Meyerson et al. 2012). Within the *P. mauritianus* populations there was no occurrence of the 200 bp band unique to *P. australis;* this may indicate that *P. australis* populations are more susceptible to the assimilation of *P. mauritianus* genetic material. As suggested by Kettenring et al. (2011) for the spread of *P. australis* haplotype M in North America, this may be a result of the fact that *P. mauritianus* is more likely to have viable pollen and seeds due to higher patch-level genetic diversity than *P. australis.* Such patch diversity increases the likelihood to contribute to genetic variation to the local pollen pool (Wu et al. 2015).

The hybrids identified in this study by the microsatellite markers were only in contact zones between *P. australis* and *P. mauritianus,* and are likely recent hybridization events. For *P. australis,* sample 49P (Pilanesberg, North West province) was found to have almost total introgression of alleles from *P. mauritianus* and sample 66P (Bushman's River, Eastern Cape) had about 50 % introgression of *P. mauritiaus* alleles. The hybrid *P. mauritianus* population from Mbabane, Swaziland (2M, 8M, 9M) had a mean membership of 67.2 % introgression of *P. australis.*

Phragmites australis and *P. mauritianus* have remained distinct species in South Africa despite having an overlapping distribution and the ability to hybridise; one explanation to the species remaining distinct is that they have different habitat requirements. Gordon Gray and Ward (1971) observed in KwaZulu-Natal, South Africa that *P. mauritianus* prefers areas with permanent, moving water, especially in areas with recent disturbance whereas *P. australis* is mostly found in areas with restricted drainage that are usually isolated from the main stream (Beckley 1984). These differences, may be either related to different physiological paths in the two species, or may be a result of the different ploidy level between the two species in South Africa where *P. australis* is octoploid (Clevering and Lissner 1999) and *P. mauritianus* is tetraploid (Gordon Gray and Ward 1971). Octoploids generally have increased plant biomass and tetraploids are generally found to be more opportunistic with high plasticity (te Beest et al. 2011).

The use of molecular markers on *Phragmites* spp. in South Africa also gave an understanding of the reed's ecology in terms of its reproductive strategy and seed dispersal. Unlike what was previously believed, it is generally sexual reproduction and thus viable seeds that help *Phragmites* spp. spread and persist (Paul et al. 2011; Kettenring and Mock 2012). This was found to be true for both *P. australis* and *P. mauritianus* because both had polyclonal reed stands. Polyclonal stands are an indication of a relatively young stand that has had numerous seeds germinate or a site with frequent disturbance (Lambertini et al. 2008; Hazelton et al. 2014). *Phragmites australis* generally first colonises a new area by sexual reproduction resulting in a complex spatial distribution where different clones intermingle; initially clonal diversity is high however overtime only a few clones that are dominant or better adapted to the local conditions remain (Koppitz 1999, Keller 2000, Lambertini et al. 2008).

The diversity of a reed stand was also found to play an important role in outcrossing potential as viable seed production is greater when more genotypes are present (Kettenring et al. 2011). *Phragmites mauritianus* was found to have both a higher number of polyclonal populations and genetic diversity compared to *P. australis;* this may indicate a higher production of viable seeds and thus more reliance on sexual reproduction (Hazelton et al. 2014). This will have important implications in sites where the two reeds compete as *P. mauritianus* may therefore be able to more readily encroach on *P. australis.* Evidence for this was found by Gordon Gray and Ward (1971), who observed that *P. mauritianus* in KwaZulu-Natal, South Africa can move into *P. australis* stands, particularly when there has been a disturbance.

In conclusion, the results from this study suggest that both *Phragmites* spp. are native

to South Africa according to their haplotype and genetic diversity. In addressing the expansion of *P. australis* it is therefore likely that reed encroachment may be a result of anthropogenic activities which can alter the ecology of riparian areas by, for example, changing hydrologic regimes, causing eutrophication, and increasing oxygen and salinity in soils (Keller 2000, Lynch, Saltonstall 2002, Hauber et al. 2011). Russell (2003) proposed that reed encroachment in the Wilderness Lakes, Western Cape, South Africa was a result of a multitude of factors including water stabilisation, reduced disturbance by large herbivores and changes to the fire regime. *Phragmites australis* on a global scale has been found to thrive in disturbed habitats, which can allow them to move into previously unoccupied habitats (Kulmatiski *et al.* 2010; Lambert & Casagrande 2006; Meyerson et al. 2010; Wu et al. 2015). Furthermore, the threat of expansion is not only restricted to *P. australis*; anthropogenic factors will also influence the distribution of *P. mauritianus* as the reeds are also known to thrive in areas of degradation with increased sedimentation being a key driver (Van Coller et al. 1997).

A cosmopolitan plant like *Phragmites* spp. must always be addressed in a global context even when dealing with local levels (Lambertini et al. 2012b). For this study, including global phylogeographic information when investigating the two *Phragmites* spp. in South Africa allowed insight into the haplotypes present. The evidence suggests that *P. australis* and *P. mauritianus* stands in South Africa are likely native African haplotypes, can hybridise and vary in their genetic characteristics. The study was also able to highlight that *P. australis* populations are closely related to the *Phragmites* Mediterranean and European lineages and that long-distance dispersal between South Africa and the Mediterranean/Europe has occurred. Further work is now needed to investigate the link between these regions and conclusively address the direction of dispersal; it will be important to include Mediterranean and European samples to compare allelic phenotypes and genetic diversity. Lastly this study suggests that reed expansion recorded in South Africa is not a result of a cryptic invasion of exotic genotypes but instead could possibly be a result of anthropogenic activities.

3. CHAPTER 3: INVESTIGATING THE GENETIC DIVERSITY AND ORIGIN OF *ARUNDO DONAX* POPULATIONS IN SOUTH AFRICA

3.1 Introduction

Alien species are often categorised as being either– archaeophytes (species introduced at the beginning of the Neolithic period, 1500 AD) or neophytes (taxa spread after 1500 AD) (Pyšek et al. 2004). However, labelling a species as such is complex and necessitates looking at the phylogeography of the species. For example, archaeophytes can become neophytes with the introduction of non-native genotypes as has been seen in *P. australis* in North America (chapter 2). For *A. donax,* the species has long been accepted as an archaeophyte in areas such as the Mediterranean region however with improved molecular work it has become apparent that the reed can become a neophyte when there has been recent and multiple introduction to new areas (Hardion et al. 2014b). This has important implications on the genetic diversity of the species and the subsequent invasive potential in the introduced range.

Arundinoideae is one of the most unresolved grass subfamilies, historically known as the dustbin group by taxonomists (Barker et al. 1995; Linder et al. 1997; Hardion et al. 2012a). For *A. donax* this is further complicated by the fact that the reeds are a 'cryptogenic species' and thus its true origin is highly debated as the biogeographic and evolutionary origin of the species is obscured through ancient cultivation (Mariani et al. 2010). In recent years with *A. donax* posing a major invasive threat in many parts of the world, there has been renewed interest in resolving these uncertainties and genetically characterising the plant. From this, two lineages of *A. donax* have been identified based on genetic analyses - Europe and Asian/Middle East populations (Mariani et al. 2010). In Asian populations, *A. donax* was found to have viable seeds and thus there is a relatively high degree of genetic variation (Hardion et al. 2012a). In Europe however, *A. donax* stands are sterile and thus there is lower genetic diversity compared to Asian populations (Lewandowski et al. 2003). It is believed that these populations reflect a genetic subset of populations from Asia and thus represent a genetic bottleneck or founding event (Ahmad et al. 2008, Hardion et al. 2012). This most likely occurred when particular genotypes were selected and spread so that overtime a single clone was being cultivated worldwide (Ahmad et al. 2008).

To investigate the proposed theory that *A. donax* populations have radiated out from Asia and represent a single clonal lineage, a number of studies were conducted to investigate *A. donax* haplotypes and genetic diversity. Khudamrongsawat et al. (2009), using RAPD and isozyme analysis, found moderate levels of genetic diversity in invasive stands of *A. donax* in

California, U.S.A. In contradiction to these findings, other studies found almost no variation (Ahmad et al. 2008; Hardion et al. 2012a). SRAP and transposable element (TE)-based molecular markers found that populations in North America were identical to the European lineage of *A. donax* (Ahmad et al. 2008) and Hardion et al. (2012) found that *A. donax* populations in the Mediterranean have low genetic variation using AFLPs. Lastly, Tarin et al. (2013) determined only one clonal lineage in North America but found evidence of differentiation within this clone based on microsatellite markers that may reflect multiple introductions. On the whole, evidence supports the theory that there has been a human-mediated movement of selected genotypes, which has resulted in low genetic diversity and most likely a single clone being found in most areas worldwide. Any variation found is likely a reflection of areas where there have been multiple introductions.

Differences in the genetic diversity of *A. donax* across studies can largely be attributed to the molecular techniques that were used. The choice of genetic markers is thus critical in accurately determining plant origin, spread and period of establishment. Due to the genealogical nature of genetic material, neutral genetic markers should be used to uncover movement patterns of organisms (Barker et al. 1998; Roderick and Navajas 2003; Ahmad et al. 2008). In addition, multi-locus genetic approaches such as microsatellite markers are optimal in determining genetic variation and displaying fine-scale population-level differences (Roderick and Navajas 2003; Ahmad et al. 2008).

The use of molecular-based techniques has proven to be an important tool in managing IAS (Gaskin et al. 2011). In particular, the use of molecular techniques to discern the native range of invasive weeds has aided in efforts to limit the introduction of vectors, help understand the adaptive potential and population structure of invasive species and can thus be used to inform biological control programmes (Roderick 2004; Gaskin et al. 2011). The application of molecular-techniques has shown to have great potential in biological control as there is growing evidence that herbivores can be sensitive to plant genotype (Lambert and Casagrande 2007; Bhattarai 2015; Cronin et al. 2016). For example, a study by Goolsby et al. (2006) used genetics to investigate the role of co-evolution on the invasive Old World climbing fern, *Lygodium microphyllum* (Lygodiaceae) and the phytophagous mite, *Floracarus perrepae* Knihinicki & Boczek (Eriophyidae) in the U.S.A. Herbivore transfer experiments determined that *F. perrepae* were most effective at inducing galls in *L. microphyllum* plant haplotypes from the same native range (Goolsby et al. 2006). The determination of a strong geographical pattern helped guide biological control to optimise host exploitation in the correct region of origin.

To date, no work has been carried out on how southern African *A. donax* populations are genetically structured compared to populations elsewhere. With a biological control programme being proposed, this study will provide baseline data using molecular-techniques on *A. donax* in South Africa with the aim of contributing to the knowledge of the founder history of this invasive species to obtain a better understanding of its reproduction and dispersal mechanisms. In South Africa, *A. donax* is known to have had multiple introductions from the 1700s (Guthrie 2007) and thus would expected to have moderate levels of genetic diversity. To further understand *A. donax* level of genetic diversity, results will be compared to the native and cosmopolitan *P. australis* and endemic *P. mauritianus* (chapter 2). Lastly, the study will help link the herbivore assemblages found (chapter 4) to plant origin.

3.2 Materials and methods

3.2.1 Sampling and DNA extraction

Leaf tissue was collected from the young apical leaves of *A. donax* during the growing season in 2014. Sampling was intended to collect from across the whole distribution of *A. donax* in South Africa (Table 3-1). Each reed stand was considered one population. Within each stand 15 - 30 leaves from different plants were collected. Fresh leaves were dried in silica gel according to the protocol of Chase and Hills (1991). DNA was extracted using the Qiagen DNeasy® Plant Mini Kit. The Qiagen protocol was modified whereby leaf tissue was ground dry in liquid nitrogen before the addition of the extraction buffer.

Table 3-1 Sampling sites for the collection of *A. donax* genetic material.

Sample number	Population Number	Site Location	Province	GPS coordinates
1	1	Nelspruit (1A-MP-1)	Mpumalanga	25°26'03.57''S; 30°52'02.49''E
2	2	Nelspruit – scrap yard (2A-MP-1)	Mpumalanga	25°01'52.73''S; 31°01'25.43''E
3	3	Hazyview (3A-MP-1)	Mpumalanga	25°27'17.05''S; 30°53'07.36''E
4,5,6,7,8	4	Hazyview – garage (4A-MP-1-5)	Mpumalanga	25°03'12.51''S; 31°07'51.18''E
9	5	Kruger National Park (5A-MP-1)	Mpumalanga	24°58'48.04''S; 31°28'53.93''E
10	6	Shakaskraal (1A-KZN-1)	KwaZulu-Natal	29°27'28''S; 31°13'00''E
11,12,13,14,15	7	La Mercy (2A-KZN-1-5)	KwaZulu-Natal	29°38'47''S; 31°07'18''E
16	8	Zimbali (3A-KZN-1)	KwaZulu-Natal	29°33'38''S; 31°10'19''E
17	9	Verulam (4A-KZN-1)	KwaZulu-Natal	29°38'44.74''S; 31°03'54.94''E
18	10	Ballito (5A-KZN-1)	KwaZulu-Natal	29°28'17''S; 31°14'28''E
19,20,21,22,23	11	Hout Bay (1A-WC-1-5)	Western Cape	34°02'05.64''S; 18°21'13.93''E
24	12	Constantia (2A-WC-1)	Western Cape	34°01'24.92''S; 18°26'18.85''E
25	13	Jonkerhoek Farm, Stellenbosch (3A-WC-1)	Western Cape	33°56'41.96''S;18°53'53.21"E
26	14	Spier Farm, Stellenbosch (4A-WC-1)	Western Cape	33°58'30.82''S;18°47'07.84"E
27,28	15	Neethlingshof Estate, Stellenbosch (5A-WC-1)	Western Cape	33°56'53.32''S;18°47'59.17"E
29	16	Rustler's Valley, Ficksburg (1A-FS-1)	Free State	28°43'12.51''S, 28°01'33.79''E
30,31,32	17	Clocolan (2A-FS-1,2 and 3A-FS-1)	Free State	28°51'30.10''S, 27°32'27.38''E
33	18	Marquard (4A-FS-1)	Free State	28°42'11.75''S, 27°26'35.50''E
34,35,36,37,38	19	Marquard (5A-FS-1-5)	Free State	28°39'39.22''S, 27°25'52.26''E
39	20	Fitzroy Street, Grahamstown	Eastern Cape	33°18'1.81''S, 26°31'40.07''E
40	21	Rhodes University, Grahamstown	Eastern Cape	33°18'41.95''S, 26°30'48.10''E

3.2.2 cpDNA

Plastid DNA diversity was screened on three intergenic spacers: *trn*T (UGU) to *trn*L (UAA) (Taberlet et al. 1991), *rbc*L to *psa*I and *trnS*(GCU)-*psbD* (Saltonstall 2001) using the methods of Hardion et al. (2014b) (Table 3-2). PCR set up and sequencing followed the same protocol as carried out on *Phragmites* spp. (refer to chapter 2: section 2.1.2).

Table 3-2 Primer sequences used to obtain *A. donax* haplotypes.

Primer	Sequence	Length of obtained sequence
*trn*T(UGU)"a "-*trn*L(UAA) "b"[1]	5'CATTACAAATGCGATGCTCT 5'TCTACCGATTTCGCCATATC	500 bp
*rbc*L-*psa*I[2]	5'TGTACAAGCTCGTAACGAAGG 5'CTAAGCCTACTAAAGGYACG	1080 bp
*trn*S-*psb*D[2]	5'GCCGCTTTAGTCCACTCAGC 5'CTATGGGGTCACARCCSAGG	850 bp

[1]Taberlet et al. 1991[2]Saltonstall 2001

3.2.3 Microsatellites

Three microsatellite primers developed by Tarin et al. (2013) for *A. donax* stands in the native (Old World) and introduced range (North America) were used (Table 3-3). Polymerase chain reactions (PCRs) contained ten picomoles of forward and reverse primers, 12.5 µl of Promega MasterMix (Madison, WI, USA) (reaction concentration of 1 U of *Taq*, 1.5mM $MgCl_2$ and 0.2 µM dNTPS), 2 µl of Promega Magnesium chloride and 7 µl of template DNA per reaction. Promega nuclease-free water was added to reach a final volume of 25 µl. Amplification was run in one of the following machines: Labnet Multi Gene II or Applied Biosystems 2720 thermal cycler. The PCR cycling protocol was 98 °C for 30 seconds, 30 cycles of 98 °C for 10 seconds, 55-62 °C for 30 seconds, 72 °C for 15 seconds, followed by a final extension at 72°C for 5 minutes. Primers were fluorescently labelled by Applied Biosystems™, South Africa. PCR products were diluted 20x with Promega nuclease free water and sent to Inqaba Biotec™, Johannesburg, South Africa for analysis. Capillary electrophoresis of DNA fragments was done using an ABI 3500® genetic analyser at Inqaba Biotec™.

To determine an error rate for the microsatellite analysis as recommended by Bonin et al. (2004) a subset of samples were duplicated (about 30 %). Lastly, to best avoid subjectivity in scoring of peaks, any peaks that were ambiguous and in particular with stutter peaks, such samples were scored as missing data.

Table 3-3 Primer sequences for the three microsatellite primer pairs used for *A. donax* according to Tarin et al. (2013).

Primer	Dye	Sequence	Size	Annealing temperature (°C)
Ad_B7	6-FAM	B7-F: 5'CTATGCATAAGTTTAGATCTACAACTAG B7-R: 5'GGTTTTGGCGACAATAAGATAGTTC	134-196 bp	55
Ad_A3	VIC	A3-F: 5'CACAGGCGCGTGATTGACC A3-R: 5'CAGATCCGACAGGACGAGATG	102-149 bp	55
Ad_B1	NED	B1-F: 5'CATGGATGCCAACTCCTTCAAC B1-R: 5'GGAAATACTTCATTCTAGTTAGAGATAGA	103-161 bp	55

3.2.4 Data analysis

Chloroplast DNA sequences chromatograms were examined and contiguous sequences were assembled and manually edited in GeneStudio™ ver. 2.2.0.0. (GeneStudio, Inc.). Alignment of sequences was done in MEGA ver. 5.2.2 using ClustalW set to default parameters (Kumar et al. 2012) and included all worldwide haplotypes downloaded from GenBank (Table 3-4).

For microsatellite data, chromatogram alignment was first constructed with Geneious ver. 8.1.7 (Kearse et al. 2012). The chromatogram ladders were all aligned to ensure peaks were registered in the same position. The dataset was entered into a diploid binary matrix (1 = presence, 0 = absence of homologous alleles) and analysed using GenAlEx ver. 6.5 (Peakall and Smouse 2012).

3.3 Results

3.3.1 cpDNA

The analysis of chloroplast sequences for *A. donax* found that all samples from across South Africa were identical to Hardion et al. (2014b) haplotype M1. *Trn*T-*trn*L samples aligning with TL5 (Accession number: KF169820) and *rbc*L-psaI samples aligning with L16 (Accession number: KF169810) and *trnS*-psb*D* samples aligning with accession number: KF169824 (Table 3-4). No variation in the chloroplast sequences was found across all samples.

Of the four biogeographical clusters determined by Hardion et al. (2014b), the South African *A. donax* groups with the Middle East biogeographic cluster (Figure 3-1). Haplotype

M1 is the most common haplotype worldwide and was found in 28 samples in the Mediterranean and Irano-Touranian regions; the haplotype was also found in New Caledonia, Peru and North America (Hardion et al. 2014b). The most closely related haplotypes are M2, M3 and M4, which are found in Afghanistan and Pakistan in the Indus Valley (Hardion et al. 2014b).

Table 3-4 Haplotype codes, combination and GenBank accession numbers for *Arundo donax* (Hardion et al. 2014b).

Haplotype	***psaA*-ORF**	***trnC-rpoB***	***rbcL-psaI***	***trnS-psbD***	***trnT-trnL***
C1	A03	KJ996117	L15	KJ996130	KJ996141
C2	A05	KJ996116	L15	SD5	TL6
C3	A05	KJ996120	L15	SD5	TL6
E1	A03	CB5	L17	KJ996126	KJ996137
E2	A03	CB5	L17	KJ996127	TL7
E3	A03	CB5	L17	KJ996128	TL7
E4	A03	CB5	L17	KJ996131	KJ996142
M1	A02	KF169805	L16	KF169824	TL5
M2	A02	CB4	L16	KJ996133	KJ996144
M3	A02	KJ996119	L16	KJ996134	KJ996145
M4	A02	CB4	L16	KJ996135	TL5
W1	A03	CB6	KJ996123	KJ996128	KJ996139
W2	A02	CB6	L16	KJ996132	KJ996143

Code for GenBank accession number: A02, KF169815; A03, KF169816; A05, KJ996112; CB4, KF996118; CB5, KJ996114; CB6, KJ996115; L15, KJ996124; L16, KF169810; L17, KJ996122; SD5, KJ996129; TL5, KF169820; TL6, KJ996140; TL7, KJ9961

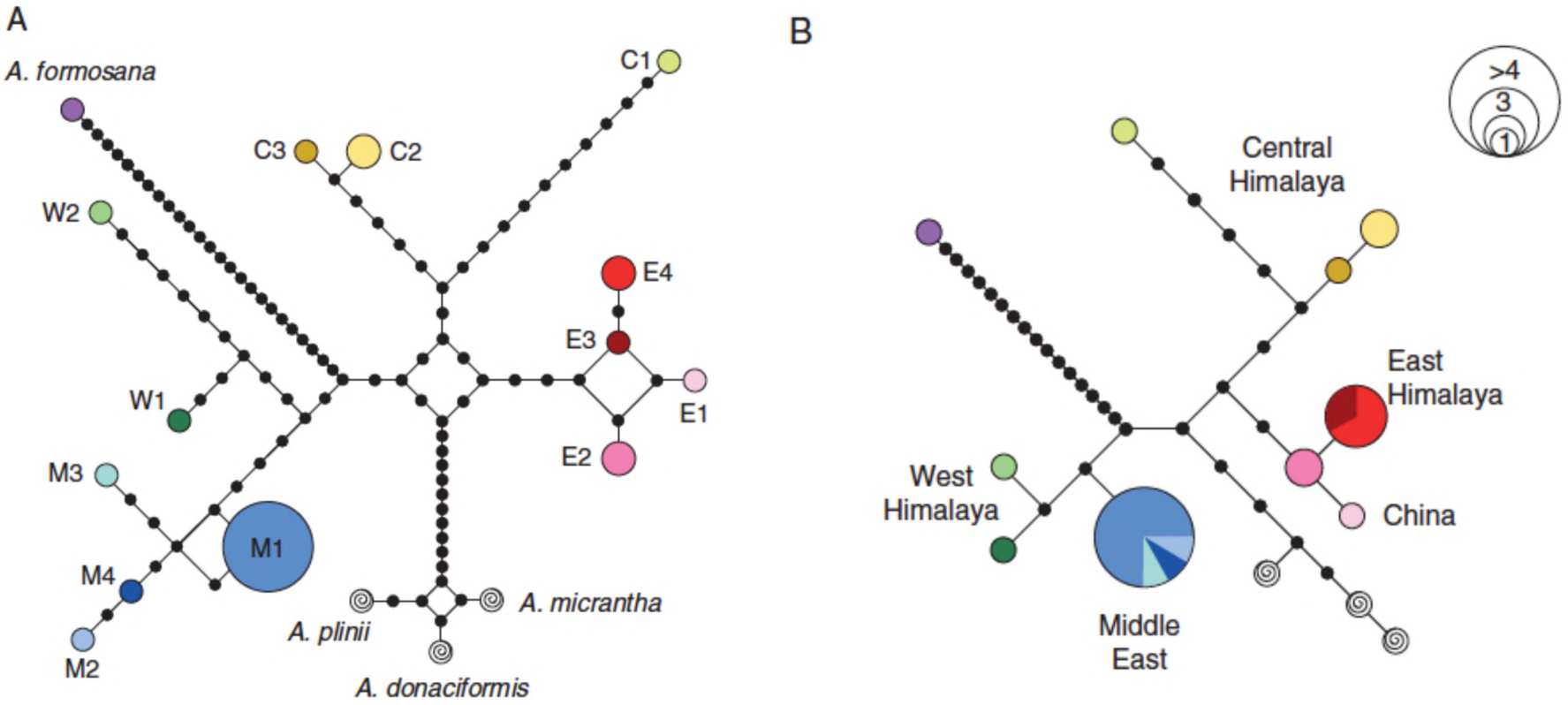


Figure 3-1 A) Combined plastid DNA network for the genus *Arundo* from Hardion et al. (2014b), 13 haplotypes were determined based on substitutions and mini-/microsatellites variation, and B) based on substitutions only, 9 haplotypes are distinguished according to Hardion et al. (2014b). Haplotypes diversity can be divided into four biogeographic clusters which differentiate along the Himalayas/ Middle East (four haplotypes M), Western Himalaya (two haplotypes W), Central Himalaya (three haplotype C) and Eastern Himalaya/China (four haplotypes E). Haplotypes corresponds to (Table 3-4). South African populations were found to be haplotype M1 corresponding to an ancestral lineage in the Himalayas/Middle East.

3.3.2 Microsatellites

All plants sampled across South Africa shared a single multilocus genotype. All populations were found to share the same genotype and in addition all replicated samples were found to have no variation in the peaks amplified. This indicates that all sampled populations can be considered members of the same clone (Tarin et al. 2013). The study found an error rate of zero. The lower error rate compared to *P. australis* and *P. mauritianus* (chapter 2) is likely due to the same ABI genetic analyser being used for all samples for *A. donax*.

Compared to both the Old World (native range) and introduced range (New World), South African samples were found to have a low allelic diversity (Table 3-5). There was no genetic diversity found across all populations of *A. donax* in South Africa. Tarin et al. (2013) also found no genetic diversity within populations in North America and Israel (Table 3-6).

Table 3-5 The genetic diversity of *A. donax* in the Old World (native range) compared to the introduced ranges in North America and South Africa according to Tarin et al. (2013).

Marker	**Number of alleles**		
	Old World	**North America**	**South Africa**
B7	29	16	1
B1	24	13	1
A3	21	18	2

Table 3-6 Comparison of the genetic diversity of *A. donax* New World populations (introduced) and Old World populations (native range). Also, a comparison of clonality within populations (separate genets in separate locations) and how these compare to populations in South Africa from this study (Tarin et al. 2013). Nei's genetic diversity (Div.), evenness (Eve.), Shannon's corrected index (Shc.).

	Size (n)	**No. of genotypes**	**Div.**	**Eve.**	**Shc.**
New World	159	6	0.243	0.220	0.266
Old World	203	129	0.929	0.120	2.218
Within-population genetic diversity					
Israel	6	1	0	1	0
California, U.S.A.	5	1	0	1	0
Texas, U.S.A.	14	1	0	1	0
South Africa	47	1	0	1	0

3.4 Discussion

Arundo donax is believed to represent one of the world's oldest plant invasions (Hardion et al. 2014b). For thousands of years *A. donax* has been a favoured plant for a variety of uses and thus selected genotypes were chosen and distributed worldwide (Goolsby et al. 2009a). Upon introduction to these adventive ranges *A. donax* no longer reproduces sexually and thus relies on vegetative growth (Khudamrongsawat et al. 2009). All of these factors have contributed to a lack of genetic diversity in *A. donax* outside of the Asian/Middle East populations (Flores and Wood 2009; Khudamrongsawat et al. 2009; Saltonstall et al. 2010). The results from this study found similar results to such previous work, with only one haplotype M1 being found across South Africa and populations being found to be genetically uniform.

The Mediterranean region is the source of about 60 % of all naturalised alien grasses in southern Africa (Milton 2004). The success of these Mediterranean plants can be attributed to bioclimatic suitability (Groves and Di Castri 1991) and also due to the fact that with European settlers there was a high volume of introductions of plants brought in multiple times (Milton

2004). *Arundo donax* is one such plant that was taken from populations in the Mediterranean to be used as for erosion control and as a building material in the late 1700s (Perdue 1958; Milton 2004). Support for this Mediterranean origin was found in this study with populations being found to share the most widely distributed haplotype M1 found in the Mediterranean and worldwide (Hardion et al. 2014b).

Records show that *A. donax* was most likely introduced multiple times since the 1700s (Guthrie 2007), however only one microsatellite phenotype was found across all populations in South Africa. Such low genetic diversity is surprising considering the history of the plant. Two theories can be drawn from this 1) a specific clone was selected from the Mediterranean 2) a lack of sexual reproduction may have resulted in a decay in genotypic diversity over time until one genotype drifted to fixation in the populations, which has been found in other clonal plants (Parker 1979; Le Roux et al. 2007). Mariani et al. (2010) found no spatial pattern of genetic variation in the Mediterranean region. Furthermore, studies of haplotype M1 have determined that populations of *A. donax* in North America and France have the same DNA profile (Ahmad et al. 2008). Unlike other plant species the distribution of genotypes is for the most part not a natural process but instead mediated by human activity (Mariani et al. 2010). When the same genotypes are found across large distances this can be interpreted as a recent dispersal through trade for anthropogenic purposes (Mariani et al. 2010). Therefore, in South Africa, the distribution of *A. donax* most likely reflects the human mediated spread of a single clone.

Arundo donax genetic diversity reflects a genetic bottleneck and is thus a good model for studying genetically depauperate species (Ahmad et al. 2008). Genetically depauperate plant species can be defined based on the overall species nuclear diversity H being lower than 0.05; this corresponds to the heterozygosity of a locus whose most frequent allele exceeds 0.97 (Vendramin et al. 2008). Genetically depauperate species present challenges to long established views of genetics and the importance of genetic diversity (Vendramin et al. 2008). Species that can survive and even thrive following severe population bottleneck represent the exception rather than the norm (Godt et al. 1997). There is little doubt that genetic variation is the raw material to allow for adaptation however the link between neutral diversity, quantitative trait variation and adaptability are not always clear (Vendramin et al. 2008). Therefore, determining the genetic diversity of a species does not always provide adequate understanding of its evolution (Vendramin et al. 2008). It is unknown how species with little diversity will adapt to novel environmental conditions (Vendramin et al. 2008). For *A. donax*, such genetic

uniformity is rare considering the cosmopolitan nature of the plant; habitats are more likely to be occupied by differentially adapted clones rather than single clones (Godt et al. 1997). One of the few other examples of this is with the stone pine, *Pinus pinea* L. that has only one Mediterranean-wide haplotype (Vendramin et al. 2008). Like with *A. donax*, the plant's distribution and ability to spread is more a result of a suitable disperser (attributed to human movement for cultivation dating back to 3000 BP) rather than genetic variation (Fallour et al. 1997; Vendramin et al. 2008).

Genetically depauperate invasive alien species raise important questions on the role of genetic diversity in invasive potential. Founding plant populations generally have depauperate genetic diversity and after range expansion can generate low intrapopulational genetic variation (Burdon and Marshall 1981; Le Roux et al. 2007). It is now believed that their success and ability to adapt can largely be attributed to phenotypic plasticity rather than genetic differentiation (Thompson et al. 1991), termed a 'general-purpose-genotype' (GPG) (Van Doninck et al. 2002). Successful clones are believed to possess more broadly adapted (general purpose) genotypes compared to sexual taxa (Baker 1967). When species are introduced to an adventive range they can only survive if plasticity allows for genetic assimilation or post drift allele frequencies to adapt towards an alternative fitness peak (Le Roux et al. 2007).

The 'general-purpose-theory' is likely a good model to explain the expansion of *A. donax* in South Africa given its lack of genetic diversity. *Arundo donax* can tolerate a wide range of environmental conditions including moisture, temperature and salinity (Tracy and DeLoach 1998; Saltonstall et al. 2010) and in addition is one of the fastest growing plants worldwide, having growth rates of up to 5 cm per day under ideal conditions (Lewandowski et al. 2003; Seawright et al. 2009). This high phenotypic plasticity is believed to allow the plant to persist and invade adventive ranges worldwide (Quinn and Holt 2008). There are a number of examples of genetically depauperate invasive alien grasses including the cordgrass, *Spartina angelica* C.E. Hubb. with low genetic diversity in Britain and France (Thompson et al. 1991) and fountaingrass, *Pennisetum setaceum* (Forssk.) Chiov. with each species having only a single genotype it is likely that plasticity is the mechanism allowing these species to become invasive in the introduced range (Thompson et al. 1991; Le Roux et al. 2007).

Determining the genetic diversity and haplotypes present in *A. donax* populations in South Africa has important implications for the future of the biological control program. Firstly, determining the haplotype present in South Africa allowed insight into the ancestral lineage of these populations. The nearest relative of the invasive haplotype M1 is in

Afghanistan and Pakistan along the Indus Valley (haplotypes M2, M3 and M4) (Hardion et al. 2014b). Searching for biological control agents suitable for South Africa should thus focus in on monophagous herbivores in this region. This area should be bioclimatically matched to Mediterranean clone (M1) (Hardion et al. 2014b). Secondly, the genetic diversity should give an indication of how the biological control agents will adapt to the plant. Plants with higher genetic diversity are able to adapt and resist herbivory (Fritz and Simms 1992). Therefore, plants that have low genetic diversity are more likely to suffer from herbivory as they have limited evolutionary potential for adaptability and defence against agents (Muller-Scharer et al. 2004; Tarin et al. 2013).

Neutral markers cannot provide information on adaptive evolution such as fitness-related traits, however they can provide important information on the number of introductions, geographic and genetic origins and pathways of spread of invasive genotypes in the introduced ranges (Ahmad et al. 2008). Using such genetic techniques in this study has determined that *A. donax* in South Africa originates from clones in the Mediterranean region and has no genetic diversity which is most likely a result of introductions from the same source population. The distribution of *A. donax* in South Africa is therefore heavily embedded in the plant's history as a resource for building and crafts (Guthrie 2007). Lastly, identification of *A. donax* haplotype in South Africa will help inform management strategies and guide a biological control programme.

4. CHAPTER 4: TESTING THE ENEMY RELEASE HYPOTHESIS ON TALL-STATURED GRASSES IN SOUTH AFRICA, USING *ARUNDO DONAX, PHRAGMITES AUSTRALIS* AND *PHRAGMITES MAURITIANUS* AS MODELS

4.1 Introduction

The diversity of herbivores associated with plant species in a community is a critical part of understanding their historical biogeography (Futuyma and Agrawal 2009). A regional flora has been assembled over time by invasion from other areas, followed by within-region diversification, and thus the composition of herbivore fauna will likely have a historical explanation (Futuyma and Agrawal 2009). Observing plant origin and herbivory was termed as coevolution by Ehrlich and Raven (1964), which explores the processes behind the interactions that occur when two groups of organisms share a close ecological relationship. Investigations involving coevolution have played an important role in understanding biological invasions that have essentially disrupted these long-term relationships. One theory is the Enemy Release Hypothesis (hereafter referred to as ERH) that outlines the importance of this loss of coevolution when species are moved beyond their native range (Lake and Leishman 2004; Lambert and Casagrande 2006; Mukherjee et al. 2012). The ERH predicts that when species are introduced to an adventive range there is a release from coevolved specialist herbivores and thus introduced plants have a competitive advantage over native plant species (Puliafico et al. 2008; Mukherjee et al. 2012). Alien invasive plants are therefore expected to have simpler herbivore communities with a reduced herbivore load (Frenzel and Brandl 2003; Heleno et al. 2009).

The key assumptions of the ERH are that a) herbivores can regulate plant populations; b) specialist herbivores that are host specific to the plant are not present in the adventive range; c) host-switching of native herbivores is rare; d) native plant species have more pressure from generalist herbivores than do introduced species (Keane and Crawley 2002; Cripps et al. 2006). In a plant's native range there is a balance of selective pressures from specialist and generalist herbivores that shape populations (Orians and Ward 2010). Specialist herbivores are believed to be key regulators of plant growth, survival and reproduction (Bernays 1998; DeWalt et al. 2004). When species are introduced to new areas, this dynamic system is disturbed because specialist herbivores do not occur outside of their host plant's ranges and native herbivores in the adventive range will not switch to new hosts (Orians and Ward 2010). The result is that exotic species escape herbivory and will have reduced herbivore abundance, comprised of mostly polyphagous generalists compared to native species which will have both specialist and

generalist herbivore natural enemies (Frenzel and Brandl 2003; Van Lenteren et al. 2003; Orians and Ward 2010).

Much evidence for the ERH has come from classical biological control studies, which provide good opportunities to investigate the role of herbivores in regulation of plant populations (McClay and Balciunas 2005). Since ERH is a key assumption in biological control, it is valuable to evaluate the role of enemy release in a plant invasion before biological control is initiated. A pre-introductory survey is a method used to assess an invasive plant in its adventive range prior to release of agents (Dudley et al. 2006). Such surveys provide baseline information on a number of important components of an invasive species including establishing a list of herbivores associated with the target weed in the adventive range (Tewksbury et al. 2002). The inclusion of pre-introductory surveys in biological control programmes can also serve as evidence for investigating whether or not the ERH is a factor influencing a plant's invasiveness (Keane and Crawley 2002).

Pre-introductory surveys can also serve as a means of determining if biological control agents are already established in the adventive range (Dudley et al. 2006). In South Africa, pre-introductory surveys have proven to be effective in this regard, with one of the best examples being with the biological control of *Sesbania punicea* Cav. (Papilionaceae) (Hoffmann and Moran 1991). In the early 1980s exploratory surveys for agents in the native distribution of *S. punicea* identified four weevil species that were later imported to quarantine in South Africa (Hoffmann and Moran 1991). While the agents were being investigated, pre-introductory surveys in South Africa discovered that one of the agents, *Trichapion lativentre* Bèguin-Billecocq (Curculionidae) was already established in South Africa from an earlier unintentional introduction (Hoffmann and Moran 1991). With the agent already present a major augmentative biological control programme resulted in *T. lativentre* becoming widespread and abundant across the country in just three years, resulting in considerable control of *S. punicea* (Hoffmann and Moran 1991).

The benefits of doing a pre-introductory survey prior to release of biological control agents have also been highlighted with the *A. donax* biological control programme in North America. Surveys for the herbivores associated with *A. donax* were carried out in California, U.S., to set up an ecological framework for the biological control programme (Dudley et al. 2008). The survey provided pivotal information by recording a number of important species on *A. donax* including potential biological control agents that were already established (Dudley et al. 2008). A biological control programme is being considered in South Africa and to date

no pre-introductory survey has been carried out, there is therefore no record of the herbivore assemblage that is already established on the plant in South Africa.

With most evidence coming as essentially a by-product of biological control investigations there has been criticism that the ERH needs more targeted and direct studies (Keane and Crawley 2002). Results obtained from investigations of herbivores in native and adventive ranges have generally supported the ERH because significant reductions in communities were found in native ranges (Keane and Crawley 2002). However, there has been a lack of studies that compare herbivore assemblages with those associated with native congeneric plant species (Keane and Crawley 2002). The inclusion of native analogous species should ensure that results are less ambiguous and give a more realistic understanding of the role of ERH on a species in a given area. In all cases the validity of the ERH's assumptions vary across species, it is therefore important to do species-specific studies to predict which groups of species are most likely to benefit from a release from natural enemies (Keane and Crawley 2002).

In this study, a pre-introductory survey for *A. donax* was carried out. To investigate the ERH the two native analogous species *P. australis* and *P. mauritianus* were included. The structural, genetic and mechanical attributes of a plant will influence the acceptability of plants to herbivores (Ehrlich and Raven 1964). As such, *Phragmites* species are expected to have a similar herbivore community as they are phylogenetically related (same tribe), ecologically similar, sympatric, C3 perennials, and aquatic species (Tracy and DeLoach 1998; Lambert et al. 2010). Investigating the herbivores on the native *Phragmites* spp. species will also play an important role in establishing baseline data for these tall-statured grasses in South Africa. To date, studies of insects associated with *Phragmites* spp. have largely been restricted to work on the species as wild hosts for important agricultural crops notably maize and sugarcane (Le Rü et al. 2006a). In addition, *P. mauritianus* being an endemic African species will allow insight into how herbivore communities may vary between invasive, cosmopolitan and endemic species.

To establish baseline data for the biological control programme in South Africa, a pre-introductory survey was carried out on *A. donax* including the native reeds *P. australis* and *P. mauritianus*. It is predicted that *A. donax* will have a less diverse herbivore community, devoid of specialists and thus have an escape from herbivory (Frenzel and Brandl 2003; Heleno et al. 2009). The *Phragmites* spp. being determined to be native to South Africa (chapter 2), are expected to have a greater diversity of herbivores with a higher species load of both generalist and specialist herbivores. Furthermore, *P. australis* is a cosmopolitan plant and *P. mauritianus*

is endemic to the African continent, this therefore provides a good opportunity to investigate differences in both period of establishment and geographical range. The results should lend an understanding to the role of herbivory on *A. donax's* invasive potential. The pre-introductory survey will identify natural enemies that may have already become established in the country. This will inform biological control researchers about which potential biological control agents to consider. Lastly, investigating an introduced, cosmopolitan and endemic species will allow links to be made between the species genetic diversity (chapter 2 and 3) and herbivore diversity associated with each plant.

4.2 Materials and methods

The study of the herbivores associated with the reed species was done in three parts 1) a long-term study (hereafter referred to as LTS) in a localised area to determine patterns of herbivore assembly over time 2) a once-off nation-wide survey (hereafter referred to as NWS) across South Africa to record all herbivores associated with the reeds across different biomes 3) an extensive literature review to produce a list of all known herbivores recorded on *A. donax, P. australis* and *P. mauritianus* in sub-Saharan Africa.

Site selection – long-term study

The first part of the study looked exclusively at *A. donax* and *P. australis* over a two-year period from June 2011 until March 2013. *Phragmites mauritianus* does not occur in the region where the LTS was conducted. To examine the herbivores associated with *A. donax* and *P. australis,* monthly insect surveys were carried out on reed stands in the Free State Province, South Africa (Table 4-1 and Figure 4-1). Five study sites were chosen according to where *A. donax* and *P. australis* grow in close proximity. The Free State province is the centre of both reeds' distributions in South Africa. Sites were located in the Eastern Free State, a high-lying part of the province about 50 km from the Maloti Mountains in Lesotho. With high elevation, the area has wet hot summers and dry cold winters, with mean temperatures in summer ranging from 12-27 °C and in winter ranging from -2-17 °C (World Weather Online 2016). Average annual rainfall is highly variable and ranges from 300 mm to over 900 mm (Moeletsi et al. 2011).

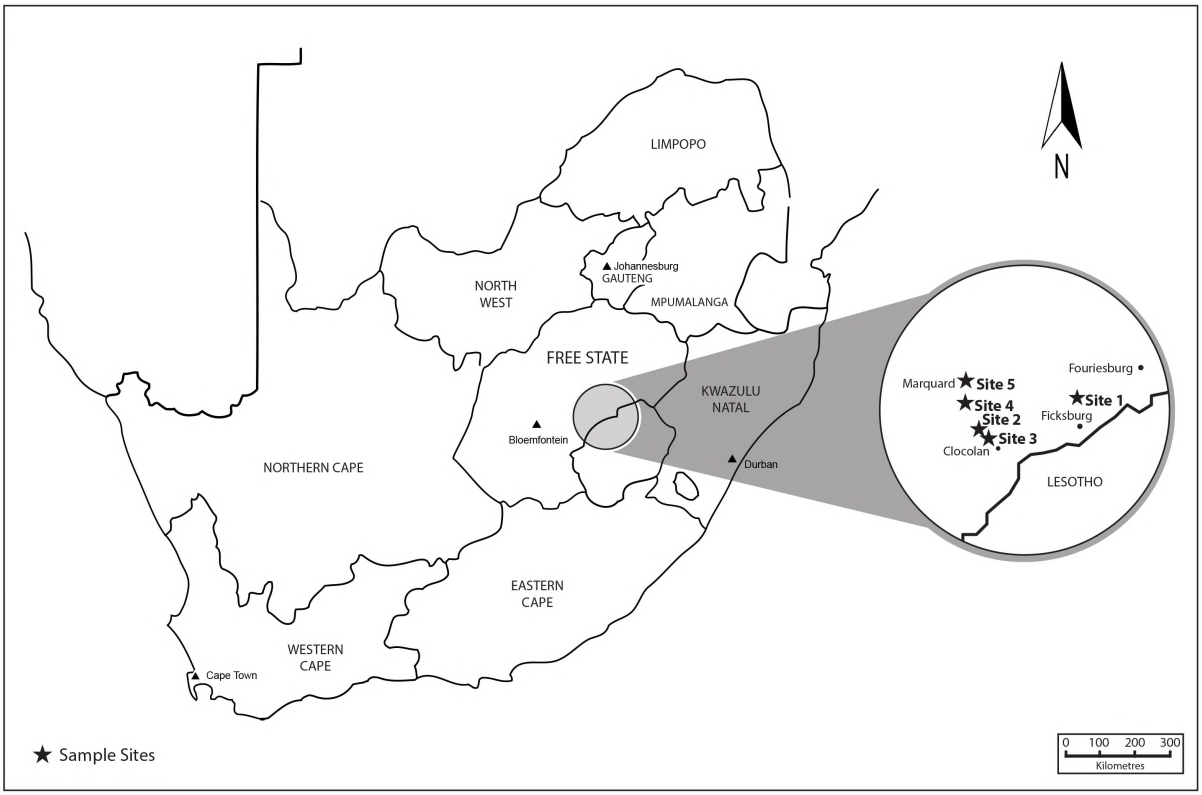


Figure 4-1 Map of the Free State province, South Africa showing the five sampling sites for the LTS.

Table 4-1 Five paired sites in the Free State, South Africa. Coverage scale: 1= patch size less than 50 m^2, 2 = restricted to a dam or small wetland system (<1 km), 3 = cover large wetland system (>1 km), 4 = cover flowing river system (>1 km). *Ad = A. donax, Pa = P. australis.*

Site	GPS Point	Elevation (m)	Distance between reeds stands (km)	Coverage scale		Surrounding land use
				Ad	*Pa*	
1	28°43'15S, 28°01'29E	1825	0.53	1	2	Grazing land
2	28°51'10S, 27°30'18E	1645	0	2	2	Mixed farming
3	28°51'31S, 27°31'25E	1611	1.1	1	2	Mixed farming
4	28°42'11S, 27°26'40E	1500	0	2	2	Crop farming
5	28°40'05S, 27°25'29E	1505	2.02	1	4	Urban

Site selection – nation-wide survey

The second part of the study was a once-off NWS across the reed's distribution in South Africa. *Phragmites mauritianus* was included in regions that the species is present. Paired sites were chosen in four provinces representing four of South Africa's nine biomes –1) fynbos,

Western Cape Province, 2) Indian Ocean Coastal Belt, KwaZulu-Natal Province, 3) Highveld grassland, Free State Province, 4) Lowveld savanna, Mpumalanga Province (Table 4-2 and Figure 4-2). Sites were selected according to areas where all three reed species grow in close proximity (roughly within 5 km of each other). In KwaZulu-Natal, in site 1, one group of plants were sampled as *P. australis* however later after genetic work in chapter 2 were determined to be *P. mauritianus*. The results from this sampling site were included with the results from site 1M-KZN and for this site no *P. australis* plants were sampled.

1) Fynbos

The fynbos region is a winter-rainfall area with a Mediterranean-type climate that is characterised by warm, dry summers and wet, temperate winters (Guthrie 2007). Rainfall is highly variable ranging from 200 to 2000 mm per year (Guthrie 2007). The fynbos biome has the greatest number of vegetative units of all the biomes in South Africa which equates to its floristic diversity (Rutherford et al. 2006). The region also has the highest number of species of which most are endemic (Rutherford et al. 2006). Mean summer and winter temperatures range from 15-29 °C and 7-19 °C respectively (World Weather Online 2016).

2) Indian Ocean Coastal Belt

The Indian Ocean Coastal Belt is a unique biome to South Africa characterised by surviving patches of forest that represent the southernmost extent of coastal (sub) tropical forest of the wet, tropical and subtropical seaboard of East Africa (Rutherford et al. 2006). The biome is highly disturbed with large areas of dense savanna or grassland vegetation in areas of deforestation (Rutherford et al. 2006). The climate is regulated by the offshore Mozambique current and receives year-round rain (Schulze et al. 2008). The climate is mild and sub-tropical (World Weather Online 2016). Summer months are warm and wet, while winter is moist to dry (World Weather Online 2016). Mean summer and winter temperatures range from 20-29 °C and 11-24 °C respectively (World Weather Online 2016).

3) Grassland

The grassland biome is a summer-rainfall area found in the cooler, elevated interior of South Africa (Rutherford et al. 2006). The biome is poorly represented elsewhere in Africa and is known for many species endemic to southern Africa (Rutherford et al. 2006). The area of grassland sampled in the Free State is characterised by wet hot summers and dry cold winters

(Moeletsi et al. 2011). The area has a higher incidence of frost than other grassland areas (Rutherford et al. 2006). Average annual rainfall is highly variable and ranges from 300 mm to over 900 mm (Moeletsi et al. 2011). Mean summer and winter temperatures range from 12-27 °C and -2-17 °C respectively (World Weather Online 2016).

4) Savanna

The savanna biome is a summer-rainfall area in the north and eastern areas of South Africa representing the most extensive biome in Africa (Rutherford et al. 2006). The savanna is climatically similar to the grassland biome however has higher temperatures (Rutherford et al. 2006). More than half of the savanna borders on grassland (Rutherford et al. 2006). The rivers in this study originate from the Drakensberg Mountains in the grassland biome and are heavily utilised for agriculture, forestry and human settlements (Garner 2005; Holmes et al. 2005). Mean summer and winter temperatures range from 17 - 28 °C and 8 - 23 °C respectively (World Weather Online 2016).

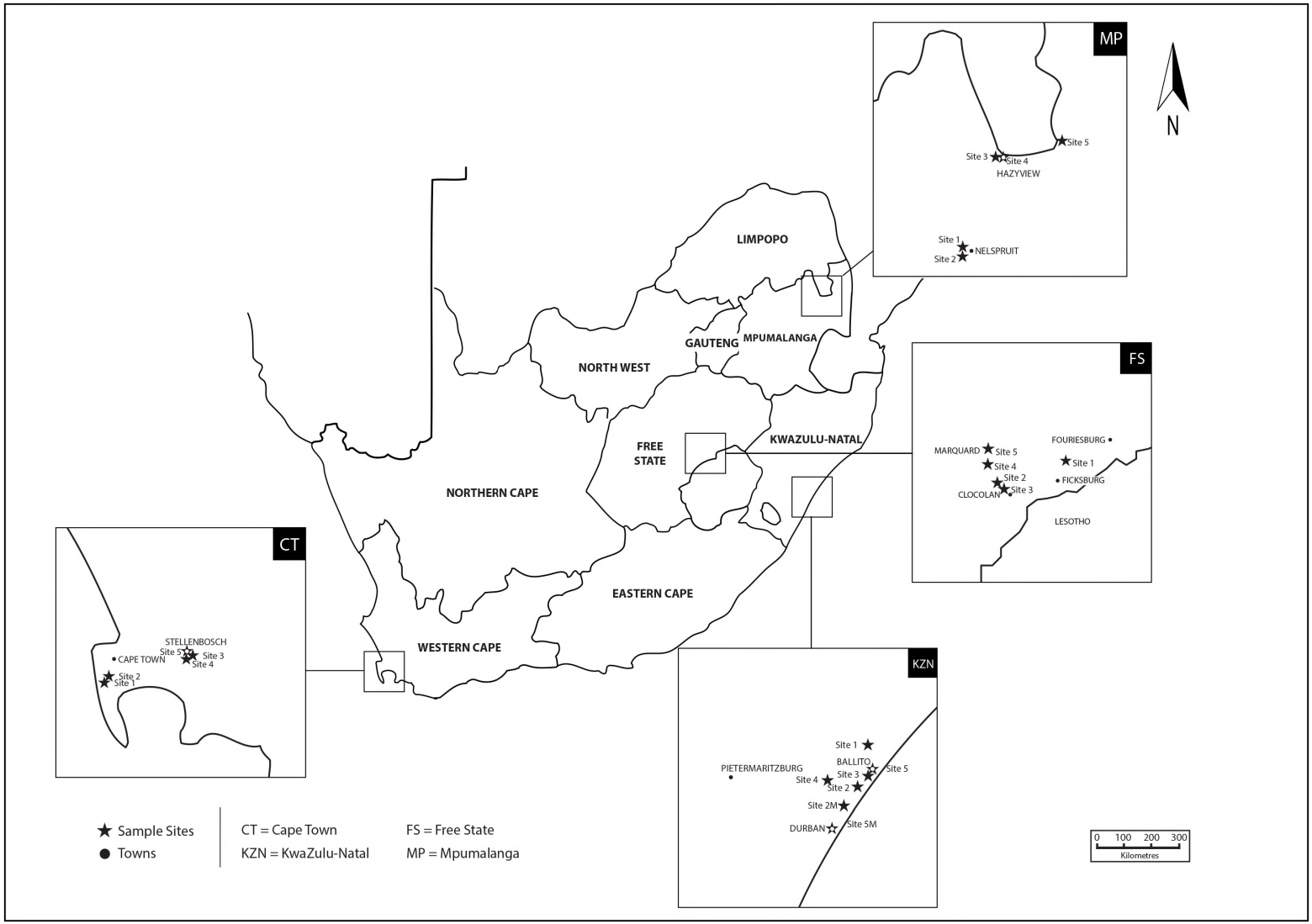


Figure 4-2 Map of South Africa, the highlighted areas focusing in on the four sampled regions during the NWS.

Table 4-2 Paired sites across South Africa. Coverage scale: 1= patch size less than 50 m^2, 2 = restricted to a dam or small wetland system (< 1 km), 3 = cover large wetland system (>1 km), 4 = flowing river system (> 1 km). *Ad*/A= *A. donax, Pa*/P = *P. australis, Pm*/M= *P. mauritianus*, KZN = KwaZulu-Natal, CT = Cape Town, FS = Free State, MP = Mpumalanga Provinces.

Site	GPS Point	Elevation (m)	Distance between each reed species (km)			Coverage scale	Surrounding land use
			Ad	*Pa*	*Pm*		
1A-KZN	29°27'28''S 31°13'00''E	22	-	0	0	1	Sugarcane farming
1M-KZN	29°27'28''S 31°13'00''E	22	0	0	-	4	Sugarcane farming
2A-KZN	29°38'47''S 31°07'18''E	10	-	0.45	-	1	Sugarcane farming
2P-KZN	29°38'47''S 31°07'18''E	5	0.45	-	-	4	Sugarcane farming
2M-KZN	29°42'45''S 31°05'35''E	14	-	-	-	3	Indigenous coastal forest
3A-KZN	29°33'38''S 31°10'19''E	16	-	0.23	0.6	1	Sugarcane farming
3P- KZN	29°33'38''S 31°10'19''E	8	0.23	-	0.81	2	Sugarcane farming
3M- KZN	29°33'38''S 31°10'19''E	7	0.6	0.81	-	4	Sugarcane farming
4A- KZN	29°38'41''S 31°03'55''E	21	0	0	0	1	Sewage works
4P- KZN	29°38'41''S 31°03'55''E	21	0	0	0	4	Sewage works

4M- KZN	29°38’41’’S 31°03’55’’E	21	0	0	0
5A- KZN	29°28’50’’S 31°14’10’’E	93	-	1.08	-
5P- KZN	29°28’17’’S 31°14’28’’E	74	1.08	-	--
5M- KZN	29°47’48’’S 30°57’57’’E	60	-	-	-
1A-CT	34°02’05.64’’S 18°21’13.93’’E	6	-	0	-
1P-CT	34°02’05.64’’S 18°21’13.93’’E	6	0	-	-
2A-CT	34°01’24.92’’S 18°26’18.85’’E	53	-	0.09	-
2P-CT	34°01’24.92’’S 18°26’18.85’’E	51	0.09	-	-
3A-CT	33°56’41.89’’S 18°53’51.52’’E	167		5.62	
3P-CT	33°58’33.98’’S 18°56’40.24’E	273	5.62		
4A-CT	33°58’30.82’’S 18°47’07.84”E	32	-	0.13	-
4P-CT	33°58’30.82’’S 18°47’07.84”E	34	0.13	-	-
5A-CT	33°56’36.68’’S 18°48’07.61”E	103	-	3.52	-
5P-CT	33°56’51.80’’S 18°45’55.06”E	116	3.52	-	-

1	Sewage works
1	Urban
2	Abandoned construction site
4	Urban
4	Urban
4	Urban
1	Suburban park
2	Suburban park
1	Vineyard
2	Nature reserve
2	Vineyard
2	Vineyard
2	Vineyard
2	Vineyard

1A-MP	25°26’22,87’’S 30°55’06.70’’E	687	-	-	5.16	1	Roadside
1M-MP	25°26’03.57’’S 30°52’02.49’’E	691	5.16	-	-	2	Passion fruit (*Passiflora edulis* Sims. (Passifloraceae)) farm
2A-MP	25°01’56.99’’S 31°02’04.99’’E	694	-	-	0	1	Macadamia nut (*Macadamia* sp. (Proteacae)) farm
2M-MP	25°01’52.73’’S 31°01’25.43’’E	694	0	-	-	**2**	Residential
3A-MP	25°27’17.05’’S 30°53’07.36’’E	525	-	-	1.1	**2**	Residential area/scrap yard
3M-MP	25°27’17.05’’S 30°53’07.36’’E	521	1.1	-	-	4	Residential area/scrap yard
4A-MP	25°03’52.41’’S 31°07’46.59’’E	566	-	-	2.3	1	Petrol garage
4M-MP	25°02’10.75’’S 31°07’05.83’’E	482	2.3	-	-	3	Golf course
5A-MP	24°58’48.04’’S 31°28’53.93’’E	297	-	-	0	1	National Park
5M-MP	24°58’48.04’’S 31°28’53.93’’E	297	0	-	-	4	National Park

4.2.1 Data collection

Long-term study

Monthly sampling began in June 2011 and ended in March 2013. At each site ten reeds were selected randomly from both *A. donax* and *P. australis* stands. Reeds were first searched for ectophagous herbivores and these were then collected and immatures were reared to the adult stage. Reeds were then removed by digging up the roots. Stem diameter, height, and the number of lateral shoots were recorded. Stem diameter was taken at 0.5 m above roots to ensure consistency and also to take a measurement close to the first internode as stem diameter remains constant in a growth season at this point (van der Toorn and Mook 1982). Reed density was recorded in 0.5 meter-square quadrats at three random points every month.

Herbivore damage from the most abundant herbivores on *A. donax* and *P. australis* was noted on the reeds by determining presence or absence of injury such as feeding scars, galls or emergence holes. For *A. donax* one randomly selected lateral shoot was chosen per reed and further examined for any damage. The quantity of damage points was then recorded.

Plant parts were separated into stems, lateral shoots, roots and flowers, and put into emergence boxes. Any herbivores that emerged were collected and identified. Voucher specimens of unidentified insects were sent to taxonomists at the South Africa National Collection (ARC-PPRI) for identification. Species found to be most abundant and damaging were deposited in the National Collection of Insects (ARC-PPRI), Pretoria.

Nation-wide survey

Sampling was carried out in the summer months of November 2014 until January 2015. At each site five reeds were randomly selected for *A. donax, P. australis* and *P. mauritianus* stands. Reeds were first searched for ectophagous herbivores and these were collected. Reed growth parameters were measured using the same methods used in the LTS. Plants were then dissected to record and collect endophagous species. All immatures were reared to their adult stage. Lepidopteran stem borers were reared on artificial diet according to Onyango and Ochieng-Odero (1994). Larvae were kept separately in plastic vials until adult emergence. A search period of 30 minutes in each site for each reed species was also carried out. During this search, the reed stands were assessed for damage and any herbivores found associated were collected. For *A. donax* lateral shoots were examined following the protocol of the LTS. Lastly, as done in the LTS, unidentified herbivores were sent to the ARC-PPRI.

Literature review

An extensive literature review was carried out to compile all known records of herbivores on *A. donax, P. australis* and *P. mauritianus* in Africa. Particular attention was given to plants with similar plant architecture to the tall-statured grasses most notably, sugarcane, *Saccharum* spp. (Poaceae), maize, *Zea mays* (Poaceae), sorghum, *Sorghum bicolor* (Poaceae). All known resources and specialists were consulted (Table 4-3).

Table 4-3 List of all research institutes, specialists and literature consulted to determine any records of herbivores on *A. donax, P. australis* and *P. mauritianus*.

Research Institute	**Expert Consulted**	**Field**
The South African Sugarcane Research Institute (SASRI)	Prof. Des Conlong	Entomologist
Agricultural Research Council- Plant Protection Research Institute (ARC-PPRI)	Mrs. Ros Urban	Taxonomist - Diptera:Tephritidae
	Dr. Michael Stiller	Taxonomist - Hemiptera:Auchenorrhyncha
	Mr. Ian Miller	Taxonomist – Hemiptera: Sternorrhyncha
	Ms. Beth Grobbelaar	Taxonomist – Coleoptera
Integrated Pest Management (IPM) sub-program	Prof. Johnnie Van den Berg	Manager of IPM program. Head of Zoology Department, North West University, South Africa
Institut de recherché pour le developpement (IRD)	Dr. Bruno Pierre le Ru	Head of noctuid stem borer biodiversity team
Databases searched	**Website**	**Date accessed**
Centre for Agriculture and Bioscience International (CABI)	www.cabi.org	January 2016
AfroMoths	www.afromoths.net	November 2015
ScaleNet	www.scalenet.info	July 2015
Books		
Prinsloo GL, Uys VM (eds). 2015. Cereals and sugarcane. In: *Insects of cultivated plants and natural pastures of southern Africa*. Cape Town: The Entomological Society of Southern Africa. pp. 88–156.		

4.2.2 Data analysis

The software programme Estimate S (Version 9.1.0) (Colwell 2009) was used to calculate species diversity. The two indices used for this study were the Shannon Index and Simpson's Diversity Index. The Shannon Index is a nonparametric diversity descriptor that is used to assess the number of rare species across samples in terms of equitability (Peet 1974).

The Simpson Index was used to compare the abundance across samples where the most plentiful species were focused on and can be regarded as a measure of 'dominance concentration' (Whittaker 1972; Hill 1973; Peet 1974). Indices were calculated by the following formulae:

Simpson's D diversity:

$$\lambda = \sum_{i=1}^{R} p_i^2$$

Shannon's H diversity:

$$H' = -\sum_{i=1}^{R} p_i \ln p_i$$

Where p_i is the proportion of the individuals in species *i*, n_i the number of individuals in species *i*, and *N* the total sample size.

Means of diversity indices were compared for statistical significances using a student's t-test (two groups, data normally distributed) or by the Kruskal and Wallis one-way analysis of variance (ANOVA) on ranks with a Dunn post hoc test (three groups, data not normally distributed or uneven variance) using SigmaPlot software (Version 12.5). The significance level for all tests was $p < 0.01$.

To test if adequate sampling events were carried out and if all associated herbivores were collected, the Chao 2 and Incidence-Based Coverage (ICE) estimators were used (Chao et al. 2009). Data on species occurrence or incidence was used because not all species abundances could be accurately quantified. The ICE estimator is a modified version of Chao 2, which is used to ensure sampling does not overestimate species richness. The two estimators were calculated using the following formulae:

Chao 2 estimator:

$$S_2 = S_{obs} + \frac{Q_1^2}{2Q_2}$$

ICE estimator:

$$S_{ace} = S_{common} + \frac{S_{rare}}{C_{ace}} + \frac{F_1}{C_{ace}}\gamma_{ace}^2$$

Where S_{obs} is the number of species in the sample and Q_1 is the number of singletons and Q_2 are the number of doubletons occurring in two samples, S_{common} are the species that occur more

than 10 times in the sampling. S_{rare} are those species which occur 10 times or less, C_{ace} is the sample incidence coverage estimator, and finally S_{ace} is the estimated coefficient of variation for $F1$ for rare species (Chazdon et al. 1998).

Means calculated for *A. donax*, *P. australis*, and *P. mauritianus* plant parameters and herbivore damage recorded were compared for statistical significances by a Kruskal- Wallis one-way analysis of variance (ANOVA) on ranks with a Dunn post hoc test (three groups, data not normally distributed or uneven variance) using SigmaPlot software (Version 12.5). The significance level for all tests was $p < 0.01$.

4.3 Results

4.3.1 Reed growth parameters

Arundo donax, *P. australis* and *P. mauritianus* have similar plant architectures however they varied in terms of plant biomass with *A. donax* having significantly greater stem height and stem diameter (Table 4-4.). *Arundo donax* reeds were an average of about two and half meters taller than the *Phragmites* spp. reeds reaching maximum heights of 9.9 m (Table 4-4.). Stems of *A. donax* were also significantly thicker than *Phragmites* spp., with most *A. donax* plants having a 1 cm greater diameter.

Lateral shoot branching divided the two *Phragmites* spp. whereby *P. mauritianus* had on average of about 7.08 ± 0.87 (SE) lateral shoots compared to 0.61 ± 0.15 (SE) for *P. australis*. The high incidence of branching in *P. mauritianus* was about the same rate as with *A. donax*. *Phragmites australis* branching was found to occur generally only when the reeds had damage to the stems whereas *P. mauritianus* will tend to branch naturally as they mature similarly to *A. donax*. Reed density varied significantly across all three reeds with *P. australis* having the most dense stands (Table 4-4.).

Table 4-4 Comparison of plant parameters for *A. donax*, *P. australis* and *P. mauritianus*. Significant differences of each plant parameter are denoted using different letter superscripts.

Plant Parameters	*A. donax*	*P. australis*	*P. mauritianus*
Mean height (m) ± SE	4.12 ± 0.06^{a}	2.25 ± 0.03^{b}	2.40 ± 0.15^{b}
Maximum height (m)	9.9	5.8	5.7
Mean density (m^2) ± SE	8.38 ± 0.22^{a}	12.90 ± 0.45^{b}	6.22 ± 0.55^{c}
Mean number of lateral shoots (per stem) ± SE	7.65 ± 0.21^{a}	0.43 ± 0.03^{b}	7.10 ± 0.87^{a}
Mean stem diameter (cm) ± SE	2.07 ± 0.02^{a}	1.03 ± 0.12^{b}	1.55 ± 0.07^{c}

4.3.2 Arthropod diversity

For the LTS, most herbivores found on *A. donax* and *P. australis* were singletons being found only once at one site in one sampling event. Such species were most likely incidental visitors with no real association with the plants, as riparian plants are used by both aquatic and terrestrial arthropods for resting, refuge and reproduction (Novotný and Basset 2000; Herrera and Dudley 2003). These incidental visitors and non-phytophagous species were excluded from further analyses. The remaining are considered to be herbivores associated with the plants. Seven species were found associated with *A. donax* and only six species with *P. australis*. There was no significant difference found in the species diversity of these herbivores using the Shannon and Simpson's indices (Figure 4-3).

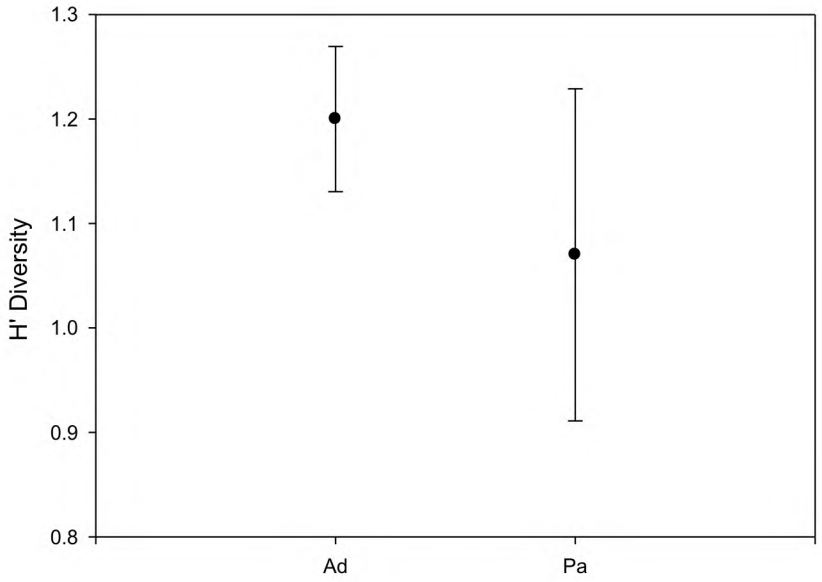
Coverage-based richness estimators were used to test the robustness of sampling. For *A. donax* the Chao 2 and ICE estimators predicted that eight herbivores are likely to be associated with the plant but it is possible that this number could be as high as 20 (Chao 2 upper 95 % CI) (Figure 4-4). With this study finding seven herbivores on *A. donax*, there is potential if sampling continues to find at least one more herbivore but possibly up to 14 more species could be recorded. For *P. australis* the Chao 2 and ICE estimators predicted that six herbivores are likely to be found in all five sites (Figure 4-4). With six species being recorded in this study the analysis suggests that sampling was sufficient and most likely recorded most herbivores in this sampling area.

The NWS sampling technique resulted in recording fewer incidentals compared to the LTS. The surveys also determined that, unlike what was found in the local area, *A. donax* has fewer herbivores associated, with only five species being determined to be herbivores and no new species being found. *Phragmites australis* sampling found three additional herbivores

across South Africa with a total of nine species being recorded (Table 4-6). Three new herbivores were recorded feeding on *P. australis,* two stem borers, *Sesamia incerta* Walker (Lepidoptera: Noctuidae), *Sesamia capensis* (Lepidoptera: Noctuidae) and a fly, *Atherigona* sp. (Diptera: Chloropidae) (RH992). *Phragmites mauritianus* was found to have ten herbivore species (Table 4-6). Five herbivores were found to also occur on *P. australis* however five herbivores were found only on *P. mauritianus*; a cecidomyiid, an Eriophyid mite, *Pirateola piscator* Fletcher (Lepidoptera: Noctuidae), *Anatrichus erinaceous* Loew (Diptera: Chloropidae) and a *Tetramesa* sp. (Hymenoptera: Eurytomidae) (Table 4-7).

Coverage-based richness estimators found that the NWS was sufficient in sampling for herbivores on *A. donax* and *P. australis* however further sampling of *P. mauritianus* would likely determine more herbivores. For *A. donax* the Chao 2 and ICE estimators predicted that five herbivores is the likely number of herbivores associated with *A. donax* with the possibility of only one more species (Chao 2 upper 95 % CI) (Figure 4-6). For *P. australis,* the Chao 2 and ICE estimators predicted that nine herbivores are likely to be found across the sites but that there is the possibility for up to fifteen more species to be found (Figure 4-6). For *P. mauritianus* the Chao 2 and ICE estimators predicted that twelve herbivores are likely to be found in all five sites with the possibility of up to twenty (Figure 4-6).

a



b

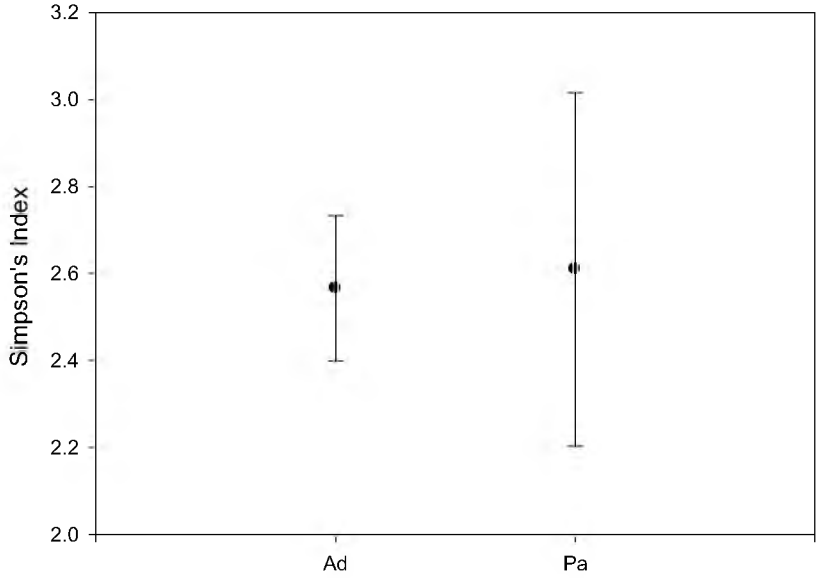
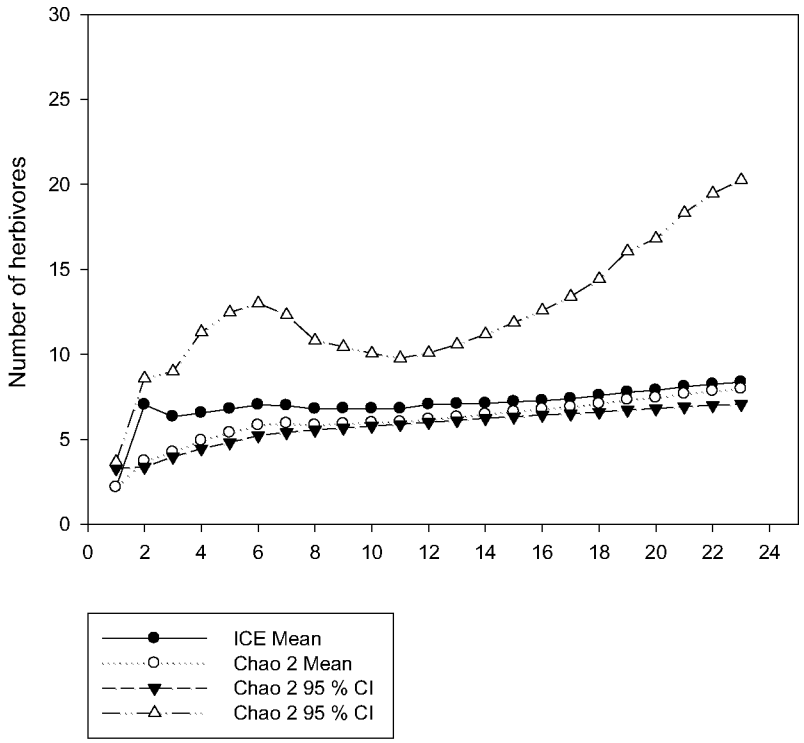


Figure 4-3 a. Mean Shannon's Index (excluding incidentals) at each of the five sites in the LTS comparing *A. donax* (Ad) and *P. australis* (Pa). Error bars represent S.E. of the mean. No significant difference in diversity was found between the two reeds ($t = 0.746$, $p = 0.477$). **b.** Mean Simpson's Index (excluding incidentals) at each of the five sites in the LTS comparing *A. donax* (Ad) and *P. australis* (Pa). Error bars represent S.E. of the mean. No significant difference in diversity was found between the two reeds ($t = 0.100$, $p = 0.923$).

a ***A. donax***



b ***P. australis***

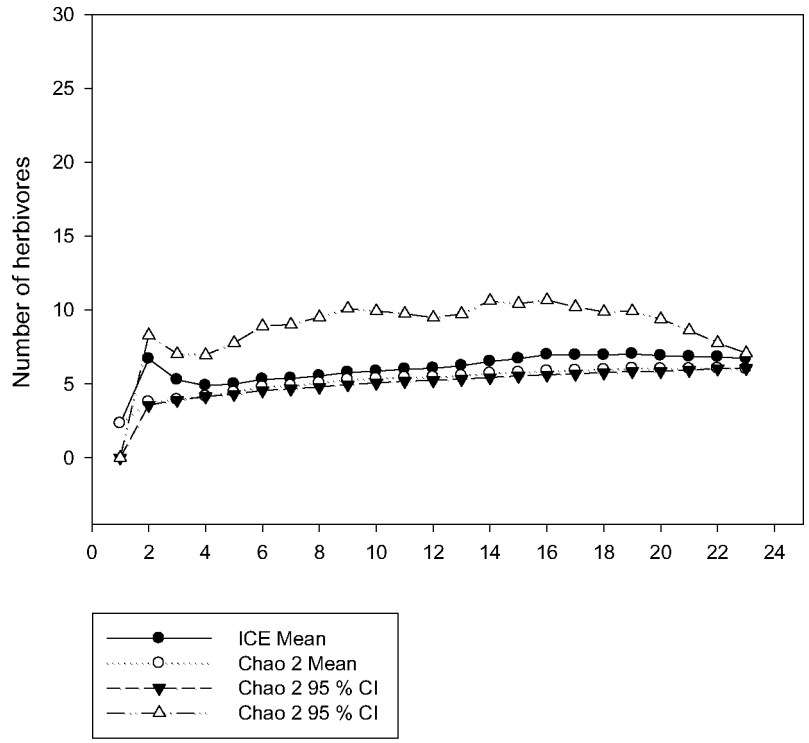


Figure 4-4 a. Incidence data for the total number of herbivores on *A. donax* found with increasing sampling events for the LTS for all five sites using the Chao 2 and ICE estimators (Colwell 2013). Both estimators predict that sampling should find about eight species for all sites with as many as twenty if sampling continues (Chao 2 upper 95% CI); b. Incidence data for the herbivores on *P. australis* found with increasing sampling events for the LTS for all five sites using the Chao 2 and ICE estimators (Colwell 2013). Both estimators predict that six species are expected to be the total number of herbivores to be found at all five sites.

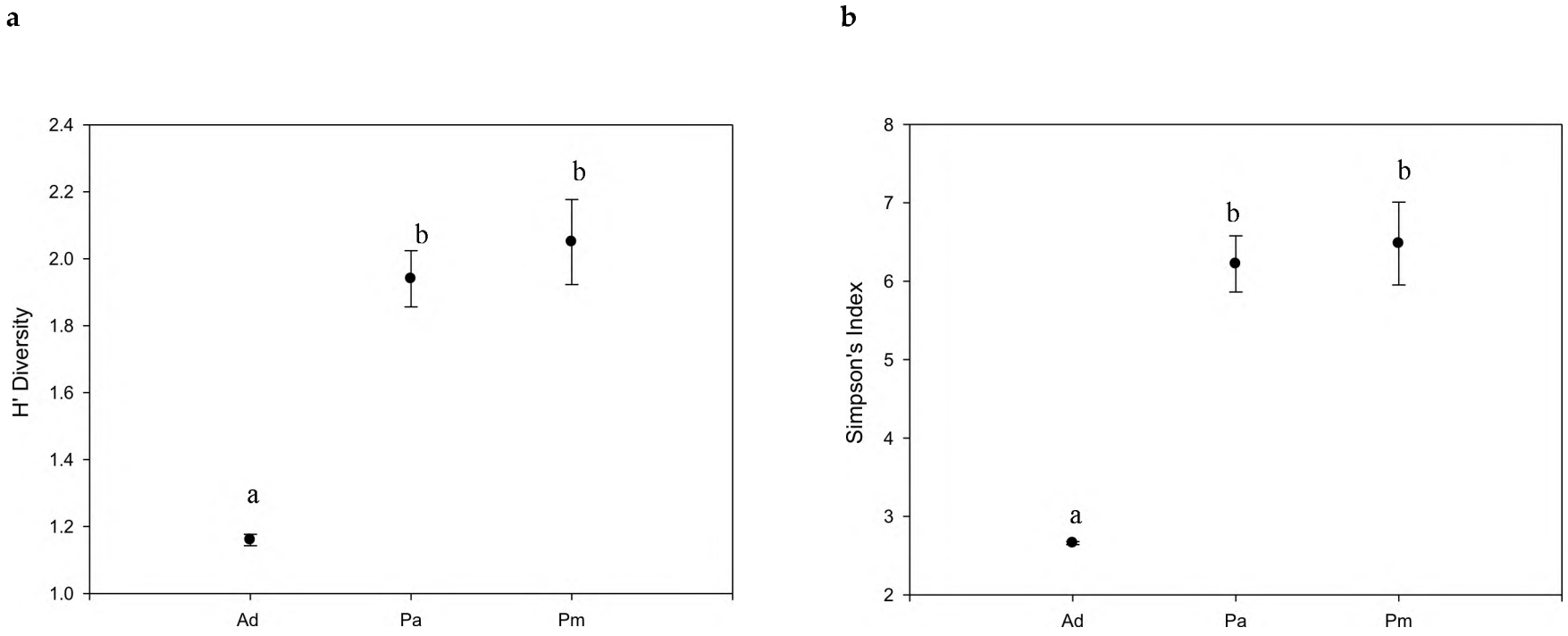


Figure 4-5 a. Mean Shannon's Index including only herbivore species at all sites comparing *A. donax* (Ad), *P. australis* (Pa), and *P. mauritianus* (Pm). Error bars represent S.E. of the mean ($H = 21.428$, $p < 0.01$). b. Mean Simpson's Index including only herbivore species at all sites comparing *A. donax* (Ad), *P. australis* (Pa), and *P. mauritianus* (Pm). Error bars represent S.E. of the mean. Means compared by Kruskal and Wallis one-way ANOVA, those followed by the same letter are not significantly different.

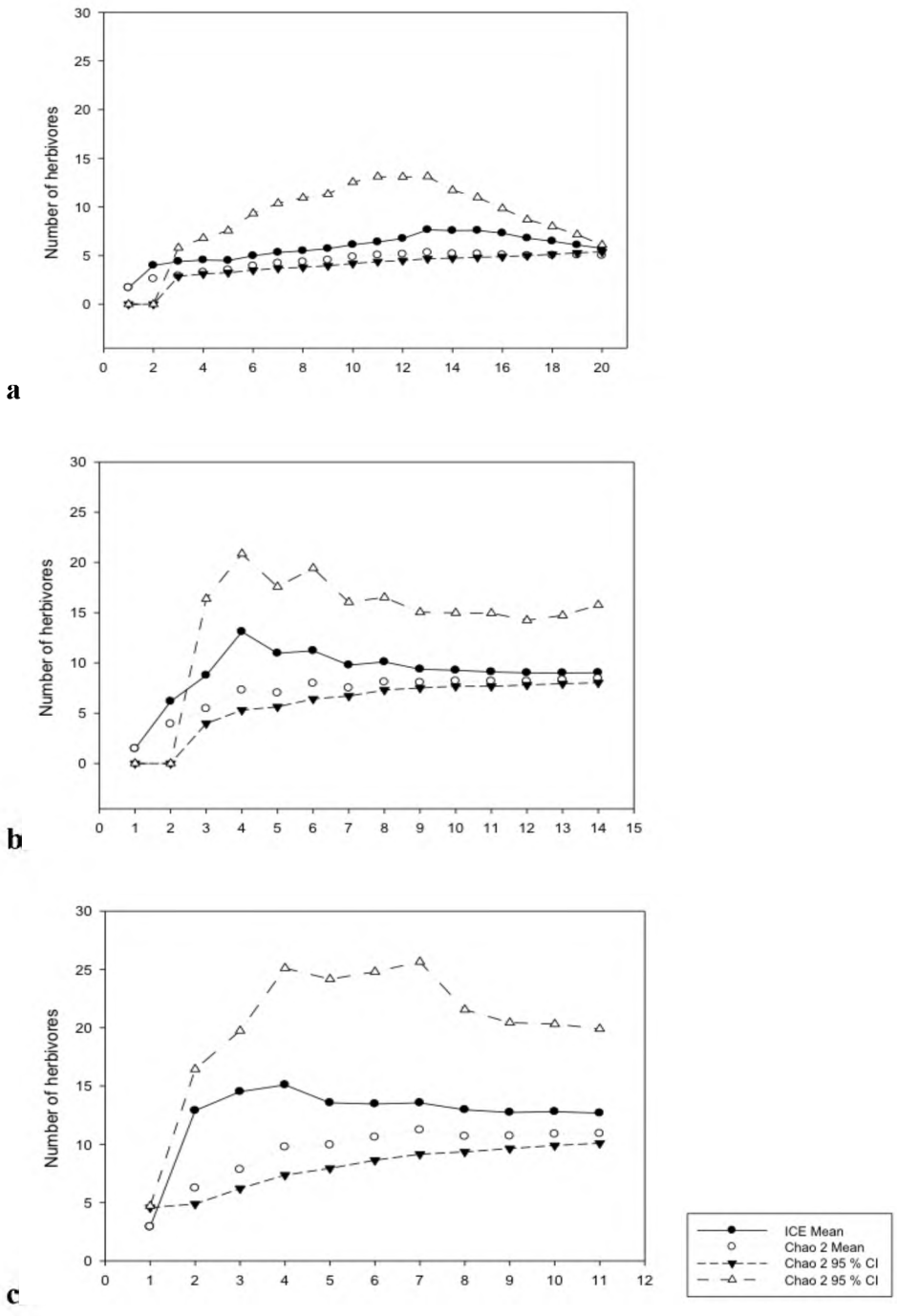


Figure 4-6 Incidence data for the total number of herbivores collected in all NWS with increasing sampling events using the Chao 2 and ICE estimators (Colwell 2013). a. *A. donax*: both estimators predict that five species are expected to be the total number of herbivores to be found at all five sites, with as many as six if sampling continues (Chao 2 upper 95% CI). b. *P. australis*: both estimators predict that twelve species is expected to be the total number of herbivores to be found at all five sites, with as many as twenty if sampling continues (Chao 2 upper 95% CI). c. *P. mauritianus*: both estimators predict that twelve species is expected to be the total number of herbivores to be found at all five sites, with as many as twenty if sampling continues (Chao 2 upper 95% CI).

4.3.3 List of herbivores on tall-statured grasses

To date there is no current list of herbivores associated with the tall-statured grasses *A. donax*, *P. australis* and *P. mauritianus* in South Africa. In this section the results from the study are combined with a literature review of all herbivores recorded to date on *A. donax* (Table 4-5), *P. australis* (Table 4-6) and *P. mauritianus* (Table 4-7). All literature of herbivores associated with the reeds in sub-Saharan Africa was included.

For *A. donax*, four herbivores were found recorded in the literature and not found during sampling; the stem borers, *Busseola fusca* Fuller (Noctuidae), *Chilo partellus* Swinhoe (Crambidae), *Sciomesa* sp. (Noctuidae), and *Sesamia calamistis* Hampson (Noctuidae). For *P. australis*, seven herbivores were found in the literature and not during surveys; the lepidopteran stem borers *Donacaula forficella* Thunberg (Crambidae), *Eldana saccharina* Walker (Pyralidae) *Cosmopterix lienigiella* Zeller (Cosmopterigidae), *B. fusca, Carelis biluma* Nye (Noctuidae), *C. partellus*, and a *Sciomesa* sp. (Noctuidae), (Table 4-6). For *P. mauritianus*, ten herbivores were not found in this study yet have been recorded; *D. forficella, B. fusca*, *S. calamistis*, *Sciomesa* sp. (Noctuidae), *Busseola* sp. (Noctuidae), *C. partellus, Carelis* sp. (Noctuidae), Crambinae sp., *Phycitinae* sp. (Pyralidae), and a cossid moth.

Table 4-5 Herbivores associated with *A. donax* excluding incidental visitors. Species feeding habit is described by part of the plant damaged, feeding guild and host-specificity. Accessioned specimens are referred to by Rhodes University accession numbers (AcRH). The incidence rate is calculated as the percentage of sampling events the herbivore was recorded in. * denotes herbivores found in literature review and not found in this study. NWS Site 1 = KwaZulu-Natal, Site 2 = Free State, Site 3 = Western Cape, site 4 = Mpumalanga.

	Plant part damaged	Long-term study					Incidence	Nation-wide survey				Incidence	Guild	Host specificity	Origin	Source
		Sites						Sites								
Hemiptera		1	2	3	4	5		1	2	3	4					
Family																
Lygaeidae *Dimorphopterus zuluensis* (Slater) (RH938-943)	Stems	*	*	*	*	*	0.10	-	-	-	-	-	Sap suckers	Oligophagous	Tropical Africa	This study
Aphididae *Melanaphis donacis* (Passerini) (RH938)	Lateral shoots and stems	*	*	*	*	*	0.74	*	*	*	*	0.75	Sap-suckers (phloem-feeding)	Polyphagous	Old World	This study
Aphididae *Hyalopterus pruni* (Geoffroy) Mealy plum aphid*	Leaves												Sap-suckers (phloem-feeding)	Polyphagous	Cosmop olitan	1
Thysanoptera																
Phlaeothripidae *Haplothrips gowdeyi* (Franklin) (RH930)	Lateral shoots		*	*			0.02	-	-	-	-	-	Pollen-feeding	Polyphagous	Tropical Africa	This study
Coleoptera																
Curculionidae Sp. 1 (RH922-927)	Lateral Shoots	*	*	*	*	*	0.07	-	-	-	-	-	-	-	-	This study
Lepidoptera																

	Crambidae *Chilo partellus* (Swinhoe, 1885)*	Stems												Stem borer	Oligophagous	Asia	2; this study
	Noctuidae *Sesamia capensis* (RH944-949)	Lateral shoots and stems	*	*	*	*	*	0.07			*		0.025	Stem borer	-	-	This study
	Noctuidae *Sesamia calamistis** (Hampson)	Stems												Stem borer	Polyphagous	Africa	2
	Noctuidae *Busseola fusca* (Fuller, 1901)*	Stems												Stem borer	Polyphagous	Sub-Saharan Africa	2
	Noctuidae Sciomesa*	Stems												Stem borer		Sub-Saharan Africa	3
	Noctuidae Sp. 1	Stems							*			*	0.05	Stem borer			This study
Diptera																	
	Chloropidae Sp. 1 (RH950 and RH995)	Stems and lateral shoots		*				0.05		*	*		0.08	Borer - shoot fly-	-	-	This study
Hymenoptera																	
	Eurytomidae *Tetramesa romana*	Lateral shoots and stems	*	*	*	*	*	0.4	*	*	*	*	0.65	Gall maker	Monophagous	Mediterranean	This study

[1]Blackman 2007;[2]Van Den Berg and Rebe 2001,[3]Le Ru et al. 2006

Table 4-6 Herbivores associated with *Phragmites australis* excluding incidental visitors. Species feeding habit is described by part of the plant damaged, feeding guild and host-specificity. Accessioned specimens are referred to by Rhodes University accession numbers (AcRH). The incidence rate is calculated as the percentage of sampling events the herbivore was recorded in. * denotes herbivores found in literature review and not found in this study. NWS Site 1 = KwaZulu-Natal, Site 2 = Free State, Site 3 = Western Cape.

	Plant part damaged	Long-term study					Incidence	Nation-wide survey			Incidence	Guild	Host specificity	Origin	Source
		Sites						Sites							
Hemiptera		1	2	3	4	5		1	2	3					
Lygaeidae *Dimophopterus zuluensis* (Slater) (RH938-943)	Stems	*	*	*	*	*	0.26		*		0.14	Sap suckers	Oligophagous	Tropical Africa	1; This study,
Aphididae *Hyalopterus pruni* (Geoffroy) Mealy plum aphid (RH948)	Leaves	*	*	*	*	*	0.60	*	*	*	0.21	Sap suckers (phloem-feeding)	Polyphagous	Cosmopolitan	This study
Thysanoptera															
Phlaeothripidae	Root and flowers	*			*		0.02	_	_	_	_		-	-	This study
Lepidoptera															
Crambidae *Chilo partellus* (Swinhoe, 1885)*	Stems											Stem borer	Oligophagous	Asia	3
Crambidae *Donacaula forficella* (Thunberg, 1794)*	Stems											Stem borer	Oligophagous	Cosmopolitan	4
Cosmopterigidae *Cosmopterix lienigiella* (Zeller, 1846)*	Leaves											Leaf-miner	Monophagous	Cosmopolitan	5
Noctuidae *Sesamia calamistis* (Hampson) (RH-949)	Stems	*	*	*	*	*	0.17		*		0.04	Stem borer	Polyphagous	Africa	This study; 2

	Noctuidae *Sesamia incerta* (Walker)	Stems	-	-	-	-	-	-		*	0.04	Stem borer			This study
	Noctuidae *Sesamia capensis*	Stems								*	0.18	Stem borer			This study
	Noctuidae *Sciomesa**	Stems										Stem borer		Sub-Saharan Africa	6
	Noctuidae *Carelis biluma* (Nye) comb. n. (Sub-genus biluma)*	Stems										Stem borer		Madagascar	7
	Noctuidae Sp. 1	Stems							*		0.04	Stem borer			This study
	Noctuidae *Busseola fusca* (Fuller)*	Stems										Stem borer	Polyphagous	Sub-Saharan Africa	6
	Pyralidae *Eldana saccharina* (Walker 1865)*	Stems										Stem borer	Polyphagous	Africa	8
Diptera															
	Chloropidae Sp. 1 (RH934, 992)	Lateral shoots and stems		*			*	0.05		*		0.07	Borer - shoot fly-	-	-	This study
	Muscidae *Antherigona* sp. (RH1011)	Lateral shoots and stems							*		*	0.18	Borer - shoot fly-	-	-	This study
Acari															
	Tarsonemidae *Steneotarsonemidae* sp.	Stem and flowers	*	*	*	*	*	0.45		*	*	0.18	Gall maker	-	-	This study

[1]Slater and Wilcox 1973; [2]Van Den Berg and Rebe 2001; [3] Prinsloo and Uys 2015; [4]Vári et al. 2002; [5]Meyrick 1912; [6]Le Ru et al. 2006; [7]Moyal et al. 2010; [8]Assefa et al. 2008

Table 4-7 Herbivores associated with *Phragmites mauritianus* excluding incidental visitors. Species feeding habit is described by part of the plant damaged, feeding guild and host-specificity. Accessioned specimens are referred to by Rhodes University accession numbers (AcRH). The incidence rate is calculated as the percentage of sampling events the herbivore was recorded in. * denotes herbivores found in literature review and not found in this study. NWS site 1 = KwaZulu-Natal, site 2 = Mpumalanga.

	Plant part damaged	Nation-wide survey		Incidence	Guild	Host specificity	Origin	Source
		Sites						
		1	2					
Hemiptera								
Lygaeidae *Dimorphopterus zuluensis* (Slater) (RH938-943)	Stems	*	*	0.2	Sap suckers	Oligophagous	Tropical Africa	1; This study,
Aphididae *Hyalopterus pruni* (Geoffroy) Mealy plum aphid (RH973-975)	Leaves	*	*	0.23	Sap suckers (phloem-feeding)	Polyphagous,	Cosmopolitan	This study
Lepidoptera								
Crambidae *Chilo partellus* (Swinhoe, 1885)*	Stem				Stem borer	Oligophagous	Asia	2
Crambidae *Donacaula forficella* (Thunberg, 1794)*	Stems				Stem borer	Oligophagous	Cosmopolitan	3
Crambidae Craminae*	Stems				Stem borer			2
Noctuidae *Pirateolea piscator* (Fletcher D.S. 1961)	Stems		*	0.14	Stem borer	Polyphagous	Tropical Africa	This study

	Noctuidae *Busseola fusca* (Fuller, 1901)*	Stem		
	Noctuidae *Sesamia calamistis**	Stem		
	Noctuidae *Carelis**	Stem		
	Noctuidae Sciomesa*	Stem		
	Noctuidae Busseola*	Stem		
	Noctuidae Sp. 1	Stem	*	*
	Pyralidae Phycitinae*	Stem		
	Cossidae*	Stem		
Diptera				
	Cecidomyiidae Sp 1. (RH985)	Lateral shoots	*	*
	Chloropidae Sp. 2 (RH1026)	Lateral shoots and stems		*
	Chloropidae *Anatrichus erinaceus* (Loew 1860) Grass fly (RH996)	Lateral shoots and stems		*
	Muscidae *Atherigona* sp. (RH1011)	Lateral shoots and stems	*	*
Hymenoptera				
	Eurytomidae *Tetramesa* sp. (RH1057)			*

	Stem borer	Polyphagous	Sub-Saharan Africa	4
	Stem borer	Polyphagous	Africa	2
	Stem borer			2
	Stem borer			2
	Stem borer			2
0.10	Stem borer			This study
	Stem borer			2;5
	Stem borer			2;5
0.41	Gall maker	-	-	This study
0.14	Borer - shoot fly			This study
0.05	Borer - shoot fly	Polyphagous	Paleotropical, Tropical Africa to Oriental region[7,8]	This study
0.50	Shoot fly	Oligophagous	-	This study
0.05	-	-	-	This study

Acari								
	Eriophyidae Sp. 1	Stems and flowers	*	0.1	Gall maker	-	-	This study

[1]Slater and Wilcox 1973; [2] Le Ru et al. 2006;[3]Vári et al. 2002; [4]Reddy 1987; [5]Ong'amo et al. 2006; [6]Fanshawe 1972; [7]Gautam et al. 2009; [8]Khan 1991.

4.3.4 Potential biological control agents

A potential biological control agent was found on *A. donax,* the galling wasp, *Tetramesa romana. Tetramesa romana* is already released as a biological control agent in North America (Dudley et al. 2006; Goolsby and Moran 2009). *Tetramesa romana* was found to be the most widespread, damaging and abundant herbivore found on *A. donax* in both the LTS and NWS (Table 4-10). *Tetramesa romana* damages *A. donax* by producing galls in the lateral shoots and occasionally in the stems.

In both the LTS and NWS surveys, *T. romana* was widely distributed (Table 4-8, Table 4-9 and Table 4-10). Damage (number of exit holes) was highly variable across sites however was overall relatively low compared to surveys in North America (Table 4-8, Table 4-9 and Table 4-10) (Dudley et al. 2008). The number of adult *T. romana* found varied between the LTS and NWS. In the LTS, *T. romana* adults recorded from the emergence boxes was low with only an average of 0.22 adults being recovered per culm (Table 4-10). However, in the NWS, an average of 6 *T. romana* larvae were found per culm (Table 4-10). The increased mean number of *T. romana* recorded in the NWS reflects the significantly higher abundance of wasps in the Mpumalanga region (Table 4-9). Furthermore, an average of about one larva was found per lateral shoot in the NWS (Table 4-10). *Tetramesa romana* populations were highly dependent on the season, being most abundant in November and December in the LTS (Figure 4-7). In both surveys, the presence of *T. romana* galls was found to be correlated with *A. donax* plant physiology, whereby plants with *T. romana* were found to be significantly taller (LTS: $U = 8000$, $p < 0.001$; NWS: $U = 595$, $p < 0.001$) and had significantly more lateral shoots (LTS: $U = 29881.5$, $p < 0.001$; NWS: $U = 404$, $p < 0.001$). Stem diameter was also found to be greater with *T. romana* galling, however this was only significant for the LTS (LTS: $U = 46146$, $p < 0.001$; NWS: $t = 1.82$, $p = 0.071$).

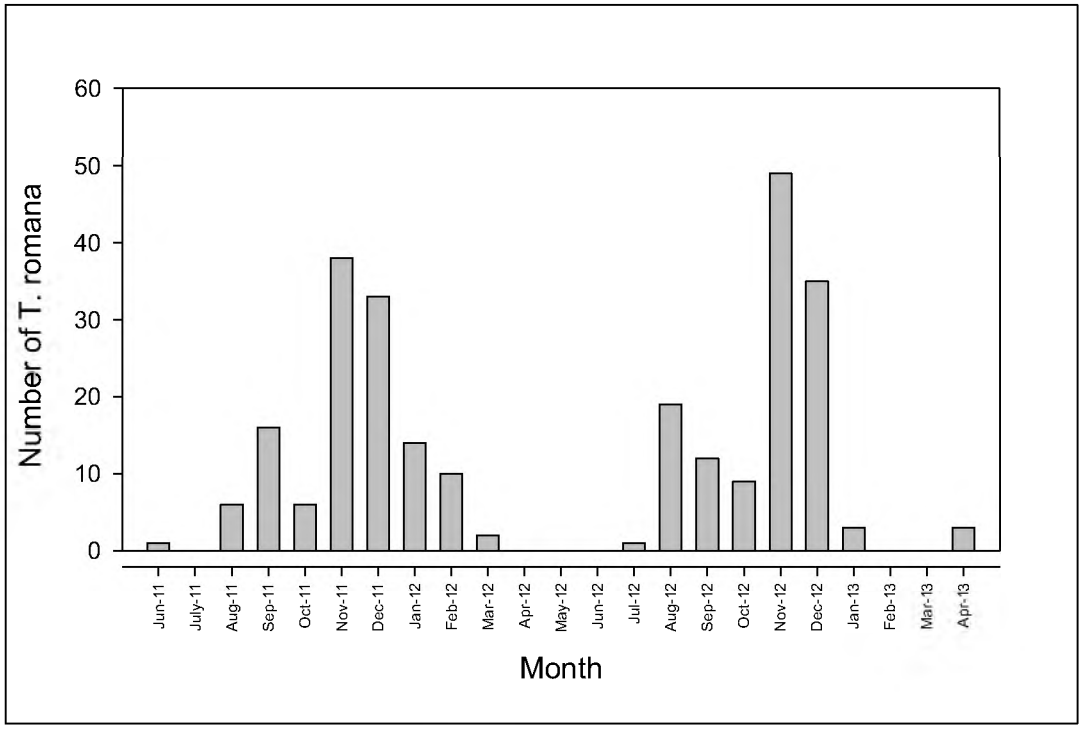


Figure 4-7 The total number of *T. romana* adults recorded from emergence boxes in each site across the two year LTS.

Table 4-8 *Tetramesa romana* recorded abundance and injury in the LTS.

	Site 1	**Site 2**	**Site 3**	**Site 4**	**Site 5**
Mean number of emergence holes ± SE	0.60 ± 0.12	0.86 ± 0.14	2.35 ± 0.18	0.69 ± 0.11	1.91 ± 0.16
Percent of lateral shoots with emergence holes	10%	16%	36%	22%	11%
Percent of plants with *T. romana* present	45%	42.86%	57.86%	25.71%	40.77%

Table 4-9 *Tetramesa romana* recorded abundance and injury in the NWS.

	Site 1	**Site 2**	**Site 3**	**Site 4**
Mean number of emergence holes ± SE	0.66 ± 0.57	0	1.2 ± 0.67	0.41 ± 0.24
Mean number of larvae per lateral shoot ± SE	1.05 ± 0.62	2.36 ± 0.87	1.13 ± 0.39	0.41 ± 1.01
Average number of *T. romana* per culm ± SE	4.6 ± 2.87	4.4 ± 2.87	5.92 ± 2.31	9.48 ± 4.7
Percent of lateral shoots with larvae ± SE	12%	16%	16%	60%
Percent of plants with *T. romana* present ± SE	40%	20%	36%	68%

Table 4-10 Comparison of *T. romana* abundance and injury in the LTS and NWS.

	LTS	NWS
Mean number of emergence holes in lateral shoots ± SE	0.88 ± 0.07	0.61 ± 0.30
Percent of lateral shoots with: LTS - emergence holes NWS – *T. romana* larvae	19 %	26 %
Percent of plants with *T. romana* present	42.44 %	41 %
Mean number of larvae per lateral shoot ± SE	_	1.13 ± 0.35
Average number of *T. romana* per culm ± SE	0.22 ± 1.46*	6.1 ± 1.60

*based on the number of *T. romana* adult wasps recorded from the emergence boxes (10 reeds per box).

4.3.5 Enemy Release Hypothesis

To investigate the ERH the results from this study were compared with herbivore assemblages found on *P. australis* and *A. donax* in their native and introduced ranges. *Phragmites mauritianus* was not included as it is endemic to the African continent and thus cannot be compared with other regions. For *A. donax* species in the introduced ranges of South Africa and North America were compared to those found in the native range in Europe and Asia; *A. donax* in South Africa was found to have a similar number of herbivores to North America but much fewer species compared to the native range in Europe and Asia (26 herbivores) (Table 4-11). In the introduced distribution, *A. donax* has lost almost all of its native herbivores, however in both South Africa and North America one native monophagous species has established (Table 4-5).

For *P. australis,* comparisons of herbivore assemblages were made with North America and Europe, which are areas that the reed is also considered native. Fewer herbivores were found in South Africa compared to both regions (Table 4-11). In South Africa, *P. australis* had a depauperate herbivore community with only one species being monophagous. In North America, despite also having a lower number of herbivores compared to Europe, twelve species were found to be monophagous. In Europe and Asia, however highly diverse herbivore communities have been recorded with a total of 154 herbivores recorded with 54 of these herbivores being monophagous.

Table 4-11 Comparison of the number of herbivores associated with *A. donax,* and *P. australis* in Europe, North America and South Africa. For *A. donax,* the native range is found in Europe and Asia and the number of species is compared to the two introduced ranges in North America and South Africa. For *P. australis,* all areas included are considered the native range. *Ad* = number of species recorded on *A. donax, Pa* = number of species recorded on *P. australis.*

Location	Host Range	*Ad*	*Pa*
Eurasia	Monophagous	1[1]	54[2]
	Oligophagous	2[1]	18[2]
	Polyphagous	23[1]	61[2]
	Unknown	-	21[2]
North America	Monophagous	1[3]	12[2]
	Oligophagous	1[3]	3[2]
	Polyphagous	1[3]	8[2]
	Unknown	-	3[2]
South Africa	Monophagous	1	0
	Oligophagous	1	1
	Polyphagous	2	3
	Unknown	4	6

[1]Tracy and DeLoach 1998,[2]Tewksbury et al. 2002,[3]Dudley et al. 2008

4.4 Discussion

The radiation of insect clades that are associated with specific plant clades has been found to show a degree of temporal correspondence; thus plant origin is closely linked to the diversification of the herbivores feeding on the plant (Futuyma and Agrawal 2009). Studies that compare introduced plants to conspecific species in their native range are important as it is relative to these populations that invaders gain and lose interactions with enemies, mutualists and competitors (Mitchell et al. 2006). To investigate such a link between plant origin and herbivory three reed species, *A. donax, P. australis* and *P. mauritianus* considered invasive, native but cosmopolitan and endemic to South Africa were assessed according to the ERH. Overall, the herbivore assemblages found on *A. donax* in South Africa when compared to the native analogous *Phragmites* spp. species did not meet the assumptions of the ERH; *A. donax* has two specialist herbivores from its native range, shares a native herbivore with the *Phragmites* spp. and is damaged by the same feeding guilds. The pre-introductory survey was also beneficial in determining a potential biological control agent *T. romana* as being abundant across all sampling sites. Lastly, the study was the first to compile a list of herbivores associated with each reed species in South Africa.

Assessing herbivory on tall-statured grasses requires looking at the overall number of species or species richness, species diversity and determining the feeding guilds, as each measure will add up to giving a clearer picture of herbivore pressure on plant populations. Looking at species richness found on the tall-statured grasses, a total of seven herbivores were

found on *A. donax* and both *Phragmites* spp. had ten herbivores. According to the Chao estimators the overall sampling effort was sufficient for all three reed species. However, from results in the LTS for both *P. australis* and *P. mauritianus* there is potential to find more herbivores with up to five and ten more species respectively. This suggests that additional sampling in more geographic and climatic ranges may be beneficial. For *A. donax,* the LTS found that there is potential for more herbivores if sampling continues; however, the level of error was reduced during the NWS with estimates indicating no more species are likely to be found. This is most likely due to the sampling method, during the NWS no emergence boxes were used and a period of active searching was carried out, which reduced the chances of including any erroneous incidentals. However, the inclusion of the LTS was advantageous in determining a number of low incidence herbivores that were not detected in the once-off NWS which highlights the advantage of incorporating records of both seasonal patterns and also geographical variations in herbivory.

To understand community composition of herbivores it is important to not only assess species richness but include measures of species diversity as this will link species numbers and their abundance (Menhinick 1964; Peet 1974; Spellerberg and Fedor 2003). In the LTS, *A. donax* was found to have a slightly higher Shannon index and about the same level of diversity for the Simpson's index compared to *P. australis.* However, in the LTS *A. donax* had significantly lower species diversity to both *P. australis* and *P. mauritianus*. Like seen with the species richness, this higher level of diversity seen in the LTS is most likely an artefact of the sampling method. Overall the *Phragmites* spp. have a higher level of herbivore diversity compared to *A. donax* and the two *Phragmites* spp. have similar levels of species and abundances to one another.

For *P. australis* and *P. mauritianus,* despite sampling in areas where the two species co-occur, many herbivores were found to be restricted to one reed species with only four herbivores being found on both *P. australis* and *P. mauritianus. Phragmites mauritianus* was found to have five herbivores that were not recorded on any of the *P. australis* plants; all of these herbivores were found to be galling herbivores and thus are likely to be specific to the particular host plant (Quiring et al. 2006). *Phragmites* herbivores are known to be sensitive to plant genotype and can even respond at the subspecies level (Blossey 2003). For example, *Lasioptera hungarica* Möhn (Diptera: Cecidomyiidae) a European *P. australis* herbivore has only been found to feed on the invasive European *P. australis* haplotypes in North America (Blossey 2003). As such although the *Phragmites* spp. are related and chemically similar, they

nonetheless differ in the identity and levels of compounds that issue from similar biosynthetic pathways which influences their herbivore communities (Futuyma and Agrawal 2009).

All three reeds had herbivores that occupied the same feeding guilds – sap-suckers, stem borers and gall makers. Investigating the feeding habits of the herbivores we can infer to some extent the level of pressure on plant populations. Each feeding guild has a varying impact on a plant as they target different life stages and parts of the plants (Andrew and Hughes 2005). For example, younger plants generally support more external herbivory (sap-sucking and chewing) and more mature plants will generally support larger endophagous herbivores (miners and stem-borers) (Andrew and Hughes 2005). With the tall-statured grasses having similar guilds of insects it is likely that all three reeds will have similar modes of damage from herbivory however the resultant pressure to populations will vary according to a number of factors including herbivore abundance.

All three reed species had pressure from sap-sucking herbivores with aphid species associated with them; *A. donax* had the exotic herbivore *M. donacis* and both *Phragmites* spp. had the cosmopolitan herbivore *H. pruni*. *Hyalopterus pruni* prefers *Prunus* sp. (Rosaceae) but will colonise the edges of *Phragmites* stands during summer months before moving on to their preferred host (Tscharntke 1992; Mook and Wiegers 1999). In the NWS *H. pruni* was found relatively equally on both *P. australis* and *P. mauritianus* being found in 25 % and 23 % of sites respectively. On *A. donax,* the introduced aphid, *M. donacis* native to southern France was found feeding on the apical shoots and less mature distal leaves (Dudley and Lambert 2007; Blackman and Eastop 2008). *Melanaphis donacis* was found across all sites with 75 % of *A. donax* sites in the NWS. Both aphid species were found to reach high populations during certain times, however it is unlikely that they were causing any major impacts on the reeds. For *M. donacis* previous studies in North America have found that even at high densities the aphids have a low impact on *A. donax* (Dudley and Lambert 2007). However, aphids can have secondary consequences as they have the potential to spread infections; for example, *H. pruni* is a vector of several viruses such as the plum pox virus (Isac et al. 1998; Li et al. 2015).

Another sap-sucking herbivore found in the study was the Chinch bug, *D. zuluensis*. *Dimorphopterus zuluensis* has previously been recorded as restricted to feeding on *Phragmites* with its main hosts being *P. australis* and *P. mauritianus* (Slater and Wilcox 1973). However, in the LTS, *D. zuluensis* was recorded feeding on *A. donax* in a number of sites. Although *Phragmites* spp. are the main hosts, the native *D. zuluensis* was found to have a behavioural expansion to utilise *A. donax,* mainly during autumn months (February, March, April). Some Blissinae species are known to shift to a secondary host plant when the primary host plant dries

up, however these species are unlikely to have population that persist on the secondary host plant (Slater 1976). This was confirmed during NWS with no *D. zuluensis* being found on any *A. donax* plants highlighting that feeding will likely only occur when *Phragmites* spp. are scarce and will most likely not establish sustainable populations on *A. donax*. The occurrence of native specialists utilising genetically similar introduced plants has been reported in a number of other species (Bush 1969; Opler 1978; Connor et al. 1980; Agrawal et al. 2005; Dostal et al. 2013).

Stem borers were found on all three reed species and are likely impacting plant populations as internal stem-feeding is a known to cause substantial fitness costs (Cronin et al. 2015). Lepidopteran stem borers are major pests of agricultural crops in sub-Saharan Africa and many use reed species as wild hosts (Moolman et al. 2014). African stem borers, with the exception of the cereal crop stem borer *C. partellus*, are all native and have co-evolved with native grasses and sedges (Getu et al. 2001). In this study, a total of five stem borers were recorded on all three reeds with *P. australis* having the highest diversity.

The stem borers recorded in this study are all widespread throughout Africa with *P. piscator* and *S. calamistis* both being reported as far north as Cameroon (Ong'amo et al. 2014). However, despite widespread distribution a general low incidence rate on wild hosts of stem borers has been found; it has generally been found that borers prefer cereal crop hosts and are relatively specialised to a few host species (Le Ru et al. 2006; Ong'amo et al. 2014). For example, *S. calamistis* was found only on *P. australis* in this study but is known to prefer wild sorghum, *Sorghum verticilliflorum* (Steud.) Stapf and *Sorghum rigidifolium* Stapf (Prinsloo and Uys 2015). However, host-switching to new hosts such as the tall-statured grasses *A. donax, P. australis* and *P. mauritianus* can occur if there has been habitat destruction and therefore utilising new species is a common mechanism to survive non-cropping seasons (Ndemah et al. 2007). This most likely explains the occurrence of stem borers on *A. donax* in South Africa found in this study and the literature despite the reed not being native to the region.

In addition to stem borers, all three reed species were attacked by shoot flies that were found to bore through the lateral shoots and stems of the reeds. Shoot flies are common herbivores on grasses as they thrive on the extensive vegetative parts of both *Phragmites* spp. and *A. donax* (Nartshuk 2014). Shoot flies can impact tall-statured grasses as they can reach large numbers and feed on the embryonic tissue in the apical cone and can thus influence plant growth parameters (Nartshuk 2014). Like with the stem borers, the shoot flies found are known pests of important agricultural crops (Prinsloo and Uys 2015). For example, the muscid, *Atherigona* sp. found on both *P. australis* and *P. mauritianus* is an important pest of sorghum which is the preferred host (Prinsloo and Uys 2015). Similarly, *A. erinaceus* found only on *P.*

mauritianus in Mpumalanga is a known pest of sorghum and pearl millet, *Pennisetum glaucum* (Poaceae) (Prinsloo and Uys 2015).

For all three tall-statured grasses, galling herbivores were found in all sites sampled and were found to reach high abundances. These galling herbivores could not be identified down to species level however it is likely that they are monophagous on *Phragmites* spp., as gall inducing phytophagous species are generally specialists as they must be finely adapted to the internal environmental of the host (Quiring et al. 2006). On *P. australis* the mite, *Steneotarsonemus* sp. was found to be the most abundant herbivore in the LTS with a mean of 27.8 % of plants sampled having galls present. Two galling herbivores were also found on *P. mauritianus,* a galling Eriophyid mite and a Cecidomyiid galling midge (Sp. 1 AcRH985). The Cecidomyiid fly morphologically resembles the galling midge *Giraudiella inclusa* (Diptera: Cecidomyiidae) that attacks *P. australis* in Europe (Tscharntke 2008) and thus these populations could represent a southern African distribution. *Giraudiella inclusa* galling impacts *P. australis* populations by causing internodes to elongate, increasing shoot diameter and the number of side shoots (Tscharntke 2008), it is likely that the Cecidomyiid flies are having a similar impact in South Africa (Tscharntke 2008). The discovery of these specialist species may have important implications to control efforts on invasive reed populations, particularly in North America. In the U.S., biological control of European *P. australis* haplotypes is being considered as some herbivores have been found to be specific to plant genotype (Schwarzländer and Häfliger 2000; Tewksbury et al. 2002; Häfliger et al. 2005; Blossey 2014). Further research on the herbivore's host specificity may determine their potential as biological control agents.

For *A. donax,* the galling wasp *T. romana* was the most widespread and abundant herbivore. *Tetramesa romana* was also found to be established in North America and after host specificity and impact testing was subsequently used for augmentative biological control to increase their field populations (Dudley et al. 2006; Goolsby and Moran 2009). Therefore, like with the survey by Hoffmann and Moran (1991) on *S. punicea,* the study was able to record the widespread establishment of a potential biological control agent. Overall, *T. romana* was found to have relatively high abundances, despite a low emergence rate in the LTS, the NWS determined that 41 % of plants had *T. romana* damage and each culm had an average of four *T. romana* larvae. Yet compared to surveys in North America, populations of *T. romana* in South Africa are still relatively low; surveys in California found an average adult wasp of 9.2 ± 1.4 (SE) larvae per stem (Dudley et al. 2006).

The aim of releasing a biological control agent is to introduce a herbivore that can overcome a plant's defense mechanisms and negatively impact its population growth (Goolsby

et al. 2006). Therefore, before *T. romana* can be considered for potential augmentative control it will be important to assess their impact on *A. donax* populations in South Africa. This study did not measure *T. romana* impact but does give a preliminary understanding of the correlation between wasp populations and *A. donax* stands. *Tetramesa romana* galls were found to be more prevalent on reeds that had increased height, diameter and lateral shoots production. Previous studies on *T. romana* impact on *A. donax* have found that wasps can increase lateral shoot production and leaf area (Dudley and Lambert 2007). Our findings may reflect such pressure on *A. donax* plant productivity. However, the correlation of increased wasp abundance and plant height is most likely a consequence of a longer time period for gall formation as *T. romana* generally target younger and thinner side shoots (Dudley et al. 2008). Despite this potential pressure on *A. donax*, studies of *T. romana* impact have found that at low densities the wasps do not significantly affect *A. donax* physiology (Moore et al. 2010). Furthermore, according to Blossey (2014) when considering potential biological control agents for *P. australis* in North America, any herbivores that are already established should not be considered for augmentative biological control as their impact is considered insignificant. Initiating augmentative biological control using *T. romana* in South Africa may therefore have negligible results.

With the overall herbivore load determined on *A. donax, P. australis* and *P. mauritianus* it is possible to test if the assumptions of the ERH have been met for *A. donax* in South Africa. In this study *A. donax* did have a reduced number of herbivores compared to Europe. Yet, *A. donax* in South Africa was found to have more herbivores compared to North America with a range of feeding guilds. This higher level of herbivory than expected may be attributed to multiple introductions of *A. donax* to the country and also a long period of establishment (Bernays and Graham 1988), which likely facilitated the occurrence of herbivory from native specialist herbivores and the accidental introduction of species. It is a complicating factor when species move across with invasive species as even though herbivory may still be lower in the introduced range, it does not represent enemy-release *sensu stricto* (Allen et al. 2015).

For both *Phragmites* spp., despite being considered native to South Africa a low herbivore diversity was found. For *P. australis* only ten species were found to be damaging the reed compared to over 150 species associated with the reed in Europe (Tewksbury et al. 2002). Such a result was unexpected as with *P. australis* being native to South Africa (chapter 2) and Europe, it would be expected that they would have a similar assemblage of herbivores. In North America, species richness of herbivores on *P. australis* was also found to be low compared to Europe (Tewksbury et al. 2002) however, this lack of herbivore specialists has been attributed to the cryptic invasion of genotypes from Europe (Schwarzländer and Häfliger 2000). However,

from chapter 2 it was determined that a cryptic invasion has not occurred and thus this lack of herbivory is unlikely to be linked to plant genotype. Furthermore, with the inclusion of the known endemic species *P. mauritianus* it was possible to compare herbivore assemblages and it was found that the two species share similar species richness and diversity. A study by Weyl (2015) found a similar pattern of herbivory in *Myriophyllum spicatum* L. (Haloragceae) populations in South Africa; *M. spicatum* is believed to be native however was found to have a complete lack of herbivores. Such results raise important questions on the diversification of herbivores on tall-statured grasses in Africa and the plant's evolutionary history in the region.

The literature review found a number of species that were not sampled in the two surveys. There was an overall bias in the literature for studies of pests of cereal crops with wild grasses being hosts (van den Berg and Rebe 2001; Ong'amo et al. 2006). Therefore, most records of herbivores on *Phragmites* spp. in Southern Africa are stem borers, particularly lepidopterans. A total of 13 stem borers on all three reeds were recorded in the literature but were not found in the two surveys. Although this does indicate that continued sampling may determine a higher diversity of stem borers, these surveys have found that the abundance of stem borers in wild hosts is very low and many species do not complete their life cycles on reeds (Le Rü et al. 2006b; Prinsloo and Uys 2015). In recent years, low incident rates have now questioned the role of wild hosts as reservoirs for stem borer pests (Blair 2015). Such results may explain not finding these species during the LTS and NWS. The herbivore lists produced from this pre-introductory survey will likely lend useful information for crop management of cereal crops in South Africa as these lists can provide information on pest occurrence and distribution.

Studies of an invasive plant and its native congeners are advantageous as they can address contributions of prior evolutionary history and post-introduction evolution to invasion (Guo et al. 2014). This study investigated the ERH with site-specific research that included native congeneric species and thus a clearer picture of the role of herbivory on tall-statured grasses in South Africa. The assumptions of the ERH were for the most part not met in this study as although *A. donax* had reduced species richness and diversity compared to the *Phragmites* spp. there has still been some recovery of herbivores due to the long history and multiple introductions in South Africa. The study also provided baseline data for the biological control programme against *A. donax* with the potential biological control agent, *T. romana* being found to be widespread and abundant in South Africa. Lastly, the inclusion of *Phragmites* spp. has given an understanding on the diversification of herbivores on tall-statured grasses in

South Africa; raising important questions on the link between species richness and plant nativity.

5. CHAPTER 5: GENERAL DISCUSSION

Riparian areas in South Africa are 'critical transition zones' for surrounding ecosystems (Ewel et al. 2001; Hoffman and Rohde 2011). However very little is known about these systems and in particular the dominant vegetation that influences their functioning. This thesis intended to improve our understanding of these systems by establishing baseline data on the three dominant tall-statured grasses *P. australis, P. mauritianus* and *A. donax* to investigate their status in terms of both plant origin (chapter 2 and 3) and herbivore pressure (chapter 4).

In this chapter, a discussion of the plant-herbivore interactions on tall-statured grasses and how this is reflected in both plant origin and population genetics is reviewed. Finally, the implications of this research on guiding the management of *A. donax, P. australis* and *P. mauritianus* is explored with particular focus on the potential of biological control on *A. donax.*

5.1.1 Plant origin and herbivory in tall-statured grasses in South Africa

This study offered a unique opportunity to explore the link between plant origin and herbivore assemblage. The plant/herbivore interface has a strong phylogenetic signal; most phytophagous insects have retained associations with the same plant taxa for millions of year, rarely moving onto other taxa (Ehrlich and Raven 1964; Futuyma and Agrawal 2009). Therefore, according to the 'species-time hypothesis', species richness of herbivores on plant species should increase with the length of time the host and potential colonisers are in contact (Strong et al. 1977; Rosenzweig 1995; White et al. 2006). The inclusion of genetic information on an exotic (*A. donax*) (chapter 3), cosmopolitan (*P. australis*) and indigenous (*P. mauritianus*) (chapter 2) plant species proved to be important in understanding how herbivore assemblages have evolved with tall-statured grasses in South Africa. *Arundo donax* was found to have reduced species richness as predicted by the ERH (chapter 4) however with the inclusion of the native *Phragmites* spp., the reduced herbivory does not simply reflect plant origin as an overall low diversity of herbivores was found. Unlike what was predicted, the native reed species have not accumulated diverse herbivore communities as it has done in other areas such as Europe.

Grasses have simple architectonic complexity and high chemical defenses, which has generally resulted in low herbivore load for most grass species (McWilliams 2004; Moran and Goolsby 2009). However, this has generally not been found to be true for tall-statured grasses, with herbivore surveys in native regions finding relatively high species richness of herbivores (Tracy and DeLoach 1998; Tscharntke 1999; Dudley et al. 2008). By including herbivore

survey data from other introduced and native regions (chapter 4) it is clear that South African reed species have considerably lower herbivore diversity. The low herbivore diversity on, *P. australis* and *P. mauritianus* was unexpected and raises many questions on the plant-insect interactions on reeds in riparian areas in South Africa. For this group of plants, the time of establishment is clearly not the driving factor in influencing herbivory and instead must be linked to limits in the geographical range. There are a number of theories that exist to explain depauperate herbivore communities on plant taxa; the most supported being species at the edge of their distribution (Le Corre and Kremer 1998), phylogenetic isolation (Agrawal and Kotanen 2003), chemical defenses and abiotic conditions (Bhattarai 2015; Cronin et al. 2016), with climate being one of the most important factors (Maron and Vila 2001; Reich and Oleksyn 2004; Gilbert et al. 2014).

The distribution of plant taxa is not static and has evolved over time, many plants today have radiated from source populations and are considered at the edge of their distribution (Le Corre and Kremer 1998). The consequence of these historical changes in plant distribution often impact herbivore associations and their diversity (Futuyma and Agrawal 2009). For example, Paterson et al. (2014) assessed herbivore assemblages on *Pereskia aculeata* Mill. (Cactaceae) in the native range and found lower species richness in the northern (Venezuela and Caribbean) and southern (Misiones Province, Argentina) limits compared to the plant's central distribution (Brazil). It is suggested that the Brazilian populations may represent the plant's true origin and thus may explain the higher species richness (Paterson et al. 2014). In this study, both *Phragmites* spp. were found to have only one haplotype across their distribution in South Africa (chapter 2) which is significantly lower diversity compared to other regions. Low haplotype diversity may reflect a possible ancient radiation out from more diverse populations in Europe. For example, northern red oak, *Quercus rubra* L. (Fabaceae) has lower haplotype diversity in areas that are at the edge of its distribution (Birchenko et al. 2009). However, even if South African populations have radiated out from a true origin, records show that *Phragmites* spp. have been established in South Africa since the Holocene period (Scott 1982; Quick 2014) and thus there has been ample time for herbivore associations to form.

Depauperate herbivore communities have often been found to be association with phylogenetic isolation, whereby more closely related plant species generally share more herbivore species than more distantly related plants (Agrawal and Kotanen 2003; Agrawal et al. 2005; Novotný and Basset 2005; Brändle et al. 2008; Gilbert et al. 2012; Dinnage 2013; Castagneyrol et al. 2014). Phylogenetic distance between neighbouring plant species can interfere with the ability of a herbivore to locate, colonise, and exploit the host plant (Yguel et

al. 2011; Le Guigo et al. 2012). For example, *M. spicatum* is believed to have no herbivores in South Africa due to not having phylogenetically related plant species within the same range (Weyl 2015). However, this should not apply to tall-statured grasses in South Africa, as all three species are genetically similar, abundant nation-wide and are known to share a similar assemblage of herbivores.

Another important factor attributed to herbivore associations is latitudinal gradient whereby species interactions are generally stronger and more specialised at lower latitudes promoting greater diversification rates and species richness (Harley 2003; Pennings and Silliman 2005; Schemske et al. 2009). Latitudinal gradient has been found to be important in herbivore richness in *Phragmites*. Cronin et al. (2015) found that native *P. australis* species richness in North America was higher at lower latitudes. Latitudinal differences in herbivory in *P. australis* was found to be related to plant genotype which influenced phenotypic plasticity (Bhattarai 2015; Cronin et al. 2016). The influence of latitude on herbivory has been found in other dominant riparian grass species. For example, *Spartina alterniflora* Loisel (Poaceae) was found to vary in levels of herbivory in leaf-chewing phytophagous species across latitude in the U.S.A. with northern latitudes having more palatable populations but less herbivory in terms of species richness and abundance (Pennings and Silliman 2005). For South African populations however, *Phragmites* spp. latitude is relatively low (about 25-35°) compared to North American (about 30-50°) and European populations (about 35-70°) that were found to have higher herbivory. However, latitudinal gradient may still be playing a role on *Phragmites* spp. herbivore richness along the gradient of the reed's distribution in Africa. With South African populations representing the highest latitude in African populations they may have evolved adaptations that have allowed them to resist herbivory compared to lower latitude populations on the continent.

One of the main differences found at different latitudes for *P. australis* was changes in plant chemical defenses, particularly total phenolics (Bhattarai 2015; Cronin et al. 2016). Different geographical conditions will result in varying environmental conditions that impact plant defense including ultraviolet light, precipitation patterns, CO_2 and temperature (Bidart-Bouzat and Imeh-Nathaniel 2008). The secondary metabolites of a plant involved in plant defense largely drive herbivore associations and host specialisation (Basset 1992; Futuyma and Agrawal 2009). Chemical defenses are known to be high in tall-statured grasses, for example the higher silica content in wild grasses is known to disturb feeding of lepidopteran stem borers such as *S. calamistis* and *E. saccharina* (Epstein 1999; Kvedaras and Keeping 2007). *Arundo donax* has a carbon to nitrogen ratio of 22:1 which is generally too high to be palatable for most

herbivores (Spencer et al. 2007). If South African tall-statured grass genotypes have evolved high chemical defenses, it may be that herbivores are selecting more palatable plant taxa that are available in riparian habitats. Generalist herbivores tend to first select the most palatable plants and then only start switching to less palatable plants once these ones become unavailable (White and Whitham 2000). This interaction is known as the associational resistance theory, and refers to a reduction in herbivory when a plant is associated with floristically complex plant assemblages (Root 1973; Brown and Ewel 1987; Andow 1991). South African riparian areas are known for high diversity of flora and fauna (Cowling and Hilton-Taylor 1997; Rutherford et al. 2006); it is therefore likely that there is a wide variety of more palatable vegetation that herbivores may be utilising. Studies of pests in agricultural crops, most notably sugarcane (*Saccharum* spp.) and sorghum (*S. bicolor* L. (Poaceae)), have found that despite wild grass hosts being widely distributed and abundant, pests are rarely found to utilise them (Van Den Berg and Rebe 2001; Le Rü et al. 2006; Le Rü et al. 2006; Moolman et al. 2014). This may reflect the associational resistance theory (Tahvanainen and Root 1972).

Lastly, climatic conditions will play a major role on herbivore diversity. The literature on herbivore assemblages on *A. donax* and *P. australis* (no previous studies have looked at herbivore assemblages on *P. mauritianus*) has mostly concentrated in the northern hemisphere, particularly in areas where the reeds will die-back in winter months. Growing season temperatures and length are known to play a major role in influencing herbivore assemblages (Maron and Vila 2001; Reich and Oleksyn 2004; Gilbert et al. 2014). The length of the growing season (summer months) will influence plant physiology such as leaf nitrogen (N) and phosphorous (P) (Reich and Oleksyn 2004). Shorter growing seasons have generally resulted in plants having a reduced ability to compensate for herbivory (Maron and Vila 2001). For example, the Platte thistle, *Cirsium canescens* Nutt. (Asteraceae) in areas with a short growing season are less able to tolerate herbivory particular on predation of seeds (Louda and Potvin 1995). In South Africa, it is only in the Free State Province that winters would result in complete suppression of growth of the reeds and thus most plants sampled will be able to maintain growth throughout the year. This may have further led to tall-statured grasses having an increased ability to defend themselves against herbivores and thus maintain relatively low species richness.

5.1.2 Management of *Phragmites* spp. in South Africa

The thesis provided the first list of herbivores associated with *P. australis* and *P. mauritianus* along with an evaluation of their haplotypes and genetic diversity in South Africa

(chapter 2). This baseline data has provided critical insight into *Phragmites* spp. populations that will allow clearer management goals to be set.

Phragmites spp. in South Africa have been increasingly been reported to be expanding in range and abundance in many riparian habitats, this has been particularly true for *P. australis* (Kotschy et al. 2000; Council for Scientific and Industrial Research 2010). This study however has found no evidence of any cryptic invasions of non-native haplotypes (chapter 2). Therefore, unlike in North America the expansion of the reeds can most likely be attributed to anthropogenic activities such as changes to hydrology and pollution (Kotschy et al. 2000; Council for Scientific and Industrial Research 2010; Hoffman and Rohde 2011). In such cases *P. australis* and *P. mauritianus* can be labelled expansive and not invasive (Pyšek et al. 2004).

Phragmites spp. in areas where the reeds are increasing are likely impacting ecosystem services. *Phragmites* spp. are efficient colonisers of disturbed soils and are dominant climax species, as they can form extensive monocultures (Gordon Gray and Ward 1971; Ailstock and Center 2000; Chu et al. 2011). Dense *P. australis* stands can result in a loss of ecosystem services by altering edaphic and trophic conditions; expansive stands can alter nutrient cycling and accumulate leaf litter which causes increased redox potentials, reduced evaporation, suppression of germination from other species and lowering of water levels (Chambers et al. 1999; Windham and Lathrop 1999; Blossey et al. 2002; Mal and Narine 2004; Engloner 2009). In southern Africa, *P. australis* expansion has been attributed to a reduction in biodiversity and water levels (Kotschy et al. 2000; Russell and Kraaij 2008). In the Wilderness Lakes in the Western Cape, for example, *P. australis* expansion was associated with a decline in native plant species such as the matting rush, *Juncus kraussii* Hochst (Juncaceae), *Scirpoides* sp. (Cyperaceae) and low scrub/fynbos (Russell and Kraaij 2008). *Phragmites mauritianus* stands are also expansive in many disturbed habitats in South Africa (Gordon Gray and Ward 1971); although their impacts have not yet been assessed, it is likely that they will have many of the same consequences as *P. australis.*

When *Phragmites* spp. form dense monospecific stands, their impact on riparian health in many cases warrants control efforts. Control efforts should be centred around reducing the size and spread of reeds stands however not eradicating the species as *Phragmites* spp. are native and provide a wide range of benefits to riparian ecosystems (Hellings and Gallagher 1992; Russell and Kraaij 2008). However, the native status of the plants is a complicating factor in terms of management and control options. In evaluating the potential for biological control, ethical boundaries dictate that exotic insects should not be considered for the control of a native plant (McFadyen 1998). Yet biological control has been considered for European *P. australis*

haplotypes in North America by a number of researchers after determining that some herbivores are specific to plant genotype (Schwarzländer and Häfliger 2000; Tewksbury et al. 2002; Häfliger et al. 2005; Blossey 2014). Yet, even in North America where there are known cryptic invasions, biological control is seen as too high risk and is not recommended (Cronin et al. 2016). Therefore, in South Africa with no evidence of any cryptic invasions (chapter 2) biological control should not be explored.

Control methods are therefore restricted to either manual removal or chemical application however current laws in South Africa prohibit (National Environmental Management: Biodiversity Act (NEMBA) (Act No 10 of 2004)) such approaches on native species (Council for Scientific and Industrial Research 2010). With the increasing threat of reed bed expansion, it will be necessary to address these restrictions and seek approvals with the responsible government departments and authorities (Department of Agriculture, Forestry and Fisheries (DAFF) and the Department of Environmental Affairs (DEA)) to begin allowing control efforts to take place. Another possible route to explore is in utilisation of these reeds to control populations. For example, many reed beds are under control in the KwaZulu-Natal Province due to community harvesting of reeds (Van Rooyen et al. 2004). Lastly, controlling the reeds will also necessitate addressing the major anthropogenic factors that are disrupting the health of riparian ecosystems most notably pollution, eutrophication and water flow.

Both this thesis and global phylogeographic studies of *Phragmites* have revealed the complexity of the genus and have shown that populations are never static. It is important to acknowledge that the populations determined in this study are likely to change in the future. Firstly, the study did not find non-native haplotypes in South Africa however this only reflects the current situation and in time there is always potential for introduction of new haplotypes that may pose invasive risks. Secondly, the abundance and distribution of *Phragmites* spp. is also likely to change with future climate change scenarios, it is generally agreed that climate change will have a positive feedback impact on *Phragmites* spp. (Caplan et al. 2014; Eller et al. 2014a; Meyerson et al. 2016). Many of the predicted impacts of climate change, most notably increased atmospheric CO_2 levels and the intrusion of saltwater into inland coastal areas, are expected to favour reed growth (Eller et al. 2014a, 2014b). *Phragmites australis* and *P. mauritianus* will differ in their tolerances and adaptability due to varying genotypes and thus we can expect changes to each species' distributions.

Lastly, the discovery that hybridisation has occurred between *P. australis* and *P. mauritianus* has important implications to riparian systems. One of the most important questions that arises from hybridisation is in the ecological ability of the progeny and in

particular their reproductive capacity and ability to adapt (Meyerson et al. 2012). There are a number of cases of hydrophyte hybrids having extreme vegetative vigour allowing them to compete or even displace parental species (e.g. *Eleocharis* and *Ranunculus*) (Les and Philbrick 1993). In such cases, the novel genotypes created can have higher fitness such as being able to deter pathogens and herbivores better than parental genotypes (Gaskin and Schaal 2002). Furthermore, hybrids can present challenges to biological control efforts as a recent evolutionary history may interfere with finding host specific biological control agents (Gaskin and Schaal 2002). Future research will be important in understanding these hybrid populations in terms of their reproductive ability, adaptability and colonising success as this gives important insight into how these hybrids may compete with *P. australis* and *P. mauritianus* stands.

5.1.3 Control against *A. donax* in South African riparian ecosystems

This study has served as a pre-introductory survey for the biological control of *A. donax* in South Africa. Establishing baseline data before a programme is initiated may offer an opportunity to improve results. The success of a biological control agent depends on a wide range of factors including climate suitability, mode of damage, genotype matching and release efforts (McFadyen 1998; Gurr and Wratten 2000; Paterson et al. 2014). Although it is impossible to predict how each factor may influence biological control, having as much information as possible will assist in making informed decisions.

The determination of the origin and genetic diversity of *A. donax* (chapter 3) has provided key information in guiding the direction of biological control. The first step to a classical biological control programme is to select agents from the plant's native range (McFadyen 1998). The study determined that *A. donax* populations belong to haplotype M1 which is known to have an ancient origin in Afghanistan and Pakistan in the Indus Valley and is now found worldwide, including North America (Hardion et al. 2014b). From this it is now possible to explore research that has already been carried out in the native range of haplotype M1. Extensive studies have been conducted to find suitable biological control agents from the native range to introduce to North America (Tracy and DeLoach 1998; Dudley and Lambert 2007; Goolsby and Moran 2009; Goolsby et al. 2009a). Much of this research will be applicable to South African populations due to a shared haplotype and thus utilising this data will save both resources on time.

Performing herbivore surveys on an IAS prior to the initiation of a biological control programme has rarely been done in the past (Dudley et al. 2008; Milbrath 2010). This thesis

has highlighted the advantageous of such an approach with the discovery of an established biological control agent, estimations of herbivore pressure and the identification of plant niches that should be targeted (chapter 4).

The study identified an established biological control agent, the galling wasp, *T. romana* and thus provided an opportunity to assess the biological control agent under natural conditions. By evaluating *T. romana* populations on *A. donax* it was found that the wasps are widespread (found in all regions) and relatively abundant (present on 41 % of sampled plants). From this, there is potential to initiate augmentative biological control and increase *T. romana* populations. However, such an approach needs careful consideration before using valuable time and resources. Firstly, a quantitative study of the impact of *T. romana* in South Africa will be beneficial in determining the level of pressure that the agents are already having on *A. donax* populations. From this, it may be that herbivory from *T. romana* has already reduced the invasive potential of *A. donax* and without these agents the plants would have posed an even greater threat to riparian areas. Secondly, it will be important to consider research from North America as augmentative biological control using *T. romana* has already been initiated there and thus lessons can be learnt from this programme, particularly in assessing if any success has come of such an approach.

The study determined that *A. donax* is under pressure from three major feeding guilds however none of these herbivores were found to feed on the plant's rhizomes. *Arundo donax* relies solely on vegetative growth in South Africa (Guthrie 2007) and thus targeting this niche may prove critical in impacting populations. Learning from work done in North America, there is potential of a rhizome feeding herbivore, *R. donacis* which has undergone host specificity testing and has since been released in North America (Goolsby et al. 2009a; Moran and Goolsby 2010; Cortes et al. 2011). This agent has been imported to South Africa to the ARC-PPRI facilities for host specificity testing (Dr. Angela Bownes, ARC-PPRI, pers. comm.). In light of the results from this study, if released, this biological control agent will provide pressure to a new niche on *A. donax* in South Africa and thus may begin to impact plant spread and abundance.

Exploring *A. donax* herbivore pressure in South Africa has also opened debate on the mechanisms behind the reed's invasive potential. According to the ERH, a loss of herbivory is one of the main factors that leads to plant's becoming invasive in the introduced range (Keane and Crawley 2002; Prior and Hellmann 2013; Heger and Jeschke 2014). For *A. donax* in South Africa, populations were found to have a lower species diversity compared to *Phragmites* spp. however still had pressure from the same feeding guilds and have abundant and widely

distributed specialists established. With a plant that is as biologically productive as *A. donax,* there is the potential that top-down regulation from herbivory is not the primary factor determining invasive success but instead bottom-up factors such as nutrient levels may play more of a role. *Arundo donax* is known to thrive in disturbed habitats where bottom-up factors such as nutrient load largely control invasive success (Lambert et al. 2014). From this, it has been found that to control *A. donax* it is necessary to adopt an integrated management approach that will include both biological control and also address the abiotic factors that are driving invasion (Oakins 2001). Biological control must still be implemented as an integral component of *A. donax* management as even if top-down pressure is not the primary driver of invasive success; biological control agents will still add a needed pressure to plant resources and thus play a role in shaping population spread and abundance.

5.1.4 Conclusions

In the past, determining the status of a species in a region based on its plant origin was often based on poor data, inappropriate criteria, or intuition (Pyšek 1995). The development of genetic techniques has vastly improved our understanding of plant origin and how plant genotypes are distributed. With this it is now possible to assess theories such as the ERH that are based on understanding plant origin, more accurately.

This thesis has demonstrated the benefits of applying genetic techniques to determining the status of tall-statured grasses in riparian ecosystems and further using this to better under plant-insect interactions. The management of riparian ecosystems in South Africa must be based on sound baseline studies. It is only then that goals can be set to both control against IAS, and protect native flora and fauna. Lastly, pre-introductory surveys can serve as an important tool in guiding future biological control programmes to ensure the most effective use of resources.

6. REFERENCES

Achenbach L, Lambertini C, Brix H. 2012. Phenotypic traits of *Phragmites australis* clones are not related to ploidy level and distribution range. *AoB PLANTS* 2012: pls017.

Adams JB, Bate GC. 1999. Growth and photosynthetic performance of *Phragmites australis* in estuarine waters: a field and experimental evaluation. *Aquatic Botany* 64: 359–367.

Agrawal AA, Kotanen PM. 2003. Herbivores and the success of exotic plants: a phylogenetically controlled experiment. *Ecology Letters* 6: 712–715.

Agrawal AA, Kotanen PM, Mitchell CE, Power AG, Godsoe W, Klironomos J. 2005. Enemy release? An experiment with congeneric plant pairs and diverse above- and belowground enemies. *Ecology* 86: 2979–2989.

Ahmad R, Liow P, Spencer DF, Jasieniuk M. 2008. Molecular evidence for a single genetic clone of invasive *Arundo donax* in the United States. *Aquatic Botany* 88: 113–120.

Ailstock MS, Center E. 2000. Adaptive strategies of common reed *Phragmites australis*. In: *Proceedings: The Role of Phragmites in the Mid-Atlantic Region.* Richmond, Virginia. pp. 1–7.

Allen WJ, Young RE, Bhattarai GP, Croy JR, Lambert AM, Meyerson LA, Cronin JT. 2015. Multitrophic enemy escape of invasive *Phragmites australis* and its introduced herbivores in North America. *Biological Invasions* 17: 3419–3432.

An J, Wang Q, Yang J, Liu J. 2012. Phylogeographic analyses of *Phragmites australis* in China: Native distribution and habitat preference of the haplotype that invaded North America. *Journal of Systematics and Evolution* 50: 334–340.

Andow DA. 1991. Vegetational diversity and arthropod population response. *Annual Review of Entomology* 36: 561–566.

Andrew NR, Hughes L. 2005. Herbivore damage along a latitudinal gradient: relative impacts of different feeding guilds. *Oikos* 108: 176–182.

Angelini LG, Ceccarini L, Bonari E. 2005. Biomass yield and energy balance of giant reed (*Arundo donax* L.) cropped in central Italy as related to different management practices. *European Journal of Agronomy* 22: 375–389.

Armstrong J, Armstrong W. 1988. *Phragmities australis*- a preliminary study of soil-oxidizing sites and internal gas transport pathways. *New Phytologist* 108: 373–382.

Armstrong J, Armstrong W, Beckett PM. 1992. *Phragmites australis*: Venturi- and humidity-induced pressure flows enhance rhizome aeration and rhizosphere oxidation. *New Phytologist* 120: 197–207.

Armstrong J, Armstrong W, Beckett PM, Halder JE, Lythe S, Holt R, Sinclair A. 1996. Pathways of aeration and the mechanisms and beneficial effects of humidity- and Venturi-induced convections in *Phragmites australis* (Cav.) Trin. ex Steud. *Aquatic Botany* 54: 177–197.

Assefa Y, Conlong D, van den Berg J, Le Rü B. 2008. The wider distribution of *Eldana saccharina* (Lepidoptera: Pyralidae) in South Africa and its potential risk to maize production. In: *Proceedings of the Annual Congress - South African Sugar Technologists' Association*. pp. 290–297.

Baker HHG. 1967. Support for Baker's law-as a rule. *Evolution* 21: 853–856.

Balogh E, Herr JM, Czakó M, Márton L. 2012. Defective development of male and female gametophytes in *Arundo donax* L.(Poaceae). *Biomass and Bioenergy* 45: 265–269.

Barker NP, Linder HP, Harley EH. 1995. Polyphyly of Arundinoideae (Poaceae): evidence from rbcL sequence data. *Systematic Botany*: 423–435.

Barker NP, Linder HP, Harley EH. 1998. Sequences of the grass-specific insert in the chloroplast rpoC2 gene elucidate generic relationships of the Arundinoideae (Poaceae).

Systematic Botany: 327–350.
Barling RD, Moore ID. 1994. Role of buffer strips in management of waterway pollution: A review. *Environmental Management* 18: 543–558.
Barret SCH, Kohn JR. 1991. Genetic and evolutionary consequences of small population size in plants; implications for conservation. In: Falk D, Holsinger K (eds), *Genetics and conservation of rare plants*. New York: Oxford University Press. pp. 3–30.
Basset Y. 1992. Host specificity of arboreal and free-living insect herbivores in rain forests. *Biological Journal of the Linnean Society* 47: 115–133.
Bautista S. 1998. A comparison of two methods of controlling *Arundo donax*. In: Bell C (ed), *Arundo and Saltcedar: the deadly duo: Proceedings of a workshop on combating the threat from Arundo and Saltcedar*. Ontario, CA: University of California, Cooperative Extension. pp. 49–52.
Beckley LE. 1984. The ichthyofauna of the Sundays Estuary, South Africa, with particular reference to the juvenile marine component. *Estuaries* 7: 248–258.
Bell GP. 1997. Ecology and management of *Arundo donax*, and approaches to riparian habitat restoration in southern California. In: Brock J, Wade M, Pyšek P, Green D (eds), *Plant invasions: studies from North America and Europe*. Leiden, The Netherlands: Backhuys Publishers. pp. 103–113.
Bernays E, Graham M. 1988. On the evolution of host specificity in phytophagous arthropods. *Ecology* 69: 886–892.
Bernays EA. 1998. Evolution of feeding behavior in insect herbivores. *Bioscience*: 35–44.
Bhattarai GP. 2015. PhD Thesis: Biogeographical approaches for studying species invasion. Louisiana State University.
Bidart-Bouzat MG, Imeh-Nathaniel A. 2008. Global change effects on plant chemical defenses against insect herbivores. *Journal of Integrative Plant Biology* 50: 1339–1354.
Birchenko I, Feng Y, Romero-Severson J. 2009. Biogeographical distribution of chloroplast diversity in Northern Red Oak (*Quercus rubra* L.). *The American Midland Naturalist* 161: 134–145.
Blackman RL. 2007. Aphids on the world's herbaceous plants and shrubs. Vol. 1: Host lists and keys; Vol. 2: The aphids. *Entomologia Experimentalis et Applicata* 125: 325–325.
Blackman RL, Eastop VF. 2008. *Aphids on the world's herbaceous plants and shrubs*. London: John Wiley & Sons.
Blackmore S, Dudley CO, Osborne PL. 1988. An annotated check-list of the aquatic macrophytes of the Shire River, Malawi, with reference to potential aquatic weeds. *Kirkia* 13: 125–142.
Blossey B. 2003. Morphological differences between native North American *Phragmites australis* genotypes and introduced invasive European genotypes. *Estuaries and Coasts* 26: 47–62.
Blossey B. 2014. *Identification, development, and release of insect biocontrol agents for the management of Phragmites australis*. Publication CR-14-2. The US Army Engineer Research and Development Center (ERDC), Department of Natural Resoures, Cornell University.
Blossey B, Schwarzländer M, Häfliger P, Casagrande R, Tewksbury L. 2002. *Common reed*. Publication FHTET-2002-04.USDA Forest Service, Morgantown, West Virgini.
Boland JM. 2006. The importance of layering in the rapid spread of *Arundo donax* (giant reed). *Madrono* 53(4): 303-312.
Bonanno G, Giudice R. 2010. Heavy metal bioaccumulation by the organs of *Phragmites australis* (common reed) and their potential use as contamination indicators. *Ecological Indicators* 10: 639–645.
Bonin A, Bellemain E, Bronken Eidesen P, Pompanon F, Brochmann C, Taberlet P. 2004. How

to track and assess genotyping errors in population genetics studies. *Molecular ecology* 13: 3261–3273.
Borin M, Milani M, Salvato M, Toscano A. 2011. Evaluation of *Phragmites australis* (Cav.) Trin. evapotranspiration in northern and southern Italy. *Ecological Engineering* 37: 721–728.
Brändle M, Kühn I, Klotz S, Belle C, Brandl R. 2008. Species richness of herbivores on exotic host plants increases with time since introduction of the host. *Diversity and Distributions* 14: 905–912.
Brix H, Sorrel BK, Orr PT. 1992. Internal pressurization and convective gas flow in some emergent. *Association for the Sciences of Limnology and Oceanography* 37: 1420–1433.
Brown BJ, Ewel JJ. 1987. Herbivory in complex and simple tropical successional ecosystems. *Ecology* 68: 108–116.
Brown WL. 1983. Genetic diversity and genetic vulnerability-an appraisal. *Economic Botany* 37: 4–12.
Burdon JJ, Marshall DR. 1981. Biological control and the reproductive mode of weeds. *Journal of Applied Ecology* 18: 649–658.
Bush GL. 1969. Sympatric host race formation and speciation in frugivorous flies of the genus *Rhagoletis* (Diptera, Tephritidae). *Evolution* 23: 237–251.
Butler DR. 1995. *Zoogeomorphology*. Cambridge: Cambridge Univ Press.
Calheiros CSC, Quitério PVB, Silva G, Crispim LFC, Brix H, Moura SC, Castro PML. 2012. Use of constructed wetland systems with *Arundo* and *Sarcocornia* for polishing high salinity tannery wastewater. *Journal of environmental management* 95: 66–71.
Cantaluppi E, Cassani E, Puglisi D, Corno L, Munaro M, Landoni M, Adani F, Pilu R. 2016. Study on the inflorescences of *Arundo donax* L. clones sampled in Italy. *Brazilian Journal of Botany* 39(1): 275-285.
Caplan JS, Wheaton CN, Mozdzer TJ. 2014. Belowground advantages in construction cost facilitate a cryptic plant invasion. *AoB PLANTS* 6: plu020.
Castagneyrol B, Jactel H, Vacher C, Brockerhoff EG, Koricheva J. 2014. Effects of plant phylogenetic diversity on herbivory depend on herbivore specialization. *Journal of Applied Ecology* 51: 134–141.
Chambers RM, Meyerson LA, Dibble K. 2012. Ecology of *Phragmites australis* and responses to tidal restoration. In: *Tidal marsh restoration*. Washington, DC: Island Press - Center for Resource Economics. pp. 81–96.
Chambers RM, Meyerson LA, Saltonstall K. 1999. Expansion of *Phragmites australis* into tidal wetlands of North America. *Aquatic Botany* 64: 261–273.
Chambers RM, Mozdzer TJ, Ambrose JC. 1998. Effects of salinity and sulfide on the distribution of *Phragmites australis* and *Spartina alterniflora* in a tidal saltmarsh. *Aquatic Botany* 62: 161–169.
Chao A, Colwell RK, Lin C, Gotelli NJ. 2009. Sufficient sampling for asymptotic minimum species richness estimators. *Ecology* 90: 1125–1133.
Chase MW, Hills HH. 1991. Silica gel: an ideal material for field preservation of leaf samples for DNA studies. *Taxon*: 215–220.
Chazdon RL, Colwell RK, Denslow JS, Guariguata MR. 1998. Statistical methods for estimating species richness of woody regeneration in primary and secondary rain forests of northeastern Costa Rica. In: Dallmeier F, Comiskey J (eds), *Forest biodiversity research, monitoring and modeling: conceptual background and old world case studies.* Indonesia: Center for International Forestry Research. pp. 285–309.
Cheatham S, Johnston MC, Marshall L. 1995. *The useful wild plants of Texas: the southeastern and southwestern United States, the Southern plains and northern Mexico*. Austin, U.S.A.: Useful Wild Plants Inc.

Chippindall LKA. 1955. A guide to the identification of grasses in South Africa. In: Meredith D (ed), *The Grasses and Pastures of South Africa*. Johannesburg: Central News Agency.
Chu H, Cho WK, Jo Y, Kim W, Rim Y, Kim J. 2011. Identification of natural hybrids in Korean *Phragmites* using haplotype and genotype analyses. *Plant Systematics and Evolution* 293: 247–253.
Clayton WD. 1968. The correct name of the common reed. *Taxon* 17: 168–169.
Clayton WD, Vorontsova M, Harman K, Williamson H. 2006. GrassBase - The online world grass flora. [Online] Available at: http://www.kew.org/data/grasses-db/www/imp01044.htm. Accessed on: 2016-05-17.
Clevering OA, Lissner J. 1999. Taxonomy, chromosome numbers, clonal diversity and population dynamics of *Phragmites australis*. *Aquatic Botany* 64: 185–208.
Coetzee JC, Adams JB, Bate GC. 1996. A botanical importance rating system for estuaries. *Journal of Coastal Conservation* 2: 131–138.
Coffman GC. 2007. PhD Thesis: Factors influencing invasion of Giant Reed (*Arundo donax*) in riparian ecosystems of mediterranean-type climate regions. University of California.
Coffman GC, Ambrose RF, Rundel PW. 2010. Wildfire promotes dominance of invasive giant reed (*Arundo donax*) in riparian ecosystems. *Biological Invasions* 12: 2723–2734.
Colwell RK. 2009. EstimateS: statistical estimation of species richness and shared species from samples. Version 8.2.
Conert H. 1961. *Die Systematik und Anatomie der Arundineae*. Frankfort: Cramer.
Connor EF, Faeth SH, Simberloff D, Opler PA. 1980. Taxonomic isolation and the accumulation of herbivorous insects: a comparison of introduced and native trees. *Ecological Entomology* 5: 205–211.
Cortes E, Goolsby JA, Moran PJ, Marcos-García MA. 2011. The effect of the armored scale, *Rhizaspidiotus donacis* (Hemiptera: Diaspididae), on shoot growth of the invasive plant *Arundo donax* (Poaceae: Arundinoideae). *Biocontrol Science and Technology* 21: 535–545.
Cortesero AM, Stapel JO, Lewis WJ. 2000. Understanding and manipulating plant attributes to enhance biological control. *Biological control* 17: 35–49.
Cosentino SL, Copani V, D'Agosta GM, Sanzone E, Mantineo M. 2006. First results on evaluation of *Arundo donax* L. clones collected in Southern Italy. *Industrial Crops and Products* 23: 212–222.
Council for Scientific and Industrial Research. 2010. *Verlorenvlei (Version 1)*. CSIR Report CSIR/NRE/CO/ER/2010/0066/B. Estuary Management Plan, CSIR, Stellenbosch.
Cowling RM, Hilton-Taylor C. 1997. Phytogeography, flora and endemism. In: Cowling RM, Richardson DM, Pierce SM (eds), *Vegetation of Southern Africa*. Cambridge: Cambridge Univ Press. pp. 43–56.
Cripps MG, Schwarzlander M, McKenney JL, Hariet HL, Price WJ. 2006. Biogeographical comparison of the arthropod herbivore communities associated with *Lepidium draba* in its native, expanded and introduced ranges. *Journal of Biogeography* 33: 2107–2119.
Cronin JT, Bhattarai GP, Allen WJ, Meyerson LA. 2015. Biogeography of a plant invasion: plant–herbivore interactions. *Ecology* 96: 1115–1127.
Cronin JT, Kiviat E, Meyerson LA, Bhattarai GP, Allen WJ. 2016. Biological control of invasive *Phragmites australis* will be detrimental to native *P. australis*. *Biological Invasions*: 1–4.
Cyrus DP, Martin TJ, Reavell PE. 1997. Salt-water intrusion from the Mzingazi River and its effects on adjacent swamp forest at Richards Bay, Zululand, South Africa. *Water South Africa* 23: 101–108.
Danin A. 2004. *Arundo* (Gramineae) in the Mediterranean reconsidered. *Willdenowia* 34: 361–369.

Danin A, Raus T, Scholz H. 2002. Contribution to the flora of Greece: a new species of *Arundo* (Poaceae). *Willdenowia* 32: 191–194.
DeWalt SJ, Denslow JS, Ickes K. 2004. Natural-enemy release facilitates habitat expansion of the invasive tropical shrub *Clidemia hirta*. *Ecology* 85: 471–483.
Dinnage R. 2013. Phylogenetic diversity of plants alters the effect of species richness on invertebrate herbivory. *PeerJ* 1: e93.
Dlugosch KM, Parker IM. 2008. Founding events in species invasions: Genetic variation, adaptive evolution, and the role of multiple introductions. *Molecular Ecology* 17: 431–449.
Dogan Y, Nedelcheva AM, Obratov-Petković D, Padure IM. 2008. Plants used in traditional handicrafts in several Balkan countries. *Indian Journal of Traditional Knowledge* 7: 157–161.
Dostal P, Allan E, Dawson W, Kleunen M, Bartish I, Fischer M. 2013. Enemy damage of exotic plant species is similar to that of natives and increases with productivity. *Journal of Ecology* 101: 388–399.
Dudley TL. 2000. *Arundo donax* L. In: Bossard CC, Randall JM, Hoshovsky MC (eds), *Invasive plants of California's wildlands*. California: University of California Press. pp. 53–58.
Dudley TL, Lambert AM. 2007. *Biological control of invasive giant reed (Arundo donax)*. Completion Report, U.S. Fish & Wildlife Service/Santa Clara River Trustee Council, California.
Dudley TL, Lambert AM, Kirk A. 2006. Augmentation biological control of *Arundo donax*. In: *California Conference on Biological Control V*. California: Marine Science Institute.
Dudley TL, Lambert AM, Kirk A, Tamagawa Y. 2008. Herbivores associated with *Arundo donax* in California. In: Julien M, Sforza R, Bon M, Evans H, Hatcher P, Hinz H, Rector B (eds), *Proceedings of the XII International Symposium on Biological Control of Weeds*. La Grand Motte, France: CABI. pp. 138–145.
Dwire KA, Kauffman JB. 2003. Fire and riparian ecosystems in landscapes of the western USA. *Forest Ecology and Management* 178: 61-74.
Earl DA, VonHoldt BM. 2012. STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources* 4: 359–361.
Ehrlich PR, Raven PH. 1964. Butterflies and plants: a study in coevolution. *Evolution* 18: 586–608.
Eller F, Lambertini C, Nguyen LX, Brix H. 2014a. Increased invasive potential of non-native *Phragmites australis*: elevated CO2 and temperature alleviate salinity effects on photosynthesis and growth. *Global Change Biology* 20: 531–543.
Eller F, Lambertini C, Nielsen MW, Radutoiu S, Brix H. 2014b. Expression of major photosynthetic and salt-resistance genes in invasive reed lineages grown under elevated CO2 and temperature. *Ecology and evolution* 4: 4161–4172.
Ellstrand N, Schierenbeck K. 2006. Hybridization as a stimulus for the evolution of invasiveness in plants? *Euphytica* 148: 35–46.
Else JA. 1996. M.S. Thesis: Post-flood establishment of native woody species and an exotic, *Arundo donax,* in a southern Californian riparian system. San Diego State University.
Engloner AI. 2009. Structure, growth dynamics and biomass of reed (*Phragmites australis*)–A review. *Flora-Morphology, Distribution, Functional Ecology of Plants* 204: 331–346.
Epstein E. 1999. Silicon. *Annual review of plant physiology and plant molecular biology* 50: 641–664.
Evanno G, Regnaut S, Goudet J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular ecology* 14: 2611–2620.

Ewel KC, Cressa C, Kneib RT, Lake PS, Levin LA, Palmer MA, Snelgrove P, Wall DH. 2001. Managing critical transition zones. *Ecosystems* 4: 452–460.
Faiella G. 2006. *The technology of Mesopotamia*. New York: The Rosen Publishing Group.
Fallour D, Fady B, Lefevre F. 1997. Study on isozyme variation in Pinus pinea L.: evidence for low polymorphism. *Silvae Genetica* 46: 201–207.
Fanshawe DB. 1972. The biology of the reed - *Phragmites mauritianus* Kunth. *Kirkia*: 147–150.
Fish L, Mashau A, Moeaha M, Nembudani M. 2015. *Identification guide to southern African grasses. An identification manual with keys, descriptions and distributions.* Pretoria: South African National Biodiversity Institute.
Flores A, Wood M. 2009. Biocontrol battle begins against Giant Reed (*Arundo*). *Agricultural Research* 57: 12–14.
Fogli S, Marchesini R, Gerdol R. 2002. Reed (*Phragmites australis*) decline in a brackish wetland in Italy. *Marine Environmental Research* 53: 465–479.
Frenzel M, Brandl R. 2003. Diversity and abundance patterns of phytophagous insect communities on alien and native host plants in the Brassicaceae. *Ecography* 26: 723–730.
Fritz R, Simms E. 1992. Plant resistance to herbivores and pathogens: ecology, evolution, and genetics. *Science* 259: 1631–1632.
Futuyma DJ, Agrawal AA. 2009. Macroevolution and the biological diversity of plants and herbivores. *Proceedings of the National Academy of Sciences of the United States of America* 106: 18054–18061.
Galatowitsch SM, Larson DL, Larson JL. 2016. Factors affecting post-control reinvasion by seed of an invasive species, *Phragmites australis,* in the central Platte River, Nebraska. *Biological Invasions* 18(9): 2505-2516.
Gao L, Tang S, Zhuge L, Nie M, Zhu Z, Li B, Yang J. 2012. Spatial genetic structure in natural populations of *Phragmites australis* in a mosaic of saline habitats in the yellow river delta, China. *PLoS ONE* 7: e43334.
Garner RD. 2005. PhD Thesis: Vegetation response to clearing of exotic invasive plants along the Sabie River, South Africa. University of Witswatersrand, Johannesburg.
Gaskin JF, Schaal BA. 2002. Hybrid *Tamarix* widespread in US invasion and undetected in native Asian range. *Proceedings of the National Academy of Sciences* 99:11256-11259.
Gaskin JF, Bon MC, Cock MJW, Cristofaro M, De Biase A, De Clerck-Floate R, Ellison CA, Hinz HL, Hufbauer RA, Julien MH, Sforza R. 2011. Applying molecular-based approaches to classical biological control of weeds. *Biological Control* 58: 1–21.
Gautam RD, Suroshe S, Mahapatro GK. 2009. Unique record of chloropid pest in rice (*Oryza sativa*). *Indian Journal of Agricultural Sciences* 79: 841–843.
Getu E, Overholt WA, Kairu E. 2001. Distribution and species composition of stemborers and their natural enemies in maize and sorghum in Ethiopia. *International Journal of Tropical Insect Science* 21: 353–359.
Gilbert B, Tunney TD, Mccann KS, Delong JP, Vasseur DA, Savage V, Shurin JB, Dell AI, Barton BT, Harley CDG, Kharouba HM, Kratina P, Blanchard JL, Clements C, Winder M, Greig HS, O'Connor MI. 2014. A bioenergetic framework for the temperature dependence of trophic interactions. *Ecology Letters* 17: 902–914.
Gilbert GS, Magarey R, Suiter K, Webb CO. 2012. Evolutionary tools for phytosanitary risk analysis: phylogenetic signal as a predictor of host range of plant pests and pathogens. *Evolutionary Applications* 5: 869–878.
Godt MJW, Walker J, Hamrick JL. 1997. Genetic diversity in the endangered lily *Harperocallis flava* and a close relative *Tofieldia racemosa*. *Conservation Biology* 11: 361–366.
Going BM, Dudley TL. 2008. Invasive riparian plant litter alters aquatic insect growth. *Biological Invasions* 10(7): 1041-1051.

Goolsby JA, De Barro PJ, Makinson JR, Pemberton RW, Hartley DM, Frohlich DR. 2006. Matching the origin of an invasive weed for selection of a herbivore haplotype for a biological control programme. *Molecular Ecology* 15: 287–297.

Goolsby JA, Kirk AA, Moran PJ, Racelis AE, Adamczyk JJ, Cortes E, Marcos MA, Jimenez MM, Summy KR, Ciomperlik MA, Sands DPA. 2011. Establishment of the Armored Scale, *Rhizaspidiotus donacis*, a biological control agent of *Arundo donax*. *Southwestern Entomologist* 36: 373–374.

Goolsby JA, Moran P. 2009. Host range of *Tetramesa romana* Walker (Hymenoptera: Eurytomidae), a potential biological control of giant reed, *Arundo donax* L. in North America. *Biological Control* 49: 160–168.

Goolsby JA, Moran PJ, Adamczyk JJ, Kirk AA, Jones WA, Marcos MA, Cortés E. 2009a. Host range of the European, rhizome-stem feeding scale *Rhizaspidiotus donacis* (Hemiptera: Diaspididae), a candidate biological control agent for giant reed, *Arundo donax* (Poales: Poaceae) in North America. *Biocontrol Science and Technology* 19: 899–918.

Goolsby JA, Spencer D, Whitehand L. 2009b. Pre-release assessment of impact on *Arundo donax* by the candidate biological control agents *Tetramesa romana* (Hymenoptera: Eurytomidae) and Rhizaspidiotus donacis (Hemiptera: Diaspididae) under quarantine conditions. *Southwestern Entomologist* 34: 359–376.

Gordon Gray KD, Ward CJ. 1971. A contribution to knowledge of *Phragmites* (Gramineae) in South Africa, with particular reference to Natal populations. *South African Journal of Botany* 37: 1–30.

Gowda PH, Goolsby JA, Yang C, Basu S, Racelis A, Howell TA. 2011. Estimating water use by Giant Reed along the Rio Grande using a large aperature scintillometer. *Subtropical Plant Science* 63: 1–6.2011.

Gregory S V., Swanson FJ, McKee WA, Cummins KW. 1991. An ecosystem perspective of riparian zones. *BioScience* 41: 540–551.

Greuter W, Scholz H. 1996. *Phragmites* in Crete, *Cenchrus frutescens*, and the nomenclature of the common reed (Gramineae). *Taxon* 45: 521–523.

Groves RH, Di Castri F. 1991. *Biogeography of Mediterranean invasions*. Cambridge: Cambridge University Press.

Guo W, Lambertini C, Li X, Meyerson LA, Brix H. 2013. Invasion of Old World *Phragmites australis* in the New World: precipitation and temperature patterns combined with human influences redesign the invasive niche. *Global Change Biology* 19: 3406–3422.

Guo W, Lambertini C, Nguyen LX, Li X, Brix H. 2014. Preadaptation and post-introduction evolution facilitate the invasion of *Phragmites australis* in North America. *Ecology and Evolution* 4: 4567–4577.

Gurr G, Wratten S. 2000. *Biological control: measures of success*. Netherlands: Springer.

Guthrie G. 2007. M.S. Thesis: Impacts of the invasive reed *Arundo donax* on biodiversity at the community-ecosystem level. University of the Western Cape.

Haddadchi A, Gross CL, Fatemi M. 2013. The expansion of sterile *Arundo donax* (Poaceae) in southeastern Australia is accompanied by genotypic variation. *Aquatic Botany* 104: 153–161.

Häfliger P, Schwarzlaender M, Blossey B. 2005. Biology of *Platycephala planifrons* (Diptera: Chloropidae) and its potential effectiveness as biological control agent for invasive *Phragmites australis* in North America. *Biological Control* 34: 302–311.

Hansen RM. 1978. Shasta ground sloth food habits, Rampart Cave, Arizona. *Paleontological Society* 4: 302–319.

Hansson PA, Fredriksson H. 2004. Use of summer harvested common reed (*Phragmites australis*) as nutrient source for organic crop production in Sweden. *Agriculture, Ecosystems and Environment* 102: 365–375.

Hardion L, Baumel A, Verlaque R, Vila B. 2014a. Distinct evolutionary histories of lowland biota on Italian and Balkan peninsulas revealed by the phylogeography of *Arundo plinii* (Poaceae). *Journal of Biogeography* 41: 2150–2161.
Hardion L, Verlaque R, Baumel A, Juin M, Vila B. 2012a. Revised systematics of Mediterranean *Arundo* (Poaceae) based on AFLP fingerprints and morphology. *Taxon* 61: 1217–1226.
Hardion L, Verlaque R, Callmander MW, Vila B. 2012b. *Arundo micrantha* Lam. (Poaceae), the correct name for *Arundo mauritanica* Desf. and *Arundo mediterranea* Danin. *Candollea* 67: 131–135.
Hardion L, Verlaque R, Saltonstall K, Leriche A, Vila B. 2014b. Origin of the invasive *Arundo donax* (Poaceae): a trans-Asian expedition in herbaria. *Annals of Botany* 114: 455–462.
Harley CDG. 2003. Abiotic stress and herbivory inteact to set range limits across a two-dimensional stress gradient. *Ecology* 84: 1477–1488.
Hart RC. 1995. South African coastal lakes. *Wetlands of South Africa*: 103–130.
Haslam SM. 1972. *Phragmites communis* Trin.(*Arundo phragmites* L.,? *Phragmites australis* (Cav.) Trin. ex Steudel). *The Journal of Ecology* 60: 585–610.
Hauber DP, Saltonstall K, White DA, Hood CS. 2011. Genetic variation in the common reed, *Phragmites australis*, in the Mississippi River Delta marshes: evidence for multiple introductions. *Estuaries and Coasts* 34: 851–862.
Hazelton ELG, Mozdzer TJ, Burdick DM, Kettenring KM, Whigham DF. 2014. *Phragmites australis* management in the United States: 40 years of methods and outcomes. *Aob Plants* 6: plu001.
Heger T, Jeschke JM. 2014. The enemy release hypothesis as a hierarchy of hypotheses. *Oikos* 123: 741–750.
Heleno RH, Ceia RS, Ramos JA, Memmott J. 2009. Effects of alien plants on insect abundance and biomass: a food-web approach. *Conservation Biology* 23: 410–419.
Hellings SE, Gallagher JL. 1992. The effects of salinity and flooding on *Phragmites australis*. *Journal of Applied Ecology* 29: 41–49.
Henderson L. 2001. *Alien Weeds and Invasive Plants. A complete guide to declared weeds and invaders in South Africa*. Pretoria: Agricultural Research Council, Plant Protection Research Institute.
Herrera AM, Dudley TL. 2003. Reduction of riparian arthropod abundance and diversity as a consequence of giant reed (*Arundo donax*) invasion. *Biological Invasions* 5: 167–177.
Hill MO. 1973. Diversity and evenness: a unifying notation and its consequences. *Ecology* 54: 427–432.
Hoffman MT, Rohde RF. 2011. Rivers through time: historical changes in the riparian vegetation of the semi-arid, winter rainfall region of South Africa in response to climate and land use. *Journal of the History of Biology* 44: 59–80.
Hoffmann JH, Moran VC. 1991. Biological control of *Sesbania punicea* (Fabaceae) in South Africa. *Agriculture, Ecosystems & Environment* 37: 157–173.
Holmes PM, Richardson DM, Esler KJ, Witkowski ETF, Fourie S. 2005. A decision-making framework for restoring riparian zones degraded by invasive alien plants in South Africa: review article. *South African Journal of Science* 101: 553–564.
Hood WG, Naiman RJ. 2000. Vulnerability of riparian zones to invasion by exotic vascular plants. *Plant Ecology* 148: 105–114.
Hooper DU, Chapin III FS, Ewel JJ. 2005. Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecological Monographs* 75: 3–35.
Howard-Williams C, Liptrot MRM. 1980. Submerged macrophyte communities in a brackish South African estuarine-lake system. *Aquatic Botany* 9: 101–116.
Hughes DA. 2001. Providing hydrological information and data analysis tools for the

determination of ecological instream flow requirements for South African rivers. *Journal of Hydrology* 241: 140–151.
Hupp CR, Osterkamp WR. 1996. Riparian vegetation and fluvial geomorphic processes. *Geomorphology* 14: 277–295.
Hurry CR, James EA, Thompson RM. 2013. Connectivity, genetic structure and stress response of *Phragmites australis*: Issues for restoration in a salinising wetland system. *Aquatic Botany* 104: 138–146.
Isac M, Preda S, Marcu M. 1998. Aphid species - vectors of plum pox virus. *Acta Virologica* 42: 233–234.
Jansson R, Nilsson C, Dynesius M, Andersson E. 2000. Effects of river regulation on river-margin vegetation: a comparison of eight boreal rivers. *Ecological Applications* 10: 203–224.
Johnson M, Dudley T, Burns C. 2006. Seed production in *Arundo donax*. *California Invasive Plant Council- News* 14: 12–13.
Keane RM, Crawley MJ. 2002. Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology & Evolution* 17: 164–170.
Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28: 1647–1649.
Kettenring KM, McCormick MK, Baron HM, Whigham DF. 2011. Mechanisms of *Phragmites australis* invasion: feedbacks among genetic diversity, nutrients, and sexual reproduction. *Journal of Applied Ecology* 48: 1305–1313.
Kettenring KM, Mock KE. 2012. Genetic diversity, reproductive mode, and dispersal differ between the cryptic invader, *Phragmites australis*, and its native conspecific. *Biological Invasions* 14: 2489–2504.
Khan ZR, Litsinger J, AT B, Villanueva F, Fernandez N, Taylo L. 1991. *World bibliography of rice stem borers: 1794-1990*. Phillippines: International Rice Research Institute.
Khudamrongsawat J, Tayyar R, Holt JS. 2009. Genetic diversity of giant reed (*Arundo donax*) in the Santa Ana River, California. *Weed Science* 52: 395–405.
Kik C, Andel J Van, Delden W Van, Joenje W, Bijlsma R. 1990. Colonization and differentiation in the clonal perennial *Agrostis Stolonifera*. *The Journal of Ecology* 78: 949.
Kiviat E, Hamilton E. 2001. *Phragmites* use by native North Americans. *Aquatic Botany* 69: 341–357.
Kleynhans CJ. 1996. A qualitative procedure for the assessment of the habitat integrity status of the Luvuvhu River (Limpopo system, South Africa). *Journal of Aquatic Ecosystem Health* 5: 41–54.
Koppitz H. 1999. Analysis of genetic diversity among selected populations of *Phragmites australis* world-wide. *Aquatic Botany* 64: 209–221.
Kotschy KA, Rogers KH, Carter AJ. 2000. Patterns of change in reed cover and distribution in a seasonal riverine wetland in South Africa. *Folia Geobotanica* 35: 363–373.
Kotze DC, Traynor CH. 2011. Wetland plant species used for craft production in KwaZulu–Natal, South Africa: ethnobotanical knowledge and environmental sustainability. *Economic Botany* 65: 271–282.
Kulmatiski A, Beard KH, Meyerson LA, Gibson JR, Mock KE. 2011. Nonnative *Phragmites australis* invasion into Utah wetlands. *Western North American Naturalist* 70: 541–552.
Kumar S, Stecher G, Peterson D, Tamura K. 2012. MEGA-CC: computing core of molecular evolutionary genetics analysis program for automated and iterative data analysis. *Bioinformatics* 28: 2685–2686.

Kvedaras OL, Keeping MG. 2007. Silicon impedes stalk penetration by the borer *Eldana saccharina* in sugarcane. *Entomologia Experimentalis et Applicata* 125: 103–110.
Lake JC, Leishman MR. 2004. Invasion success of exotic plants in natural ecosystems: the role of disturbance, plant attributes and freedom from herbivores. *Biological Conservation* 117: 215–226.
Lambert AM, Casagrande RA. 2006. Distribution of native and exotic *Phragmites australis* in Rhode Island. *Northeastern Naturalist* 13: 551–560.
Lambert AM, Casagrande RA. 2007. Susceptibility of native and non-native common reed to the non-native mealy plum aphid (Homoptera: Aphididae) in North America. *Environmental Entomology* 36: 451–457.
Lambert AM, Dudley TL, Robbins J. 2014. Nutrient enrichment and soil conditions drive productivity in the large-statured invasive grass *Arundo donax*. *Aquatic Botany* 112: 16–22.
Lambert AM, Dudley TL, Saltonstall K. 2010. Ecology and impacts of the large-statured invasive grasses *Arundo donax* and *Phragmites australis* in North America. *Invasive Plant Science and Management* 3: 489–494.
Lambertini C. 2016. Heteroplasmy: another complexity of *Phragmites* genome to take into account. *Biological Invasions*: 1–13.
Lambertini C, Eller FP, Achenbach L, Loc NX, Guo W-Y, Brix H. 2012a. Revisiting *Phragmites australis* variation in the Danube Delta with DNA molecular techniques. In: Gâştescu P, Lewis W, Breţcan P (eds), *Water Resources and Wetlands*. Tulcea, Romania. pp. 142–150.
Lambertini C, Gustafsson MHG, Frydenberg J, Lissner J, Speranza M, Brix H. 2006. A phylogeographic study of the cosmopolitan genus *Phragmites* (Poaceae) based on AFLPs. *Plant Systematics and Evolution* 258: 161–182.
Lambertini C, Gustafsson MHG, Frydenberg J, Speranza M, Brix H. 2008. Genetic diversity patterns in *Phragmites australis* at the population, regional and continental scales. *Aquatic Botany* 88: 160–170.
Lambertini C, Mendelssohn IA, Gustafsson MH, Olesen B, Riis T, Sorrell BK, Brix H. 2012b. Tracing the origin of Gulf Coast *Phragmites* (Poaceae): a story of long-distance dispersal and hybridization. *American Journal of Botany* 99: 538–551.
Lambertini C, Sorrell BK, Riis T, Olesen B, Brix H. 2012c. Exploring the borders of European *Phragmites* within a cosmopolitan genus. *AoB PLANTS* 2012: pls020.
Launert E. 1971. *Phragmites* Adans. In: Fernandes A, Launert E, Wild H (eds), *Flora Zambesiaca*. Chicago: The University of Chicago Press. pp. 91–94.
Le Corre V, Kremer A. 1998. Cumulative effects of founding events during colonisation on genetic diversity and differentiation in an island and stepping-stone model. *Journal of Evolutionary Biology* 11: 495–512.
Lee B, Scholz M. 2007. What is the role of *Phragmites australis* in experimental constructed wetland filters treating urban runoff? *Ecological Engineering* 29: 87–95.
Le Guigo P, Rolier A, Le Corff J. 2012. Plant neighborhood influences colonization of Brassicaceae by specialist and generalist aphids. *Oecologia* 169: 753–761.
Le Roux JJ, Wieczorek AM, Wright MG, Tran CT. 2007. Super-genotype: global monoclonality defies the odds of nature. *PLoS ONE* 2: e590.
Le Rü BP, Ong'amo GO, Moyal P, Muchugu E, Ngala L, Musyoka B, Abdullah Z, Matama-Kauma T, Lada VY, Pallangyo B. 2006a. Geographic distribution and host plant ranges of East African noctuid stem borers. *Interntional Journal of Entomology* 42: 353–361.
Le Rü BP, Ong'amo GO, Moyal P, Ngala L, Musyoka B, Abdullah Z, Cugala D, Defabachew B, Haile T, Matama TK, Lada VY, Negassi B, Pallangyo K, Ravolonandrianina J, Sidumo A, Omwega CO, Schulthess F, Calatayud P, Silvain JF. 2006b. Diversity of lepidopteran

stem borers on monocotyledonous plants in eastern Africa and the islands of Madagascar and Zanzibar revisited. *Bulletin of Entomological Research* 96: 555–563.
Les DH, Philbrick CT. 1993. Studies of hybridization and chromosome number variation in aquatic angiosperms: evolutionary implications. *Aquatic Botany* 44: 181–228.
Lewandowski I, Scurlock JMO, Lindvall E, Christou M. 2003. The development and current status of perennial rhizomatous grasses as energy crops in the US and Europe. *Biomass and Bioenergy* 25: 335–361.
Lewontin RC. 1995. The apportionment of human diversity. In: *Evolutionary biology*. Chicago: Springer. pp. 381–398.
Li CX, Shi M, Tian JH, Lin XD, Kang YJ, Chen LJ, Qin XC, Xu J, Holmes EC, Zhang YZ. 2015. Unprecedented genomic diversity of RNA viruses in arthropods reveals the ancestry of negative-sense RNA viruses. *eLife* 4: e05378.
Linder HP, Verboom GA, Barker NP. 1997. Phylogeny and evolution in the Crinipes Group of grasses (Arundinoideae: Poaceae). *Kew Bulletin* 52: 91–110.
Loreau M, Hector A. 2001. Partitioning selection and complementarity in biodiversity experiments. *Nature* 412: 72–76.
Louda SM, Potvin MA. 1995. Effect of inflorescence-feeding insects on the demography and lifetime fitness of a native plant. *Ecology* 76: 229–245.
Loveless MD, Hamrick JL. 1984. Ecological determinants of genetic structure in plant populations. *Annual Review of Ecology and Systematics* 15: 65–95.
Lynch EA, Saltonstall K. 2002. Paleoecological and genetic analyses provide evidence for recent colonization of native *Phragmites australis* populations in a Lake Superior wetland. *Wetlands* 22: 637–646.
Mabberley DJ. 1997. *The plant-book*. Cambridge: Cambridge University Press.
Macnae W. 1963. Mangrove swamps in South Africa. *The Journal of Ecology* 51: 1–25.
Mal TK, Narine L. 2004. The biology of Canadian weeds. 129. *Phragmites australis* (Cav.) Trin. ex Steud. *Canadian Journal of Plant Science* 84: 365–396.
Malanson GP. 1993. *Riparian landscapes*. Cambridge: Cambridge University Press.
Mariani C, Cabrini R, Danin A, Piffanelli P, Fricano A, Gomarasca S, Dicandilo M, Grassi F, Soave C. 2010. Origin, diffusion and reproduction of the giant reed (*Arundo donax* L.): a promising weedy energy crop. *Annals of Applied Biology* 157: 191–202.
Maron JL, Vila M. 2001. When do herbivores affect plant invasion? Evidence for the natural enemies and biotic resistance hypotheses. *Oikos* 95: 361–373.
Mascia F, Fenu G, Angius R, Bacchetta G. 2013. *Arundo micrantha*, a new reed species for Italy, threatened in the freshwater habitat by the congeneric invasive A. donax. *Plant Biosystems* 147: 717–729.
McClay AS, Balciunas JK. 2005. The role of pre-release efficacy assessment in selecting classical biological control agents for weeds—applying the Anna Karenina principle. *Biological control* 35: 197–207.
McFadyen RE. 1998. Biological control of weeds. *Annual Review of Entomology* 43: 369–393.
McKee J, Richards A. 1996. Variation in seed production and germinability in common reed (*Phragmites australis*) in Britain and France with respect to climate. *New Phytologist* 133: 233–243.
McWilliams JD. 2004. *Arundo donax*. [Online] Available at: http://www.fs.fed.us/database/feis/plants/graminoid/arudon/all.html. Accessed on: 2014-01-16.
Meadows ME, Baxter AJ. 2001. Holocene vegetation history and palaeoenvironments at Klaarfontein Springs, Western Cape, South Africa. *The Holocene* 11: 699–706.
Meadows RE, Saltonstall K. 2007. Distribution of native and introduced *Phragmites australis* in freshwater and oligohaline tidal marshes of the Delmarva peninsula and southern New

Jersey. *Journal of the Torrey Botanical Society* 134: 99–107.
Menhinick EF. 1964. A comparison of some species-individuals diversity indices applied to samples of field insects. *Ecology* 45: 859–861.
Meyerson LA, Cronin JT. 2013. Evidence for multiple introductions of *Phragmites australis* to North America: detection of a new non-native haplotype. *Biological Invasions* 15: 2605–2608.
Meyerson LA, Cronin JT, Pyšek P. 2016. *Phragmites* as a model system. *Biological Invasions*.
Meyerson LA, Lambertini C, McCormick MK, Whigham DF. 2012. Hybridization of common reed in North America? The answer is blowing in the wind. *AoB PLANTS* 2012: pls022.
Meyrick E. 1912. New South African Microlepidoptera. *Annals of the South African Museum* 10: 349–379.
Milbrath LR. 2010. Phytophagous arthropods of invasive swallow-wort vines (*Vincetoxicum* spp.) in New York. *Environmental entomology* 39: 68-78.
Milton SJ. 2004. Grasses as invasive alien plants in South Africa: working for water. *South African Journal of Science* 100: 69–75.
Minchinton TE, Bertness MD. 2003. Disturbance-mediated competition and the spread of *Phragmites australis* in a coastal marsh. *Ecological Applications* 13: 1400–1416.
Mitchell CE, Agrawal AA, Bever JD, Gilbert GS, Hufbauer RA, Klironomos JN, Maron JL, Morris WF, Parker IM, Power AG. 2006. Biotic interactions and plant invasions. *Ecology Letters* 9: 726–740.
Moeletsi ME, Walker S, Landman WA. 2011. ENSO and implications on rainfall characteristics with reference to maize production in the Free State Province of South Africa. *Physics and Chemistry of the Earth, Parts A/B/C* 36: 715–726.
Mook JH, Wiegers J. 1999. Distribution of the aphid *Hyalopterus pruni* Geoffr. within and between habitats of Common Reed *Phragmites australis* (Cav.) Trin. ex Steudel as a result of migration and population growth. *Limnologica-Ecology and Management of Inland Waters* 29: 64–70.
Moolman J, van den Berg J, Conlong D, Cugala D, Siebert S, Le Rü B. 2014. Species diversity and distribution of lepidopteran stem borers in South Africa and Mozambique. *Journal of Applied Entomology* 138: 52–66.
Moore GW, Watts DA, Goolsby JA. 2010. Ecophysiological responses of giant reed (*Arundo donax*) to herbivory. *Invasive Plant Science and Management* 3: 521–529.
Moran PJ, Goolsby JA. 2009. Biology of the galling wasp *Tetramesa romana*, a biological control agent of giant reed. *Biological Control* 49: 169–179.
Moran PJ, Goolsby JA. 2010. Biology of the armored scale *Rhizaspidiotus donacis* (Hemiptera: Diaspididae), a candidate agent for biological control of giant reed. *Annals of the Entomological Society of America* 103: 252–263.
Moran PJ, Goolsby JA. 2014. Effect of nitrogen fertilization on growth of *Arundo donax* and on rearing of a biological control agent, the shoot gall-forming wasp *Tetramesa romana*. *Biocontrol Science and Technology* 24: 503–517.
Moyal P, Le Rü B, Conlong D, Cugala D, Defabachew B, Matama-Kauma T, Pallangyo B, van den Berg J. 2010. Systematics and molecular phylogeny of two African stem borer genera, *Sciomesa* Tams & Bowden and *Carelis* Bowden (Lepidoptera: Noctuidae). *Bulletin of Entomological Research* 100: 641–659.
Mukherjee A, Jones JW, Cuda JP, Kiker G, Overholt WA. 2012. Effect of simulated herbivory on growth of the invasive weed *Hygrophila polysperma*: Experimental and predictive approaches. *Biological Control* 60: 271–279.
Muller-Scharer H, Schaffner U, Steinger T. 2004. Evolution in invasive plants: implications for biological control. *Trends in Ecology & Evolution* 19: 417–422.
Mutlu B. 2002. *Phragmites frutescens* H. Scholz (Gramineae), A New Record For The Flora

of Turkey. *Hacettepe Journal of Biology and Chemistry* 31: 23–26.
Naiman RJ, Decamps H. 1997. The ecology of interfaces: riparian zones. *Annual Review of Ecology and Systematics* 28: 621–658.
Naiman RJ, Rogers KH. 1997. Large animals and system-level characteristics in river corridors. *BioScience* 47: 521–529.
Nartshuk EP. 2014. Grass-fly larvae (Diptera, Chloropidae): Diversity, habitats, and feeding specializations. *Entomological Review* 94: 514–525.
Ndemah R, Schulthess F, Le Rü B, Bame I. 2007. Lepidopteran cereal stemborers and associated natural enemies on maize and wild grass hosts in Cameroon. *Journal of Applied Entomology* 131: 658–668.
Nei M. 1973. Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences of the United States of America* 70: 3321–3323.
Nei M, Maruyama T, Chakraborty R. 1975. The bottleneck effect and genetic variability in populations. *Evolution* 29: 1–10.
Neto AB, Santos RA, Freire FL, Camargo SS, Carius R, Finger F, Beyer W. 1997. Relation between mechanical and structural properties of silicon-incorporated hard aC: H films. *Thin Solid Films* 293: 206-211.
Neumann FH, Stager JC, Scott L, Venter HJT, Weyhenmeyer C. 2008. Holocene vegetation and climate records from Lake Sibaya, KwaZulu-Natal (South Africa). *Review of Palaeobotany and Palynology* 152: 113–128.
Nguyen LX, Lambertini C, Sorrell BK, Eller F, Achenbach L, Brix H. 2013. Photosynthesis of co-existing *Phragmites* haplotypes in their non-native range: are characteristics determined by adaptations derived from their native origin? *AoB Plants* 5: plt016.
Novotný V, Basset Y. 2000. Rare species in communities of tropical insect herbivores: pondering the mystery of singletons. *Oikos* 89: 564–572.
Novotný V, Basset Y. 2005. Host specificity of insect herbivores in tropical forests. *Proceedings. Biological Sciences / The Royal Society* 272: 1083–90.
Oakins AJ. 2001. Thesis: An assessment and management protocol for *Arundo donax* in the Salinas Valley Watershed. California State University.
Ong'amo GO, Le Gall P, Ndemah R, Le Rü BP. 2014. Diversity and host range of lepidopteran stemborer species in Cameroon. *African Entomology* 22: 625–635.
Ong'amo GO, Rü BP Le, Dupas S, Moyal P, Muchugu E, Calatayud P-A, Silvain J-F. 2006. The role of wild host plants in the abundance of lepidopteran stem borers along altitudinal gradient in Kenya. *International Journal of Entomology* 42: 363–370.
Onyango FO. 1994. Continuous rearing of the maize stem borer *Busseola fusca* on an artificial diet. *Entomologia Experimentalis et Applicata* 73: 139–144.
Opler PA. 1978. Interaction of plant life history components as related to arboreal herbivory. *The Ecology of Arboreal Folivores*: 23–31.
Orians CM, Ward D. 2010. Evolution of plant defenses in nonindigenous environments. *Annual Review of Entomology* 55: 439–459.
Papayannis T, Pritchard D. 2011. *Culture and wetlands in the Mediterranean: an evolving story*. Athens: Mediterranean Institute for Nature and Anthropos (Med-INA).
Papazoglou EG, Karantounias GA, Vemmos SN, Bouranis DL. 2005. Photosynthesis and growth responses of giant reed (*Arundo donax* L.) to the heavy metals Cd and Ni. *Environment international* 31: 243–249.
Parker ED. 1979. Ecological implications of clonal diversity in parthenogenetic morphospecies. *Integrative and Comparative Biology* 19: 753–762.
Paterson ID, Vitorino M, de Cristo S, Martin G, Hill M. 2014. Prioritisation of potential agents for the biological control of the invasive alien weed, *Pereskia aculeata* (Cactaceae), in South Africa. *Biocontrol Science and Technology* 24: 407–425.

Paucă-Comănescu M, Clevering OA, Hanganu J, Gridin M. 1999. Phenotypic differences among ploidy levels of *Phragmites australis* growing in Romania. *Aquatic Botany* 64: 223–234.
Paul J, Kirk H, Freeland J. 2011. Genetic diversity and differentiation of fragmented reedbeds (*Phragmites australis*) in the United Kingdom. *Hydrobiologia* 665: 107–115.
Peakall R, Smouse PE. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research--an update. *Bioinformatics* 28: 2537–2539.
Peet RK. 1974. The measurement of species diversity. *Annual Review of Ecology and Systematics* 5: 285–307.
Pennings SC, Silliman BR. 2005. Linking biogeography and community ecology: Latitudinal variation in plant-herbivore interaction strength. *Ecology* 86: 2310–2319.
Perdue RE. 1958. *Arundo donax*—source of musical reeds and industrial cellulose. *Economic Botany* 12: 368–404.
Pilu R, Bucci A, Badone FC, Landoni M. 2012. Giant reed (*Arundo donax* L.): a weed plant or a promising energy crop? *African Journal of Biotechnology* 11: 9163–9174.
Prentis PJ, Wilson JR, Dormontt EE, Richardson DM, Lowe AJ. 2008. Adaptive evolution in invasive species. *Trends in Plant Science* 13: 288-294.
Prinsloo GL, Uys VM (eds). 2015. Cereals and sugarcane. In: *Insects of cultivated plants and natural pastures of southern Africa*. Cape Town: The Entomological Society of Southern Africa. pp. 88–156.
Prior KM, Hellmann JJ. 2013. Does enemy loss cause release? A biogeographical comparison of parasitoid effects on an introduced insect. *Ecology* 94: 1015–1024.
Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genotype data. *Genetics* 155: 945–959.
Puértolas L, Damásio J, Barata C, Soares AMVM, Prat N. 2010. Evaluation of side-effects of glyphosate mediated control of giant reed (*Arundo donax*) on the structure and function of a nearby Mediterranean river ecosystem. *Environmental Research* 110: 556–564.
Puliafico KP, Schwarzlaender M, Harmon BL, Hinz HL. 2008. Effect of generalist insect herbivores on introduced *Lepidium draba* (Brassicaceae): implications for the enemy release hypothesis. *Journal of Applied Entomology* 132: 519–529.
Pyšek P, Prach K. 1993. Plant invasions and the role of riparian habitats: a comparison of four species alien to central Europe. *Journal of Biogeography* 20: 413–420.
Pyšek P, Richardson DM, Rejmánek M, Webster GL, Williamson M, Kirschner J. 2004. Alien plants in checklists and floras: towards better communication between taxonomists and ecologists. *Taxon* 53: 131–143.
Quick L. 2014. *Paleoenvironmental research at Verlorenvlei*. University of Cape Town, Department Environmental and Geographical Sciences.
Quinn LD, Holt JS. 2008. Ecological correlates of invasion by *Arundo donax* in three southern California riparian habitats. *Biological Invasions* 10: 591–601.
Quinn LD, Rauterkus M, Holt JS. 2007. Effects of nitrogen enrichment and competition on growth and spread of Giant Reed (*Arundo donax*). *Weed Science* 55: 319–326.
Quiring D, Flaherty L, Johns R, Morrison A. 2006. Variable effects of plant module size on abundance and performance of galling insects. In: *Galling Arthropods and Their Associates*. Japan: Springer. pp. 189–197.
Racelis AE, Goolsby JA, Penk R, Jones WK, Roland TJ. 2010. Development of an inundative, aerial release technique for the Arundo wasp, biological control agent of the the invasive *Arundo donax* L. *Southwestern Entomologist* 35: 495–501.
Rahmatullah M, Ferdausi D, Mollik MAH, Jahan R, Chowdhury MH, Haque WM. 2010. A survey of medicinal plants used by Kavirajes of Chalna area, Khulna district, Bangladesh. *African Journal of Traditional, Complementary and Alternative Medicines* 7: 91–97.

Rao V, Hodgkin T. 2002. Genetic diversity and conservation and utilization of plant genetic resources. *Plant Cell, Tissue and Organ Culture* 68: 1–19.
Reddy KVS. 1987. Sorghum stem borers in eastern Africa. In: *International Workshop on Sorghum Stem Borers*. India. pp. 33–40.
Reich PB, Oleksyn J. 2004. Global patterns of plant leaf N and P in relation to temperature and latitude. *Proceedings of the National Academy of Sciences of the United States of America* 101: 11001–11006.
Resh VH, Brown AV, Covich AP, Gurtz ME, Li HW, Minshall GW, Reice SR, Sheldon AL, Wallace JB, Wissmar RC. 1988. The role of disturbance in stream ecology. *Journal of the North American Benthological Society.* 7:433-55.
Richardson DM, Holmes PM, Esler KJ, Galatowitsch SM, Stromberg JC, Kirkman SP, Pyšek P, Hobbs RJ. 2007. Riparian vegetation: degradation, alien plant invasions, and restoration prospects. *Diversity and Distributions* 13: 126–139.
Rieger JP, Kreager DA. 1989. Giant reed (*Arundo donax*): a climax community of the riparian zone. In: *Protection, Management, and Restoration for the 1990's: Proceedings of the California Riparian Systems Conference.* Berkeley, California: US Department of Agriculture, Forest Service, Pacific Southwest Forest and Range Experiment Station. pp. 222–225.
River Health Programme. 2003. *State-of-rivers report: Diep, Hout Bay, Lourens and Palmier river systems*. Department of Water Affairs and Forestry, Pretoria.
Roderick GK. 2004. Tracing the origin of pests and natural enemies: genetic and statistical approaches. *Genetics, Evolution, and Biological Control*: 97–112.
Roderick GK, Navajas M. 2003. Genes in new environments: genetics and evolution in biological control. *Nature Reviews Genetics* 4: 889–899.
Root RB. 1973. Organization of a plant-arthropod association in simple and diverse habitats: The fauna of collards (Brassica Oleracea). *Ecological Monographs* 43: 95–124.
Rosenzweig ML. 1995. *Species diversity in space and time*. Cambridge: Cambridge University Press.
Rouget M, Richardson DM, Nel JL, Le Maitre DC, Egoh B, Mgidi T. 2004. Mapping the potential ranges of major plant invaders in South Africa, Lesotho and Swaziland using climatic suitability. *Diversity and Distributions* 10: 475–484.
Rowntree K. 1991. An assessment of the potential impact of alien invasive vegetation on the geomorphology of river channels in South Africa. *Southern African Journal of Aquatic Sciences* 17: 28–43.
Russell IA, Kraaij T. 2008. Effects of cutting *Phragmites australis* along an inundation gradient, with implications for managing reed encroachment in a South African estuarine lake system. *Wetlands Ecology and Management* 16: 383–393.
Rutherford MC, Mucina L, Powrie LW. 2006. Biomes and bioregions of southern Africa. In: *The Vegetation of South Africa, Lesotho and Swaziland.* Pretoria: South African National Biodiversity Institute. pp. 30–51.
Saltonstall K. 2001. A set of primers for amplification of noncoding regions of chloroplast DNA in the grasses. *Molecular Ecology Notes* 1: 76–78.
Saltonstall K. 2002. Cryptic invasion by a non-native genotype of the common reed, *Phragmites australis*, into North America. *Proceedings of the National Academy of Sciences of the United States of America* 99: 2445–2449.
Saltonstall K. 2003a. A rapid method for identifying the origin of North American *Phragmites* populations using RFLP analysis. *Wetlands* 23: 1043–1047.
Saltonstall K. 2003b. Microsatellite variation within and among North American lineages of *Phragmites australis*. *Molecular ecology* 12: 1689–1702.
Saltonstall K, Lambert A, Meyerson LA. 2010. Genetics and reproduction of common

(*Phragmites australis*) and giant reed (*Arundo donax*). *Invasive Plant Science and Management* 3: 495–505.
San Miguel A, Roy J, Gury J, Monier A, Coissac E, Ravanel P, Geremia RA, Raveton M. 2014. Effects of organochlorines on microbial diversity and community structure in *Phragmites australis* rhizosphere. *Applied Microbiology and Biotechnology* 98: 4257–4266.
Schemske DW, Mittelbach GG, Cornell H, Sobel JM, Roy K. 2009. Is there a latitudinal gradient in the importance of biotic interactions? *Annual Review of Ecology, Evolution, and Systematics* 40: 245–269.
Scholz H, Böhling N. 2000. *Phragmites frutescens* (Gramineae) re-visited . The discovery of an overlooked, woody grass in Greece, especially Crete. *Willdenowia* 30: 251–261.
Schulze RE, Maharaj M, Warburton ML, Gers CJ, Horan MJC, Kunz RP, Clark DJ. 2008. South African atlas of climatology and agrohydrology. *Water Research Commission, Pretoria, RSA. WRC Report* 1489: 8.
Schwarzländer M, Häfliger P. 2000. Shoot flies, gall midges, and shoot and rhizome mining moths associated with common reed in Europe and their potential for biological control. In: *Proceedings of the Xth International Symposium on Biological Control of Weeds.* Bozeman, Montana: Montana State University.
Scott L. 1982. A late quaternary pollen record from the Transvaal bushveld, South Africa. *Quaternary Research* 17: 339–370.
Seawright EK, Rister ME, Lacewell RD, McCorkle DA, Sturdivant AW, Yang C, Goolsby JA. 2009. Economic implications for the biological control of *Arundo donax*: Rio Grande Basin. *Southwestern Entomologist* 34: 377–394.
Shafroth PB, Stromberg JC, Patten DT. 2002. Riparian vegetation response to altered disturbance and stress regimes. *Ecological Applications* 12: 107–123.
Silva CMN, Silva L, Oliveira N, Geraldes P, Hervías S. 2011. Control of giant reed *Arundo donax* on Vila Franca do Campo Islet, Azores, Portugal. *Conservation Evidence* 8: 93–99.
Slater JA. 1976. Monocots and chinch bugs: a study of host plant relationships in the Lygaeid subfamily Blissinae (Hemiptera: Lygaeidae). *Biotropica* 8: 143–165.
Slater JA, Wilcox DB. 1973. The chinch bugs, or Blissinae of South Africa (Hemiptera: Lygaeidae). *Memoirs of the Entomological Society of Southern Africa.*
Sousa WP. 1984. The role of disturbance in natural communities. *Annual Review of Ecology and Systematics* 15(1): 353-391.
Spatz H, Beismann H, Brüchert F, Emanns A, Speck T. 1997. Biomechanics of the giant reed *Arundo donax*. *Philosophical Transactions of the Royal Society B: Biological Sciences* 352: 1–10.
Spellerberg IF, Fedor PJ. 2003. A tribute to Claude Shannon (1916–2001) and a plea for more rigorous use of species richness, species diversity and the 'Shannon–Wiener' Index. *Global Ecology and Biogeography* 12: 177–179.
Spencer D, Sher A, Thornby D, Liow PS, Ksander G, Tan W. 2007. Non-destructive assessment of *Arundo donax* (Poaceae) leaf quality. *Journal of Freshwater Ecology* 22: 277–285.
Spencer DF, Tan W, Liow P, Ksander GG, Whitehand LC, Weaver S, Olson J, Newhouser M. 2008. Evaluation of glyphosate for managing Giant Reed (*Arundo donax*). *Invasive Plant Science and Management* 1: 248–254.
Spencer DF, Tan W, Liow PS, Ksander GG, Whitehand LC. 2009. Evaluation of a late summer imazapyr treatment for managing giant reed (*Arundo donax*). *Transactions of the American Fisheries Society* 131: 234-244.
Spencer DF, Tan W, Whitehand LC. 2010. Variation in *Arundo donax* stem and leaf strength: implications for herbivory. *Aquatic Botany* 93: 75–82.
Stiuca R. 2008. The autumn migration of birds in the delta. [Online] Available at:

http://old.rri.ro/arh-art.shtml?lang=1&sec=170&art=16706. Accessed on: 2016-02-09.
Strong DR, McCoy ED, Rey JR. 1977. Time and the number of herbivore species: the pests of sugarcane. *Ecology* 58: 167–175.
Sturgeon KE. 1954. A revised list of grasses of southern Rhodesia. *Rhodesia Agricultural Journal* 51: 4–5.
Taberlet P, Gielly L, Pautou G, Bouvet J. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17: 1105–1109.
Tahvanainen J, Root R. 1972. The influence of vegetational diversity on the population ecology of a specialized herbivore, *Phyllotreta cruciferae* (Coleoptera: Chrysomelidae). *Oecologia* 10: 321–346.
Takahashi R, Nishio T, Ichizen N, Takano T. 2007. Salt-tolerant reed plants contain lower Na+ and higher K + than salt-sensitive reed plants. *Acta Physiologiae Plantarum* 29: 431–438.
Tarin D, Pepper AE, Goolsby JA, Moran PJ, Arquieta AC, Kirk AE, Manhart JR. 2013. Microsatellites uncover multiple introductions of clonal giant reed (*Arundo donax*). *Invasive Plant Science and Management* 6: 328–338.
te Beest M, Le Roux JJ, Richardson DM, Brysting AK, Suda J, Kubešová M, Pyšek P. 2011. The more the better? The role of polyploidy in facilitating plant invasions. *Annals of Botany* 277.
Tewksbury L, Casagrande R, Blossey B, Häfliger P, Schwarzländer M. 2002. Potential for biological control of *Phragmites australis* in North America. *Biological Control* 23: 191–212.
The Plant List. 2013. *Arundo*. [Online] Available at: http://www.theplantlist.org/1.1/browse/A/Poaceae/Arundo/. Accessed on: 2016-05-18.
Tho B, Sorrell BK, Lambertini C, Eller F, Brix H. 2016. *Phragmites australis*: how do genotypes of different phylogeographic origins differ from their invasive genotypes in growth, nitrogen allocation and gas exchange? *Biological Invasions*: 1–14.
Thompson JD, McNeilly T, Gray AJ. 1991. Population variation in *Spartina anglica* C. E. Hubbard. II. Reciprocal transplants among three successional populations. *New Phytologist* 117: 129–139.
Tickner DP, Angold PG, Gurnell A, Owen Mountford J. 2001. Riparian plant invasions: hydrogeomorphological control and ecological impacts. *Progress in Physical Geography* 25: 22–52.
Tomimatsu H, Nakano K, Yamamoto N, Suyama Y. 2014. Effects of genotypic diversity of *Phragmites australis* on primary productivity and water quality in an experimental wetland. *Oecologia* 175: 163–172.
Tracy JL, DeLoach CJ. 1998. Suitability of classical biological control for giant reed (*Arundo donax*) in the United States. In: *Arundo and Saltcedar Management Workshop Proceedings*. California: University of California Cooperative Extension. pp. 73–109.
Tscharntke T. 1992. Fragmentation of *Phragmites* habitats, minimum viable population size, habitat suitability, and local extinction of moths, midges, flies, aphids, and birds. *Conservation Biology* 6: 530–536.
Tscharntke T. 1999. Insects on common reed (*Phragmites australis*): community structure and the impact of herbivory on shoot growth. *Aquatic Botany* 64: 399–410.
Tscharntke T. 2008. Changes in shoot growth of *Phragmites australis* caused by the gall maker *Giraudiella inclusa* (Diptera: Cecidomyiidae). *Oikos* 54: 370–377.
Turesson G. 1922. The species and the variety as ecological units. *Hereditas* 3: 100–113.
Turpie J., Heydenrych B. 2000. Economic consequences of alien infestation of the Cape Floral Kingdom's Fynbos vegetation. In: *The Economics of Biological Invasions*.Cheltenham. Edward Elgar Publishing Limited. pp.152-182.
Vachon N, Freeland JR. 2011. Phylogeographic inferences from chloroplast DNA: quantifying

the effects of mutations in repetitive and non-repetitive sequences. *Molecular Ecology Resources* 11: 279–285.
Vári L, Martin K, Kroon DM. 2002. *Classification and checklist of the species of Lepidoptera recorded in Southern Africa*. Pretoria: Simple Solutions.
Vasquez EA, Glenn EP, Brown J, Guntenspergen GR, Nelson SG. 2005. Salt tolerance underlies the cryptic invasion of North American salt marshes by an introduced haplotype of the common reed *Phragmites australis* (Poaceae). *Marine Ecology Progress Series* 298: 1–8.
van Coller A, Rogers K, Heritage G. 1997. Linking riparian vegetation types and fluvial geomorphology along the Sabie River within the Kruger National Park, South Africa. *African Journal of Ecology* 35: 194–212.
van den Berg J, Rebe M. 2001. Developing habitat management systems for gramineous stemborers in South Africa. *Insect Science and its Application* 21: 381–388.
van der Merwe CG, Schoonbee HJ, Pretorius J. 1990. Observations on concentrations of the heavy metals zinc, manganese, nickel and iron in the water, in the sediments and in two aquatic macrophytes, *Typha capensis* (Rohrb.) N. E. Br. and *Arundo donax* L., of a stream affected by goldmine and industrial effluent. *Water South Africa* 16: 119–124.
van der Putten WH. 1997. Die-back of *Phragmites australis* in European wetlands: an overview of the European Research Programme on reed die-back and progression (1993-1994). *Aquatic Botany* 59: 263–275.
van der Toorn J, Mook JH. 1982. The influence of environmental factors and management on stands of *Phragmites australis*. I. Effects of burning, frost and insect damage on shoot density and shoot size. *Journal of Applied Ecology* 19: 477–499.
van Doninck K, Schön I, De Bruyn L, Martens K. 2002. A general purpose genotype in an ancient asexual. *Oecologia* 132: 205–212.
van Lenteren JC, Babendreier D, Bigler F, Burgio G, Hokkanen HMT, Kuske S, Loomans AJM, Menzler-Hokkanen I, Van Rijn PCJ, Thomas MB. 2003. Environmental risk assessment of exotic natural enemies used in inundative biological control. *Biocontrol* 48: 3–38.
van Rooyen MW, Tosh CA, Van Rooyen N, Matthews WS, Kellerman MJS. 2004. Impact of harvesting and fire on *Phragmites australis* reed quality in Tembe Elephant Park, Maputaland. *Koedoe* 47: 31–40.
van Wilgen BW, Nel JL, Rouget M. 2007. Invasive alien plants and South African rivers: a proposed approach to the prioritization of control operations. *Freshwater Biology* 52: 711–723.
Veldkamp JF. 1994. Miscellaneous notes on southeast-Asian Gramineae .9. Setaria and Paspalidium. *Blumea* 39: 373–384.
Vendramin GG, Fady B, González-Martínez SC, Hu FS, Scotti I, Sebastiani F, Soto A, Petit R. 2008. Genetically depauperate but widespread: The case of an emblematic Mediterranean pine. *Evolution* 62: 680–688.
Vernersson T, Bonelli PR, Cerrella EG, Cukierman AL. 2002. *Arundo donax* cane as a precursor for activated carbons preparation by phosphoric acid activation. *Bioresource Technology* 83: 95–104.
Versfeld DB, Le Maitre DC, Chapman RA. 1998. *Alien invading plants and water resources in South Africa: a preliminary assessment*. WRC Report no. TT99/98, Council Scientific and Industrial Research, Pretoria.
Victorin M. 1995. *Flore Laurentiene*. Montreal: University of Montreal Press.
Waisel Y. 1972. *Biology of halophytes*. New York: Academic Press.
Warren RS, Fell PE, Grimsby JL, Buck EL, Rilling GC, Fertik RA. 2001. Rates, patterns, and impacts of *Phragmites australis* expansion and effects of experimental *Phragmites* control

on vegetation, macroinvertebrates, and fish within tidelands of the lower Connecticut River. *Estuaries* 24: 90–107.
Washitani I. 2001. Plant conservation ecology for management and restoration of riparian habitats of lowland Japan. *Population Ecology* 43: 189–195.
Weyl PSR. 2015. PhD Thesis: Friend or foe? : Resolving the status of the submerged macrophyte *Myriophyllum spicatum* L. (Haloragaceae) in southern Africa. Rhodes University.
White EM, Wilson JC, Clarke AR. 2006. Biotic indirect effects: a neglected concept in invasion biology. *Diversity and Distributions* 12: 443–455.
White JA, Whitham TG. 2000. Associational susceptibility of cottonwood to a box elder herbivore. *Ecology* 81: 1795–1803.
Whittaker RH. 1972. Evolution and measurement of species diversity. *Taxon* 21: 213–251.
Windham L, Lathrop RG. 1999. Effects of *Phragmites australis* (common reed) invasion on aboveground biomass and soil properties in brackish tidal marsh of the Mullica River, New Jersey. *Estuaries* 22: 927–935.
World Commission on Dams. 2000. *Dams and Development*. London: Earthscan Publications Ltd.
World Weather Online. 2016. Yearly Weather Summary. [Online] Available at: http://www.worldweatheronline.com. Accessed on: 2016-01-27.
Wu CA, Lowry DB, Cooley AM, Wright KM, Lee YW, Willis JH. 2008. Mimulus is an emerging model system for the integration of ecological and genomic studies. *Heredity* 100: 220–230.
Wu CA, Murray LA, Heffernan KE. 2015. Evidence for natural hybridization between native and introduced lineages of *Phragmites australis* in the Chesapeake Bay watershed. *American Journal of Botany* 102: 805–812.
Yang C, Everitt JH, Goolsby JA. 2011. Mapping Giant Reed (*Arundo donax*) infestations along the Texas–Mexico portion of the Rio Grande with aerial photography. *Invasive Plant Science and Management* 4: 402–410.
Ye ZH, Baker AJM, Wong MH, Willis AJ. 1997. Zinc, lead and cadmium tolerance, uptake and accumulation by the common reed, *Phragmites australis* (Cav.) Trin. ex Steudel. *Annals of Botany* 80: 363–370.
Yeh FC, Boyle J. 1997. POPGENE, the user-friendly shareware for population genetic analysis. *Molecular Biology and Biotechnology* 434: 724–731.
Yguel B, Bailey R, Tosh DN, Vialatte A, Vasseur C, Vitrac X, Jean F, Prinzing A. 2011. Phytophagy on phylogenetically isolated trees: why hosts should escape their relatives. *Ecology Letters* 14: 1117–1124.
Zedler JB, Kercher S. 2004. Causes and consequences of invasive plants in wetlands: opportunities, opportunists, and outcomes. *Critical Reviews in Plant Sciences* 23: 431–452.
Zhao X, Dupont L, Schefub E, Meadows ME, Hahn A, Wefer G. 2016. Holocene vegetation and climate variability in the winter and summer rainfall zones of South Africa. *The Holocene* 26: 843–857.

7. APPENDIX

Table 0-1 Haplotype combinations of genus *Phragmites* (Guo et al. 2016, in press). World haplotypes were used to compare to South African haplotypes.

trnT-trnL	*rbcl-psaI*	Haplotype	Species	Origin	Distribution	References
10 (AF457397)	1(AY016332)	A	*Pa* ssp *americanus*		North America	Saltonstall 2002
11 (AF457398)	1(AY016332)	B	*Pa* ssp *americanus*		North America	Saltonstall 2002
13 (AF457399)	1(AY016332)	C	*Pa* ssp *americanus*		North America	Saltonstall 2002
16 (AF457402)	1(AY016332)	D	*Pa* ssp *americanus*		North America	Saltonstall 2002
2 (AY016325)	2 (AY016333)	E	*Pa* ssp *americanus*		North America	Saltonstall 2002
8 (AF457395)	2 (AY016333)	F	*Pa* ssp *americanus*		North America	Saltonstall 2002
9 (AF457396)	2 (AY016333)	G	*Pa* ssp *americanus*		North America	Saltonstall 2002
11 (AF457398)	2 (AY016333)	H	*Pa* ssp *americanus*		North America	Saltonstall 2002
5 (AY016328)	3 (AY016334)	I	*Pa.* var. *berlandieri*	?	Gulf Coast, South America, Asia/ Australia	Saltonstall 2002
5h	3 (AY016334)	I1	*P. karka*		Malaysia	Lambertini et al., unpublished

5i (HQ664450)	3 (AY016334)	I2 (Land)	*P. mauritianus; P. karka*
5e	3 (AY016334)	13	*P. karka*
5a	3 (AY016334)	14	*Pa*
1 (AY016324)	4 (AY016335)	J	*Pa*
3 (AY016326)	4 (AY016335)	K	*Pa*
5 (AY016328)	4 (AY016335)	L	*Pa*
5c	4 (AY016335)	L1 (vfM2)	*Pa*
5f	4 (AY016335)	L2	*Pa*
4b	4 (AY016335)	M	*Pa*
4a	4 (AY016335)	M1 (Delta)	*Pa* ssp. *altissimus*

Africa	Greece, Mexico, Burkino Faso, Uganda, Gulf Coast, South America	Lambertini et al. 2012b
	Southeast Asia	Lambertini et al. 2012c
	Southeast Asia	Lambertini et al. 2012c
	Asia/ Australia	Saltonstall 2002
	Africa, Asia, Europe	Saltonstall 2002
	Europe, Asia/ Australia	Saltonstall 2002
Eurasia?	Europe, North America	Vachon and Freeland 2011; Lambertini et al. 2012c; Meyerson and Cronin 2013
	North Europe	Lambertini et al. 2012c
Eurasia	Europe, Asia, Africa, North and South America, New Zealand	Saltonstall 2002
Mediterranean Region	Mediterranean Region and Mississippi Delta	Hauber et al. 2011

4c	4 (AY016335)	M3	*Pa* ssp. *altissimus*
4d	4 (AY016335)	M4	*Pa* ssp. *altissimus*
4e	4 (AY016335)	M5	*Pa* ssp. *altissimus*
4d	4c	M6	*Pa* ssp. *altissimus*
6 (AF457393)	4 (AY016335)	N	*Pa*
7 (AF457394)	4 (AY016335)	O	*Pa*
7c	4 (AY016335)	O1	*Pa*
7a	4 (AY016335)	O2	*Pa*
7b	4 (AY016335)	O3	*Pa*
1 (AY016324)	5 (AF457382)	P	*Pa*
1b	5 (AF457382)	P2	*Pa*
5 (AY016328)	5 (AF457382)	Q	*Pa*

Middle and South Europe	Lambertini et al. 2012c
North Africa, Middle East, South Europe	Lambertini et al. 2012c
Greece	Lambertini et al. 2012c
Jordan	Lambertini et al. 2012c
France	Saltonstall 2002
Europe, Asia, Australia	Saltonstall 2002
North Europe	Lambertini et al. 2012c
North and South Europe	Lambertini et al. 2012c
East Asia	Lambertini et al. unpublished
East Russia, East Asia, Australia	Saltonstall 2002
East and Northeast Asia, New Zealand	Lambertini et al. 2012c
Asia, Australia	Saltonstall 2002

5 (AY016328)	6 (AF457383)	R	*Pa*
2 (AY016325)	7 (AF457384)	S	*Pa* ssp. *americanus*
5 (AY016328)	8 (AF457385)	T	*Pa*
5 (AY016328)	9 (AF457386)	U	*P. karka, P. frutescens*
5b	9 (AF457386)	U1	*P. karka*
5e	9 (AF457386)	U2	*P. karka*
5i	9 (AF457386)	U3	*P. frutescens*
5 (AY016328)	10 (AF457387)	V	*P. mauritianus*
5g	10 (AF457387)	V1	*P. mauritianus*
5d	10 (AF457387)	V2	*P. mauritianus*
15 (AF457401)	11 (AF457388)	W	*P. japonicus*
15 (AF457401)	12 (AF457389)	X	*Pa*
14 (AF457400)	13 (AF457390)	Y	*Pa*
8 (AF457395)	14 (AF457391)	Z	*Pa* ssp. *americanus*

Africa	Saltonstall 2002
North America	Saltonstall 2002
Europe	Saltonstall 2002
Asia, Australia	Saltonstall 2002
East Asia	Lambertini et al. 2012c
Southeast Asia	Lambertini et al. 2012c
South Europe	Lambertini et al. 2012c
Africa	Saltonstall 2002
South Africa	Lambertini et al. 2012c
Central Africa	Lambertini et al. 2012c
Northeast Asia	Saltonstall 2002
South America	Saltonstall 2002
North America	Saltonstall 2002
North America	Saltonstall 2002

8 (AF457395)	15 (AF457392)	AA	*Pa* ssp. *americanus*
17 (AY714215)	2 (AY016333)	AB	*Pa* ssp. *americanus*
18 (AY714216)	2 (AY016333)	AC	*Pa* ssp. *americanus*
19 (JF271679)	4 (AY016335)	AD (Greeny 2)	*Pa*
H20 (SLJ01, JN224811)	H21 (SLJ01, JN224812)	AE	*Pa*
1 (AY016324)	H21 (SLJ01), JN224812)	AF	*Pa*
H20 (SLJ01, JN224811)	5 (AF457382)	AG	*Pa*
21 (JQ265821)	4 (AY016335)	AH	*Pa*
3 (AY016326)	21b (HQ664451)	AI (Greeny 3)	*Pa*
3 (AY016326)	21b (HQ664451)	AJ	*Pa*
4b (AY016327)	21a	AJ1	*Pa*

	North America	Saltonstall 2002
	North America	Meadows and Saltonstall 2007
	North America	Meadows and Saltonstall 2007
North America or Europe?	Gulf Coast, Europe	Hauber et al. 2011
	Australia	Hurry et al. 2013
	Australia	Hurry et al. 2013
	Australia	Hurry et al. 2013
	North Europe	Lambertini et al. 2012c
South Africa?	South Africa, Gulf Coast	Lambertini et al. 2012c
	Europe	Lambertini et al. 2012c
	Middle East	Lambertini et al., unpublished

4f	22 (JQ265822)	AK	*Pa*
22 (HQ449553)	4 (AY016335)	AL	*Pa*
22a	4 (AY016335)	AL1	*Pa*
23	11	AM	*P. japonicus*
1b	23	AN	*Pa*
5	24	AO	*P. mauritianus*
12	10	AP	*P. mauritianus*
1 (AY016324)	16 (JF503246)	H28	*Pa*
1 (AY016324)	17 (JF503247)	H29	*Pa*
4 (AY016347)	18 (JF503248)	H31	*Pa*
7 (AF457394)	18 (JF503248)	H32	*Pa*
4 (AY016327)	19 (JF503249)	H33	*Pa*
19 (JF503245)	5 (AF457382)	H30	*Pa*

East Europe	Lambertini et al. 2012c
North Africa	Lambertini et al. 2012c
South Europe	Lambertini et al., unpublished
Northeast Asia	Lambertini et al. 2012c
East Asia	Lambertini et al. 2012c
Southern Africa	Lambertini et al. 2012c
Southern Africa	Lambertini et al. 2012c
East Asia	An et al. 2012
East Asia	An et al. 2012
East Asia	An et al. 2012
East Asia	An et al. 2012
East Asia	An et al. 2012
Europe	An et al. 2012

vfM3 (GQ468795)	4 (AY016335)	vfM3	*Pa*	North America	Vachon and Freeland 2011
vfE4 (GQ468793)	4 (AY016335)	vfE4	*Pa*	East Asia	Vachon and Freeland 2011

Table 0-2 *Phragmites australis* populations and the resulting phenotype (- denotes samples with missing data).

Sample number	Site Location	Population	Phenotype
1,	Port St. John's	1	A
2	Port St. John's	1	A
3	Port St. John's	1	A
7	Port St. John's	1	A
45	Port St. John's	1	A
46	Port St. John's	1	A
47	Port St. John's	1	A
9	Kosi Bay	2	B
41	Kosi Bay	2	C
42	Kosi Bay	2	C
43	St. Lucia	3	D
44	St. Lucia	3	E
37	Durban	4	F
38	Durban	4	F
49	Pilanesberg Nature Reserve	5	G
53	Golden Gate Nature Reserve	6	H
55	Rustler's Valley	-	-
59	Hartebeespoort Dam	7	I
61	Stellenbosch	8	J
62	Knysna	9	K
63	Kars River	10	L
64	Rocherpan Nature Reserve	11	M
65	Cape Point	12	N
66	Bushmans River	13	O
68	Plettenberg Bay	14	P
69	Bot River - Kleinemond	15	Q
70	Buffels River	16	R
71	Somerset West	17	S
72	Berg River -Franshoek	18	T
73	Three Waters Dam	19	U
74	Marquard	20	V
75	Baviaans River	21	W
76	Ladybrand	22	X
77	Wilderness Lagoon	23	Y
78	Agulhas National Park	24	Z
79	Clocolan	25	AA
97	Clocolan (3P-FS-1)	25	-
80	Marine Drive	26	AB
81	Alexandria	27	AC
82	Ratelfontein	28	AD
83	Seekoei	29	AE
84	Eland's Bay	30	AF
85	La Mercy (2P-DB-1)	31	AG
86	Zimbali (3P-DB-1)	32	AH
87	Ballito (5P-DB-1-5)	33	AI

88	Ballito (5P-DB-1-5)	33	AI
89	Ballito (5P-DB-1-5)	33	AI
90	Ballito (5P-DB-1-5)	33	AI
91	Ballito (5P-DB-1-5)	33	AI
92	Clocolan (2P-FS-1-5)	34	AJ
93	Clocolan (2P-FS-1-5)	34	AJ
94	Clocolan (2P-FS-1-5)	34	AJ
95	Clocolan (2P-FS-1-5)	34	AK
96	Clocolan (2P-FS-1-5)	34	AJ
98	Hout Bay (1P-CT-1)	35	AL
99	Constantia (2P-CT-1-5)	36	AM
100	Constantia (2P-CT-1-5)	36	AM
101	Constantia (2P-CT-1-5)	36	AM
102	Constantia (2P-CT-1-5)	36	AM
103	Constantia (2P-CT-1-5)	36	AM
104	Jonkershoek Nature Reserve (3P-CT-1)	37	AN
105	Spier Wine Estate (4P-CT-1)	38	AO
106	Mulderbosch Wine Estate (5P-CT-1)	39	AP

Table 0-3 *Phragmites australis* populations and the resulting phenotype (- denotes samples with missing data).

Sample number	Site Location	Population	Phenotype
1M	Little Gowrie	1	A
6M	Little Gowrie	1	B
7M	Little Gowrie	1	C
2M	Mbabane - Swaziland	2	D
8M	Mbabane - Swaziland	2	E
9M	Mbabane - Swaziland	2	E
3M	Umhlanga	3	F
10M	Umhlanga	3	F
11M	Umhlanga	3	F
12M	Umhlanga	3	G
14M	Umhlanga	3	F
4M	Sozisa - Swaziland	4	H
5M	Sozisa - Swaziland	4	I
13M	Sozisa - Swaziland	4	I
15M	Shakaskraal	5	J
16M	Shakaskraal	5	J
17M	Shakaskraal	5	J

18M	Shakaskraal	5	J
19M	Shakaskraal	5	J
31M	Shakaskraal	5	K
20M	Zimbali	6	L
21M	Umgeni	7	M
22M	Nelspruit	8	-
23M	Nelspruit- scrap yard	9	N
33M	Nelspruit- scrap yard	9	N
34M	Nelspruit- scrap yard	9	O
35M	Nelspruit- scrap yard	9	P
36M	Nelspruit- scrap yard	9	P
37M	Nelspruit- scrap yard	9	P
38M	Nelspruit- scrap yard	9	Q
24M	Hazyview	10	R
39M	Hazyview	10	S
25M	Sabie Sun-Hazyview	11	T
40M	Sabie Sun-Hazyview	11	T
26M	Kruger National Park	12	U
27M	Kruger National Park	12	U
28M	Kruger National Park	12	V
29M	Kruger National Park	12	V
30M	Kruger National Park	12	V
41M	Kruger National Park	12	W
32M	Nelspruit – Hippo Lodge	13	X
42M	Namibia 1	14	Y
43M	Namibia 2	15	Z
44M	Namibia 3	16	AA

Table 0-4 *Phragmites australis* sites and their assigned population according to STRUCTURE (Pritchard *et al.* 2000).

Samples	Population	Location	Membership probability (population average)	
			K=1 (red)	K=2 (green)
1,2,3,7,45,46,47	1	Port St. Johns, Eastern Cape	0.988	0.012
9,41,42	2	Kosi Bay, KwaZulu-Natal	0.973	0.027
44	4	St. Lucia, KwaZulu-Natal	0.515	0.485
37,38	3	Bird Park, KwaZulu-Natal	0.983	0.017
49	5	Pilanesberg,North-West	0.149	0.851
53	6	Golden Gate, Free State	0.029	0.971
59	7	Hartebeespoort Dam, Gauteng	0.218	0.782
61	8	Stellenbosch	0.012	0.988
62	9	Knysna, Western Cape	0.024	0.976
63	10	Kars River, Western Cape	0.010	0.990
64	11	Rocherpan Nature Reserve, Western Cape	0.059	0.941
65	12	Cape Point	0.114	0.886
66	13	Bushman's River, Eastern Cape	0.033	0.967
68	14	Plett Bay	0.331	0.669
69	15	Bot River, Western Cape	0.022	0.978
70	16	Buffels River, Western Cape	0.014	0.986
71	17	Somerset West, Western Cape	0.625	0.375

72	18	Berg River, Western Cape	0.015	0.985
73	19	Three Waters Dam, Western Cape	0.184	0.816
74	20	Marquard, Free State	0.035	0.965
75	21	Baviaans River, Western Cape	0.036	0.964
76	22	Ladybrand, Free State	0.169	0.831
77	23	Wilderness, Western Cape	0.020	0.980
78	24	Agulhas, Western Cape	0.029	0.971
79,97	25	Clocolan, Free State	0.574	0.426
80	26	Marine Drive, Western Cape	0.021	0.979
81	27	Alexandria, Eastern Cape	0.053	0.947
82	28	Ratelfontein, Northern Cape	0.036	0.964
83	29	Seekoei	0.034	0.966
84	30	Eland's Bay, Western Cape	0.392	0.608
85	31	La Mercy, KwaZulu-Natal	0.984	0.016
86	32	Zimbali, Kwa-Zulu Natal	0.313	0.687
87,88,89,90,91	33	Umgeni River, Kwazulu-Natal	0.974	0.026
92,93,94,95,96	34	Clocolan Free State	0.014	0.986
98	35	Hout Bay, Western Cape	0.009	0.991
99,100, 103	36	Constantia, Western Cape	0.054	0.946
104	37	Jonkershoek Nature Reserve, Western Cape	0.037	0.963
105	38	Spier, Stellenbosch	0.039	0.961
106	39	Mulderbosch, Stellenbosch	0.021	0.979

Table 0-5 List of *P. mauritianus* samples and the presence or absence of the waxy bands, Waxy100 and Waxy200. Black indicates the presence of a band.

Samples	Population	Location	Membership probability (population average)		
			k=1	k=2	k=3
1	1	Little Gowrie, Mpumalanga	0.111	0.521	0.368
6	1	Little Gowrie, Mpumalanga	0.020	0.051	0.929
7	1	Little Gowrie, Mpumalanga	0.048	0.831	0.121
2,8,9	2	Mbabane, Swaziland	0.015	0.023	0.962
3,10,11,14	3	Umhlanga, KwaZulu-Natal	0.978	0.012	0.010
12	3	Umhlanga, KwaZulu-Natal	0.414	0.072	0.513
4	4	Sozisa, Swaziland	0.013	0.758	0.228
5,13	4	Sozisa, Swaziland	0.015	0.235	0.750
15,16,17,18,19,31	5	Shakaskraal, KwaZulu-Natal	0.017	0.956	0.027
20	6	Zimbali, KwaZulu-Natal	0.096	0.847	0.057
21	7	Umgeni River, KwaZulu-Natal	0.845	0.093	0.062
22	8	Nelspruit, Mpumalanaga	0.075	0.588	0.337
23,33,34,38	9	Nelspruit (Scrap yard), Mpumalanga	0.845	0.112	0.043

35,36,37	9	Nelspruit (Scrap yard), Mpumalanga	0.030	0.607	0.363
24	10	Hazyview, Mpumalanga	0.597	0.354	0.050
39	10	Hazyview, Mpumalanga	0.231	0.742	0.027
25,40	11	Hazyview (Sabie Sun), Mpumalanga	0.024	0.884	0.093
26,27,28,29,30	12	Kruger National Park, Mpumalanga	0.974	0.015	0.011
41	12	Kruger National Park, Mpumalanga	0.036	0.938	0.027
32	13	Crocodile River, Nelspruit, Mpumalanga	0.413	0.491	0.096
42	14	Ngalwana Village, Zambezi	0.334	0.544	0.122
43	15	Barotse Floodplains	0.066	0.845	0.089
44	16	Lungwebungu Mouth	0.251	0.652	0.096