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The invasion ecology of *Pontederia cordata* L. (Pontederiaceae) in South Africa

THESIS

Submitted in fulfilment of the requirements for the degree of

MASTER OF SCIENCE

by

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Abstract

Pontederia cordata L. (Pontederiaceae) is a tristylous invasive macrophyte – originating from North and South America – that has caused detrimental environmental, agricultural and socio-economic impacts in South Africa (SA). This novel study investigates the invasive ecology of *P. cordata* in SA by determining population genetics, pollination ecology and floral traits. Preliminary field surveys suggest that only one of three tristylous forms of *P. cordata* is invading SA and no seeds have been observed in any invasive populations. This study therefore determined the population genetics, mode of spread of *P. cordata* in SA and possible reasons for the lack of seed production, as well as providing suggestions for future control and management strategies.

Inter Simple Sequence Repeats of leaf samples from invasive populations in SA and the native range of the United States of America (USA) were performed to determine the population genetics of *P. cordata*. The clarification of population structure of an alien invasive plant can provide insight into founder effects, introduction events and modes of spread and is important for the development of management plans such as biological control. Results from the genetic analyses indicated that *P. cordata* populations have low genetic diversity within and amongst invasive populations in comparison to native populations. This suggests that high gene flow and sexual reproduction is not present in invasive populations, and that only a single or very few introductory events have occurred in SA. Furthermore, invasive *P. cordata* populations shared the highest genetic similarity with native samples from Belle Haven, Virginia, USA, and thus further sampling and future genetic surveys should be conducted in this area to identify source populations to survey for potential biological control agents.

Following these findings, I investigated whether sexual reproduction and seed production is absent from invasive *P. cordata* populations in SA as speculated. Floral traits from populations throughout all the invaded provinces were measured and, along with pollen grain measurements, it was determined that only short-morphed plants are present in SA. It was speculated that the absence of native pollinators in the invasive range may be responsible for the absence of sexual reproduction. However, a pollination study confirmed the presence of generalist insect pollinators. Thereafter,

artificial pollination experiments on 8 865 flowers were conducted to determine whether an incompatibility system was present which prevented seed production. No seeds were produced and it was concluded that illegitimate pollination of the short-morphed plants prevented seed production and rhizomes are responsible for the invasion of *P. cordata* throughout SA.

The implications of these findings and possible management strategies such as biological control is discussed in Chapter 4. These findings suggest that control programmes should target the plants rhizomes to prevent and reduce spread. Preventing the introduction of medium- and long-morphed plants into SA is crucial to prevent *P. cordata* from producing seeds and intensifying invasion further through both asexual and sexual spread.

Acknowledgements

I would like to acknowledge and express my sincere gratitude to the following people and organisations:

The National Research Foundation (NRF) and Department of Environmental Affairs (DEA) for their funding. The Centre for Biological Control (CBC), Rhodes University and the late Elias and Ada Levenstein for their funding and bursaries for this project and conferences. Without any of this help, my project would not have been possible. I am extremely grateful!

My supervisor, Prof. Julie Coetzee, for her patience, encouragement and valuable feedback. Thanks for all the reassurance and not giving up on me!
My co-supervisor, Prof. Sjirk Geerts, for his constant encouragement, enthusiasm and valuable advice. Those long emails were worth it!
My genetics advisor, Dr. Iain Paterson, for his valuable troubleshooting advice, quick responses and guidance on my genetics work.

Dr. Shelley Edwards for allowing me to use the laboratory and for her assistance in the Zoology and Entomology Laboratory (ZELM) at Rhodes University.
Prof. Brad Ripley for allowing me to utilize his WinDIAS Analysis System and laboratory at the Botany Department, Rhodes University.
Prof. Martin Hill for his motivating Friday tea speeches and encouragement and support towards the CBC team. Thank you for allowing me to be part of the CBC team, I will never forget some of those soap box talks before teatime snacks! I would also like to thank the students and staff at CBC and the Botany Department at Rhodes University for their friendship and helpfulness, with special thanks to Mrs. Jeanne van der Merwe for her assistance with my administrative work for field trips and purchase orders.

I would also like to thank the following people for their assistance during my raw data collection: Shirley Pinchuck and Marvin Randall at the Electron Microscopy Unit at Rhodes University. Dr. Craig Peters, Dr. Tracey Nowell and Dr. Riaan Strauss for their valuable advice and use of materials. Dr. Lesley Henderson for *P. cordata* population co-ordinates. Prof. Lyn Gettys and her colleagues, as well as the Dustan family,

Megan Reid, Phillip Ivey, Prof. Geerts and Prof. Coetzee for collecting and sending me *P. cordata* leaf samples, flower samples and/or flower pictures. A special thanks to Maretha and the Waainek team for all their help with collecting and maintaining *P. cordata* plants. The Central Analytical Facilities (CAF) at Stellenbosch University for processing my ISSR samples, with a special thanks to Mrs. Rene Veikondis.

Finally, I would like to thank my partner, Tyron for his love and support through the final stretch of this project and who has begun to learn that science can be cool! My sincerest gratitude goes to my parents, Jenifer and Richard, my sister Savannah and my brother Rory, my grandparents Mike and Doreen, Nicole and my aunt Kathy for their constant love and encouragement. This thesis is dedicated to my parents, who have sacrificed a lot for their children and have taught me that through hard work and determination, anything is possible.

CHAPTER 1

Introduction and Literature Review

1.1. Biological invasions

Biological invasions occur when organisms that have spread outside their native regions survive and proliferate in new areas (Caffrey *et al*., 2010). These invasions can pose a serious threat to the conservation of biodiversity due to their negative impacts (Caffrey *et al*., 2010). Research on biological invasions is therefore important to prevent, detect, understand and control invasions, especially since increased globalization has facilitated the spread of invasive or potentially invasive species (Meyerson & Mooney, 2007). If an introduced species cannot establish and naturalize successfully, then it cannot become invasive and problematic in a new area (Tobin, 2018). Some non-native species survive with human assistance but cannot establish in the wild, for example, a horticultural plant species may lack obligatory mutualists which prevents the species from being pollinated or reproducing (Zenni & Nuñez, 2013).

Introduced species that do not become invasive are generally unharmful, beneficial or do not have a serious impact in the area (Schlaepfer *et al*., 2011). One example of a non-native species having a potential benefit on its environment are honeysuckle plants (*Lonicera* spp.) in the eastern and Midwestern parts of North America (Gleditsch & Carlo, 2010). Honeysuckle plants provide nectar and abundantly produce fruit which native birds rely on as a major food source, however the plants may have some adverse effects on nesting of native birds (Gleditsch & Carlo, 2010).

Introduced species that do establish, naturalize and become invasive can cause immense destruction and act as drivers for biotic changes (Novacek & Cleland, 2001). A classic example of a highly invasive species that drove biotic changes is the Nile Perch (*Lates niloticus*), a large piscivorous predator introduced into Lake Victoria in the 1950s (Kaufman, 1992; Marshall, 2018). This species was introduced as a food source, however it aggressively predated and caused the extinction of many cichlids' species (Kaufman, 1992; Marshall, 2018). Furthermore, the invasion of the Nile Perch

resulted in increased nutrient cycling caused by excessive population growth (nutrient loading) and disruption of food chains, altogether leading to eutrophication of the lake (Kolding *et al*., 2008; Marshall, 2018).

The spread of an invasive species may significantly influence a country's native species growth and survival by having a higher competitive ability for resources, a more efficient reproductive system or opportunistically growing in disturbed areas (Daehler, 2003). Successful establishment of an invasive species may be explained by the "enemy release hypothesis" (Keane & Crawley, 2002; Catford *et al*., 2009). The enemy release hypothesis predicts that a non-native species introduced into an invaded area may escape from its natural enemies such as predators, herbivores or diseases and can therefore rapidly proliferate and spread (Keane & Crawley, 2002). For example, St John's wort, *Hypericum perforatum* L. (Hypericaceae), is a perennial that invades disturbed areas, including pastures, and grows into dense stands that inhibit livestock grazing (Crompton *et al*., 1988). Vilà and Marco (2004) compared herbivory of St John's wort from native ranges in Europe and from invaded areas in western North American. They found that the invasive populations in North America had less herbivor damage and grew more abundantly compared to native European populations, indicating that the invasive populations experienced less attack by pests in their new ranges (Vilà *et al*., 2003).

Contrary to the enemy release hypothesis, some introduced species have not become invasive because they lack mutualistic relationships in the new habitat (Zenni & Nuñez, 2013). *Ficus microcarpa* L. f. (Moraceae) an introduced monoecious fig tree in Florida only became widely invasive once its mutualistic wasp pollinator, *Eupristina verticillata* Waterston (Agaonidae), was introduced into the region (Zenni & Nuñez, 2013; Wang, 2014). *Ficus microcarpa* is also an introduced tree in South Africa, however it remains non-invasive due to the absence of *E. verticillata* in the country (van Noort *et al*., 2013).

1.2. Invasive plants

The spread of alien invasive plants can be linked to dispersal vectors such as: industrial trading for ornamental purposes, internet trade, indirect transport via humans, wind or bird dispersal (Coutts *et al*., 2011; Martin & Coetzee, 2011; Faulkner *et al*., 2020). Two of the main causes for the spread of aquatic invasive plants are the

global aquarium trade and the ornamental garden trade, inadvertently contributing to the harmful distribution of these plants that exclude and out-compete indigenous florae (Martin & Coetzee, 2011; Olden *et al*., 2020).

The global aquarium trade and ornamental garden trade has subsequently accelerated the transport of exotic aquatic species beyond their natural dispersal barriers at a pace that far exceeds naturally occurring introductions over geological time scales (Havel *et al*., 2015). The subsequent results of these introductions cause devastating impact, especially since they are generally traded as vegetative fragments with high survival probability and thus do not require specific conditions for seed germination (Peres *et al*., 2018). Peres *et al*., (2018) determined that in Brazil, 287 species of aquatic plants from 58 families are commercialized for online trade through illegal e-commerce of aquatic plants and out of the 287 traded species only 34% were of native Brazillian flora, and 11 species were native in all Brazillian biomes. It was also found that 188 species were exotic, with several highly invasive species in many parts of the world present, such as *Pontederia crassipes* (Martius) [≡*Eichhornia crassipes* (Martius) Solms-Laubach] (Pontederiaceae), *Egeria densa* Planch. (Hydrocharitaceae), *Hydrilla verticillata* (L.f.) Royle (Hydrocharitaceae) and *Myriophyllum aquaticum* (Vell.) Verdc. (Haloragaceae) (Peres *et al*., 2018). Another study by Maki and Galatowitsch (2004) determined that federal noxious weeds and exotic species could be obtained 98% of the time during sale and transport of aquatic plants from horticultural traders throughout the Unites States of America into Minnesota. Misidentification and incidental inclusion of the wrong species were also issues raised in the transport of aquatic plants, whereby 93% of the time aquatic plant traders across the Unites States of America accidentally included aquatic plants not requested in their orders (Maki & Galatowitsch, 2004). Similar findings were present during surveys in South Africa, whereby a variety of invasive aquatic plants such as water lettuce (*Pistia stratiotes* L. (Araceae)), red water fern (*Azolla filiculoides* Lamarck (Azollaceae)) and Kariba weed (*Salvinia molesta* D.S. Mitchell (Salviniaceae)) amongst others were traded by pet stores and aquarium traders who were often unknowing or uninformed about the invasiveness and regulations prohibiting sales of these species (Martin & Coetzee, 2011). The resultant outcome of these risky trades can be devastating. Often lack of knowledge regarding alien invasive plants can result in overflow or disposal of the invasive plant into ditches, dams and streams that could

lead to subsequent plant invasion. One example of the negative impacts caused by an invasive aquatic plant is the heterosporous aquatic fern, *A. filiculoides*, that was introduced as a pond plant from South America and became invasive throughout South African waterbodies in the 1980's (Hill *et al*., 2008). *Azolla filiculoides* became a rapid coloniser on open water surfaces and was easily dispersed to other waterbodies by waterfowl and vehicles driving through shallow infested waters (Hill *et al*., 2008). *Azolla filiculoides* spread predominantly by vegetative fragments, forming dense mats that prevent photosynthesis and oxygen diffusion from the air, thereby eliminating submerged plant, fish and invertebrate populations and degrading waterbodies to a point of ecosystem collapse (Gratwicke & Marshall, 2001). Mechanical and chemical control of *A. filiculoides* proved undesirable in the invaded aquatic environments and thus biological control was considered, and in 1997 the frond-feeding weevil, *Stenoplemus rufinasus* Gyllenhal (Coleoptera: Curculionidae) from Florida, United States of America was released in South Africa (Hill, 1998). The release of the frond-feeding weevil to control *A. filiculoides* was extremely successful, and the weed no longer poses a threat to the aquatic ecosystems of South Africa (McConnachie *et al*., 2004; Hill *et al*., 2008).

A variety of control mechanisms can be implemented to control invasive plants such as: mechanical removal by mowing or digging out plants; manual removal by mulching and hand pulling; chemical application by spraying herbicides; and implementation of a biological control programme, whereby a natural enemy (insect, parasite or pathogen) usually from the invasive species' origin specifically targets the plant (Watts *et al*., 2015). Myers and Bazely (2005) discussed the following advantages and disadvantages of these control mechanisms for alien invasive plants: manual and mechanical control can be labour intensive and expensive to implement and can also be ineffective if there is a large belowground biomass that can resprout or if the invasion sites are inaccessible. Chemical control is extremely expensive and can be highly damaging to the environment. Manual, mechanical and chemical controls are generally short-term solutions to alien plant invasions; however, they can promote job creation. Biological control is generally the most cost effective, long-term solution, however one of its disadvantages is that the biological control agents take time to build up their populations and disturbances or disease might cause populations to decline.

Integrated control methods using biological control together with other controls can therefore produce successful results (Orr, 2009).

Early detection, rapid response and monitoring changes to species invasions is also important for effective exotic plant management strategies to prevent further spread and ecosystem damage (Wilson *et al*., 2017). While prevention of species invasion is the first line of defense, early detection and rapid response to invasions is important to minimize the invasions impact (Reaser *et al*., 2009). Once an invasive or exotic species is detected in an area, resources should be directed into managing and monitoring the area (Guillera-Arroita *et al*., 2014). Managing a species during early stages of its invasion can save resources and reduce long term costs by preventing it from causing further negative environmental and socio-economic impact (Guillera-Arroita *et al*., 2014). Removing an invasive species is not always straightforward, and if eradication success is declared too soon, then the invasive species may reinvade due to weakened control pressure (Reaser *et al*., 2009). Long-term monitoring of invasive species, especially when a control mechanism is in place is therefore essential to determine if further control strategies are needed, as well as providing valuable information about how the ecosystems and communities are responding (Blossey, 1999). In a biological control context, post-release evaluation and surveys are paramount to success, and provide information about whether invaded sites are being successfully controlled and restored, or whether more biological control agents need to be reared for further release on the invaded sites to maintain control pressure. (Carson *et al*., 2008; Morin *et al*., 2009; Schaffner *et al*., 2020).

1.3. Plant invasions in South Africa

South Africa has an extensive history of invasive plants that reduce native biodiversity and degrade terrestrial and freshwater ecosystems (Levine *et al*., 2003; Van Wilgen *et al.,* 2020). The economic damages that alien invasive species have caused in South Africa are estimated to have cost billions of Rands each year and, as more species are introduced, the problems and impacts are increasing (Van Wilgen & Wilson, 2018).

In the past, many freshwater ecosystems in South Africa were anthropogenically modified due to an increased water demand from urbanization, industrialization and population growth (Naidoo, 2005). Historically, South Africa contained an abundance

of rivers and streams but large, standing waterbodies were uncommon. The anthropogenic demand for freshwater subsequently drove the construction of many standing man-made waterbodies (DEA, 2012), however few indigenous aquatic plants grew in these ecosystems. Furthermore, increased urbanization and industrialization led to an increase in pollutants directly or indirectly flowing in these freshwater systems (DEA, 2012). Increased nitrogen and phosphates thus led to degradation and eutrophication of South African freshwater systems, which provided ideal habitats for alien invasive aquatic macrophytes. Aquatic macrophytes are aquatic photosynthetic organisms that can be seen by the naked eye, and can be categorized into floating macrophytes (on the surface of a waterbody), submerged macrophytes (below the surface of a waterbody) or emergent macrophytes (rooted plants growing up through the surface of the water) (Chambers *et al*., 2007).

Floating invasive macrophytes invaded South Africa without difficulty due to an absence of indigenous floating macrophyte competitors. Consequently, slow flowing water bodies exhibited nutrient-rich niches that lacked native plant barriers, allowing for invasive macrophytes such as *P. crassipes* to spread abundantly (Coetzee *et al*., 2011). *Pontederia crassipes,* commonly known as waterhyacinth, is a notorious floating aquatic weed that aggressively spreads and overruns waterbodies, especially in eutrophic waters (Van Wyk & Van Wilgen, 2002). Floating invasive macrophytes such as water hyacinth have caused many freshwater ecosystem disturbances, negative ecological impacts such as increased habitats for mosquitos, and regime shifts through opportunistic expansion into South African waterbodies (Coetzee *et al*., 2011). Other examples of invasive floating macrophytes in South Africa include red water fern (*A. filiculoides*), water lettuce (*P. stratiotes*) and parrot's feather (*M. aquaticum*) (Henderson & Cilliers, 2002).

Measures to control and reduce floating macrophyte invasions have been successfully implemented in many cases, thereby presenting submerged and emergent invasive macrophytes the opportunity to spread in their absence (Coetzee *et al*., 2011). More recent invasions from invasive macrophytes spreading through South African freshwater ecosystems includes a submerged African Oxygen Weed *E. densa,* a floating macrophyte *Nymphaea mexicana* Zucc. (Nymphaceae) and emergent

macrophytes such as *Iris pseudocorus* L. (Iridaceae), *Nasturtium officinale* W.T. Aiton. (Brassicaceae) and *Pontederia cordata* L. (Pontederiaceae) (Hill & Coetzee, 2017).

Invasions from emergent macrophytes are known to compete with indigenous macrophytes and can have a negative effect on species richness and diversity (Michelan *et al.*, 2009). *Sagittaria platyphylla* (Engelm.) J.G. Sm (Alismataceae) is an emergent rooted macrophyte from the United States of America that has caused considerable negative impacts on aquatic ecosystems in South Africa (Ndlovu *et al*., 2020). In Australia, *S. platyphylla* causes significant damage to irrigation infrastructure and blocks waterways, drains and channels which causes highly detrimental impacts due to increased water levels and blockage during flooding periods (Adair *et al*., 2012). *Sagittaria platyphylla* also causes significant impacts on recreational activities and provides habitats to invasive fish such as the European carp by forming dense stands (Adair *et al*., 2012). In South Africa, *S. platyphylla* has spread rapidly over 120 kilometers and 72 sites – since its first detection in 2008 – by vegetative stem fragments, tubers and stolons and inflorescences' producing thousands of achenes (small one-seeded fruit) (Ndlovu *et al*., 2020). *Sagittaria platyphylla* and other invasive emergent freshwater macrophytes cause a deterioration in native habitat quality and heterogeneity. This is caused by the macrophytes forming impenetrable monotypic infestations and changing light diffusion and dissolved oxygen which can cause anaerobic conditions in waterbodies and wetlands (Kovalenko *et al*., 2009).

1.4. Pontederia cordata

Pontederia cordata is another invasive emergent macrophyte in South Africa that belongs to the Pontederiaceae family. Named after the pickerel fish that inhabits the submerged parts of the plant, *P. cordata* is commonly known as pickerelweed (Eckenwalder & Barrett, 1986). *Pontederia cordata* is indigenous to Central, North and South America. The taxonomy of *P. cordata* comprises several synonyms and varieties. The most common varieties listed are *Pontederia cordata* var. *cordata* L., *Pontederia cordata* var. *ovalis* (Martius) Solms and *Pontederia cordata* var. *lanceolata* (Nutt.) Griseb (ITIS Report, 2017). Synonyms include *Pontederia cordata* L. var. *lancifolia* (Muhl. ex Elliot) Torrey, *Pontederia cordata* L. var. *albiflora* Raf and

Pontederia cordata L. var. *angustifolia* (Pursh) Torrey & Elliot (Lowden, 1973; Melton & Sutton, 1991; ITIS Report, 2017).

Pontederia cordata plants are 1 - 2 m in height and can be identified by tristylous flowers on a spike that emerges above tall green leaves with varying shape and size (Lowden, 1973). Distinguishing leaf variability has been identified between two of the three varieties - *P. cordata* var. *lanceolata* produces leaves that are lanceolate in shape and are typically narrow to broad, whereas *P. cordata* var. *cordata* produce oval to triangular cordate shaped leaves (Melton and Sutton, 1991). *Pontederia cordata* var. *ovalis* is similar in leaf morphology to *P. cordata* var. *lanceolate* because it also has ovate or 'lanceolate' lamina (Smith, 1898). Other closely related *Pontederia* species, such as *Pontederia parviflora* Alex. (Pontederiaceae) produces white flowers with a yellow nectar guide in the posterior perianth lobes, whereas *P. cordata* produces blue-purple flowers and yellow nectar guides on the middle upper lobe (Fig. 1.1) (Lowden, 1973; Pellegrini *et al*., 2018). An individual *P. cordata* flower consists of a short corolla with three upper and three lower lobes spreading outwards, and nectar guides present as two yellow marks (Henderson & Cilliers, 2002).The flower organs generally develop into one style and two sets of three stamens at variable style lengths (Henderson & Cilliers, 2002).

Pontederia cordata possesses a unique floral trimorphism in the form of tristyly (Fig. 1.2), whereby three types of flowering plants may be present in a population (Ornduff, 1966). The nature of tristyly in a population may occur as follows: flowers containing long and mid-length anthers and a short stigma (Fig. 1.2.A); flowers containing long and short-length anthers and a mid-length stigma (Fig. 1.2.B); flowers containing mid-length and short-length anthers and long stigma (Fig. 1.2.C) (Ornduff, 1966). This reproductive scheme ensures efficient cross breeding by promoting pollination of stigmas from anthers at the equivalent level from other plants (Price & Barrett, 1984). Tristyly combined with a self-incompatability system therefore reduces the occurrence of self-pollination and maximises gene flow and seed production within a population (Ornduff, 1966).

Figure 1.1. *Pontederia cordata* L. (Pontederiaceae) (Watercolour painting by M. Stones, 1991).

(https://louisianadigitallibrary.org/islandora/object/lsu-sc-msw:124/datastream/JPG/view).

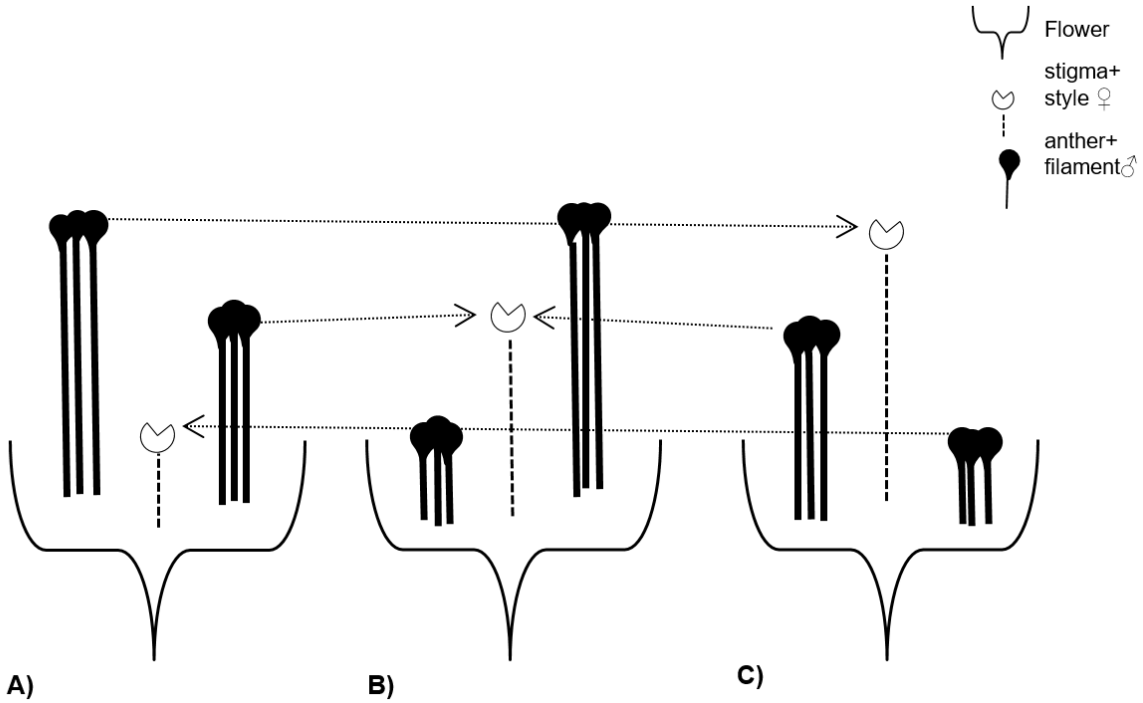


Figure 1.2. Diagrammatic representation of floral morphology: three different style morphs in tristylous breeding system of *Pontederia cordata*. **A)** short-styled morphology **B)** mid-styled morphology **C)** long-styled morphology. Arrows indicate legitimate cross-pollination by pollinators.

Populations *of P. cordata* typically form large colonies in shallow waterbodies such as ponds, streams, wetlands and riverbanks. The plant's long fibrous roots (Fig. 1.3) can form dense vegetative mats that block drainage ditches and spread throughout wetlands and irrigated crop fields (Melton & Sutton, 1991). These roots also possess aerenchyma that increases oxygen transfer and root porosity, resulting in a tolerance towards infertile soil and variable water quality (Li *et al*, 2013; Gu *et al*, 2015). *Pontederia cordata* also thrives in nutrient rich sediments, altogether giving this species a competitive advantage over plants that lack these adaptable characteristics (Melton & Sutton, 1991).

Figure 1.3. Dense, fibrous roots of *Pontederia cordata* (photo by S. Wansell, 2019).

Pontederia cordata populations may also reproduce via clonal growth through rhizome propagation (Melton & Sutton, 1991). Since clonal plants possess the same genetic identities, flowers in a rhizome-connected population may all possess the same tristylous floral morphology (Ornduff, 1966). It is therefore essential for a pollinator to carry pollen from a different tristylous individual to prevent self-pollination. Self-pollinated plants generally produce very few seeds, are more vulnerable to diseases, and tend to have an earlier senescence of the flowers' pistil (Scribailo & Barrett, 1994). Pollinators of *P. cordata* in its native range in eastern Ontario, Canada, include two bumble bees, *Bombus impatiens* Cresson (Apidae) and *Bombus vagans* Smith (Apidae) and a specialist bee, *Melissodes apicata* Lovell and Cockerell (Anthophoridae) - an insect that is structurally adapted for pollen collection from *P. cordata* flowers (Harder & Barrett, 1993). Pollen is collected on different parts on the insect's body and subsequently deposited onto corresponding stigmas of flowers with suitable style-morphs, resulting in successful cross-pollination (Harder & Barrett, 1993).

1.5. Invasion of *Pontederia cordata* in South Africa

South Africa is a water-scarce country, and the ability of *P. cordata* to form dense monospecific stands and reduce surface water runoff in vital waterbodies may become highly problematic. *Pontederia cordata* has recently been labelled as a Category 1b alien invasive species on South Africa's National List of Invasive Species under the National Environmental Management: Biodiversity Act 10/2004 (Department of Environmental Affairs, 2014). A category 1b alien invasive species requires compulsory control as part of an invasive species control programme (Department of Environmental Affairs, 2014). Category 1b invasive plants are to be removed and destroyed because they have a high invasive potential (Department of Environmental Affairs, 2014). No permits are to be issued out for these plants, and thus far only one plant trader has been issued with a notice in connection with *P. cordata* to withdraw the listed invasive species from trade (Van Wilgen & Wilson, 2018).

Control and removal of *P. cordata* is crucial, as rapid spread of this alien invasive in South Africa may have already caused deleterious effects on the invaded areas hydrology and native ecosystems by reducing water flow, increasing water uptake and outcompeting indigenous plants (Chamier *et al.,* 2012). Appropriate disposal of *P. cordata* is necessary to ensure that the plants do not spread further once removed. It is speculated that plants have been spreading via rhizome fragmentation since no seed set has been observed in any of the populations in South Africa. Propagation can easily occur when rhizomes break off from a cluster of plants, thus *P. cordata* is an invasive species that can easily be traded horticulturally and has ideal hardy characteristics to continue growing once established in an area (Yu *et al*., 2016).

Many invasive populations throughout eight South African provinces have already been recorded. Populations are often found in wetlands, waterbodies such as dams, on riverbanks, tributaries and urban environments such as drainage systems and ponds. The following sites have been recorded as field observations during this study to obtain a brief overview of the extent of invasion in some areas in South Africa.

Eastern Cape Province

Hogsback Arboretum is a popular tourist attraction in Hogsback (32.5952° S, 26.9323° E) in the Eastern Cape. The Arboretum is a preserved area with beautiful waterfalls and many hikes, however, the main causeway at the entrance of the Arboretum is highly infested with *P. cordata* on both sides of the bridge (Fig. 1.4.a). Water no longer flows through the infested area, and dense rhizomes and large monospecific stands outcompete indigenous plants. Similarly, another hiking trail next to a flowing river approximately 5 km away from Hogsback Arboretum also contains a dense *P. cordata* population. This infestation is also growing along a causeway and restricting water flow (Fig. 1.4.b).

Many populations have also been found in urban environments. Two separate populations of *P. cordata* were found growing abundantly at the Boardwalk Casino and Entertainment World located next to the Nelson Mandela Bay beachfront in Port Elizabeth (33.9608° S, 25.6022° E) (Fig. 1.3.c). *Pontederia cordata* populations at the Boardwalk are approximately 1-1.5m in height and prevent access to some of the ponds. The Boardwalk is situated in a region that is experiencing serious drought and water restrictions, thus efficient water management and saving is paramount (Colvin *et al*., 2016). Field observations have shown that vigorous and aggressive growth of *P. cordata* have already began outcompeting indigenous plants growing alongside the banks of the ponds.

Pontederia cordata has also been observed growing in many garden ponds. A population of *P. cordata* was found growing in a resident's koi pond in Gonubie (32.9423° S, 28.0098° E), a suburb of East London (Fig. 1.4.d). Gonubie has many small causeways, drainage ditches and streams, thus growing this highly invasive species in ponds is a serious risk because overgrown plants that cannot be controlled are often removed and dumped in such areas. Furthermore, attractive pond plants such as *P. cordata* with showy lilac-purple flowers may easily be shared amongst avid gardeners that are unaware of the plant being a Category 1b invasive (NEMBA, 2004).

Pontederia cordata is known to be used as a pond stabiliser and is frequently used to decorate or stabilise waterbodies in golf courses and fish farms. The Royal Port Alfred golf course in Port Alfred (33.5864° S, 26.8851° E) has a small dam where *P. cordata*

has been proliferating. The dam is situated in a low-lying area that has been flooded in the past, thus it is speculated that rhizomes from dumped garden refuse led to infestation of the dam.

Figure 1.4. Infestations of *Pontederia cordata* in the Eastern Cape province, South Africa. a) dense flowering population at the Hogsback Arboretum, Hogsback. b) infestation at a causeway along a hiking trail, Hogsback. c) invasive populations at the Boardwalk, Port Elizabeth. d) flowering plants found in an ornamental fishpond, Gonubie, East London (photos by S. Wansell, 2019).

Kwa-Zulu Natal province

The Underberg Country Club and golf course in Underberg, Kwazulu-Natal (KZN) (29.7929° S, 29.4918° E) has a large dam with *P. cordata* populations surrounding almost the entire perimeter (Fig. 1.5.a). Expansion and branching off from these populations could be seen towards the lower lying dams and it was speculated that plants or rhizomes broke off and washed over the pathway during a flood event (Fig. 1.5.b). Himeville Nature Reserve situated in the small town of Himeville (29.7496° S, 29.5137° E) just outside Underberg in KZN had a large infestation of *P. cordata* in their main high lying dam (Fig. 1.5.c). This population was different to other *P. cordata* populations in South Africa because they produced long, thin lanceolate shaped leaves, whereas field observations of other populations recorded mainly lighter green, cordate shaped leaves. It may therefore be possible that more than one variety of *P. cordata* is present in South Africa, such as *P. cordata* var. *lanceolata* in the Himeville Nature Reserve, KZN because of the lanceolate leaf morphology, whereas other populations mentioned in other provinces might be *P. cordata* var. *cordata* because of their cordate shaped leaves. Alternatively, phenotypic plasticity could be contributing to the variable leaf morphology present in different populations throughout the country.

Populations of *P. cordata* were also observed growing in garden ponds in Pietermaritzburg (29.6006° S, 30.3794° E) and a few residents have even admitted to removing and sharing plants once growth became too abundant in their ponds. The KZN National Botanical Garden in Pietermaritzburg has an irrigation canal that *P. cordata* was spreading through.

Figure 1.5. Invasion of *Pontederia cordata* in Kwazulu-Natal Province. **a)** Underberg Golf Course highly infested with *Pontederia cordata* surrounding the top dam **b)** growth and expansion of *P. cordata* across the bridge into the lower lying dam **c)** Himeville Nature Reserve dam with large *Pontederia cordata* population (Photos by S. Wansell, 2019)

Western Cape Province

The Western Cape Province is renowned for its rich biodiversity, however many urbanized areas in the Western Cape are ideal hotspots for invasion (Rebelo *et al*., 2011; Gaertner *et al*., 2017). Field observations conducted on *P. cordata* populations in Jonkershoek (-33.956784° S, 18.915221° E) recorded dense monospecific stands with flowers producing no seeds (Fig. 1.6.) (S. Geerts, pers. comm. 2018). Similarly, a population at the Paarl Arboretum (33.75952° S, 18.97677° E) contained no seeds or single plants (S. Geerts, pers. comm. 2018).

Field observations the following year showed that the populations in Paarl Arboretum were no longer abundant due to the deep waterbodies drying up, furthermore, the

dense populations in Jonkershoek were mostly cleared and replanted with native species (S. Geerts, pers. comm. 2018) .

Figure 1.6. Invasion of *Pontederia cordata* in the Western Cape province. **a)** *Pontederia cordata* inflorescence in bloom and post-bloom with no seed set **b)** a flowering population in Jonkershoek (photo by S. Geerts, 2018) **c)** a dense population of *Pontederia cordata* in Jonkershoek (photo by P. Ivey, 2018).

Gauteng and North West Provinces

Another urbanized area that is highly infested with *P. cordata* populations is the Johannesburg Botanical Gardens and Emmarentia Dam (26.1503° S, 28.0058° E) in Gauteng. These dense monospecific stands were found throughout the perimeters of the dams as well as clusters in the shallow areas (Fig. 1.7.a-c). Many other aquatic invasive plants also invaded the areas, including *I. pseudacorus* and *N. mexicana*. There are reports that campaigns in Johannesburg have been implemented to control alien species invasions (Jobo, 2018), however if invasive plants such as *P. cordata* and *I. pseudacorus* are not completely removed, they will continue to resprout and spread via underground rhizomes (Jaca & Mkhize, 2015).

Populations of *P. cordata* have also been observed at Pecanwood Estate (25.7684° S, 27.8575° E) located next to the Hartbeestpoort dam. Flooding from these ponds containing *P. cordata* plants could therefore be catastrophic and could lead to rapid spread into the eutrophic dam that is already infested with *P. crassipes* (Kitunda, 2017).

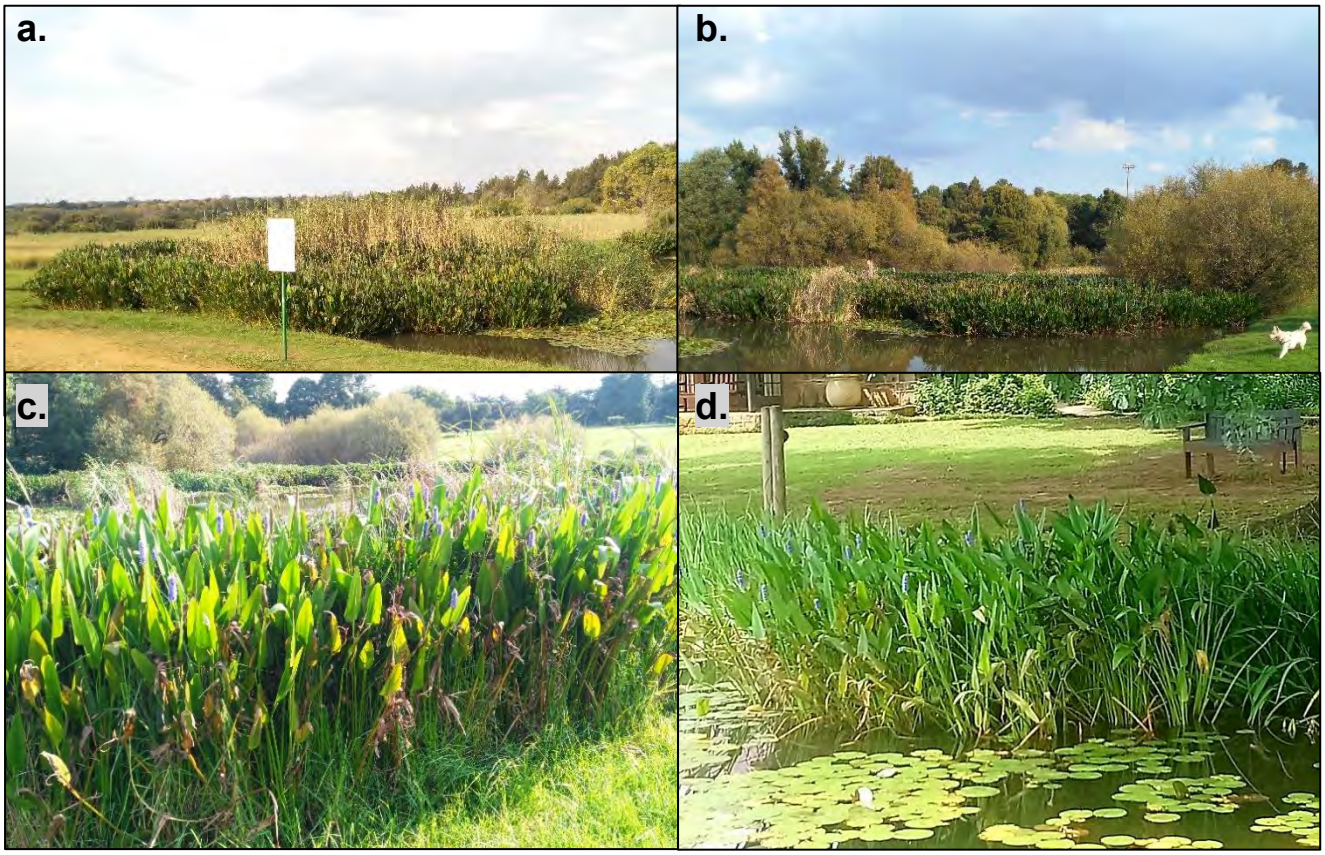


Figure 1.7. Invasive populations of *Pontederia cordata* in Gauteng and North West Province. **a-c)** dense populations of *Pontederia cordata* in Emmerentia dam, Johannesburg, Gauteng **d)** population of *Pontederia cordata* at Pecanwood Estate, Hartbeestpoort, North West Province

Developing a control strategy against the spread of *P. cordata* in South Africa is crucial to halt the invasion of *P. cordata* spreading abundantly throughout South Africa. Furthermore, understanding the invasion ecology of *P. cordata* is the first step to developing such a strategy.

1.6. Overview of study aims and thesis structure

The aim of this study is to answer the following key questions about *P. cordata* invasion in South Africa:

Are the invasive *P. cordata* populations in South Africa genetically diverse or is one source population spreading clonally throughout the country?

Chapter 2 investigates the population genetics of *P. cordata* populations in South Africa compared to some native range populations. Investigating the population genetics of *P. cordata* would allow the determination of the genetic diversity of the populations in South Africa. It may be possible that the plants spreading throughout South Africa are clonal or a horticultural variety with little genetic diversity. Alternatively, a high genetic diversity and multiple introductions could attribute to the successful expansion of *P. cordata*. These findings could contribute valuable information to the formation of a biological control programme and provide insight into effective control measures.

Chapter 3 investigates the invasion and reproduction characteristics of *P. cordata* in South Africa. Understanding the ecology of *P. cordata* involves investigating how the invasive species adapts to variable habitat conditions. Generally, local adaptation and phenotypic plasticity aid in the survival and spread of invasive plant populations (Levine *et al*., 2003). *Pontederia cordata* may have these adaptive mechanisms through the following characteristics: clonal growth via rhizomes that cause rapid lateral expansion to form dense stands and outcompete native plants, and a tristylous reproductive system that ensures legitimate cross-pollination and higher seed production. Further investigation of these adaptive characteristics on *P. cordata* populations in South Africa would, therefore, provide valuable insight on the mechanism of invasion and how to combat this invasive species before it reaches its full invasive potential. The questions addressed in Chapter 3 are therefore:

Are plants spreading throughout South Africa via sexual reproduction or is the absence of one or more morphed flowers causing a self-incompatibility system and thus no seed production?

Pontederia cordata populations typically have a long flowering period, thus it may be possible that seeds are being produced over a long period of time (Ornduff, 1966). South Africa may have an ideal climate for seed germination since optimal germination of its seeds generally occur at temperatures higher than 20 ℃ (Whigham & Simpson, 1982). Obtaining flowers from populations distributed throughout South Africa would allow one to investigate whether there are different floral morphologies (short, medium and long-morphed flowers) present, a characteristic that could lead to rapid production of seeds that could spread throughout freshwater ecosystems in the country. However, it is highly probable that populations are spreading vegetatively by asexual propagation of rhizomes, since anecdotal evidence suggest only short-morphed flowers are present and no seeds are produced in South Africa. Performing pollination experiments would determine whether seed production is possible or whether this is prevented due to the lack of multiple floral morphs of this tristylous species.

Is an absence of *P. cordata* pollinators preventing seed set?

The types of pollinators that pollinate *P. cordata* populations in South Africa are largely unknown, as little research has been conducted on the reproductive aspects of *P. cordata* in the invaded range. Further investigation is necessary to determine whether established populations in South Africa are being pollinated by native insects, and whether any seeds or fruit can be produced as a result of this pollination.

CHAPTER 2.

Population genetics of *Pontederia cordata* in South Africa

2.1. Introduction

Phylogenetics and population genetics study the genetic connections and interactions between taxa and between individuals respectively, and broaden our understanding of evolutionary relationships, highlight inter- and intraspecies genetic distribution and variation (Avise & Wollenberg, 1997; Cutter, 2013). This in turn can aid in the conservation of ecosystems and studies on biodiversity (Kresovich & McFerson, 1992; Mondini *et al*., 2000). Moreover, performing genetic analyses on invasive species can contribute to a better understanding of the relationships and ecology of the invader (Kresovich & McFerson, 1992; Mondini *et al*., 2009). This includes components of life histories that have contributed to successful invasions and the phylogenetic placement of an invasive species into geographic regions (Mondini *et al*., 2009). A comparative genetic study by Crawley *et al*., (1996) on a wide range of successful invasive plants species in the British Isles found that there was a low number of naturalised invasive plants originating from tropical and sub-tropical areas due to the lack of frost hardiness. Alternatively, invasive plants that originated from similar geographic and climatic regions invaded far more abundantly as they possessed traits that allowed them to withstand frost (Crawley *et al*., 1996). By performing these analyses, it was also found that the invasive plants generally grew bigger than native plants as a result of a lack of natural enemies, thereby having a competitive advantage over native plants (Crawley *et al*., 1996).

The study of phylogenetics and population genetics may also help researchers in understanding invasive species and their invasion success through taxonomic clarification, assessment of evolutionary relationships, ploidy levels and genetic adaptation and admixture (Le Roux *et al*., 2010; Gaskin *et al*., 2011; Handley *et al*., 2011; Beest *et al*., 2012). These studies can also help manage invasions through the development of biological control programmes of target invasive species with monophagous enemies because they can help elucidate invasive species reproduction schemes and hybridization events as well as compare the diversity of invasive species to source populations (Prentis *et al*., 2008; Wilson *et al*., 2009). Moreover, molecular approaches may improve biological control success by clarifying

biological control agents' population structure and even uncovering cryptic species. Through genetic analyses and interbreeding studies on a biological control agent for *P. crassipes* it was determined that, what was first thought as one single species of sap-feeding mirid for biological control in South Africa, were actually two reproductively isolated species (Taylor *et al*., 2011; Paterson *et al*., 2016). Furthermore, Paterson *et al.,* (2019) determined that these two mirid species had high host specificity for *P. crassipes*, however they exhibited different thermal tolerances and thus the correct species should be released in sites with suitable thermal environments for more effective biological control of *P. crassipes*. The positive implications from performing these genetic studies include discovering more potential agents for biological control programmes, as well as decreasing the potential danger of cryptic species that may cause unanticipated non-target plant damage by expanding our knowledge of the biological control agents themselves (Paynter *et al*., 2008; Smith *et al*., 2018).

2.1.1. The importance of genetics in understanding and managing alien invasive plants

The distribution of an invasive species in a foreign area is typically caused by an invasive individual being released once and spreading, or a series of localised releases (Kolar & Lodge, 2001). In the past, it may have been hard to distinguish between these two factors; however, the development of molecular techniques has enabled one to find the source of an invasive species in its native range and subsequently link invasive populations to the source (Paterson *et al*., 2009). A study by Ndlovu *et al*., (2013) used nuclear and plastid DNA markers to construct phylogenetic relationships between *Acacia pycnantha* Benth. (Fabaceae) populations in native regions of eastern Australia and invasive populations that have spread to western Australia, South Africa and Portugal. Through this phylogenetic approach, plastid genealogies depicted two distinct dryland and wetland forms of *A. pycnantha* in eastern Australia. Invasive *A. pycnantha* populations in Portugal and western Australia resembled the wetland form, whereas most of the invasive populations in South Africa were of the dryland form (Ndlovu *et al*., 2013). These findings had important implications when determining a biological control strategy, since the biological control agent, *Trichilogaster signiventris* Girault. (Pteromalidae), sourced from a native *A. pycnantha* dryland region in south Australia successfully controlled invasive *A. pycnantha* plants in South Africa that originated from the same region and

shared similar genotypic characteristics (Ndlovu *et al*., 2013). Identification of the source of an invasive species can therefore provide insight into host-specificity and compatibility in biological control strategies (Ndlovu *et al*., 2013).

Finding the source of a species in its native range may be possible since native populations generally have a degree of genetic distinctiveness (Lockwood *et al*., 2007; Gaskin *et al*., 2011). Genetic diversity is the variation of alleles in a gene pool (Banks *et al*., 2013). Patterns of genetic variation (or the lack thereof) may therefore provide insight into population history, such as genetic bottlenecks caused by disturbance or invasion of a species into a new area (Ward *et al*., 2008). By determining the genetic diversity and structure of individuals in native and invasive populations, it may be possible to find the origin of the invasive species through clustering of similar genotypes (Ward *et al*., 2008; Gaskin *et al*., 2011). Selection of a biological control agent from the original native plant population is the best strategy to ensure high efficiency of a selected biological control agent, since local adaptations and co-evolution of insects on the original population should be the most damaging to populations that have spread from this source (Paterson *et al*., 2009; Ndlovu *et al*., 2013). One such example of co-evolution is *Lygodium microphyllum* (Pteridophyta: Lygodiaceae), a species of scrambling fern indigenous to the wet tropical and subtropical regions of the Old World including Australia, Africa, Asia and Oceania and its co-evolved herbivore, an eriophyid mite, *Floracarus perrepae* Knihinicki & Boczek (Eriophyidae). A study by Goolsby *et al*., (2005) on *L. microphyllum* invading the Florida Everglades used chloroplast intron sequencing and herbivore transfer experiments to determine the origin of *L. microphyllum* invasion and the relationship between the different haplotypes of *L. microphyllum* and phytophagous mites. The haplotype match of *L. microphyllum* in the Everglades was found to originate from the region of northern Queensland, Australia/ Papua New Guinea (Goolsby *et al*., 2005). They subsequently determined that the locally adapted mites associated with the haplotype of *L. microphyllum* invading the Everglades originated from the same region of northern Queensland as the weed, and that these specific mites would be the best prospect for biological control in the Everglades because they are the most damaging to the specific haplotype (Goolsby *et al*., 2005). They established that all *F. perrepae* mite populations were locally adapted to the haplotypes of *L. microphyllum* from where they were collected, and that using mites locally adapted to the correct *L. microphyllum*

genotype would be the greatest prospect for biological control programmes since they caused direct impact on the plants fitness and optimal host exploitation (Goolsby *et al*., 2005).

While in some cases it is essential to know the exact geographic origin of an invasive weed population to select the most effective locally adapted genotype of the biological control agent, there are also instances where this is not necessarily important. Better prioritisation of biological control agents can also be determined though climatic matching, predicted host range and mode of damage by the agent on the weed (Paterson *et al*., 2014). Through these prioritisation methods, native enemies that are unsuitable for biological control programmes may be efficiently excluded, thereby reducing the cost of performing host specificity trials during the biological control programme development. In South Africa in the 1990s, invasive *Pereskia aculeata* Miller (Cactaceae) populations had limited impact by the biological control agent, *Phenrica guerini* Bechyné (Chrysomelidae, Alticinae) release in 1991 (Klein, 1999). *Pereskia aculeata* continued to spread and cause negative environmental and economic consequences. Molecular techniques were thus used to identify the source of South African *P. aculeata* populations which, through genotype matching, were most closely matched to populations from Rio de Janeiro in Brazil (Paterson *et al*., 2009). Further studies using climatic matching of the invaded range in South Africa to *P. aculeata*'s indigenous region were conducted (Paterson *et al*., 2014). In South Africa, *P. aculeata* is the most damaging and prolific invading climber in eastern subtropical coastal regions, thus natural enemies found in similar climatic conditions in its native region may be more suitable and likely to establish and persevere as biological control agents because they are already locally adapted to the climate (Paterson *et al*., 2014). Native enemies that had the best mode of damage for the selected weed were also surveyed for, such as targeting vegetative parts of the plant that would cause the most damage and ultimately plant mortality.

2.1.2. Commonly used molecular techniques and genetic markers

Genetic markers have been developed over time to enable the direct examination of DNA sequences of individuals, allowing genetic variation caused by mutations, insertions, shifts and deletions of DNA to be detected (Ward *et al*., 2008; Banks *et al*., 2013). Molecular techniques utilise genetic markers such as nuclear DNA-based markers, mitochondrial genomes (mtDNA) and chloroplast genomes (cpDNA) to

assess the genetic variability, and patterns of genetic differentiation in invasive species genomes (Demesure *et al*., 1995; Gaskin *et al*., 2011). DNA-based molecular markers generally involve the use of restriction enzymes and Polymerase Chain Reactions (PCR). Restriction enzyme procedures utilise restriction endonucleases to digest and cut amplified DNA to produce variable polynucleotide fragments depending on nucleotide deletions, insertions or mutations (DeBry & Slade, 1985). An example of a molecular marker technique that uses restriction endonuclease enzymes is Restriction Fragment Length Polymorphism (RFLP) – a process that involves DNA polymorphism detection through the binding of a chemically-labelled DNA probe to a Southern blot of DNA digested by restriction enzymes, thereby producing a profile of DNA fragments with variable lengths that may infer genetic variability (Bardakci, 2001). Desplanque *et al*., (1999) performed a genetic analysis using RFLPs on wild beets and weed beets, *Beta vulgaris* L. (Amaranthaceae) invading cultivated sugar-beet fields in northern France to determine the relationship among them. It was found that in seed producing areas, a high exchange of genes and hybridization occurred, however the outcome could produce weed beets if transgenes were introduced from wild to cultivated beets (maternal route) or spread hybridized beets into the wild if cultivated beets spread transgenes to wild beets (paternal route) (Desplanque *et al*., 1999). Altogether it was concluded that assessing possible gene flows is essential in risk assessment of transgenic beet farming and can highlight possible relationships between wild and cultivated plant species (Desplanque *et al*., 1999).

While RFLP techniques are advantageous because it does not require prior knowledge of the subject's genome, it can be expensive, complex, and generally requires large quantities of high quality DNA (Gaskin *et al*., 2011). Other DNA markers such as Random Amplified Polymorphic DNA (RAPD) used in plant phylogenetic and population genetic studies are generally faster and less laborious to perform on smaller quantities of plant genomic material (Deragon & Landry, 1992; Mondini *et al*., 2009).

RAPD is a PCR-based technique that involves the random amplification of genomic DNA using short primers (Stammers *et al*., 1995). The resultant amplified segments of genomic DNA produce polymorphisms, which are subsequently used as genetic markers in the construction of genetic maps to identify individuals at a species level (Williams *et al*., 1990). RAPD analysis was used in an invasion biology study to

examine the genetic diversity of invading Japanese knotweed, *Fallopia japonica* (Hout.) R. Decr. (Polygonaceae) populations in Britain (Hollingsworth & Bailey, 2000). Due to resultant identical RAPD profiles and the lack of any fertile male *F. japonica* individuals in Britain, Hollingsworth and Bailey (2000) concluded that populations were not multi-clonal, but were in fact a single widespread clone. RAPD can therefore be a useful analytical tool in invasive biological studies, however a notable disadvantage of RAPD is its sensitivity to reaction conditions and thus the outcomes of a procedure may produce different patterns based on the temperature profile, DNA purity and other such PCR parameters (Fritsch & Rieseberg, 1996; Qian *et al*., 2001).

Amplified Fragment Length Polymorphism (AFLP) markers are less sensitive to reaction conditions and can be used to identify individuals at a sub-species level on resultant genetic maps (Vos *et al*., 1995; Althoff *et al*., 2007). AFLP uses an approach similar to both RFLP and RAPD techniques, whereby whole genomic DNA is digested by restriction endonucleases and the resultant DNA fragments are bound to oligonucleotides (Vos *et al*., 1995). The DNA templates are then amplified using a combination of primers with different extensions to create a representative fraction of the genome. This is subsequently analysed by corresponding the fragments to unique positions on the genome (Vos *et al*., 1995; Meudt & Clarke, 2007). One of the main disadvantages of AFLP analysis is the reduced accuracy of results when genomic DNA is not completely digested by restriction endonucleases, which will in turn lead to amplification of uncut fragments (Vos *et al*., 1995; Mueller & Wolfenbarger, 1999). Additionally, DNA fragments produced by AFLP that are the same size may not have come from the same sequence or genomic region, thereby allowing a possibility of homoplasy and co-migration of fragments (Althoff *et al*., 2007; Meudt & Clarke, 2007). This technique is thus best used for phylogenetic studies investigating intraspecies patterns and not studies on significantly different taxa (Althoff *et al*., 2007).

Wu *et al*., (2010) used AFLP markers to evaluate the genetic diversity of invasive *Veronica hederifolia* L. (Plantaginaceae), commonly known as ivy-leaved speedwell, in eastern China. By performing AFLP analyses, they found that a high amount of genetic diversity existed within and between invasive *V. hederifolia* populations. Wu *et al*. (2010) therefore recommended that an eradication strategy and higher surveillance should be implemented since the invasive species appeared to be highly

competitive in new areas and could have broad adaptability because of high gene flow and genetic variation (Wu *et al*., 2010).

Inter-Simple Sequence Repeats (ISSR) are also commonly used to distinguish individuals at subspecies level (Lakshmanan *et al*., 2007). These markers are useful because they show inter- and intra-genomic diversity through variation within unique genomic regions (Godwin *et al*., 1997). ISSR analysis was used in a study by Paterson and Zachariades (2013) to confirm the existence of two genetically distinct southern African and Asian/West African biotypes of *Chromolaena odorata* (L.) R.M. King & Robinson (Asteraceae) in invaded countries, as well as the source of the biotype invading southern Africa. Based off a phylogenetic network constructed from the ISSR analysis, Paterson and Zacharaides (2013) determined that two biotypes of *C. ordorata* in invaded regions originate from two separate native regions and that the biotype invading southern Africa originated from Cuba and Jamaica (Paterson & Zachariades, 2013). Based on these results, surveys for potential biological control organisms are focused on populations in Cuba and Jamaica (Paterson & Zachariades, 2013). Similarly, an extensive phylogeographic study was also carried out on *C. odorata* by Shao *et al*., (2018) using different molecular techniques – chloroplast and nuclear DNA sequences and microsatellite DNA markers. The results concluded that the Asian/West African biotype may originate from Trinidad and Tobago and that, synonymous to the study by Paterson and Zachariades (2013), the southern African biotype originates from Cuba and Jamaica (Shao *et al*., 2018). They also suggested that potential biological control agents for the two biotypes should come from these source regions (Paterson & Zachariades, 2013; Shao *et al*., 2018). Different molecular techniques produce synonymous results and less costly techniques such as ISSR work just as efficiently as more complex DNA markers and sequencing techniques if they are used for appropriate questions and are interpreted correctly with the strengths and weaknesses of the relevant technique in mind.

Another analysis using ISSR was performed by Ye *et al*., (2004) on *C. odorata* to investigate the genetic variability of invasive populations in China. *Chromolaena odorata* populations in China displayed low genetic variation and Ye *et al*., (2004) suggested that this information should be considered when developing a biological control programme, since genetic uniformity in plants may result in a uniformed

response to a biological control agent (Ye *et al*., 2004). Ten years later, Yu *et al*., (2014) performed a study on *C. odorata* across vast regions of tropical and subtropical Asia using chloroplast DNA fragments and nuclear Internal Transcribed Spacers (ITS). Similar to the study performed by Ye *et al.,* (2004), Yu *et al.,* (2014) revealed extremely low genetic variability amongst *C. odorata* populations and that there is likely to be a dominant invasive genotype in Asia that is more invasive than genotypes elsewhere.

ISSR and AFLP are thus some of the best molecular techniques used in the phylogenetics of invasive plants as they allow for reliable genomic mapping and differentiation between closely related individuals in invasive species populations, without great expense and laborious complexity (Godwin *et al*., 1997; Mondini *et al*., 2009).

2.1.3. Phylogenetics of invasive aquatic plants

The need for reliable genetic source information has spurred the use of molecular techniques to analyse aquatic plants (Ward *et al*., 2008; Maréchal, 2019). These may differ slightly from population genetics of terrestrial plants because aquatic plants, especially invasive aquatic plants, frequently have different modes of reproduction, highly variable gene flow and local adaptations (Hu *et al*., 2017). Gene flow of invasive aquatic plants may be influenced by the discontinuous nature of their aquatic habitats such as enclosed, still waterbodies or sporadic running waters (Barrett *et al.,* 1993; Abbasi *et al*., 2016). Furthermore, the type of propagules in aquatic plants may affect genetic diversity, and the transport of vegetative fragments compared to seed dispersal may lead to more effective spread and successful gene establishment (Barrett *et al*., 1993). Consequently, asexual reproduction could explain the extensive geographical ranges of many clonal aquatic species (Barrett *et al*., 1993).

In a global scale population genetic survey conducted on the notorious waterweed, *P. crassipes* by Zhang *et al*., (2010), AFLP analyses presented low levels of genetic diversity in the majority of invasive populations and it was found that *P. crassipes* generally invades through clonal propagules. These findings have contributed to the understanding of the ecology, genetics of invasions and ultimately the invasive success of *P. crassipes*, as it was concluded that *P. crassipes* may have key life-history traits that promote naturalisation in foreign areas, rather than high levels of genetic variation and local adaptation (Zhang *et al*., 2010). Similarly, an invasive

macrophyte species *Egeria densa* Planch. (Hydrocharitaceae) was studied to examine genetic variability between invaded clonal populations in the United States of America and Chile, and native populations in Brazil, Uruguay and Argentina (Carter & Sytsma, 2001). The results showed little genetic variability between the populations, suggesting that a bottlenecking event could have occurred in the invasion history of the species, or that low genetic diversity is already present in native populations (Carter & Sytsma, 2001).

Many invasive aquatic plants reproduce asexually through clonal propagation (You *et al*., 2016). This may enable them to spread rapidly in favourable conditions and invade natural ecosystems, however, one of the main disadvantages that invasive species may face when spreading clonally is low genetic diversity (Dong *et al*., 2014). Low genetic diversity limits evolution and local adaptation of clonal plants, thereby limiting defences against pathogens and herbivores – this may be advantageous for biological control strategies, as biological control agents may effectively establish and prove more damaging on these populations (Ward *et al*., 2008; Paterson *et al*., 2009).

Assessing patterns of genetic diversity in invasive and native populations of aquatic invasive plants can allow one to determine the origin of invasion, especially in clonal species (Ward *et al*., 2008). A phylogenetic study by Madeira *et al*., (2007) on the highly invasive monoecious aquatic macrophyte *H. verticillata* in South Africa was performed to determine the origin of introductions. The resultant sequences of *H. verticillata* in South Africa matched identically with Indonesian and Malaysian specimens, which led to the hypothesis that the populations in South Africa are the product of horticultural trade from these regions (Madeira *et al*., 2007). These results may also have important implications for preventative measures of further introductions of *H. verticillata* in South Africa and may aid in the development of further biological control strategies as previously mentioned.

2.1.4. The phylogenetic analysis of *Pontederia cordata*

Pontederia cordata belongs to Pontederiaceae, the same family as *P. crassipes* – a Category 1b invasive weed in South Africa (NEMBA, 2014) and one of the world's worst invasive aquatic weeds (Holm *et al*., 1977). In recent years, *P. cordata* has also been categorised as a Category 1b invasive weed in South Africa (NEMBA, 2014) and has become invasive at sites throughout the country (Fig. 2.1). The pathway of

introduction of *P. cordata* into South Africa and dispersal mechanisms within the country remain unknown, however it is speculated that it may be a single introduction with subsequent asexually spreading via rhizomes, since no seeds have been observed in South Africa.

Figure 2.1. Distribution of invasive *Pontederia cordata* populations (red markers) in South Africa (records obtained from the SAPIA and SANBI databases, 2016).

Localities of *P. cordata* populations are locally distributed around points of introduction in urban areas, especially since it is likely that dispersal pathways originated from the horticultural trade (Price & Barrett, 1982). Characteristics of *P. cordata* species in South Africa include lilac to blue flowers and lanceolate to heart-shaped leaves that are similar to the features of *P. cordata* var. *lanceolata* (Nuttall) Grisebach and *P. cordata* var. *cordata*, respectively (Lowden, 1973; Melton & Sutton, 1991). *Pontederia cordata* comprises three main varieties, *P. cordata* var. *cordata*, *P. cordata* var. *lanceolata* (Nuttall) Grisebach (usually synonymic with *P. cordata* var. *lancifolia*

(Muhlenberg) Torrey), *P. cordata* var. *ovalis* (Martius) Solms and other synonymic names. Almost all the synonymic names of variety and form are based on leaf morphology (Lowden, 1973), however leaf variation in populations and individual plants have also been observed in the field which makes taxonomic identification based on leaf morphology difficult. *Pontederia cordata* is indigenous to North America (Fig. 2.2) throughout Ontario (Canada) to Nova Scotia and New England, south to Florida, north to Minnesota and west to Oklahoma and Missouri in the United States of America (Lowden, 1973; USDA-NRCS PLANTS Database, 2018). *Pontederia cordata* is also distributed in Cuba and throughout South America, including Colombia, Brazil, Paraguay, Argentina and Uruguay (Witt & Luke, 2017).

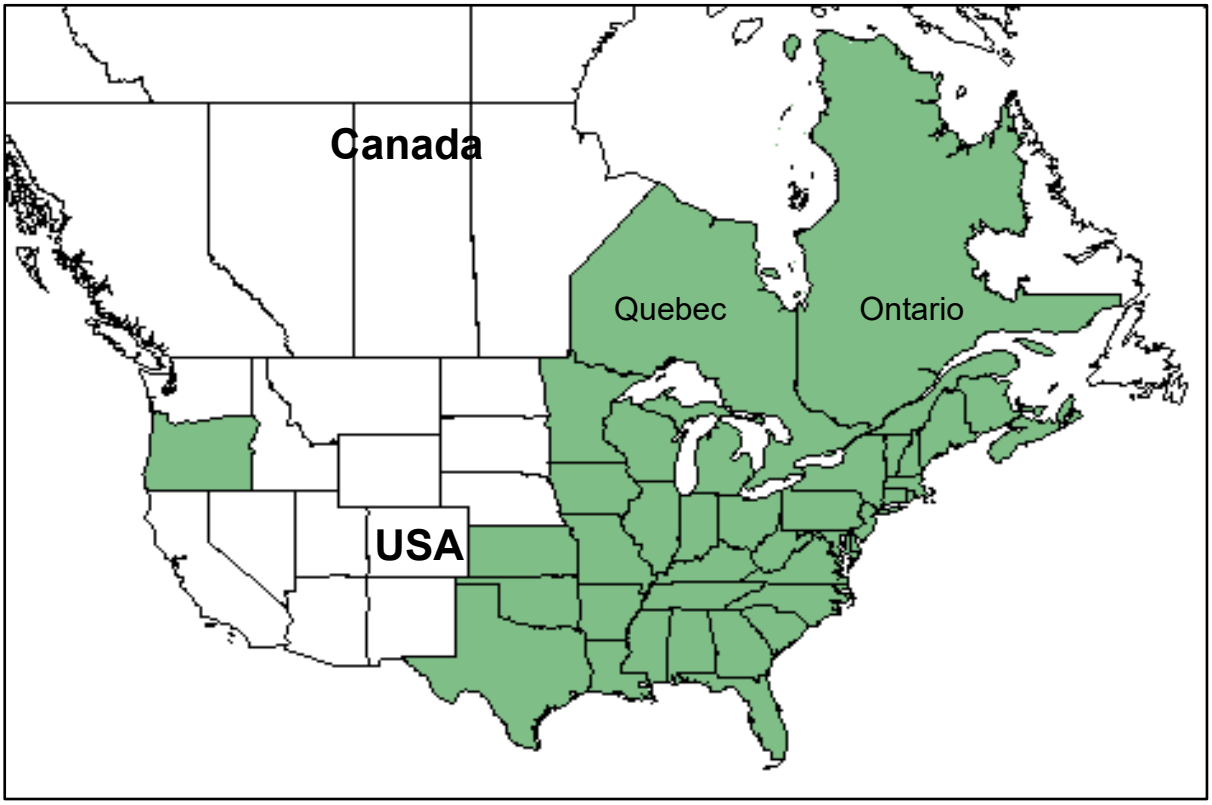


Figure 2.2. Map of the United States of America and Canada showing indigenous *Pontederia cordata* distribution (native *Pontederia cordata* populations highlighted green) (USDA-NRCS PLANTS Database, 2018).

Performing genetic analyses on samples from native regions in North America and invasive regions throughout South Africa would potentially allow one to determine source populations and may provide insight into the dispersal of the invasive species. Invasive *P. cordata* populations in South Africa may be the result of a single introduction spreading vegetatively/clonally with low genetic diversity. Alternatively, there may be high genetic diversity between the invasive populations, however, sexual propagation may not be viable due to ''illegitimate'' cross-pollination and self-incompatibility (Ornduff, 1966).

The aim of this study is therefore to investigate the relationship between native North American populations and invasive populations using ISSR to provide insight into the genetic diversity and mode of reproduction and spread of *P. cordata* in South Africa. This will also help in formulating management plans for the species, and more specifically, what biological control approaches can be taken to control the invasive populations.

2.2. Materials and methods

2.2.1. Sampling and leaf preservation

Introduced *P. cordata* populations were collected from natural and artificial (pond) waterbodies in South Africa covering the widest geographic distribution possible in order to obtain an extensive range of genetic variability. Native populations were sampled in the United States of America (Table 1). Within each *P. cordata* population, approximately 5 g of healthy, young leaf material was harvested and placed into zip-lock plastic bags. Young leaf material was harvested every metre for ten metres. The samples were individually wiped with distilled water to remove any contaminants and then wrapped in sterile tissue paper. Samples were then dried using silica gel blue according to Chase and Hills (1991) in sealed plastic bags.

Table 1. Localities, number of samples and co-ordinates of *Pontederia cordata* sample sites in South Africa and the United States of America.

Province/State	Locality	Geographic co-ordinates:	No. of samples used in analysis
South Africa (invasive range)			
KwaZulu-Natal	Tongaat	-29.5881° S, 31.0851° E	10
KwaZulu-Natal	Underberg golf course	-29.78613° S, 29.48936° E	1
North West Province	Pecanwood Estate, Hartbeestpoort	-25.76842 ° S, 27.85751° E	1
Gauteng	Emmarentia Botanical Gardens, Johannesburg	-26.16180° S, 27.99983° E	2
Gauteng	Glenhazel, Johannesburg	-26.1389° S, 28.1020° E	1
Western Cape	Jonkershoek	-33.95678° S, 18.91522° E	1
Western Cape	Westlake	-34.07560° S, 18.45124° E	1
Western Cape	Heldervue	-34.06067° S, 18.82819° E	1
Western Cape	Eden/Wilderness	-33.96573° S, 22.60981° E	1
Eastern Cape	Royal Port Alfred Golf Course	-33.60039° S, 26.89169° E	1
Eastern Cape	St Francis Marine	-34.14840° S, 24.87567° E	9
Eastern Cape	Grahamstown/Makhanda old golf course	-33.29694° S, 26.49930° E	1
Eastern Cape	Hogsback Arboretum	-32.59505° S, 26.94683° E	1
United States of America (native range)			
Virginia	Belle Haven Marina Boat Launch	38.77749°N, -77.04886° W	10
Louisiana	Blind River	30.09390°N, -90.76240° W	1
Florida	Coconut Creek	26.30958°N, -80.20349° W	10
Florida	Apopka Lake	28.68390°N, -81.67351° W	1
North Carolina	Apex Town Lake	35.74876°N, -78.81778° W	1
Texas	Lennox Foundation Preserve	25.84933°N, -97.38910° W	1
Virginia	Cone Marsh Wildlife Management Area, Louisa county	41.38032°N, -91.39565° W	1
Florida	Okeechobee Lake	26.93292°N, -81.05025° W	1
North Carolina	Wolf Village NCSU campus	35.78862°N, -78.68630° W	1
Florida	Everglades	26.32060°N, -80.33004° W	1
Florida	Kissimee Lake, Florida	27.989917°N, -81.281753° W	1
Texas	Lewisville Aquatic Ecosystem Research facility	33.06900°N, -96.95800° W	2
Georgia	Seminole Lake	27.84148°N, -82.77382° W	1

2.2.2. DNA extraction

Extraction of *P. cordata* DNA from the collected samples was performed using the Qiagen DNeasy® Plant Mini Kit (Qiagen Inc., 2006) following the manufacturer's protocol to obtain total genomic DNA. Individual dried leaf tissue samples were ground up in a mortar and pestle with liquid nitrogen before following the Qiagen protocol. DNA was extracted from 1-2 dry leaf samples from the native range, except for St Francis Marine and Tongaat where 9 and 10 dry leaf samples were used for extraction, respectively. These two populations were geographically widespread and appeared to be large populations that could have contained variable genetic diversity. Extracted DNA samples were stored at -20 ºC until further use.

2.2.3. Inter-Simple Sequence Repeats PCR protocol and analysis

Eight universal ISSR primers (Table 2) were tested and two of these primers, HB13 and HB15, produced a high number of replicable bands. Primers were designed by A. Wolfe and H. Ballard (Wolfe *et al., 1998,* McCauley & Ballard, 2002, Yockteng *et al.,* 2003). Primers were labelled with the fluorescent dye, 6-FAM, by the primer manufacturers, so that capillary electrophoresis could be utilized to measure fragment lengths.

Table 2. ISSR primer sequences tested during genetic analysis of *Pontederia cordata.* Primers in bold text produce the highest number of replicable bands and were selected for the study.

Primer Name:	**Primer Sequence (5' to 3')**
17898A	CACACACACACAAC
17898B	CACACACACACACACAGT
17899A	CACACACACACAAG
844B	CTCTCTCTCTCTCTCTGC
HB10	GAGAGAGAGAGACC
HB13	**GAGGAGGAGGC**
HB15	**GTGGTGGTGGC**
814	CTCTCTCTCTCTCTCTTG

PCR reactions of 20 µL was prepared for each sample using 10 µL iTaq Universal green SYBR SuperMix™ (BioRad), 0.8 µL primer diluted from stock solution (1:9 with ddH_2O), 0.8 µL MgCl (included in the iTaq), 5.4 µL ddH_2O and 3 µL template DNA

(ranging from 5 – 15 ng/ µL). The PCR tubes were then run in a thermocycler according to Table 3.

Table 3. PCR protocol for ISSR using DNA from *Pontederia cordata* leaf samples.

Steps:	Cycles:	Temperature (°C)	Time:
Initial denaturation	1	94	2 min.
Denaturation	35	94	30 sec.
Annealing		44	45 sec.
Extension		72	1 min. 30 sec.
Final Extension	1	72	20 min.
Hold	1	4	∞

Reactions were replicated from the PCR step in different thermocyclers to verify reproducibility and the PCR products were sent to Centre Analytical Facility in Stellenbosch, South Africa for processing into electropherograms by capillary electrophoresis.

2.2.4. Data processing

GeneMarker

The electropherograms (.fsa files) were analysed in GeneMarker® v 2.7.4 (SoftGenetics LLC). Only electropherogram peaks or 'bands' that were present in both replicates were verified and scored. The absence or presence of bands were used as a scoring process, these scores (as a binary matrix) first originated as electropherograms that were processed in Genemarker according to the following programme settings:

Analysis Type: AFLP, Size Standard: GS500, Standard Color: Orange

The Raw Data Analysis settings selected (default settings): Auto Range (frame), Saturation Repair, Spike Removal, Pull-up Correction, and Baseline Subtraction. The size call selected was Local Southern.

The Allele Call settings selected: Auto Range selected starting at 120 bps (unreliable peaks or 'noise' ranged from 1-120 bps), Auto Range ends at 1000 bps where peaks no longer appeared, Peak Detection Threshold - Min Intensity: 50 RFU (Relative Fluorescence Units), Max Intensity: 30000 RFU. Peak threshold lower than

20 RFU were usually not a reliable representation of true peaks and thus a slightly higher RFU of 50 was selected to ensure validity (Whitlock *et al*., 2008). Percentage >1 Global Max, Local Region % >5 Local Max and Plus-A Filter selected (default settings).

The settings mentioned above were selected as they are considered conservative and minimise genotyping errors (Whitlock *et al*., 2008). All default settings described above showed negligible effects on scoring during preliminary testing and were left as is.

The Allele Report Settings selected to obtain a summary peak table were as follows: Peak Table: only 'Grouped by Markers' and 'Show [blank space] when no allele call' were selected. Selected columns were Allele, Size, Height and Area.

The resultant allele report was saved as a .txt file and exported to RawGeno v 2.0.1 (Arrigo *et al.,* 2012) for further band scoring.

RawGeno

RawGeno is an AFLP Scoring Package that was run through R v 3.5.0 statistical platform (R Foundation for Statistical Computing). Samples were checked for integrity by removing low quality samples at the beginning on the analysis ('< 5% AFLP peaks per individual'). Non-satisfactory electropherograms such as individuals with very low numbers of successfully amplified bands that slipped through the filtering processes in GeneMarker were thus filtered out successfully in RawGeno.

Thereafter, the following Binning parameters were set: Maximum Bin Width: 1.5 bp, Minimum Bin Width:1.0 bp, Scoring Range: 100-500 bps, Low Fluorescence Bins (std RFU): 100. Electropherogram peaks lower than 100 bp were discarded as they are likely to be homoplasic and unreproducible (Arrigo *et al.,* 2012).

The binary matrix produced in RawGeno was subsequently exported and consolidated in Microsoft Excel ®.

FreeTree

A pairwise similarity index using the consolidated binary matrix was generated in FreeTree (Hampl *et al.,* 2001) using Jaccard's index and was used to generate levels of genetic variation. The generated data were processed in Microsoft Excel® by using

all within pairwise values to plot the average genetic variability of *P. cordata* between the native range (North America) and the invasive range (South Africa). A Mann–Whitney U test was conducted on the genetic distances to determine any significant differences between the invasive and native populations. A Mann-Whitney U test was conducted because of unequal population variances and unbalanced populations sizes.

PAST

The binary matrix was converted to a pairwise similarity matrix in PAST: Paleontological statistics package v. 3.26 (Hammer *et al.,* 2001) using Jaccard's coefficient for binary genetic data to produce a Principle Components Analysis (PCA) with a covariance matrix and scatterplot comparing individuals and groups of native and invasive samples. Even though Lamboy (1994) warned against using Jaccard's coefficient as it may infer bias produced by artifactual bands, Jaccard's coefficient is still the ideal coefficient to use because it excludes negative matches as a character (Jaccard, 1908). The exclusion of shared absences is important because the shared absences of bands do not necessarily imply homology and the reasons why bands are absent may be unknown (Sokal, 1963; Hollingsworth *et al*., 1999).

Structure

STRUCTUE v. 2.3.4 (Pritchard *et al.,* 2000) was used to process the binary dataset during a Bayesian model-based cluster analysis to infer genetic structure and clustering. STRUCTURE was used to assign South African and the North American samples into the appropriate number of clusters (K) or 'populations' according to their genetic similarities (Pritchard *et al.,* 2000). The correlated allele frequencies and admixed model were applied with settings of 10 000 burn-in iterations and 250 000 Monte Carlo Markov Chain iterations with 10 independent runs of the analysis. The admixed model was used because it has been demonstrated as the most efficient model to study intra-specific variation (Falush *et al.,* 2003). The optimal K-value was determined using StructureSelector (Li & Liu, 2018) that presented the number of natural clusters that the samples were partitioned into. The Peuchmaille method (Peuchmaille, 2016) and Evanno method (Evanno *et al.,* 2005) were used in StructureSelector for K-estimation. The Peuchmaille method was used as it accounts

for datasets containing uneven and even sample sizes (Puechmaille, 2016). The Peuchmaille method uses four statistical tests to estimate the optimal number of clusters, namely 'MedMeaK', 'MedMedK', 'MaxMeaK' and 'MaxMedK'. The threshold set in StructureSelector was set to a range of 0.5 – 0.8 as recommended (Peuchmaille, 2016). CLUMPAK software (Kopelman *et al.,* 2015) was used to summarize and graphically visualize the resultant STRUCTURE output.

SplitsTree

A neighbor-joining (NJ) tree with bootstrap support (10 000 replications) was constructed from the consolidated matrix in SplitsTree4 V4.16.1 (Huson & Bryant, 2006). Bootstraps were run for 10 000 runs to record any significant branching of major branches. The following settings were selected: **Character transformation**: Jaccard co-efficient; **Distance transformation:** Neighbour-joining tree; **Tree transformation**: TreeSelector (converts trees to splits); **Splits transformation**: EqualAngle algorithm.

2.3. Results

2.3.1. Mean genetic distance

The genetic distance of the native USA populations (median = 0.2139+/-0.25 (Q.R), N=496) was significantly greater than that of the invasive SA populations (median = 0.17+/- 0.31, N=465) (U = 963, $P < 0.0001$).

2.3.2. Principle Components Analysis and STRUCTURE analysis

The PCA of the native and invasive samples showed a scattered distribution of native samples (USA - black symbols) (Fig. 2.3). Invasive South African samples presented in coloured symbols were mainly distributed on one side (Component 2) of the scatterplot. Clusters of samples in South Africa separated into their own groups from each region/province, especially Tongaat, Kwa-Zulu Natal that showed tight grouping (circled green) and majority of St Francis Marine samples (red squares) that also showed grouping. Overall, South African samples were more tightly clustered together in comparison to USA samples. Component 1 and 2 of the PCA depicted 21.9% and 12.8% of the total variation, respectively (Fig. 2.3).

When comparing invasive and native *P. cordata* samples in the STRUCTURE analysis, the overall STRUCTURE analysis summary produced the number of clusters/distinct populations (K-value) = 2 for USA and South Africa (Fig. 2.4.A).

When population data were not set as priors, Puechmaille (2016) method produced K=2 at a threshold of 0.5-0.8. The Evanno *et al.,* (2005) method reported K=5 for ΔK and K=8 for mean LnP(K). When the population data were set as priors (i.e. native and invasive samples were labelled/categorized into their countries), the Puechmaille (2016) method still produced K=2. The Evanno *et al.,* (2005) method reported K=4 for ΔK and K=5 for the mean LnP(K). Since the sample size is uneven (n=63) and the Puechmaille (2016) method is said to account for and outperform other sampling methods for unevenness, K=2 was selected for the STRUCTURE output presented (Fig. 2.5.A).

Similar to the PCA plot showing scattered points from native range in USA (Fig. 2.3), the STRUCTURE output showed the same samples from USA with variable orange and blue clusters compared to South African samples, that were predominately depicted in one solid blue cluster (Fig. 2.4.A).

For samples within the invasive South African region, the overall optimal K value was K=3 or K=2 for the Puechmaille (2016) method at a threshold of 0.5, 0.6 and at a threshold of 0.7, 0.8, respectively. The Evanno *et al.,* (2005) method reported K=2 for ΔK and K=6 for the mean LnP(K). Since both methods reported K=2 during tests with and without population priors, the K=2 STRUCTURE output for South African populations is presented in Figure 2.4.B. Distinct grouping of Tongaat, KZN (Fig. 2.4.B, n=9) was depicted in green, similar to the Tongaat points clumped together in the PCA plot (Fig. 2.3), however no other distinctly different coloured bars for any of the other provinces was shown.

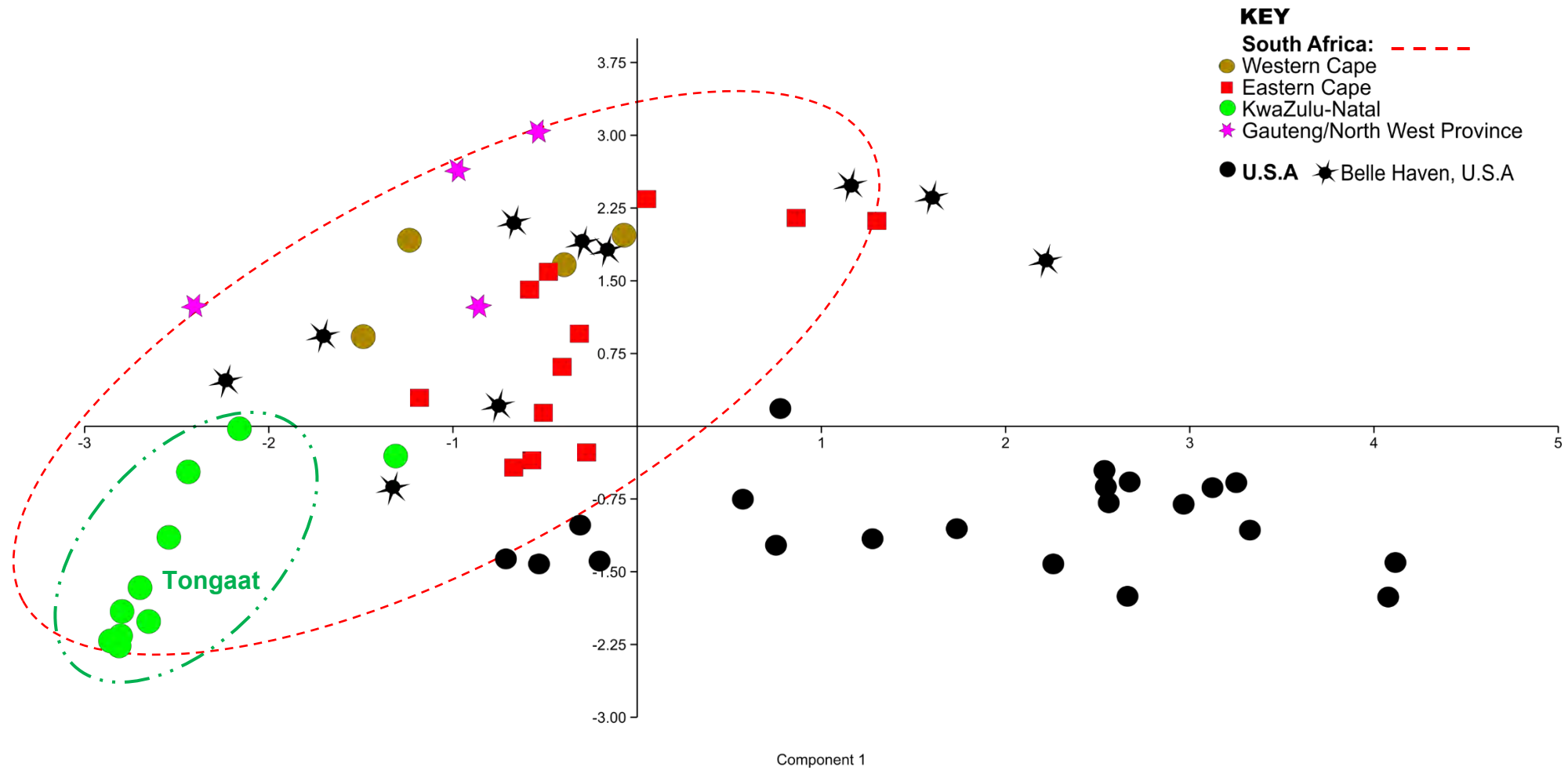


Figure 2.3. Principal Component Analysis from ISSR binary data demonstrating predominantly one genetic cluster of *Pontederia cordata* in the invasive range (coloured symbols) and scattered genetic points in the native range (black symbols). Black stars infer genetic similarity of native to invasive (colour) individuals. Black circles infer high genetic diversity amongst native individuals. 21.895% of the variation is explained by Component 1 and 12.791% by Component 2.

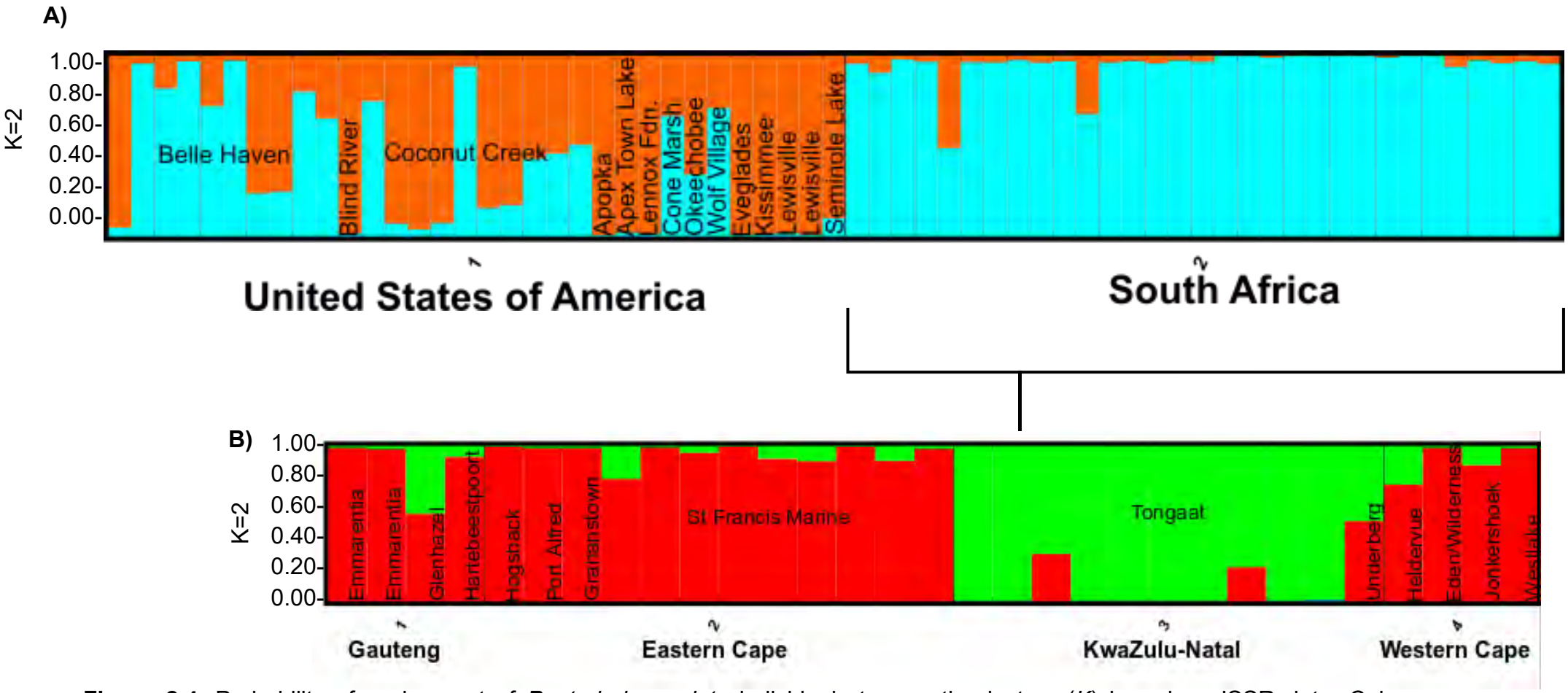


Figure 2.4. Probability of assignment of *Pontederia cordata* individuals to genetic clusters (*K*) based on ISSR data. Columns represent the probability of assignment of populations to **A)** 2 genetic clusters and **B)** 4 genetic clusters, using the Bayesian – clustering algorithm in STRUCTURE. Individuals are grouped by geographic locality (**A:** country, **B:** South African provinces) from which they were collected. n = 10 for Belle Haven, Coconut Creek and Tongaat, n = 9 for St Francis Marine and n = 4 for all other labelled samples.

2.3.3. SplitsTree Analysis

The SplitsTree tree depicted native *P. cordata* populations in the USA as one branched group with the exception of Belle Haven, Virginia, whose distribution was scattered around various sites throughout the invasive South African range (Fig. 2.5). Individuals from Tongaat, KwaZulu-Natal (blue) in the invasive range showed major branching off from the rest of the samples (Fig. 2.5). Similarly, St Francis Marine individuals from the Eastern Cape also branched off on two separate major branches, however, a few other individuals from the invasive range from Kwazulu-Natal, Gauteng and Western Cape were grouped in with St Francis Marine. No significant bootstrap values were recorded for any of the major branches.

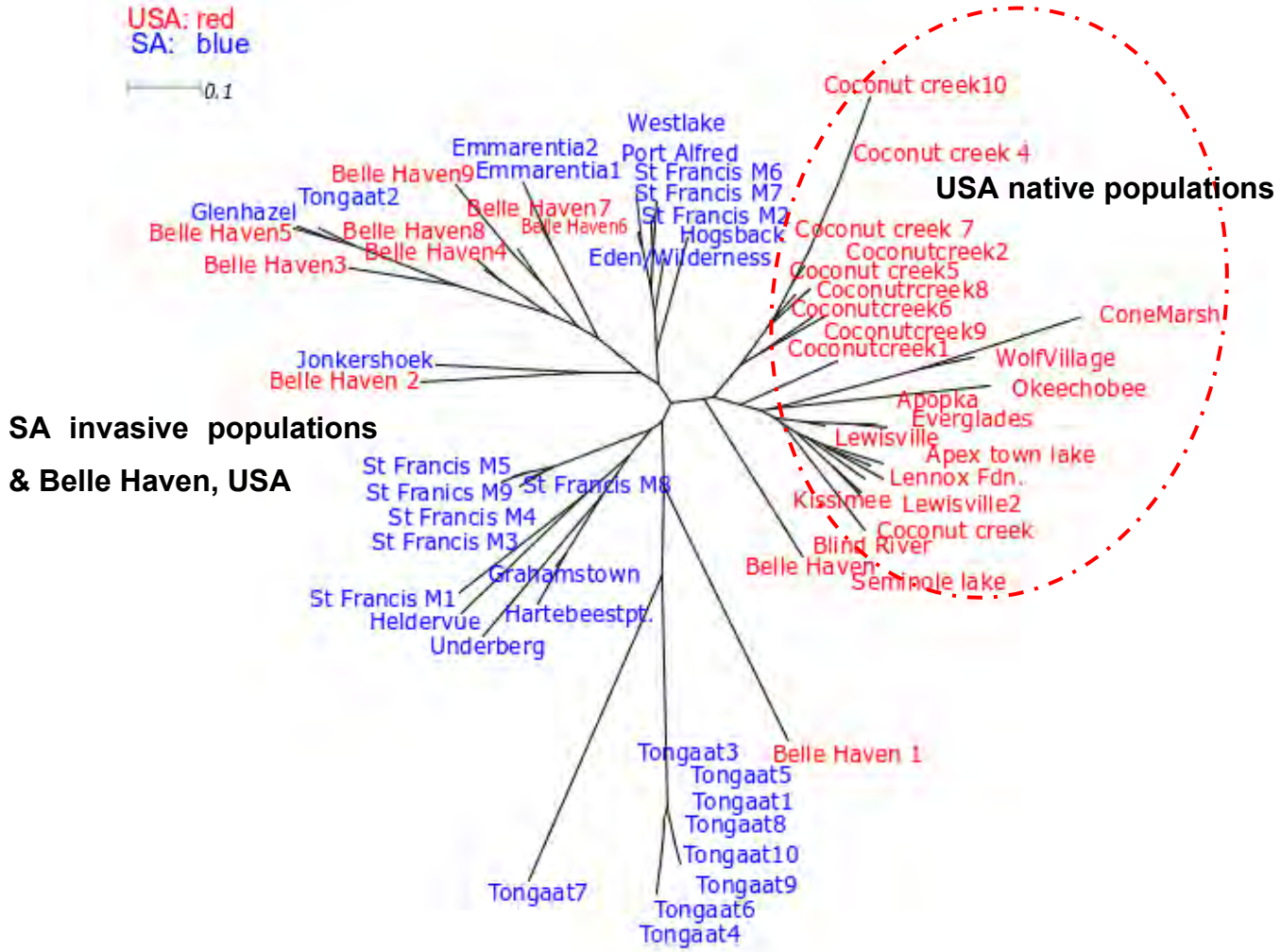


Figure 2.5. SplitsTree v4 graphical output (NeighbourNet method showing Jaccard index) for *Pontederia cordata* individuals from South Africa (Blue – invasive range) and United States of America (Red – native range).

2.4. Discussion

To date, no genetic studies have been conducted on invasive *P. cordata* populations and these novel results enhance our understanding of the population genetic consequences of colonization in South Africa and provide useful insight into a biological control programme to control this harmful invader. Understanding the genetic diversity of *P. cordata* should help us understand what part of the plant should be targeted for the most effective control and greatest reduction in spread, such as targeting flowers to inhibit sexual reproduction or targeting rhizomes to prevent clonal spread.

Through ISSR analysis (measured by mean Jaccard's genetic distances for native and invasive regions), found that *P. cordata* samples in native regions of the USA displayed a significantly higher genetic diversity compared to the invasive plants sampled in South Africa. This is to be expected, since native *P. cordata* plants have both asexual and sexual reproduction compared to invasive plants that may be predominantly spreading via asexual reproduction and could even be genetically bottlenecked (Gaskin *et al*., 2011). The STRUCTURE output computed synonymic results. Overall, there were 2 distinct clusters (K=2), one grouping of invasive individuals from South Africa and another separate grouping of native individuals from the United States of America. The native populations in the USA displayed variable coloured bands inferring greater genetic variability and gene flow than invasive individuals depicted only in solid blue bands with minimal variability. This further infers that invasive populations could be predominantly spreading asexually in South Africa, causing low genetic diversity compared to sexual and asexual reproduction in the native range.

In contrast to native populations, invasive *P. cordata* samples in South Africa were more genetically uniform. The greatest genetic uniformity presented during the PCA analysis was within *P. cordata* populations, such as tight grouping of individuals within Tongaat, KZN and clumping of individuals in St Francis Marine, E.C. *Pontederia cordata* plants within populations generally proliferate asexually via clonal rhizomes, which may result in low genetic diversity from each site. These results suggest that it is likely that invasive *P. cordata* plants may be less diverse because only a single introduction or introductions of similar genotypes from one source area occurred.

Based on our results, we also speculate that only one or a few propagules would have been introduced during an introduction at most.

Although not always the case, low genetic diversity is usually present when plants spread asexually via clonal propagules and vegetative fragments (Hollingsworth & Bailey, 2000; Meloni *et al*., 2013). Past studies on alien invasive plants suggested that low genetic diversity may limit the ability of a plant to evolve in a new habitat and become a successful invader due to founder effects, such as genetic bottlenecking and inbreeding depression causing a negative genetic impact, however newer findings are suggesting that this might not be the case (Eckert, 2000; Song *et al*., 2012; Geng *et al*., 2016). Newly introduced populations experiencing genetic bottlenecks and inbreeding depression tend to have reduced survival and fertility of offspring due to inbreeding of related individuals (Heschel & Paige, 1995; Charlesworth & Willis, 2009; Mullarkey *et al*., 2013). This is predominantly caused by a change in allele frequencies, lower heterozygosity, an increase in recessive deleterious mutations and in some cases, extinction through mutational meltdown may occur (Ellestrand & Elam, 1993; Lynch *et al*., 1995; Charlesworth & Willis, 2009). On the contrary, introduced populations may experience clonal integration, phenotypic plasticity, preadaptation and random genetic mutations which may attribute invasion success (Novak & Mack, 2005; Song *et al*., 2012; Geng *et al*., 2016).

Clonal integration is possible with certain types of asexual reproduction whereby the translocation of resources between interconnected ramets takes place, and by doing so it may be possible for clonal plants to establish and endure environmental heterogeneity, disturbances and local resource shortages (Song *et al*., 2012). A meta-analysis using 84 studies and 57 taxa conducted by Song *et al*., (2012) on the effects of clonal integration on biomass production and plant performance determined that an increased performance was observed for the recipient part of the clone (part of the clone importing resources such as water, nutrients and carbohydrates), and interestingly, the donor part of the clone exporting resources did not experience a decrease in performance. Furthermore, Wang *et al*., (2017) tested whether clonal integration may benefit alien invasive plants more than native plants from five pairs of naturally co-occurring and congeneric native and invasive clonal plants in China. Rhizomes or stolons from each pair of native and invasive plants belonged to one of four different genera (*Paspalum, Wedelia, Hydrocotyle* and *Alternanthera*), from four

different families (Poaceae, Asteraceae, Araliaceae and Amaranthaceae) (Wang *et al*., 2017). These plants underwent three different experiments simulating environmental heterogeneity with variable light, nutrients, or soil-water distribution with connected and disconnected ramets to determine biomass production (Wang *et al*., 2017). Wang *et al*., (2017) determined that clonal integration increased biomass, more so in invasive plants than native plants and this may provide invasive clonal plants a competitive advantage over native plants and contribute to the success of invasive clonal plants with low genetic diversity in heterogeneous environments. In this regard, it could be speculated that clonal integration of rhizomes could be contributing to the successful spread of invasive *P. cordata* populations with low genetic diversity in South Africa, especially within invasive populations where genetic uniformity is present.

Other important adaptive strategies for invasive plants such as *P. cordata* with low genetic diversity to establish in new heterogeneous environments is rapid post-introduction evolution and phenotypic plasticity (Geng *et al*., 2016). Phenotypic plasticity is the term used when a given genotype expresses different phenotypes under different local environmental conditions (de Kroon *et al*., 2005). This adaptive strategy may allow invasive plants to colonize diverse habitats across broad geographic areas while maintaining some level of fitness (Sultan, 2001; Richards *et al*., 2006; Pichancourt & van Klinken, 2012). Alternatively, preadaptation or 'prior' adaptation may be contributing to the invasion success of *P. cordata* in South Africa. Some degree of preadaptation is generally a requirement for an invasive species to persist in a new habitat where it has no previous history of adaptation (Jenkins & Keller, 2010; MacDougall *et al*., 2018). *Pontederia cordata* may have evolved to specific environmental conditions in its native range, such as being adapted to variable water levels or evolving in subtropical climates like southeastern United States and southeastern South America (Ritter, 2006). *Pontederia cordata*'s invaded populations along the warmer coastal areas in South Africa (Fig. 2.1) may be a similar environment to its native range, therefore *P. cordata* could possess adaptations and traits needed to succeed in its introduced range before introduction even occurred.

Evolution of *P. cordata* species in its native range would have led to the species differentiating into different populations with many different genotypes arising. However, South African populations have lower genetic diversity compared to native

USA populations which could be an indication that only a few genotypes were introduced into South Africa. Studies on low genetic diversity in successful invasive weeds have shown that high genetic diversity is not a prerequisite for successful invasions, such as one of the world's most successful aquatic plant invaders, *P. crassipes*, which has extremely low genetic diversity in the majority of its introduced populations, with approximately 80% of the introduced populations studied comprising a single clone (Zhang *et al*., 2010). Other such examples of invasive success include *Impatiens glandulifera* Royle (Balsaminaceae), an annual plant native to the Himalayas, however multiple introductions into Europe have caused this plant to become a major invasive threat to native plant communities (Hagenblad *et al*., 2015). Hagen *et al*., (2015) genetically characterised and compared invasive *I. glandulifera* populations in Europe to native populations in Kashmir, India using microsatellite markers for STRUCTURE analyses (amongst other molecular analyses) and a comparative analysis on 39 similar genetic studies of other invasive species. They found that *I. glandulifera* had repeated introductions (not specifically from Kashmir), however unusually low genetic diversity compared to native populations and even other invasive species despite multiple introductions was present (Hagenblad *et al*., 2015). It was suggested that multiple introductions and dispersal by humans and phenotypic plasticity, instead of genetic diversity, facilitated the spread of *I. glandulifera* across Europe (Hagenblad *et al*., 2015). Even though it is speculated that only a very few genotypes of *P. cordata* were introduced into South Africa , it may still be possible that, similar to Hagenblad *et al*., (2015), plasticity and dispersal by humans in the horticultural trade are facilitating the spread across the country, especially since *P. cordata* is an attractive pond plant.

Surprisingly, not all invasive plants have lower genetic diversity to their native counterparts. An invasive European wetland canary grass, *Phalaris arundinaceae* L. (Poaceae) invading North America was studied for the consequences of its multiple introductions into the country (Lavergne & Molofsky, 2007). Lavergne and Molofsky (2007) determined that the multiple uncontrolled introductions of *P. arundinaceae* from different native European regions into North America alleviated genetic bottlenecks and generated new genotypes in the invaded range. Furthermore, it was found that the invaded range had a higher genetic diversity and heritable phenotypic variation than the native range, causing invasive *P. arundinaceae* plants to have a greater ability

to colonize and spread (Lavergne & Molofsky, 2007). The impacts of these new genotypic invaders with high evolutionary potential could be catastrophic to native plant community structure and ecosystem processes throughout the invaded region (Lavergne & Molofsky, 2007). My findings during this genetic study of *P. cordata* provided insight into the genetic diversity within each population from invasive and native regions using a Principle Components Analysis, which depicted native populations from six different states in the USA, sporadically scattered apart from one another. This inferred high genetic diversity amongst native populations in the United States of America and, although only a few introductions of *P. cordata* in South Africa is expected due to low genetic diversity of invasive populations, it is imperative that additional introductions of genetic material into the country is limited. Further uncontrolled introductions of *P. cordata* plants from different regions of the native range into South Africa could lead to new genotypic invaders with greater invasive potential and disastrous impacts on native ecosystems, as demonstrated by *P. arundinaceae* invading North America (Lavergne & Molofsky, 2007).

Interestingly, the genetic results presented here also suggest that Belle Haven, Virginia was the only native population intermingled with invasive South African populations. Further investigation using a SplitsTree analysis suggested the same findings, whereby the native population that grouped together and was genetically closest to invasive South African populations was also Belle Haven, Virginia. While other native populations in the native range in the USA distinctly branch off away from the invasive populations, Belle Haven shows similar genetic uniformity with invasive *P. cordata* populations in major cities of Johannesburg and Cape Town, as well as Tongaat, a tourist destination in KwaZulu-Natal province. Although more sampling is needed in the USA and other parts of the native range, particularly South America, before one can accurately determine the area from which invasive *P. cordata* originated, Belle Haven, Virginia, USA is a good starting point for further studies.

When looking at the STRUCTURE output for invasive South African populations individually without comparing them to the USA, the K-value = 2 output for all four provinces also suggests that throughout the country, there are no greatly diverse populations, except for Tongaat, K.Z.N that showed separate clustering from the rest of the populations. Since both the PCA and STRUCTURE analyses suggest that

Tongaat is genetically distinct from other invasive sites, Tongaat's population of *P. cordata* may therefore come from a separate introductory event and may have a different source population to the other invasive populations. However, the fact that Belle Haven, Virginia is observed grouping with Tongaat in separate analyses suggests that its source population may potentially come from the area of Virginia too.

Conclusion

These findings may be the first step to developing a biological control programme for *P. cordata* in South Africa and, while more sampling is needed before one can accurately conclude where the invasive *P. cordata* source populations come from, this study may direct future sampling efforts. It is evidently clear that invasive *P. cordata* populations in South Africa have lower genetic diversity in comparison to native populations sampled, and it is likely that only one or a few individuals were introduced with slight genetic variation amongst populations.

Similar scenarios have been studied in the past, such as *Pennisetum setaceum* (Forssk.) Chiov. (Poaceae), a fountain grass native to the Mediterranean parts of North Africa and the Middle East, and invasive in North America, southern Africa, Australia, Hawaii and Fiji (Williams *et al*., 1995; Rahlao *et al*., 2014). A study by Le Roux *et al*., (2007) using ITS regions and ISSR was performed to compare patterns of invasiveness and genetic diversity to native populations of *P. setaceum*. DNA sequencing of the ITS regions showed a lack of phylogeographical differentiation within and among invasive populations, and thus ISSR rendering high intra-specific resolution was subsequently used (Le Roux *et al*., 2007). Surprisingly, the ISSR analysis also depicted a lack of differentiation among the populations investigated (Le Roux *et al*., 2007). Unlike the genetic variances depicted by *P. cordata* during this genetics study on native and invasive *P. cordata* populations, Le Roux *et al*., (2007) results on invasive *P. setaceum* showed identical ISSR banding pattern data for all samples for each primer used, indicating no variation between Egypt populations (native range) and South Africa, Namibia, Hawaii, Arizona and California populations (invasive range). Le Roux *et al*.'s (2007) findings suggest that *P. setaceum* populations share a global monoclonality, and that a fixed super-genotype exists that relies on extremely high levels of phenotypic plasticity to invade in variable environmental conditions. This single, successful asexual genotype could have resulted from genetic drift and fixation combined with decay in genotypic diversity over time. A similar study

was conducted on *Arundo donax* L. (Poaceae), a globally invasive giant reed originating from eastern Asia and widely introduced throughout the African continent, subtropical United States of America, the Mediterranean, Australia and South America (Canavan *et al*., 2017). Microsatellite markers and three chloroplast regions were used to investigate the phylogeography, plant haplotype, genetic structure and diversity of *A. donax* in South Africa to provide insight into founder effects, reproduction schemes and dispersal mechanisms for a proposed biological control programme (Canavan *et al*., 2017). The genetic structure of *A. donax* in South Africa compared to other populations in the native 'Old World' and invasive North American ranges depicted no genetic variation during chloroplast sequencing. Higher resolution microsatellite markers also depicted genetic uniformity and it was concluded that populations throughout South Africa share a single genotype from Asia, synonymous to the globally distributed haloptype in other invaded regions. Canavan *et al*., (2017) therefore suggested that biological control agents adapted to the widespread haplotype should be considered for a biological control programme.

ISSR results from this genetic study of *P. cordata* suggest similar findings to the above mentioned studies – albeit there is some presence of genetic variation, the low genetic diversity may be advantageous for a biological control programme because these invasive plants may be more vulnerable and have more of a uniformed response to a biological control agent due to limited evolutionary potential for adaptation and herbivory defences (Nissen *et al*., 1995; Ye *et al*., 2004; Canavan *et al*., 2007).

For further research into a biological control programme for *P. cordata*, more sampling in the USA should be conducted to determine the area in which biological control agents should be surveyed (source populations), however Belle Haven, Virginia, is a good starting point for further sampling because results showed that Belle Haven had the most genetically similarity to invasive individuals in South Africa. Furthermore, sampling from native regions in Canada and in South America should also be conducted to exclude any possibility that individuals originate from native regions other than the USA.

Future genetic studies on invasive *P. cordata* populations in South Africa should also consider investigating what variety of *P. cordata* is present in the country. Although *P. cordata* has many synonymic subspecies/varieties (Pellegrini *et al*., 2018), there are

only three varieties that are most common, with chloroplast genomes listed on GenBank – *P. cordata* var. *cordata*, *P. cordata* var. ovalis and *P. cordata* var. *lancifolia* (Benson *et al*., 2017; ITIS Report, 2017). No genetic studies have been conducted on *P. cordata* varieties in the invasive range to see if there are multiple varieties introduced. Thus, determining which variety is present in South Africa may have important implications for a biological control programme because biological control agent surveys should be conducted on the right variety in the native range to ensure maximum success rate and herbivore damage from the agent.

The overall outcome of the genetic study suggests that invasive *P. cordata* plants could be spreading asexually, especially within populations and thus targeting the plants rhizomes by mechanical removal and biological control may be the most promising strategy to control the spread of *P. cordata* in South Africa. However, the slight diversity of populations such as Tongaat compared to other invasive populations should not be overlooked, as it may be possible that this incidence is caused by sexual reproduction between populations instead of different introduction events occurring. Investigation into the invasion ecology and modes of reproduction of *P. cordata* in South Africa is subsequently addressed in Chapter 3.

CHAPTER 3.

Where are the seeds? Pollination ecology and floral traits of the invasive *Pontederia cordata* in South Africa.

3.1. Introduction

Alien invasive species have a highly detrimental impact on ecosystems, causing a reduction in the biodiversity of native fauna and florae and may even contribute to the local extinction of native species (Catford *et al*., 2018). In South Africa in 2017, biological invasions accounted for 25% of the reduction of native biodiversity (Van Wilgen & Wilson, 2018). Alien invasive species can also negatively impact ecosystem services, which in turn causes socio-economic losses. In 2017, the South African National Status Report on Biological Invasions reported that ZAR1.5 Billion per year is spent on the control of alien invasive plant species by the Department of Environmental Affairs alone, and that the figure would be greater if the control measure inputs from other government/semi-government entities and the private sector were included (Van Wilgen & Wilson, 2018). Turpie *et al*., (2003) reported an estimated ZAR684 million per year was lost due to ecosystem service reduction from alien species invasion in the Cape Floristic Region, Western Cape, South Africa, a global biodiversity hotspot. Water consumption by alien invasive plants in the river systems and wetlands of this region has reduced water flow, altered river flow regimes and led to a reduction in native floral diversity through competition (Turpie *et al*., 2003).

Alien invasive plants generally have common invasive characteristics that allow them to overcome abiotic and biotic barriers to establishment and spread so that they can successfully naturalise and outcompete native plants. Such characteristics include the ability to adapt to new environments and the ability to reproduce sexually and/or asexually for rapid proliferation (Van Kleunen *et al*., 2014).

It is important for alien invasive plants to have a breeding system that can be effective in newly invaded environments where there may be a lack of appropriate pollinators and/or seed dispersers to facilitate spread (Le Roux *et al*., 2020). Characteristics such as self-pollination (selfing) encourage a higher propagule pressure in new areas to ensure establishment. A plant that can self-pollinate does not have to rely on external

factors for pollination and can ensure that its stigma receives pollen for seed formation, even under adverse conditions (Wilcock & Neiland, 2002). Selfing and asexual (clonal) reproduction allows for an invasive species to overcome pollen limitations and lack of specialised pollinators, thereby escaping pollination failure (unsuccessful pollination) (Baker, 1955; Razanajatovo *et al*., 2016).

Pontederia cordata is an emergent macrophyte that can colonise areas in its native range by dispersing copious amounts of single-seeded, buoyant fruits via waterbodies (Gettys & Dumroese, 2009). *Pontederia cordata* reproduces sexually and asexually in its native region of North America, however no seed or fruit have been observed to date in South Africa. Investigating the breeding system of invasive *P. cordata* populations in South Africa may provide valuable information for risk assessments and a better understanding of how the species may be spreading, unlike the typical sexual reproductive means of common invasive plants.

Pontederia cordata has already been recorded in almost every province in South Africa (SANBI database, 2016) and thus research, monitoring and development of control programmes are essential in order to curb the species invasive potential. It is therefore important to justify that the species has a high risk of becoming a damaging, aggressive aquatic invasive species (Le Roux *et al*., 2010) in order for a rapid response strategy to be implemented.

3.1.1. The breeding system of *Pontederia cordata* in South Africa

Pontederia cordata belongs to the same family as *P. crassipes* and has been listed as a Category 1b invasive plant in South Africa (NEMBA, 2014). *Pontederia crassipes* has a high invasive potential and is present on almost every continent on earth and thus numerous studies have been conducted on its biology, introduction history and invasion ecology (Villamagna & Murphy, 2010; Ndimele *et al.,* 2011; Coetzee *et al.,* 2017). The invasion ecology of *Pontederia cordata,* a sister to this troublesome invader has received very little attention. An important aspect of *P. cordata'*s invasion ecology is its breeding system that could contribute to this species invasive traits. Understanding its breeding system may provide insight into how the plant naturalizes and spreads.

Reports of trimorphism in floral morphology studies of *P. cordata* go as far back as 1875, where it was the only known heterostyled species belonging to

monocotyledonous plants (Hazen, 1918). Presently, *P. cordata* belongs to one of only four angiosperm families where a heteromorphic breeding system known as tristyly occurs (Gettys, 2005). Tristyly is a rare and complex breeding system that ensures optimal seed production and gene flow through cross-pollination, since each plant possesses only one of three style morphs (Ornduff, 1966).

Tristylous flowers are categorised into three different morphologies determined by their style length: a long (L) morph has a long style and short and medium stamens, a medium (M) morph has a medium style and short and long stamens and a short (S) morph has a short style and medium and long stamens (Charlesworth, 1979) (Fig. 1.2). This tristylous morphology with alternate lengths of stamens and styles ensures that pollinators present pollen to the correctly matching stigma. For example, a pollinator would collect pollen from long anthers on a M or S morphed flower and deposit the pollen onto the stigma of a long style on an L morphed flower (Fig. 3.1). This form of pollination is called 'legitimate pollination' and encourages genetic diversity within and among populations (Ornduff, 1966; Barrett, 1976).

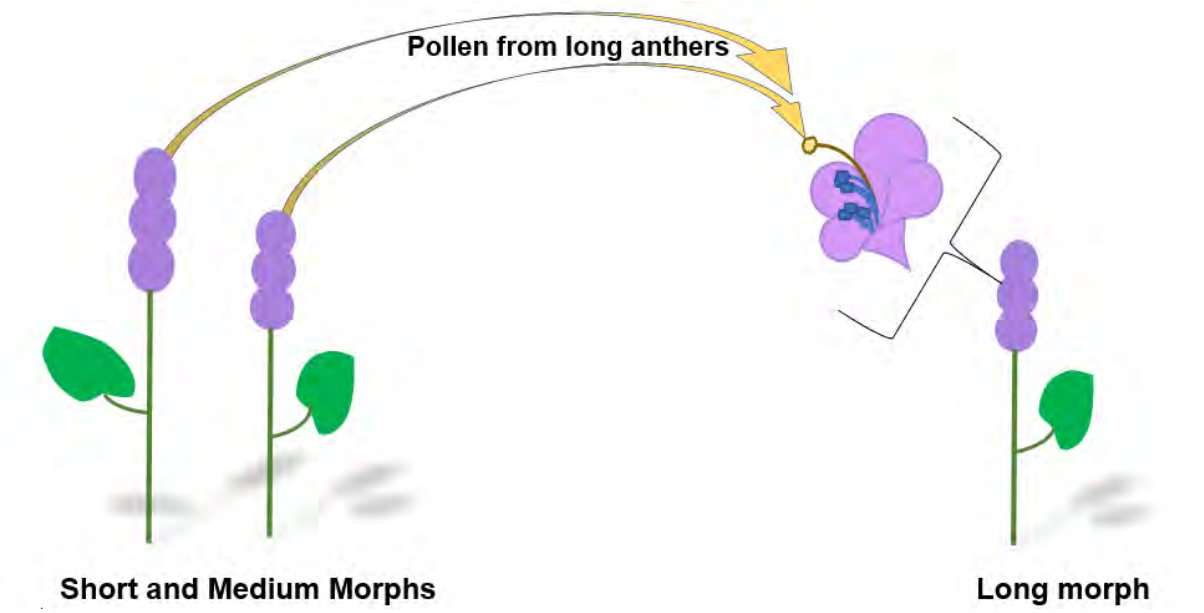


Figure 3.1. Schematic diagram of legitimate pollination of a stigma on a long style of an L-morphed *Pontederia cordata* flower with pollen from long anthers of S- and M-morphed flowers.

Moreover, there is a distinct difference and separation between the pollen sizes of L - , M - and S morphed anthers, whereby the large anthers possess the largest pollen, the medium anthers possess medium sized pollen and the short anthers produce the smallest pollen (Gettys, 2005). Thus, it may be possible to match the pollen sizes to the different morphed flowers, because the large and medium sized pollen should come from the S morphed flowers containing M and L anthers and so forth (Price & Barrett, 1982; Barrett & Glover, 1985; Gettys, 2005). Short morphed flowers in South Africa should therefore have pollen grain sizes of L and M anthers similar to that present in the native range (Price & Barrett, 1982; Barrett & Glover, 1985; Gettys, 2005) and may be a characteristic that can help confirm the presence of the short morphed plants in South Africa.

3.1.2. Illegitimate pollination and self-incompatibility

A complex tristylous breeding scheme frequently comes at a cost. 'Illegitimate pollination' and self-incompatibility may heavily reduce or prevent fruit and seed production (Barrett & Anderson, 1985). Illegitimate pollination occurs through self-pollination or pollination of incorrectly matched flower morphs, for example, pollen from a short or medium length stamen of a L morphed flower pollinating the long stigma on the same flower.

Several studies have been conducted on native *P. cordata* populations in North America to determine the extent of self-incompatibility and illegitimate pollination. Ornduff (1966) performed legitimate and illegitimate 'own-form' pollination on *P. cordata* in greenhouse experiments and found that illegitimate pollination in all three floral morphologies reduced fruit production. This confirmed the presence of an incompatibility system. Short morphed flowers depicted the strongest incompatibility with only 5.3% of illegitimately pollinated flowers bearing fruit in comparison to 61.3% of legitimate pollinated flowers bearing fruit (Ornduff, 1966). A pollination study by Barrett (1976) performed on tristylous *Pontederia rotundifolia* L. yielded similar results, showing that S morphed flowers have the strongest self-incompatibility when they are illegitimately pollinated or self-pollinated.

Self-incompatibility is not always present in tristylous species. A study on the trimorphism of a North American wetland perennial, *Decodon verticillatus* L. Ell. (Lythraceae), revealed a high degree of compatibility when self-pollinated (Eckert &

Barrett, 1993). *Decodon verticillatus* is highly clonal, and it is speculated that mutations affecting floral morphology that promote selfing and increase fitness can spread quickly across populations, thereby promoting self-compatibility (Eckert & Barrett, 1993). Changes in floral morphologies to promote self-compatibility have been observed with other tristylous species including *P. crassipes* (Barrett, 1988). *Pontederia crassipes* is one of the world's most notorious invasive aquatic weeds and has multiple strategies to proliferate, including the loss of a morph to become distylous and the ability to proliferate rapidly via asexual reproduction (Barrett, 1988). Many invasive species have such reproductive strategies to ensure efficient establishment and spread in new habitats. The ability to self-pollinate is an advantageous characteristic for an invasive plant species because introduced plants have to overcome barriers in new habitats such as the lack of appropriate pollinators or the lack of mates to ensure cross pollination (Razanajatovo *et al*., 2016). Self-pollination may therefore enable an invasive plant species to establish, naturalise and become self-sustaining (Razanajatovo *et al*., 2016).

Self-pollination may also confer serious disadvantages, such as inbreeding depression (Rodger and Johnson, 2013). *Acacia dealbata* (Fabaceae) is an alien invasive tree in South Africa that has a high tendency to self-pollinate (Henderson, 2007; Correia *et al*., 2014). Self-pollination generally occurs when pollinators visit multiple flowers on the same tree, especially in invaded areas where individuals are sparse (Rodger & Johnson, 2013). The resultant inbreeding depression caused by lack of gene flow can be a severe drawback, potentially causing problems with the development, growth and survival of seedlings (Rodger & Johnson, 2013). A study by Rodger and Johnson (2013) found that the progeny of self-fertilised *A. dealbata* plants in an isolated invaded area in South Africa displayed lower survival rates and expressed yellow leaf discolouration compared to progeny of cross-pollinated plants. Self-incompatibility in invasive plants can therefore be a low-risk invasive characteristic or 'failsafe' that promotes cross-pollination and ensures genetic diversity and fitness in a species.

3.1.3. Floral morphology of *Pontederia cordata* in South Africa

Although *P. cordata* has three different morph forms in its native range (Ornduff, 1966), preliminary field observations throughout its invasive range in South Africa have only recorded populations containing S morphed flowers. It may be possible that seed

production is unachievable, since S morphed flowers have the strongest self-incompatibility recorded (Ornduff, 1966; Gettys, 2005).

In native *P. cordata* populations in North America, flowers have specialist pollinators with correctly shaped body-forms to pollinate the flowers efficiently (Harder & Barrett, 1993; Gettys, 2005). It could therefore be possible that the generalist pollinators in South Africa are not sufficiently cross-pollinating flowers, unlike the specialist pollinators in North America. Alternatively, it may be possible that the invasive variety in South Africa is a sterile horticultural variety (Henderson, 2001) and may share similar characteristics to 'Singapore pink', a cultivar of *P. cordata* that has been traded in aquatic plant nurseries and only propagates clonally (Gettys, 2005).

Determining whether *P. cordata* is capable of sexual reproduction in South Africa will contribute to our knowledge of the invasion ecology of this species, and its subsequent management. This study therefore investigated: (1) the floral morphology of *P. cordata* populations in South Africa with the aim of determining whether the S morph dominates populations; (2) the pollen grain sizes of *P. cordata* flowers in South Africa which should correlate with morph dominance; (3) pollinators associated with invasive populations in South Africa to determine whether appropriate pollinators are present; and (4) whether *P. cordata* plants are self-compatible through pollination experiments.

3.2. Materials and methods

3.2.1. Floral morphology of *Pontederia cordata* in South Africa

Pontederia cordata flowers, from populations throughout South Africa, were measured to determine variation in floral morphology and score floral morphs as short, medium or long (Table 3.1). Flower organs included in the floral morphology analysis were pistils, filaments, anthers and petal lengths.

At each site, inflorescence samples were collected from a random starting point, 1m apart for 10m to get an average representation of the morphs present in the population. Inflorescence samples were stored in ethanol and distilled water (50% v/v). At each location, 10 inflorescences were analysed. Measurements of floral organs from 10 random flowers per inflorescence were visualized and captured using WinDIAS 3 Leaf Image Analysis System 3.2. (Delta-T Devices Ltd., 2017).

The lengths of the following floral organs were measured: stigma, style, anther, filament and petals. Measurements were recorded in ImageJ (Schindelin *et al*., 2012) and differences between sites were analysed in Statistica v.13 (TIBCO Software, 2017) using ANOVA and Principal Component Analyses.

Table 3.1. Locations, co-ordinates and variables studied at each of the *Pontederia cordata* populations in South Africa that were included in this study.

Location:	Co-ordinates	Population size	Abundance of flowers	Floral morphology analysis	Pollen grain size analysis	Pollinator analysis	Artificial pollination experiment
Pietermaritzburg Botanical Gardens, KwaZulu-Natal	29.6079° S 30.3478° E	~3m^2	<10 inflorescences	x	x	x	
Underberg golf course, KwaZulu-Natal	29.786865° S 29.491959° E	~13 x 66 m	>10 000 inflorescences	x	x	x	
Durban Botanical Gardens, KwaZulu-Natal	29.846667° S 31.006667° E	~6m^2	-	x			
Emmarentia Botanical Gardens, Johannesburg, Gauteng	26.161863° E 27.999846° S	~331 x 4m	>10 000 inflorescences	x			
Glenhazel, Johannesburg, Gauteng	26.1389° S 28.1020° E	~20m^2	~150 inflorescences	x	x		
Jonkershoek, Western Cape	33.956784° S 18.915221° E	~15m^2	~100 inflorescences	x			
Westlake, Western Cape	34.07560° S 18.45124° E	-	-	x	x		
Port Elizabeth, Eastern Cape	33.9822° S 25.6572° E	~18m^2	~5000 inflorescences	x	x	x	
Royal Port Alfred Golf Course, Eastern Cape	33.600395° S 26.891699° E	~8m^2	~2000 inflorescences	x	x	x	
Gonubie, East London, Eastern Cape	32.936281° S 28.023967° E	~1m^2	~1000 inflorescences			x	
Old golf course in Makhanda (Grahamstown), Eastern Cape	33.296944° S 26.499306° E	-	~1500 inflorescences				x
Hogsback Arboretum, Eastern Cape	32.5952° S 26.9323° E	~28 x 11m	>8000 inflorescences				x
Hogsback hiking trail, Eastern Cape	32.595056°S 26.94683°E	~6m^2	~3000 inflorescences				x

3.2.2. Pollen grain size analysis of *Pontederia cordata* in South Africa

The diameter of pollen grains from *P. cordata* populations in South Africa was measured under a scanning electron microscope in order to determine variation in pollen size. Furthermore, since only short-morphed flowers are present in South Africa, these measurements were conducted to compare pollen size with data from the native range. Stigmatic pollen loads were obtained from dried flowers of six *P. cordata* populations in South Africa (Table 3.1). Pollen stored in ethanol and distilled water (50% v/v) was prepared for scanning electron microscope (SEM) visualization using a gold plating technique according to the developer's manual (Quorum Sample Preparation System Instruction Manual, 2002). Preparation included placing the pollen and filaments on Double Sided Adhesive Carbon Tape, 8mm(W)x20m(L) (Electron Microscopy Sciences, catalog number: 77816) on SME stubs and coating them using a Q150 RS Quorum Rotary-Pumped Coater using the QT-Timed Gold vacuum cycle (Quorum Sample Preparation System Instruction Manual, 2002).

Coated samples were then analysed under a TESCAN Vega TS 5136LM SEM using Scadium software (Olympus Scadium software, 2010). Pictures of the equatorial and polar axis of dried pollen grains were captured and measurements were recorded in ImageJ (Schindelin *et al*., 2012). Differences in pollen dimensions between sites were then analysed using ANOVAs in Statistica v. 13 (TIBCO Software, 2017).

3.2.3. The pollinators of *Pontederia cordata* in South Africa

Field observations of *P. cordata* pollinators in South Africa were conducted on populations in East London Port Alfred, Underberg, Pietermaritzburg and Port Elizabeth (Table 3.1). Flowers were observed in mornings and afternoons for 2 hours for insect visitors. In total, observations for insect visitors took 10 hours cumulated across all populations. Surveys were conducted during peak flowering time in spring and summer. Pollinator species, number of flowers visited and number of inflorescences in the population was recorded. Data were analysed in Statistica using a Kruskal-Wallis test because data was not normally distributed (TIBCO Software, 2017). Kruskal-Wallis ANOVAs were used to determine differences in abundance of insect pollinators between populations, irrespective of the plant population sizes recorded during the survey.

3.2.4. Hand pollination experiments of *Pontederia cordata* in South Africa

Green house self-pollination and cross-pollination experiments were conducted, using a methodology similar to that of Barrett (1976), to determine the possibility of seed production in invasive *P. cordata* plants in South Africa. *Pontederia cordata* plants (approximately 40-50 plants) were collected from the old golf course in Makhanda labelled 'Grahamstown', Hogsback Arboretum labelled 'Hogsback site 1' and a Hogsback hiking trail labelled 'Hogsback site 2' from the Eastern Cape, South Africa (Table 3.1). Plants were separated by location and planted in plastic swimming pools (2m in diameter) in soil covering the base, and water filled to 10 cm above the soil. Plants were fertilised with Osmocote slow release fertilizer (N:P:K = 16:9:12) (Scotts-Sierra Horticultural Products), at a rate of 5mg N/l. Plants were watered once a week to ensure water saturated the soil and covered the base of the stems.

Once the plants were budding, fine-meshed pollinator exclusion bags were placed over each bud to prevent pollen flow from outside sources. The pollination experiment began as soon as the first flowers on an inflorescence opened.

For artificial illegitimate self-pollination treatments, flowers were marked and emasculated. Pollen was transferred from a flower of a plant from the same location to the stigma of the marked flower, via forceps. Pollinations were made daily on each flower and the number of flowers on each inflorescence was recorded. This process was repeated for 10 inflorescences using pollen from long filaments and 10 inflorescences using pollen from medium filaments.

For artificial cross-pollination treatments, pollen was transferred from a flower of a plant from a different collection location to the designated stigmas via forceps. Hogsback site 1 anthers were pollinated with pollen from Grahamstown flowers, Hogsback site 2 anthers were pollinated with pollen from Hogsback site 1 flowers, and Grahamstown anthers were pollinated with pollen from Hogsback site 2 (Table 3.2.). This process was repeated for 10 inflorescences using pollen from long filaments and 10 inflorescences using pollen from medium filaments. Unbagged (to include pollinators) and bagged but untreated controls were also included on 10 inflorescences per control for each population. For all pollination experiments, flowers

were observed for 4-6 weeks or until dried flower debris had fallen off the inflorescence to determine whether seed set occurred.

Table 3.2. Pollination treatments of *Pontederia cordata* flowers from three Eastern Cape populations to test for seed production

	Total number of treatments per site		
Treatment:	Grahamstown	Hogsback site 1	Hogsback site 2
Unbagged	436	525	622
Bagged	387	326	511
Self-pollination: Long anther	377	505	832
Self-pollination: Medium anther	442	455	680
Cross-pollination: Short style x Pollen from long anthers of Hogsback site 2	568	-	-
Cross-pollination: Short style x Pollen from long anthers of Grahamstown	-	444	-
Cross-pollination: Short style x Pollen from long anthers of Hogsback site 1	-	-	495
Cross-pollination: Short style x Pollen from medium anthers of Hogsback site 2	462	-	-
Cross-pollination: Short style x Pollen from medium anthers of Grahamstown	-	415	-
Cross-pollination: Short style x Pollen from medium anthers of Hogsback site 1	-	-	393

A germination experiment on the resultant dried flowers and flower debris was conducted to determine whether seeds were produced. The germination experiment was conducted in a Constant Environment room at 25 °C with a lighting setting of 10 hours light and 14 hours dark. The dried flowers and flower debris were soaked in distilled water and kept damp for 4-5 weeks to encourage germination of any seeds present (Gettys & Dumroese, 2009).

3.3. Results

3.3.1. The floral morphology of *Pontederia cordata* in South Africa

A Principal Component Analysis (PCA) comparing floral organs from invasive populations of *P. cordata* in South Africa was conducted to determine floral organ variability and whether only short-morphed flowers occur. Only short-morphed flowers are present in South African populations of *P. cordata*. The PCA comparing floral organs showed clumping of the variables, with low variability at each location (Fig. 3.2.). The floral organs of invasive *P. cordata* plants exhibited a clumped distribution and little variability was seen amongst the different South African populations (Fig. 3.3). Factors 1 and 2 made up for 49.56% and 24.27% of the total variation in the analysis, respectively (Table 3.5).

One-way ANOVAs were conducted to determine any differences between the different *P. cordata* populations in South Africa for each floral organ. Differences in *P. cordata* floral organ morphology were observed among populations (Table 3.3), but very little variation occurred within each population (Fig. 3.2).

Table 3.3. Results of ANOVAs comparing *Pontederia cordata* floral organ morphology among populations in South Africa.

Flower organ:	F-value $_{(8, 2151)}$:	*P*-value
Medium filament	257.36	<0.001
Long filament	262.83	<0.001
Medium anther	27.03	<0.001
Long anther	46.40	<0.001
Pistil	21.84	<0.001
Petal length	60.90	<0.001

The population of *P. cordata* in Glenhazel produced flowers with the longest filaments for both medium and long filaments measuring 6.6 ± 0.2 mm and 9.4 ± 0.3 mm in length, respectively (Fig. 3.4 a, b). The Emmarentia and Durban populations had similar long filament lengths. Other *P. cordata* populations that showed similarity when comparing filament lengths were Port Elizabeth and Port Alfred (both from the Eastern Cape), and Pietermaritzburg and Underberg (both from KwaZulu-Natal) (Fig. 3.4 a, b). Westlake showed no similarity of medium or long filament lengths to any other

populations surveyed (Fig. 3.4 a, b).The *P. cordata* population in Durban produced flowers with the shortest medium and long anthers in comparison to the other populations surveyed throughout South Africa (Fig. 3.4 c, d).

Petal length in *P. cordata* populations were highly variable (Fig. 3.4 f). Underberg and Jonkershoek had the smallest petals, whereas Glenhazel and Port Alfred had the largest petals (Fig. 3.4 f). Other populations such as Westlake, Port Elizabeth and Pietermaritzburg, i.e. populations from three different provinces, all had similar petal lengths (Fig. 3.4 f).

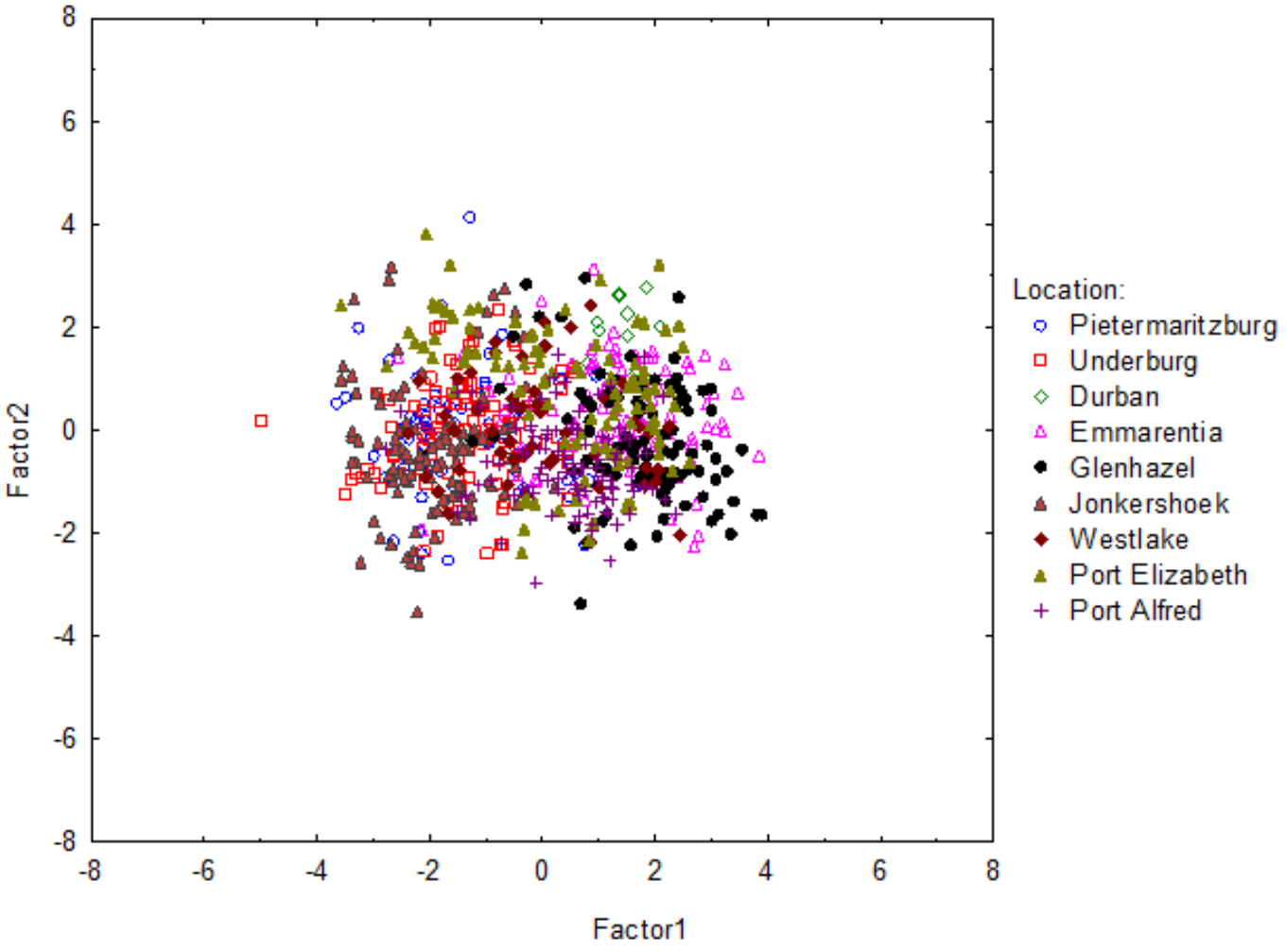


Figure 3.2. Principle Component Analysis of floral organs from *Pontederia cordata* populations in South Africa. Floral organs analyzed: pistil, medium and long filaments, medium and long anthers, and petal length.

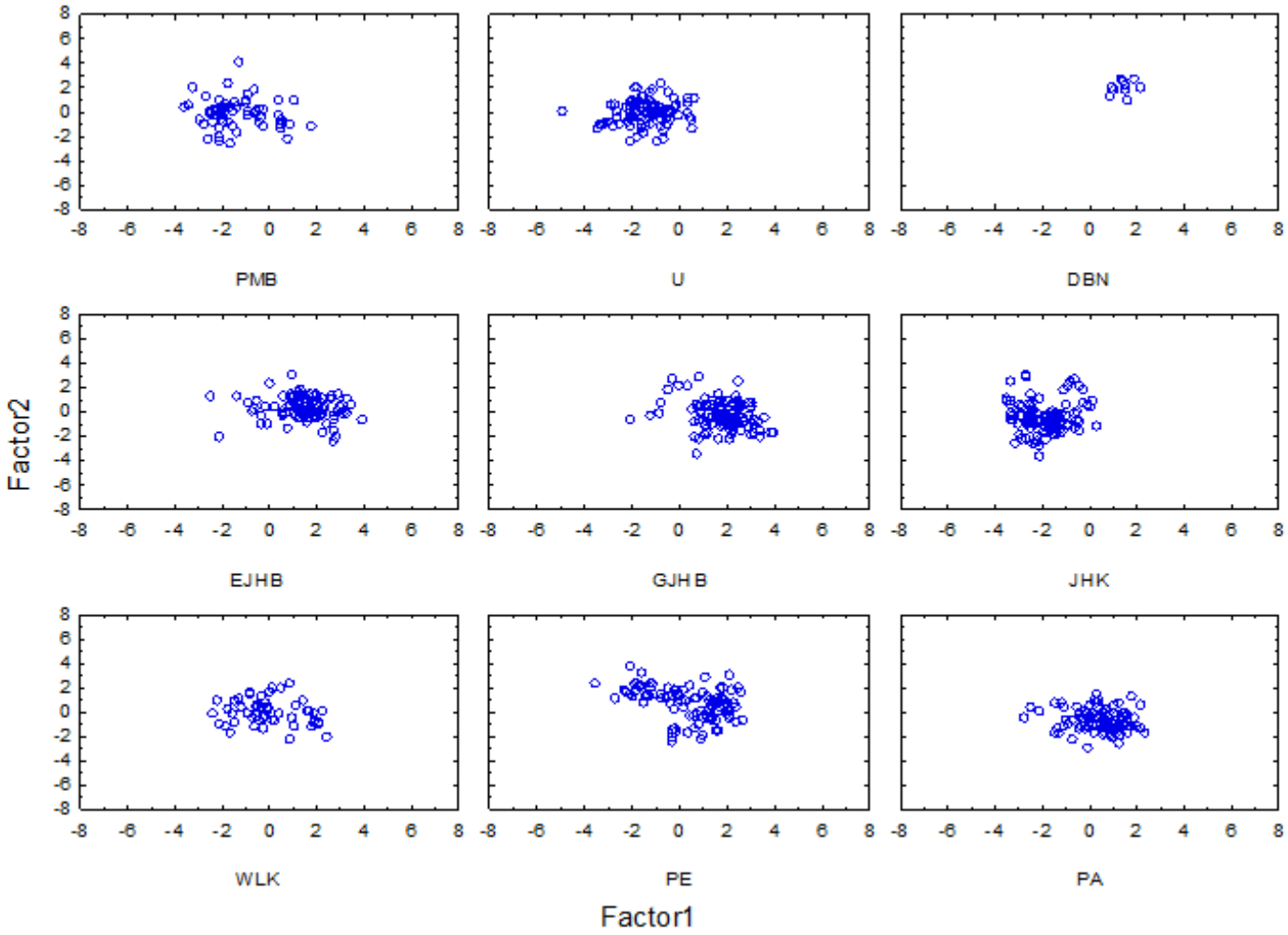


Figure 3.3. Principal Component Analysis of floral organs of *Pontederia cordata* from invasive populations in South Africa shown separately. PMB: Pietermaritzburg, KwaZulu-Natal. U: Underberg, KwaZulu-Natal. DBN: Durban, KwaZulu-Natal. EJHB: Emmarentia Botanical Gardens in Johannesburg. GJHB: Glenhazel, Johannesburg. JHK:Jonkershoek, Western Cape. WLK: Westlake, Western Cape. PE: Port Elizabeth, Eastern Cape. PA: Port Alfred, Eastern Cape.

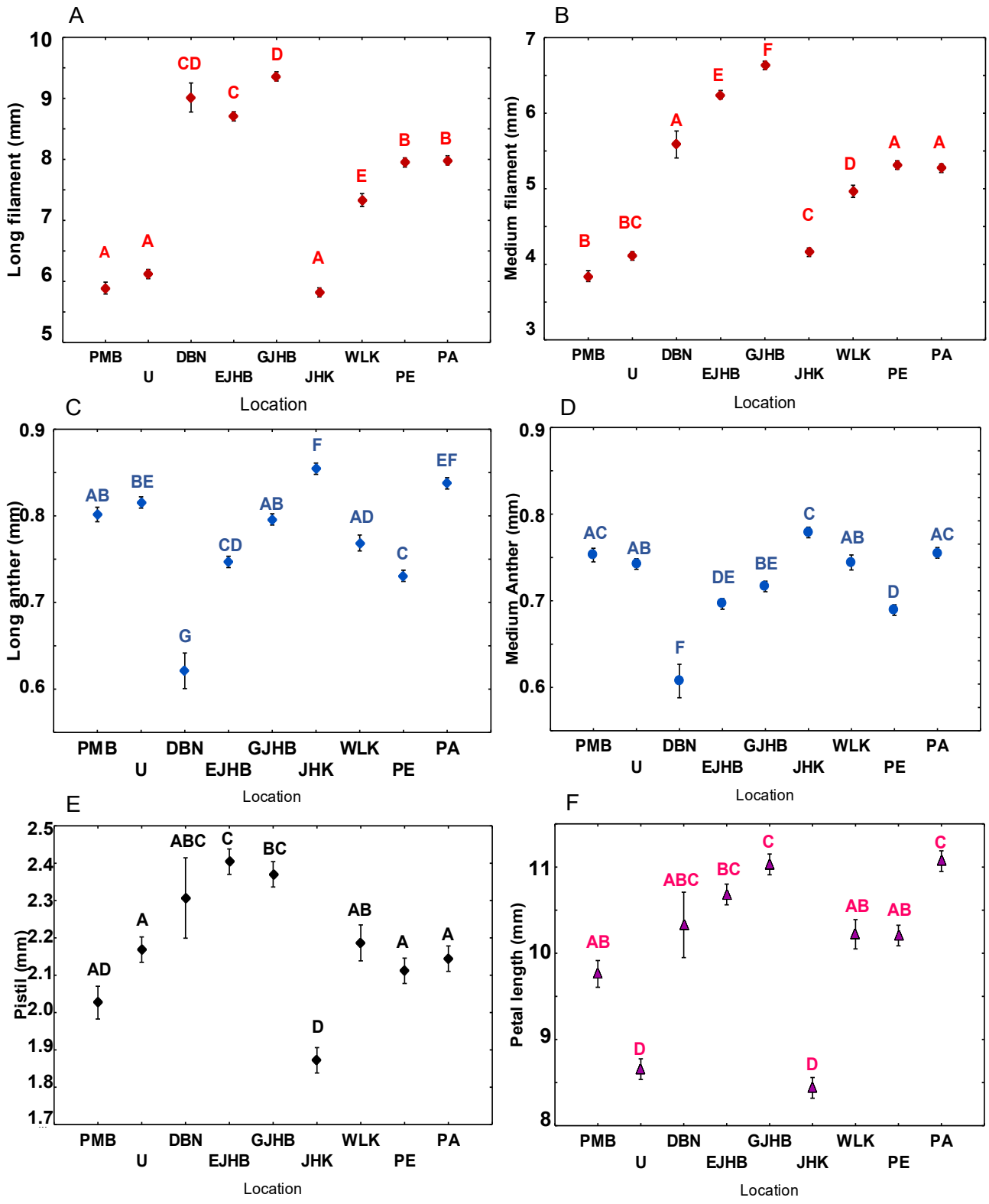


Figure 3.4. Flower organ measurements from *Pontederia cordata* from populations throughout South Africa. Means compared by ANOVA, vertical lines denote ± standard error bars. The same letters indicate population similarity (Tukey's HSD, $P<0.05$).

Locations: PMB: Pietermaritzburg, U: Underberg, DBN: Durban, EJHB: Emmarentia, GJHB: Glenhazel, JHK: Jonkershoek, WLK: Westlake, PE: Port Elizabeth, PA: Port Alfred.

Pistils were the smallest floral organ measured in every populations and varied between 1.85 ± 0.9 mm and 2.4 ± 0.8 mm (Fig. 3.3 e). Pistils were smaller in comparison to the pistils of short-morphed flowers of *P. cordata* var. *lanceolata* measured in the United States of America (native region) that were 2.7 ± 0.1 mm in height (Table 3.4).

Filament lengths recorded in invasive South African populations were smaller in size compared to *P. cordata* var. *ovalis* and *P. cordata* var. *cordata* (Table 3.4). Anther lengths were closest in size to *P. cordata* var. *cordata*, especially long anthers, however, there was no drastic variation when comparing to *P. cordata* var. *ovalis* either. Overall, the floral organ sizes of invasive South African populations were very similar to the sizes of floral organs in short-morphed flowers in the native range (Table 3.4).

Table 3.4. Comparison of floral organ sizes and pollen grain sizes from long and medium anthers of *Pontederia cordata* in South Africa (only short-morph) to the United States of America for short morphed flowers.

Floral traits	South Africa (invaded range)	United States of America (native range) for S morph flowers
Pistil length	2.17 ± 0.001 mm	Richards & Barrett, 1987: *P. cordata* var. *lanceolata* 2.7 ± 0.1 mm Hazen,1918: 2.7 to 3.0 mm Lowden, 1943: *P. cordata var. cordata* 1.0 mm *P. cordata var. ovalis* 0.8 mm
Filament length	Med: 5.15 ± 0.029 mm Long: 7.1 ± 0.039 mm	Lowden, 1943: *P. cordata var. cordata* Med:9.2; Long: 14.2 mm *P. cordata var. ovalis* Med: 6.7; Long:11.1 mm
Anther length	Med: 0.73 ± 0.02 mm Long: 0.79 ± 0.03 mm	Price & Barrett, 1982: Med: 0.85 ± 0.05 mm Long: 1.02 ± 0.06 mm Lowden, 1943: *P. cordata var. cordata* Med:0.8; Long: 0.8 mm *P. cordata var. ovalis* Med: 0.8; Long:0.9 mm
Pollen grain size Fresh (µm)	Med. anther pollen: 28.25 ± 0.68 Long anther pollen: 38.50 ± 1.2	Barrett & Glover, 1985: Med. anther pollen: 53.95 ± 3.6 Long anther pollen: 65.65 ± 3.22 Price & Barrett, 1982: Med. anther pollen: 46.2 ±2.0 Long anther pollen: 58.9 ± 7.1
Average pollen grain size (µm)	**(50% EtOH):** Med. anther:25.40 ±0.53 Long anther:34.20 ±0.52	**(Acetolyzed):** Gettys, 2005: Med. anther: 35.04 ± 0.49 Long anther: 44.97 ±0.3

Table 3.5. Factor coordinates and Eigenvalues from Principal Component Analysis of floral organs of *Pontederia cordata* in South Africa.

Flower organs:	**Factor 1**	**Factor 2**	**Factor 3**	**Factor 4**	**Factor 5**	**Factor 6**
Pistil	0.62786	-0.22303	0.74206	0.07075	-0.01974	0.00290
Petal length	0.83219	-0.27837	-0.22609	0.05029	-0.41324	-0.07467
Medium filament	0.91596	-0.03448	-0.14864	-0.05181	0.32217	-0.17678
Long filament	0.94581	-0.04510	-0.18612	-0.00544	0.10531	0.24010
Medium anther	-0.31280	-0.79955	-0.10649	0.49040	0.10494	0.00523
Long anther	-0.23632	-0.82867	-0.00470	-0.50665	0.02373	0.01355
Eigenvalues	2.97398	1.456422	0.66986	0.50744	0.29760	0.09469
% Total variance	**49.56631**	**24.27369**	**11.16440**	**8.45734**	**4.96002**	**0.57824**

3.3.2. *Pontederia cordata* pollen grain size analysis in South Africa

The morphology of the pollen grains from invasive *P. cordata* flowers showed a spheroid/biconcave shape but did not appear to be collapsed once gold coated and analyzed under a vacuum (Fig. 3.5). Fresh pollen grains were larger than processed pollen and the mean pollen grain size of long and medium anthers in South African populations (apart from the fresh pollen) was 34.20 ± 0.52 µm and 25.40 ± 0.53 µm, respectively (Table 3.4). Pollen grains from long and medium anthers, recorded in Port Alfred (Fig. 3.4a) and Port Elizabeth (Fig. 3.4b), respectively, were significantly larger than from other sample sites. Pollen grains from both long and medium anthers from Pietermaritzburg populations in KwaZulu-Natal province were significantly smaller than most of the other populations (Fig. 3.4). Pollen grains for both medium and long anthers in South African population were much smaller in comparison to North American measurements of *P. cordata* pollen (Table 3.4).

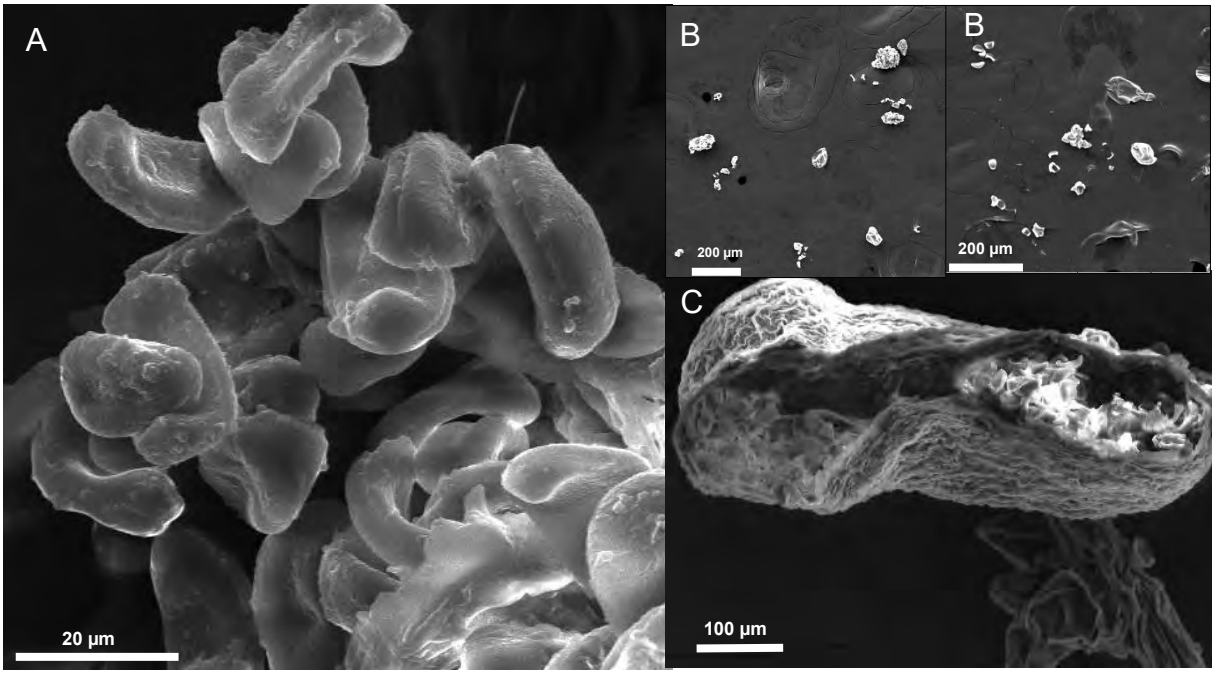


Figure 3.5. Scanning Electron Microscope images of the pollen grain morphology from flowers of *Pontederia cordata* in South Africa. A: pollen grains from a medium anther stored in alcohol. B: fresh pollen grains. C: pollen grains attached to an anther stored in alcohol.

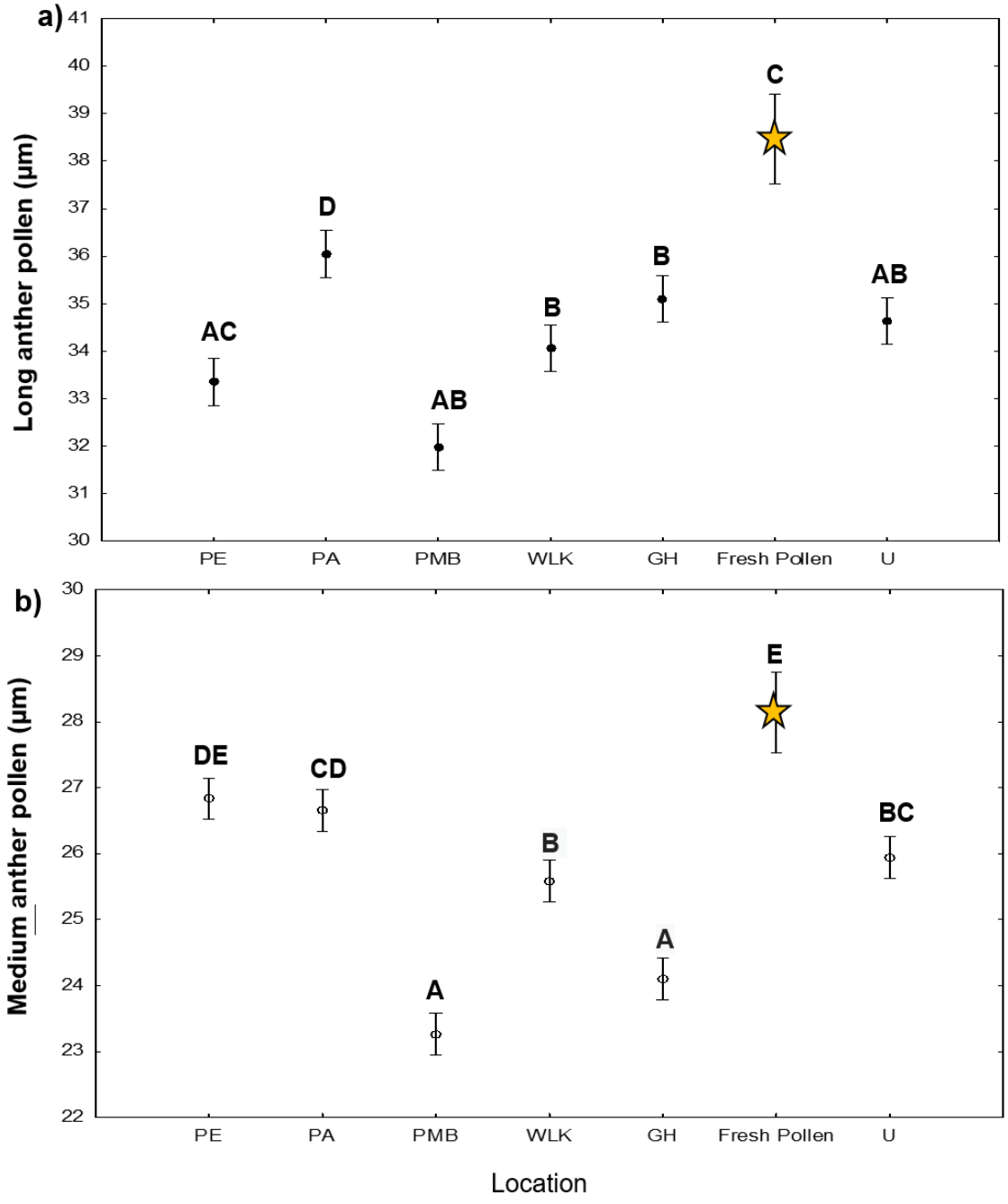


Figure 3.6. Mean pollen grain diameters from **a)** long anthers **b)** medium anthers. Vertical lines denote ± standard error bars. Flowers from short-morphed *Pontederia cordata* flowers stored in alcohol in South African populations were used. U: Underberg. PE: Port Elizabeth. PA: Port Alfred. PMB: Pietermaritzburg. WLK: Westlake. GH: Glenhazel. Fresh pollen from Hogsback indicate with star. Letters indicate population similarity using Tukey's HSD test. **a)** $F_{6, 457}$= 10.150, P < 0.0001. **b)** $F_{6, 457}$= 20.518, P< 0.0001.

3.3.3. The pollinators of *Pontederia cordata* in South Africa

Pontederia cordata flowers were visited by Hymenoptera (bees, carpenter bees and wasps), Lepidoptera (butterflies and moths) and Diptera (flies) species (Fig. 3.7). Underberg and East London had the greatest variety of insects visiting *P. cordata* flowers with a visitation rate of 1.40 and 1.23 visits per flower per hour respectively (Fig. 3.7). Bees, flies and wasps were the most frequent flower visitors throughout the populations. Pietermaritzburg had the highest abundance of bees visiting flowers (H_3,= 3.853, N = 16, *P* = 0.2778), however the abundance of butterflies, flies and wasps in Pietermaritzburg was approximately the same as the abundances of the insects in the other localities. There were no significant differences between the different pollinator insects visiting *P. cordata* flowers in every population recorded (Table 3.6).

Table 3.6. Kruskal-Wallis ANOVA values testing for differences in insect visitating rates among different populations of invasive *Pontederia cordata*.

Location:	Kruskal-Wallis test:	P-value:
Pietermaritzburg, KwaZulu-Natal	H (3, N = 16) = 3.852897	0.2778
Underberg, KwaZulu-Natal	H (5, N= 30) = 7.781782	0.1687
Port Elizabeth, Eastern Cape	H (3, N = 26) = 1.781970	0.6189
East London, Eastern Cape	H (4, N= 17) =3.313443	0.5068
Port Alfred, Eastern Cape	H (3, N= 13) =0.2971271	0.9606

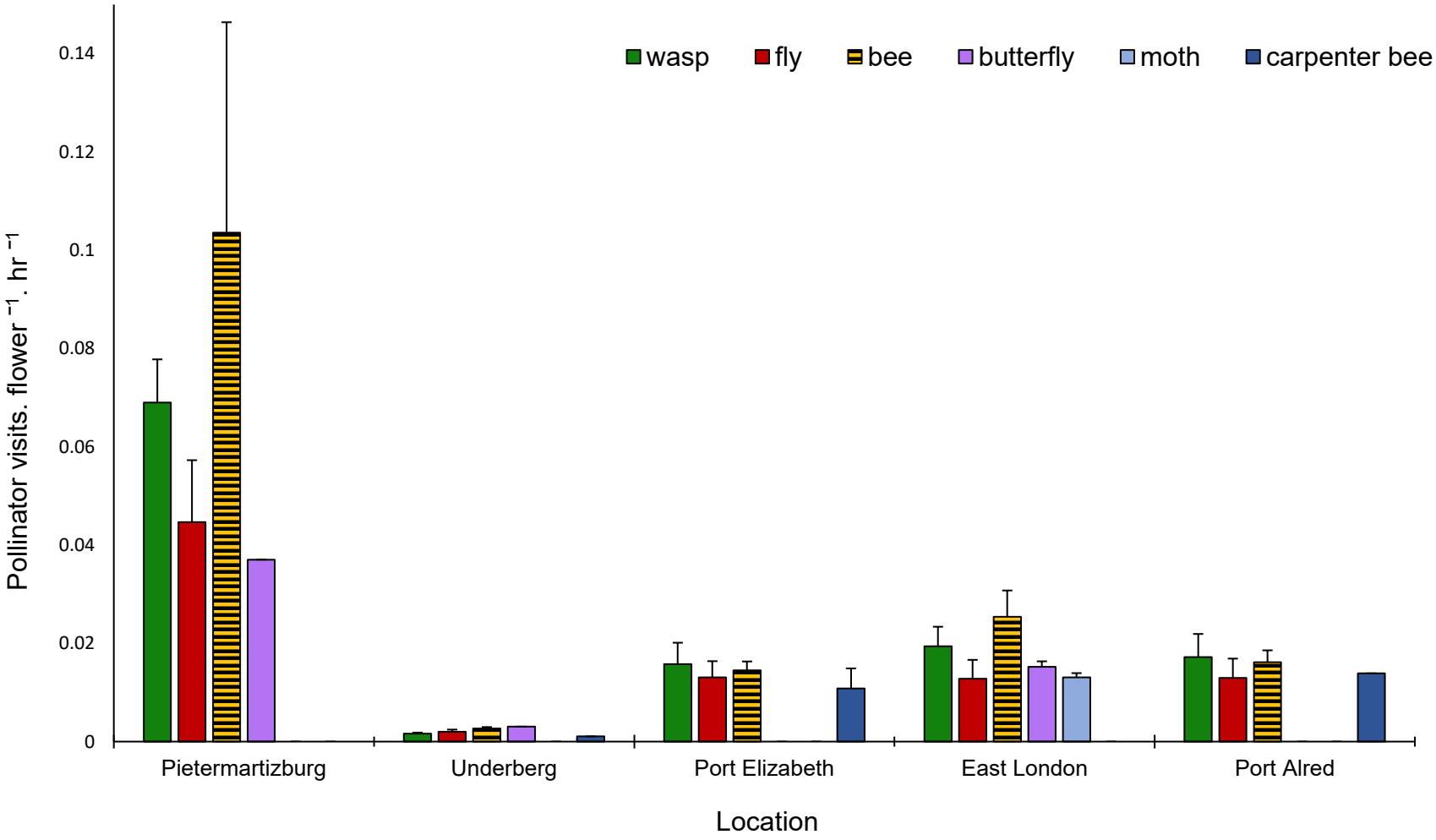


Figure 3.7. Types of insect pollinators and number of visits on *Pontederia cordata* flowers in South Africa per hour. Vertical lines denote mean ± standard error.

3.3.4. Hand pollination experiments of *Pontederia cordata* in South Africa

Because no seeds of *P. cordata* were recorded from any flowers during field surveys of invasive populations in South Africa, flowers from three South African populations were artificially pollinated in greenhouse experiments to determine whether seed production was possible. Short-morphed flowers are the only existing morph in South Africa and thus all treatments used short morphed flowers containing a short pistil and medium and long stamens (Table 3.2.). The outcome from the experiment was that no seeds were produced from any of the treatments (controls, self-pollinations and cross-pollinations). Only flower debris was recorded as a result of the pollination experiment (Fig. 3.8). The subsequent germination experiment of the flower debris to ensure that no seeds were produced resulted in no germination of any of the debris collected from the pollination experiment.

Figure 3.8. Dried *Pontederia cordata* flowers approximately 3-4 weeks after artificial cross-pollination using pollen from a medium anther.

3.4. Discussion

The most common way that alien invasive plants spread involves both sexual and asexual reproduction. *Pontederia crassipes*, a close relative of *P. cordata* spreads in such a manner (Barrett, 1988; Coetzee *et al*., 2017). I expected that *P. cordata* invaded South Africa similarly, as propagation via both forms of reproduction would explain why this species has become such a highly invasive plant over recent years. However, no sexual reproduction in *P. cordata* was recorded from this study in South Africa since results from field surveys and pollination experiments showed no seed or fruit production from invasive *P. cordata* plants.

Pontederia cordata has an incompatibility system during reproduction to ensure legitimate cross-pollination and optimal seed production (Ornduff, 1966). Legitimate pollination is present in the native range but does not seem to be present in the invasive range in South Africa. For legitimate pollination to take place, more than one floral morph (short, medium and long morph) is needed for cross-pollination (Ornduff, 1966). The first step to determine whether seeds are present in invasive populations in South Africa was to investigate what floral morphs were present. This provided insight as to whether sexual reproduction was taking place amongst different floral morphs, or whether one or more of these floral morphs were missing. Flowers from invasive populations throughout the country were analysed to determine pistil length (an indicator of the morph of a *P. cordata* plant) and I found that all invasive populations produced flowers with pistils that were indicative of short morphed plants. The pistils (< 2.5 mm) were smaller than or similar to the pistils of short-morphed native plants in United States of America except for Lowden (1943) that recorded smaller pistils (Hazen, 1918; Richards & Barrett, 1987). Furthermore, other floral organs such as filaments and anthers from invasive South African populations were also similar in length to the short-morphed floral organs in the native range and thus this inferred that the invasive *P. cordata* plants in South Africa are all short-morphed and sexual reproduction might not occur (Lowden, 1943; Price & Barrett, 1982).

There was little variability present when analysing the floral organs in invasive plants, suggesting similarity in floral morphology of *P. cordata* throughout South Africa. This suggests that *P. cordata* is not spreading via seeds and no cross-pollination is occurring, because low morphological variability in floral traits is generally linked with asexual reproduction, low genetic diversity and low gene flow (Leles *et al*., 2015).

Additionally, the low floral variability present amongst the invasive populations suggests that populations throughout South Africa may even be clonal populations that are spreading via clonal rhizomes, especially since low genetic variability is present within populations (Chapter 2). The higher intra-specific variability observed from each individual floral organ – when comparing invasive populations – showed no distinct pattern or clumping of populations, and even though Port Elizabeth and Port Alfred populations (both from the Eastern Cape), and Pietermaritzburg and Underberg populations (both from KwaZulu-Natal) showed similarity when comparing filament lengths, the other floral organs showed no similarities between these populations (Fig. 3.3). Random variability could be attributed to phenotypic plasticity – the production of alternative environmentally induced traits by regulatory gene networks in direct response to environmental stresses and heterogeneity, such as variable topography, soil, water-level and climatic conditions (Pfennig & Ehrenreich, 2014). A study by Stout *et al*., (2015) on the genetic diversity and floral width variation of invasive *Rhododendron ponticum* L. (Ericaceae) populations in Ireland compared to native populations in southern Europe determined that invasive populations have low genetic diversity and limited gene flow, however they suggested that phenotypic plasticity and local adaptation may be responsible for flower production with wider corolla tubes and increased nectar availability in invasive populations. These phenotypic variations may have been a response to new pollinator communities in the invaded range (Stout *et al*., 2015).

In this study, the pollen of invasive *P. cordata* flowers in South Africa was analysed to further match the short morphological characteristics of *P. cordata* flowers to pollen grains of short-morphed flowers in literature. Variances in pollen sizes for short morphed flowers in the native range were reported by the different past studies using slightly different analytical methods (Price & Barrett, 1982; Barrett & Glover, 1985; Gettys, 2005) and thus matching of the pollen sizes present in this study to those variable pollen sizes was not completely accurate. Despite this, the pollen from both long and medium anthers of invasive South African populations were considerably smaller than that of native North American short-morphed populations (Fig. 3.4). This could suggest that they are characteristic of short morphed flowers' pollen albeit smaller in size than native *P. cordata* pollen. The lack of other morphed plants in the invasive range in South Africa prevented the comparison of pollen from short, medium

and long morphed invasive plants and thus the identification of any pollen trimorphism in the country was not possible. Long anther pollen was, however, larger than medium anther pollen synonymous to past studies (Table 3.4: Price & Barrett, 1982; Barrett & Glover, 1985; Gettys, 2005). Pollen size variability was present amongst the different invasive populations in South Africa, however no significant variations were observed and thus all populations share the same short morphed flowers that produce this pollen.

It may also be possible that the very small, variable pollen grain sizes observed in this study may be contributing to a strong incompatibility system and the failure of seed production, or that the pollen itself may be sterile due to abnormalities in pollen development related to continuous vegetative reproduction (Smith, 1898). A study by Glover and Barrett (1985) investigated the trimorphic incompatibility of the tristylous species, *Pontederia sagittata* Presl. (Pontederiaceae), an emergent macrophyte native to Central and South America that was once thought to be a variety of *P. cordata*. Their studies in the native range were similar to this study in the invasive range, and included flower and pollen measurements, controlled pollinations, analysis of the behaviour of pollen tubes and field studies in Mexico (Glover & Barrett, 1985). Glover and Barrett (1985) determined that a very strong self-incompatibility system was present for *P. sagittata*, especially for long and short morphed flowers, and found that pollen from long anthers of short morphed flowers produced almost no seeds during self-pollinations since only 9.4% produced fruit out of 457 short morphed flowers self-pollinated by long anthers. Their study on native Mexico populations determined that short morphed flowers received the lowest number of pollen grains and the greatest amount of illegitimate pollen in some populations, and that pollen from long and medium anthers (such as the short-morphed flower) contributed the least to the pollen pool (Glover & Barrett, 1985). Furthermore, they deduced that illegitimate cross-pollinations presented cross-incompatibility and produced similar results to that of self-pollination (Glover & Barrett, 1985). They therefore speculated that a variety of mechanisms could exist that inhibit fertilization from illegitimate pollen such as: incompatible pollen from alternative anther levels causing inhibition of the growth of flower's pollen tubes, germination failure on the stigma, germination but abnormal growth of pollen tubes that fail to penetrate stigmatic tissue, incompatibility reaction in the ovary or incomplete growth of pollen tubes in the style. Their results

from the illegitimate pollinations showed cases of illegitimate pollen tubes growing extremely slowly, and in some instances the tubes did not even reach the base of the style (Glover & Barrett, 1985). Similar results were present during self-pollination of *Narcissus tazetta* L. (Amaryllidaceae) (Dulberger, 1964) in distylous native populations in Israel, whereby self-pollinated short or long styled plants proved to be almost or entirely sterile during breeding experiments, but cross-pollinated plants produced fertile seeds. Dulberger (1964) determined that self-sterility occurring from self-pollination was caused by ovule inhibition whereby the ovule broke down after fertilization or after the pollen tube penetrated the embryo sac. They determined that the ovaries swelled, degenerated and collapsed 2-3 weeks after self-pollination (Dulberger, 1964). Invasive short morphed *P. cordata* populations could be experiencing similar inhibition mechanisms to *P. sagittata* and *N. tazetta* and thus no seeds are being produced in invasive populations in South Africa. Pollen germination experiments would therefore be useful to test this.

It was also speculated that insufficient pollination caused by the absence of specialised native pollinators could be the cause of no seed production in invasive *P. cordata* populations in South Africa. A specialist solitary bee pollinator, *Melissodes apicata* Lovell and Cockerel (Anthophoridae), is co-adapted to tristylous floral polymorphisms present in *P. cordata* populations in the native range of North America (Harder & Barrett, 1993). *Melissodes apicata* has behavioural and morphological adaptations for collecting *P. cordata* pollen and nectar such as possessing proboscides with tiny hairs for collecting the appropriate pollen (Laberge, 1963; Wolfe & Barrett, 1987). A pollinator investigation was thus undertaken to determine if any insects in South Africa were pollinating *P. cordata* flowers in the invasive populations. Despite the lack of *M. apicata* in *P. cordata,* invasive populations in South Africa are still sufficiently visited by generalist pollinators.

The resultant pollinator study depicted the lowest pollinator visits in Underberg, KZN on each monitored flower compared to other locations, yet the *P. cordata* population in Underberg was one of the largest populations surveyed with numerous inflorescences (Table. 3.1.). This result may have been caused by the insect pollinators being more dispersed and having more choices of inflorescence to visit, which inadvertently caused fewer pollinators to visit the monitored flowers. Mechanical

clearing of populations in Pietermaritzburg was implemented during 2019 (SANBI Database, 2019) and thus only a few isolated plants remained for observation during the pollinator survey. Locations with fewer blooming inflorescences provided fewer choices for insect visitors and thus an increase in flower visits was observed for smaller populations such as Pietermaritzburg (Fig. 3.6). Future studies should survey populations of similar sizes to avoid such discrepancies, however the main purpose of the pollinator study was to determine that insect pollinators visit flowers of invasive *P. cordata* populations. Apart from the specialist pollinator *M.* apicata in the native range in the United States of America, there are also generalist insects that pollinate *P. cordata,* such as generalist honeybees and bumble bees, *Bombus impatiens* Cresson and *Bombus vagans* Smith (Apidae) (Harder & Barrett, 1993).The presence of generalist insects such as honeybees pollinating invasive *P. cordata* flowers in South Africa is thus an indication that the lack of specialised pollinators in the invasive range is not the reason for no seed production because pollination still occurs.

To strengthen our investigation further, 8 865 *P. cordata* flowers from invasive populations in South Africa were artificially pollinated in various treatments at optimal growth conditions to determine whether any seeds could be produced. Since no seeds were developed from any of these flowers, it was concluded that seeds are not produced in invasive populations in South Africa, most likely due to the self-incompatibility of flowers from short-morphed plants invading.

Conclusion

Results from this study indicate that seeds are not present in *P. cordata* populations in South Africa due to *P. cordata*'s incompatibility system and thus sexual reproduction is not a contributor to the invasion success of this plant throughout the country. Asexual reproduction is therefore responsible for *P. cordata*'s spread, and it is likely that plants are spreading via rhizomes. It is therefore imperative that further introduction of *P. cordata* into South Africa is prevented to avoid the possibility of another morph being introduced that could legitimately cross-pollinate the short-morphed plants to produce seeds. Although *P. cordata* is already a highly invasive macrophyte in South Africa, sexual reproduction would increase the species invasiveness and allow *P. cordata* seeds to easily spread between waterbodies compared to slower spread by rhizomes.

Self-incompatibility in angiosperms is the most effective anti-selfing mechanism present in plant reproductive biology (Khanduri *et al*., 2013). It is likely that sexual reproduction in *P. cordata* is not present in invasive South African populations due to self-incompatibility and illegitimate pollination of short morphed plants. Several studies have shown that the short-morphed flowers from *P. cordata* have the greatest incompatibility system (Ornduff, 1966; Barrett, 1976; Gettys, 2005). Ornduff (1966) reported that an infinitesimal 5.3% of illegitimately pollinated short-morphed *P. cordata* flowers studied produced seed-bearing fruit. It is therefore unsurprising that 0% of illegitimately pollinated flowers from invasive populations of short-morphed plants in this study produced seeds.

Flowers of *P. cordata* are uniovulate, and in the native range all three tristylous morphs in flowering populations are producing pollen (Barrett & Glover, 1985). In this sense, as long as insect pollinators are present and self-incompatibility is maintained, flowers are generally insensitive to fluctuations of legitimate and illegitimate pollination because some compatible pollen will always reach them, thereby maintaining fecundity (Barrett & Glover, 1985). This may be the case in the native range, however, in the invasive range there is only one type of tristylous flowering plant present amongst all populations in South Africa. Pollen loads therefore only contain one illegitimate, incompatible type of pollen for short-morphed flowering plants, and thus flowers are infecund. Further studies on the incompatibility system of invasive short-morphed plants in South Africa should analyse the behaviour of pollen tubes from these short-morphed flowers to confirm whether there are mechanisms inhibiting fertilization during illegitimate pollination.

Lastly, variable leaf morphology observed in invasive populations during field surveys in South Africa such as thin, lanceolate leaves in Himeville Nature Reserve, KZN and cordate leaves elsewhere highlight that future genetic and morphological research should focus on the varieties of *P. cordata* present South Africa. Genetic markers such as ISSR (Chapter 2) or AFLP techniques could be used to differentiate between the different varieties, which could then be linked to the morphological features of the different varieties, for example: cordate leaves which could be *P. cordata* var. *cordata* or lanceolate leaves which could be *P. cordata* var. *lanceolata*. Determining whether differences in floral morphology and leaf variability are due to phenotypic plasticity or genetic diversity will be an important next step. Identifying which varieties of *P. cordata*

are present in South Africa will also help in the development of a biological control programme since it is expected that the most efficient control and damage would come from a biological control agent locally adapted in its native range to the invasive variety present in South Africa (Paterson *et al*., 2009).

CHAPTER 4.

General Discussion

The aim of this thesis was to determine the invasion ecology of *P. cordata* in South Africa by investigating the species' population genetics, reproductive traits and invasive characteristics in the country. DNA from leaf samples throughout the invasive South African range were collected to determine the genetic diversity and structure from invasive *P. cordata* plants, and to compare these results to the genetic diversity and characteristics of DNA samples from the native range in United States of America. Results indicated that low genetic diversity was present throughout the invasive range compared to the native range, and that little genetic variability was present within each invasive population. This study provides insight into the introductory events, modes of spread and traits that could be exploited during the development of a biological control programme against *P. cordata*.

However, ecological studies provide further understanding of the genetic results and confirm the prediction that plants are not spreading by seed in South Africa. Thus, the reproduction and floral characteristics of invasive *P. cordata* populations were investigated. Analysis of floral organ traits and pollen sizes confirmed that only one type of tristylous plant was spreading throughout the country – the short-morphed plant. Artificial pollination experiments and germination experiments determined that plants in South Africa could not produce seed due to the incompatibility system present amongst short-morphed flowers. Consequently, the low genetic diversity may be present due to the lack of sexual reproduction in invasive populations. The implications, limitations and importance of these findings are herewith discussed, as well the influence that the South African public have on the spread of *P. cordata* in the country and insights into controlling and managing its spread.

4.2. Population genetics of *Pontederia cordata* in South Africa

Genetic diversity and variation may provide evidence for events such as genetic bottlenecks, singular or multiple introductory events and even insinuate modes of spread such as high gene flow indicating sexual reproduction (Novak & Mack, 2005). Genetic markers are useful tools to profile the genetic characteristics of invasive plants and can aid researchers in determining the source of invasions in the native range for

conducting native enemies surveys (Paterson *et al*, 2009; Paterson & Zachariades, 2013). Through ISSR techniques, I found that *P. cordata* plants had low genetic diversity in invasive South African populations (Chapter 2). High variability and genetic diversity were observed in every sample from the native as expected, due to high gene flow and variation in the native range's gene pool. These findings suggested that only one or very few introductions of *P. cordata* occurred into South Africa from the native range. The low genetic variability observed within each invasive *P. cordata* population in South Africa also indicated that plants were proliferating asexually, however slight variability amongst the different invasive populations suggested that the idea of sexual reproduction amongst invasive populations should not be excluded. The morphological aspects such as floral traits and the pollen ecology of *P. cordata* was therefore investigated to determine if sexual reproduction was indeed taking place, or whether plants were spreading asexually via rhizomes.

4.3. Where are the seeds? Pollination ecology and floral traits of *Pontederia cordata* in South Africa.

Self-incompatibility is a breeding scheme that prevents self pollination and enables a flowering plant to avoid the inbreeding and deleterious genetic effects that may occur as a result of self-fertilization (Barrett & Cruzan, 1994). Tristyly, a type of sexual polymorphism that is present in *P. cordata*, promotes cross pollination by insect-mediated pollination from anthers of certain levels to corresponding stigmas. Tristyly ensures the exchange of generic material amongst different styled plants and possibly maximises parental fitness and fecundity through seed production. Additionally, insect-mediated pollination of tristylous flowers reduces pollen wastage because pollen from different anthers is generally assorted onto specific areas of the insect body to be delivered to the correct stigma (Wolfe & Barrett, 1987).

Field surveys of *P. cordata* invading South Africa found no seed in any of the populations surveyed throughout the invaded provinces. This is likely due to the lack of legitimate fertilization. Floral morphology, pollination ecology and pollinator studies (Chapter 3) determined that invasive plants were not capable of sexual reproduction due to a self-incompatibility system present because of their short stylus morphology. These results were synonymous with the genetic study in Chapter 2, whereby low genetic diversity of invasive populations was determined, and it was suggested that

asexual reproduction was responsible for invasion. The floral morphology studies conducted on invasive *P. cordata* populations in South Africa determined low variability of floral organ sizes amongst invasive populations. Furthermore, the pistil, filament and anther sizes positively corresponded to the sizes of short-morphed flowers measured in the native range from past studies (Hazen, 1918; Lowden, 1943; Price & Barrett, 1982; Richards & Barrett, 1987). These findings, along with the very small pollen morphology of *P. cordata* in invasive populations confirmed that short morphed plants were present throughout South Africa.

Slight variation amongst the floral organ sizes in invasive populations of *P. cordata* were observed during the study in Chapter 3 and could be attributed to phenotypic plasticity. Interestingly, slight genetic variation was also observed amongst the different invasive populations which suggested that even though invasive populations are all short morphed and do not outbreed, they are not characterised by one singular monoclone like *P. crassipes*, that was found to be one singular genotype clone across many different invasive countries (Zhang *et al*., 2010). Unlike the short styled plants of *P. cordata* being present in the invasive range in South Africa, the intermediate (medium) style form of *P. crassipes* is prevalent and can produce high levels of seed production and fecundity in the introduced range where only singular morphed colonies are present (Coetzee *et al*., 2017). It may be possible that introduction of medium or long morphed plants of *P. cordata* into South Africa may enable *P. cordata* plants to produce seeds. This may be instigated from outcrossing different styled flowers, but also from self-fertilization and a greater self-compatibility present amongst medium and long morphed *P. cordata* plants (similar to the scenario of the mid-style form of *P. crassipes* producing seeds in the invasive range) (Barrett & Forno, 1982; Barrett & Anderson, 1985). Barrett and Anderson (1985) demonstrated that the incompatibility system of *P. cordata* from self-pollination is closely linked to the style length of the plant, such as medium morphed flowers showing more compatibility during self-pollination than short morphed plants. They further suggested that differences in incompatibility between the different style levels of *P. cordata* may be caused by pleiotropic effects of major genes controlling sub-characters of the tristylous syndrome (Barrett & Anderson, 1985). The presence of a single style morph invading South Africa that is incapable of seed reproduction has provided a window of opportunity for a management programme to be developed that can focus on reducing

biomass and rhizomes spread without having to worry about seeds. The sooner *P. cordata* invasion is controlled and reduced, the less chance of additional introductions occurring that could potentially cause outbreeding amongst invasive populations with subsequent seed production, escalated spread and further problems.

There are very few examples of pollination preventing biological invasions in the world (largely because pollination of non-invasive species is rarely studied), and even less so in South Africa (Wang, 2014; Le Roux *et al*., 2020). Often an introduced plant will self-fertilize or be pollinated by generalist pollinators in the invaded range to produce seed and overcome the reproductive barrier that may prevent establishment (Le Roux *et al*., 2020). This study determined that an alien invasive species produces no seeds due to pollination failure yet successfully invades South Africa. The introduction of other morphed *P. cordata* plants into South Africa would likely lead to legitimate cross-pollination and seed production. This may subsequently lead to a more rapid spread of *P. cordata* in the country over longer distances because *P. cordata* produces copious amounts of seeds that are smaller, buoyant and more easily dispersed than rhizomes (Gettys & Dumroese, 2009).

4.4. Insights into the development of a biological control programme for *Pontederia cordata*

Meticulous planning and implementation of strategies to eradicate and control alien invasive plants are critical to achieve significant results such as reduction in alien invasive plant's biomass and a decrease in proliferation and spread. The need for this planning has increased in recent years due to alien invasive species adapting to variable biotic and abiotic environments caused by climate change and urbanization (Jose *et al*., 2013). The study of invasion ecology and the underlying mechanisms that enable a species to successfully invade is therefore a crucial part of alien invasion sciences to understand and combat invasion (Jose *et al*., 2013). Management programmes should plan control strategies from the ecology and invasive traits of the specific alien invasive plant in its invasive habitat to develop a customized combination of control tactics for successful and cost-effective control.

One such example of integrated control management is on the famine weed, *Parthenium hysterophorus* L. (Astereaceae), an aggressive alien invasive plant native

to America that has invaded over 30 countries worldwide, including South Africa and Australia (Picman & Picman, 1984; Dhileepan & Strathie, 2009). Individual control mechanisms such as chemical, manual or mechanical control have proved futile to reduce the spread of *P. hysterophorus* due to herbicide resistance, health risks, uneconomical expenses and extreme proliferation from high seed banks (Picman & Picman, 1984; Temesgen *et al*., 2017). Integrated control based on exploiting the invasive ecology and biology of *P. hysterophorus* was thus recommended (Kebaso *et al*., 2020). One of the greatest invasive traits of *P. hysterophorus* is its highly prolific nature, whereby an individual plant can produce up to 100 000 seeds and germinate any time of the year, however its spread is solely caused by cross-pollinated seeds, and germination generally requires barren soil (Parsons & Cuthbertson, 2001). Integrated control of *P. hysterophorus* therefore targets the flowering part of the plant to stop seed production and includes the application of herbicides when plants are small and seeds have not yet been produced so that native grasses can quickly recolonize the invaded area. Alternatively, mechanical removal occurs by ploughing invaded areas before the flowering and seeding stages so that pastures can re-establish. Biotechnologists have even suggested the innovative use of *P. hysterophorus* for medicinal prospects and for biogas production by using the noxious weed as a feed source during anaerobic digestion for methane gas (Patel, 2011; Tadesse *et al*., 2014; Temesgen *et al*., 2017). To avoid constant use of herbicides that can cause herbicide resistance amongst invasive populations, the application of biological control agents was also recommended for long-term control of *P. hysterophorus*. Nine species of insects including the stem-galling moth *Epiblema strenuana* (Lepidoptera: Tortricidae) and the winter rust fungus *Puccinia abrupta* var. *partheniicola* (Basidiomycota: Puccinaceae) have provided sustainable control in Australia where *P. hysterophorus* is invasive (Dhileepan, 2001).

Management strategies for *P. cordata* using integrated control where biological control is used as the main component and other control methods such as mechanical control and herbicidal treatment (in extreme cases) may prove to be the most effective, sustainable and affordable solution to control the spread of *P. cordata.* Such strategies have been developed for *P. crassipes*, whereby integrated management strategies using biological control in synergy with other control methods that complement each other in a given area are implemented (Julien, 2001). These control methods include

mechanical or physical removal, nutrient reduction, flow manipulation in the infested waterbody and herbicide treatment (Julien, 2001). Integrated management using short term manual/mechanical removal of underground rhizomes and above ground biomass on small areas of *P. cordata* infestations such as overgrown garden ponds, and long term biological control on dense populations in larger waterbodies is therefore suggested for *P. cordata* invasions in South Africa. Alternatively, as a last resort in large, highly invaded areas or areas of economical importance where other control methods are not present or working, it may be suitable to use herbicide or bioherbicide treatment depending on the availability and authorization for use in the country. Herbicides that have been used for control of *P. crassipes* such as glyphosate, 2,4-D, diquat and paraquat have resulted in successful control in some small, single-purpose waterbodies and thus may work well on small *P. cordata* infestations (Gutierrez *et al*.,1994; Terblanche *et al*., 2012).

Once a biological control programme is developed, it is also important to consider the compatibility of biological control agents with each herbicide and surfactants used. Without appropriate integration of control methods, herbicide control operations can cause interference and high mortality of biological control agents due to surfactants and toxicity of active ingredients (Ueckermann & Hill, 2000). For example, herbicides containing diquat and 2, 4-D amine are toxic to the biological control agent *Neochetina eichhorniae* Warner (Coleoptera: Curculionidae) and *Eccritotarsus catarinensis* (Carvalho) (Hemiptera: Miridae) released on *P. crassipes* in South Africa (Ueckermann & Hill, 2000; Hill *et al*., 2012). Furthermore, prolific reinfestation of *P. crassipes* after spraying occurred because the high mortality of biological control agents and lack of untreated *P. crassipes* for insect feeding and refuge during the herbicidal applications reduced the enemy populations and control pressure on the weed (Hill & Olckers, 2001). It is therefore essential to communicate with water authorities and raise awareness of the advantages of integrated control once a biological control agent is released on *P. cordata*; and to develop management practices for invaded areas of *P. cordata* that will be the most effective and sustainable in the long term.

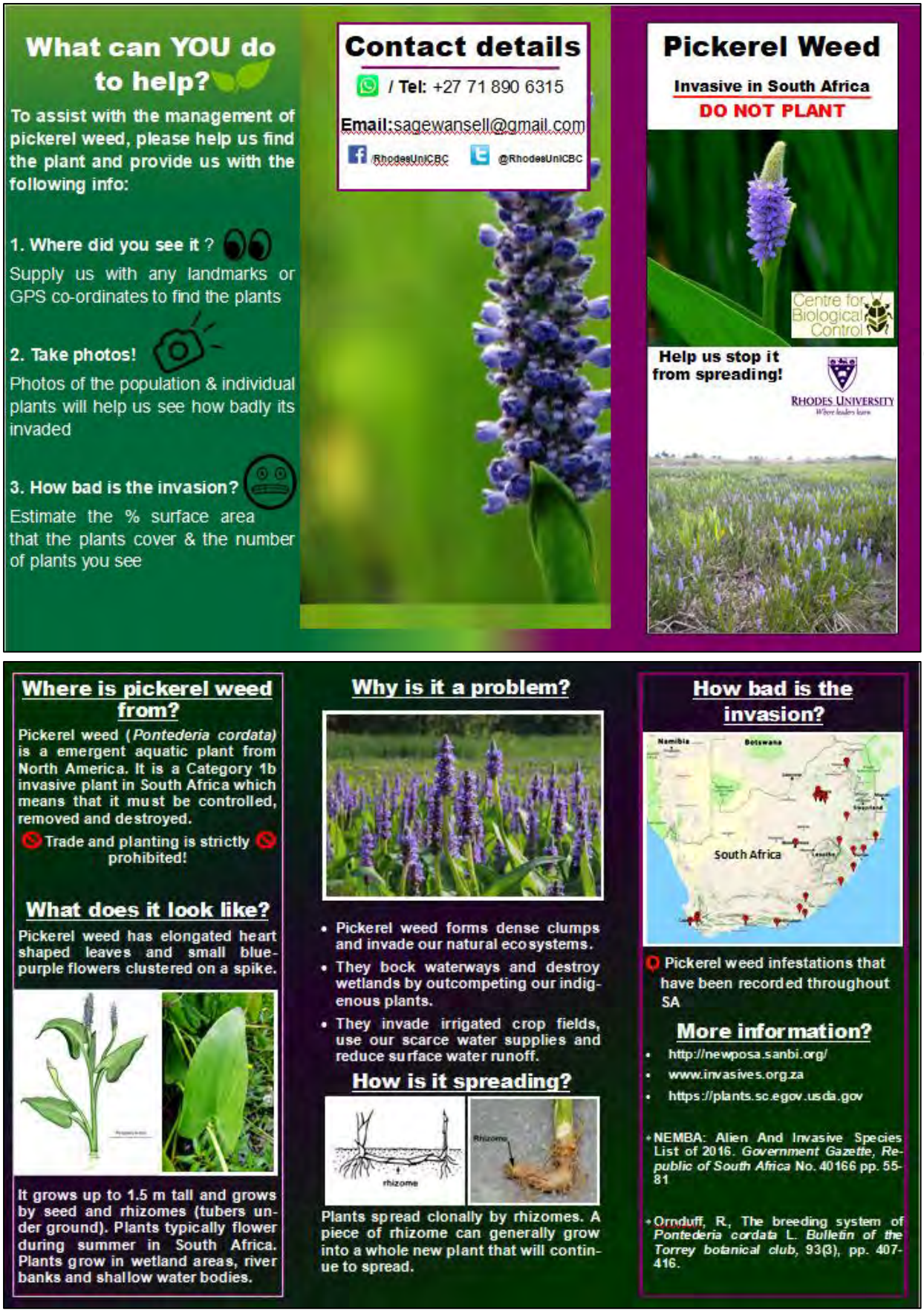
One of the most important strategies for the prevention and reduction in spread of an alien invasive species invasion is public support and awareness on the identification,

impact and control of alien invasive species (Merchante *et al*., 2011). Campaigns should focus on raising awareness amongst conservation professionals at botanical gardens and national parks, aquarists, horticultural traders and schoolteachers to teach youth and expand the knowledge about alien invasive species (Merchante *et al*., 2011). Schreck Reis *et al*., (2011) assessed the impact of raising awareness of alien invasive plant species and biological invasions during a workshop involving 5 different high schools and 170 students in Portugal. The educational workshop focussed on identification of alien invasive plants invading Portugal, impacts of invasive species, competition between the alien invasive plants and native flora, and the control of alien invasive plants (Schreck Reis *et al*., 2011). A year later they tested the students' knowledge of alien invasions compared to students that had not participated in the workshop and determined that the workshop students acquired and retained the knowledge of biological invasions (Schreck Reis *et al*., 2011). The workshop was successful in raising awareness amongst these students of the importance of prevention and control of alien invasive plants (Schreck Reis *et al*., 2011). Targeting youth for educational programmes on alien invasions can prepare future citizens to be more proactive towards alien invasive species invasion and nature conservation (Merchante *et al*., 2011).

Gardeners, horticultural traders and nurseries can be an unintentional source of introduction and spread of biological invasions, however through awareness and participation they can also play a major part in prevention and reduction in alien invasive species spread (Vanderhoeven *et al*., 2011). In Australia, numerous web applications have been developed for the identification, surveying and reporting of alien invasive plants by citizens such as the online sites 'MyWeedWatcher' and 'Weed Spotters Network Queensland' (Novoa *et al*., 2019). Citizens in South Africa can also access similar applications such as iNaturalist, whereby they can upload geotagged photos of alien invasive species onto the website which provides experts and officials information on biological invasions and newly invaded sites in southern Africa (https://www.inaturalist.org/places/south-africa). In Britain, a citizen-science study by Dehnen-Schmutz and Controy (2018) worked with gardeners to report hard to control ornamental plants invading their gardens onto a web platform to identify potentially invasive ornamental plants. Their results reported 201 records of 121 species and 104 non-native plants growing in their gardens, which subsequently led to the gardeners

restricting the plants spread and in some instances attempted to exterminate the plants (Dehnen-Schmutz & Controy, 2018). This study highlighted that raising awareness of alien invasive plants and working in collaboration with gardeners through early detection, surveillance and monitoring could help prevent and control the spread of alien invasive plants (Dehnen-Schmutz a Controy, 2018).

Posters and pamphlets containing comprehensible citizen science on the invasiveness and harmful consequences of alien plant invasion in South Africa could be used to raise awareness and support to manage the plant invasion (Novoa *et al*., 2017). The construction of a pamphlet containing information about the invasion of *P. cordata* in South Africa from this study is currently underway (Fig. 4.1).



Cover page

Figure 4.1. Pamphlet design of *Pontederia cordata* invasion in South Africa to raise public awareness.

Awareness campaigns, posters and pamphlets may reduce both deliberate and accidental spread of *P. cordata* into and within South Africa by increasing public awareness of the species invasive status and decreasing the illegal sale and trade of *P. cordata* plants. This may subsequently aid in minimizing the threat of *P. cordata* invasion.

It is speculated that the current spread of *P. cordata* in South Africa may be perpetuated by avid gardeners and horticulturists through trading and dumping of plants, asexual propagules and underground fragments in ditches, streams and other waterbodies. Furthermore, it is highly likely that fish farmers and golf course owners may be utilising *P. cordata* as a stabilizing plant for dams and waterbodies, ignorant of the species' NEMBA 1b invasive status and threat to the ecosystem (NEMBA, 2004). The status of *P. cordata* under South Africa's National List of Invasive Species under the National Environmental Management: Biodiversity Act 10/2004 is Category 1b, meaning that *P. cordata* invasion requires compulsory control as part of an invasive species control programme, and that populations should be removed and destroyed (Department of Environmental Affairs, 2014). Furthermore, trading of *P. cordata* in South Africa is not permitted due to the plants highly invasive potential. Despite these restrictions in trade and legislation, backyard trading may facilitate the spread of the species, a common problem in South Africa (Martin & Coetzee, 2011).

Using the framework by Kumschick *et al*., (2020) as a guide to suggest the regulation and listing of *P. cordata*, it is recommended that *P. cordata* is regulated as a control target and a national management programme is developed. Based off the findings in this study it is also recommended that the invasive status of *P. cordata* remains a Category 1b invasive plant (NEMBA, 2004; Department of Environmental Affairs, 2014). *Pontederia cordata* is adaptable to variable climatic and habitat conditions and is present throughout almost all provinces in South Africa, except for extremely arid areas with sparse waterbodies. Elsewhere in the world there are also cases of introduced, naturalized or invasive *P. cordata* populations (Henderson & Cilliers, 2002; Wittenberg, 2006; Stanley *et al*., 2007; Witt & Luke, 2017). The dispersal of *P. cordata*'s rhizomes between waterbodies is likely aided by humans, albeit rhizome dispersal is not necessarily as fast as seed dispersal, it has still rapidly spread and caused negative environmental and socio-economic impacts. The environmental impacts of *P. cordata* invasion include the decline of indigenous plant populations in

invaded areas and changes in community composition. Much like other alien invasive plants in South Africa, *P. cordata* populations also have a high water uptake and reduce water flow in invaded waterbodies (Chamier *et al.,* 2012). The socio-economic impacts of *P. cordata* invasion include the disappearance of livelihoods, agriculture (invasion of irrigated crop fields) and tourism from waterbodies where dense stands of *P. cordata* have formed (Melton & Sutton, 1991). If other morphed *P. cordata* plants are introduced into South Africa and enable the invasive species to become sexually reproductive, then it is probable that *P. cordata* may become as invasive and problematic as its close relative, *P. crassipes* that is listed as one of the global top 100 alien invasive species (Van Wyk & Van Wilgen, 2002; Global Invasive Species Database, 2020). Management strategies to control and reduce the spread of *P. cordata* in South Africa is therefore crucial. Moreover, preventing further introductions of *P. cordata* into the country and ending the trade of *P. cordata* by gardeners and horticultural traders would slow down the dispersal of *P. cordata* within the country.

A similar macrophyte, *Monochoria africana* (Solms) N.E.Br. (Pontederiaceae) has the potential to become an attractive cultivated aquatic plant that could replace the trade of *P. cordata* in the horticultural industry (Fig. 4.2) (Glen, 2006).

Figure 4.2. *Monochoria africana* (Solms) N.E.Br. (Pontederiaceae) (Watercolour painting by G. Condy (Glen *et al*., 2001)).

Monochoria africana is a distylous indigenous macrophyte in Eastern and South Africa and has showy blue to violet inflorescence on a tall spike, similar to *P. cordata* (Fig. 4.2) (Lyengar, 1923; Glen *et al*., 2001). *Monochoria africana* is scarce and occurs sporadically in localised areas mainly in Limpopo and Mpumalanga, South Africa (Glen, 2006). Future research into understanding *M. africana*'s biology, germination and propagation may therefore aid this plant in becoming an alternative popular macrophyte in South African waterbodies such as ponds, water features, botanical gardens and golf course dams (Glen, 2006). Moreover, because *M. Africana* is an indigenous species, it will have native enemies which should ensure that it does not spread and cause undesirable impacts.

Many urbanized areas such as the Underberg golf course in KwaZulu-Natal have prolific invasive populations of *P. cordata* growing. The managers of the Underberg golf course were unaware of the invasive status of *P. cordata*; however, they agreed that *P. cordata* is a noxious menace because they experienced frequent problems of dense overgrown populations invading throughout their shallow dam, which often had to be mechanically removed. Mechanical removal of *P. cordata* is not a long-term strategy to control the invasion of *P. cordata*, since it is often laborious, time consuming and costly (Myers & Bazely, 2005). Underground rhizomes also make it difficult to fully remove the entire infestation, and subsequent reinfestation is highly probable if overlooked (Grewell *et al*., 2019). No herbicide treatment has been specified for use on *P. cordata* in South Africa, and it is unlikely to provide any long-term solution for the spread of *P. cordata* because of the plant's rhizomes allowing for resprouting after spraying. Herbicide treatment can also be damaging to the surrounding environment and may affect non-target fauna and flora if not applied correctly (Van Bruggen *et al*., 2018; Vonk & Kraak, 2020). Furthermore, frequent herbicide applications may cause herbicide-resistance of invasive plants and thus a more holistic approach is needed in which more than one control mechanism is incorporated (Mohammadi, 2013), such as biological control combined with mechanical control in larger, more problematic areas.

Although more planning and research is needed before a biological control programme is fully developed for *P. cordata* in South Africa, there are insect species that could prove to be viable prospects as biological control agents. *Xubida infusella* Walker. (Lepidoptera; Pyralidae) is a leaf and petiole mining moth that can develop on a small

number of freshwater aquatic members of the Pontederiaceae family, including *P. crassipes*, *Eichhornia paniculata* (Spreng.) Solms-Laub, *P. rotundifolia* and *P. cordata*. *Xubida infusella* has already been released in Australia for the biological control of invasive *P. crassipes* and host-specificity studies have shown that the moth causes considerable damage to both *P. crassipes* and *P. cordata* under high and low nutrient regimes (Stanley *et al*., 2007). The life cycle of *X. infusella* involves egg masses laid amongst the plants leaves and after a week of incubation at sub-tropical temperatures (25°C) the larvae hatch and burrow downwards into the laminae and petioles. Damage to the invasive plant is caused by extensive tunnelling of the larvae in the leaves, petioles and roots, and in some cases feeding may even be carried over to the next plant. After pupation, the pupa departs from premade exit windows in the petiole to repeat the cycle, which requires 64 days to complete. The study by Stanley *et al*., (2007) showed that *X. infusella* larvae had a greater impact on *P. cordata* compared to *P. crassipes* since *P. cordata* produced the lowest biomass, daughter plants and leaf production compared to *P. crassipes* after herbivory. Herbivory damage by *X. infusella* larvae also caused *P. cordata* to have the greatest herbivory response, whereby the growth of secondary shoots was stimulated, however these shoots were extremely low in biomass (Stanley *et al*., 2007).

These findings may be a promising lead for the prospect of a potential biological control agent for *P. cordata* in South Africa because very few Pontederiaceae species are present in the country such as the rare *M. Africana* However, *X. infusella* is originally a biological control agent for *P. crassipes* and may not be the best suited biological control agent for *P. cordata.* Performance studies showed that *X. infusella* had a better performance and produced 70% more progeny on *P. crassipes* than *P. cordata.* These findings also occurred in a controlled experimental environment and may not represent the true nature and preference of *X. infusella* in the field (Stanley *et al*., 2007). Other biological control agents that have already been released in South Africa on *P. crassipes* could potentially be viable agents for *P. cordata* because they are related, and thus future research could also include performance studies and assessment of the damage of existing biological control agents on *P. cordata*.

Other native herbivores that may be of interest during biological control surveys that cause damage to the leaves and petioles of indigenous *P. cordata* plants in United States of America can be found in a literature review by Harms and Grodowitz (2009)

where an informative table is compiled of insect herbivores (potential agents) and associated aquatic and wetland plants. Based on the findings of this thesis, surveys for biological control agents should prioritize insects that damage *P. cordata's* rhizomes because asexual reproduction via rhizomes is responsible for the spread of *P. cordata* in South Africa (Chapter 3).

4.5. Limitations and recommendations

It is imperative that no more introductory events of *P. cordata* into South Africa occur, since this would introduce gene flow and greater genetic diversity into the country. Invasive *P. cordata* populations in South Africa have low genetic diversity, especially within populations (Chapter 2) and thus these populations may produce a similar herbivory response to the biological control agent (Nissen *et al*., 1995; Ye *et al*., 2004; Canavan *et al*., 2007). Biological control agents are adapted to a specific plant species because they can overcome the specific plants' defences and effectively damage the plant (Brodeur, 2012). Because invasive *P. cordata* populations have low genetic variability there is chance that a biocontrol agent will produce similar results on all these populations. On a genomic level, low genetic diversity in a plant may result in restricted allelic diversity and limited genomic material from which the plant can use to respond to herbivory, thereby decreasing the variability of the plant's response to insect damage (Spielman *et al*., 2004; Barton et al., 2014). Decreased genetic diversity within and between populations of *P. cordata* therefore bodes well for future plant management using biological control programmes.

Introduction of more than one morph into South Africa (such as medium and long morphed plants) could have catastrophic consequences, since the incompatibility system currently present amongst short morphed flowers in invasive populations would be redundant (Chapter 3). Introduced *P. cordata* plants containing medium or long morphed flowers would be able to legitimately cross-pollinate flowers from the short-morphed populations to produce viable seeds. This would increase invasiveness and promoting sexual reproduction and spread between waterbodies, thereby exacerbating the spread of *P. cordata* in the country. Furthermore, the introduction of *P. cordata* plants would increase the genetic diversity of the current invasive populations, which could alleviate any genetic bottlenecks and founder effects previously caused by only a few introductory events (Chapter 2) and asexual reproduction. If sexual reproduction between invasive *P. cordata* plants occurred,

control programmes would also have to incorporate mechanisms to inhibit flower, fruit or seed production, which would require further research and expenses.

The use of ISSRs in this study (Chapter 2) provided appropriate inter- and intra-genomic diversity and successfully showed the genetic relationship between samples from native regions of United States of America and invasive populations throughout South Africa, as well as diversity within invasive populations. However, samples used in the genetic study were limited as they did not include all sites from the invaded range, albeit samples were used from every province where *P. cordata* has invaded. Parts of the native range such as Canada, countries in South America, and a few states in the USA were not included in this study and could match more closely to the genetic characteristics of invasive populations in South Africa which could have important implications for biological control programmes. Future studies should include genetic analyses of *P. cordata* populations in these areas that were not sampled in Chapter 2. It is also recommended that more sampling around Belle Haven, Virginia should be conducted because results from Chapter 2 indicated genetic similarity of invasive *P. cordata* populations to native populations from this area. Finding the source population of invasive *P. cordata* plants would greatly improve the prospects for biological control agents in a control programme because these native enemies may be adapted to the genotype that is invading, which can improve success rates (Paterson *et al*., 2009; Paterson & Zachariades, 2013). If desired, repeating this study with a wider sampling range and higher resolution techniques could be conducted using molecular techniques such as nuclear internal transcribed spacers (ITS) regions which could provide reliable confirmation and further insight into possible genetic relationships as well as the varieties of *P. cordata* populations invading South Africa (Le Roux *et al*., 2007; Canavan *et al*., 2017).

This study confirms that no seeds are produced from the short morphed flowers in invasive populations in South Africa due to an incompatibility system (Chapter 3), however it is recommended that pollen tube growth experiments should be conducted to determine how this phenomenon is occurring (Dulberger, 1964; Glover & Barret, 1985). Future studies could even incorporate legitimate cross-pollination experiments of the invasive short-morph flowers with medium and long morph *P. cordata* plants imported from the native range and grown under strict quarantine conditions to determine the extent of seed production from legitimate pollination. This could provide

possible insight into the unfortunate event that the medium or long morphed plants are accidentally introduced into South Africa in the future.

Currently, asexual reproduction via rhizomes is responsible for the rapid spread of *P. cordata* throughout the country. No research has yet been conducted on the invasive traits of these underground rootstocks, and thus performing rhizome experiments to test the dormancy period, survival rate after fragmentation or desiccation and performance under different nutrient regimes could provide valuable insight into the asexual reproductive characteristics of *P. cordata* that has enabled it to become such a successful invader in South Africa.

4.6. Conclusion

This study has provided novel insight into the invasion ecology of *P. cordata* in South Africa by providing novel information on the genetic, floral and pollination ecology of invasive plants. Control and management strategies should focus on controlling the asexual reproductive parts of *P. cordata* plants to curb the invasion in South Africa since targeting floral organs will be redundant due to absence of seed production. An integrated management approach is recommended to control *P. cordata* in South Africa, and it is recommended that biological control in combination with mechanical control may be the most sustainable, long term solution to tackling this species invasion. Uniformed short-morphed plants and low genetic diversity amongst invasive South African populations suggest that a well-developed control strategy may work extremely efficiently throughout invasive populations due to the possibility of a uniformed response to a biological control agent. The invasion of *P. cordata* in South Africa should be addressed from multiple approaches whereby management and control strategies should also be met with awareness programmes and prevention tactics to stop spread of *P. cordata* and further introductions intro the country, especially from the two other styled plants.

Pontederia cordata is introduced and/or invasive in other African countries such as Kenya, Tanzania, Malawi, Zambia and Uganda, and countries such as Australia and in Europe, including Switzerland (Henderson & Cilliers, 2002; Wittenberg, 2006; Stanley *et al*., 2007; Witt & Luke, 2017). This study may provide information on the invasion ecology of *P. cordata* that can be comparable to other invasive populations

throughout the world and may even help to provide insight for future management strategies elsewhere.

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