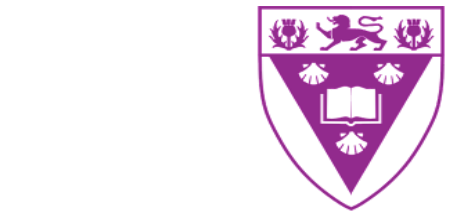


RHODES UNIVERSITY

Grahamstown • 6140 • South Africa

The Invasion Ecology of *Nymphaea mexicana* Zucc. (Mexican Water lily) in South Africa

THESIS

Submitted in fulfilment of the requirements for the degree of

MASTER OF SCIENCE

By

PRINAVIN NAIDU

Department of Botany

Rhodes University

P.O. Box 94

Grahamstown

6140

December 2018

Abstract

The Mexican water lily, *Nymphaea mexicana* Zuccarini, is an aquatic perennial, native to southern USA and Mexico, and has been introduced to South Africa via the ornamental plant trade. This species has rapid growth rates and becomes weedy in dams, ponds and rivers. It is currently listed as a NEM:BA category 1b invasive plant in South Africa. One possible management measure for this weed is biological control, but it is a novel target because no biological control programme has been initiated against it anywhere in the world. This study is intended as a baseline for the biological control programme against this plant in South Africa. Assessing the population structure and mode of reproduction of invasive alien plants is an imperative aid to determining if biological control is a suitable management option.

Using amplified fragment length polymorphism (AFLP) molecular markers, I compared the amount of genetic variability and differentiation of *N. mexicana* in its native range (USA), and invasive range (South Africa). Results indicated a large genetic distance between populations in the USA and South Africa, compared to populations within each country. The genetic variability of the invasive populations was higher than that found in the native distribution. This could be due to hybridization in the introduced range, and/or multiple introductions from different source populations. Differences in the morphology of *N. mexicana* plants in the invasive range and South Africa were also observed which confirm the results of the genetic analyses.

I also assessed the reproductive mode of *N. mexicana* cultivars/hybrids by conducting breeding system experiments and field pollinator studies. Results indicated that the cultivars are sterile, suggesting that the primary mode of reproduction is asexual via fragmentation of tubers. The main pollinators that were found to be associated with the cultivars in South Africa were honeybees, sweat bees, flies and beetles. These insect groups were the same as those that were observed in another study which was conducted on the pollinators associated with the pure *N. mexicana* in the native range in southern USA.

Mechanical and chemical control of *N. mexicana* and its multiple genotypes have been applied but have not been efficient due to the fast regeneration of shoots, especially in summer. Therefore, these two management options are not long–term solutions and will also be costly due to the widespread occurrence of the hybrids in South Africa. Thus the only cost–effective, environmentally friendly, self–sustainable and long–term management option is biological control. The significant divergence between native and invasive populations of *N. mexicana,* as well as the possibility of numerous invasive cultivars, may limit future prospects of

biological control of this species. However the differences in the root structures between native South African waterlilies, such as *N. lotus* and *N. nouchali*, and the introduced waterlilies, such as *N. mexicana* and its associated hybrids, may play a pivotal role in the success of biological control of the *N. mexicana* hybrid complex in South Africa. Natural enemies which feed on the hard tuberous roots of *N. mexicana* and its hybrids, as opposed to the soft bulbs of the native *N. nouchali* and *N. lotus*, should be prioritised.

Table of contents

List of figures

List of tables

“Education is the great engine of personal development. It is through education that the daughter of a peasant can become a doctor, that the son of a mine worker can become the head of the mine, that a child of farmworkers can become the president of a great nation. It is what we make out of what we have, not what we are given, that separates one person from another.”

“It always seems impossible until it’s done.”

–Nelson Rolihlahla Mandela

Acknowledgements

I would like to start off by thanking my supervisor Prof. Julie Coetzee for entrusting me with the *Nymphaea mexicana* project. I also want to sincerely acknowledge her patience and kindness whilst guiding me through this thesis. I owe sincere thanks to Prof. Martin Hill for allowing me to be a part of his team at the Centre for Biological Control at Rhodes University. It has been a dream of mine to obtain a degree from Rhodes University and both Julie and Martin have made this dream come true and for that I will forever hold them close to my heart. I am extremely thankful to Dr. Iain Paterson for coming on board and navigating me through rough waters. The molecular studies in this thesis would not be what it is without his guidance and for that I am forever grateful to him. Dr. Jaclyn Hill played a pivotal role in starting me off with this project and to her as well I owe sincere thanks. Dr. Rosie Mangan helped me a great deal with my molecular lab work and has passed down onto me a vast amount of molecular skills, which I hope to put to good use in the near future and I thus owe my sincere gratitude to her. I would also like to graciously thank Prof. Craig Peter from the Department of Botany at Rhodes University for assisting and guiding me through my breeding system experiments. An earnest thank you to Mrs. Jeanne van der Merwe from the Centre for Biological Control at Rhodes University for always going out of her way in assisting me with preparations for my field trips and purchase order forms for equipment.

I owe sincere thanks to my dear colleagues Dr. Sonia Nadege Kenfack – Voukeng and especially the soon to be Dr. Mavis Acheampong for continuous support throughout this thesis. Thank you to Mr. Phumudzo Ramabulana at the Westlake Conservation Centre in Cape Town and Mr. Nic Venter at Witwatersrand University in Johannesburg for sending me live plants for my breeding system experiments. A sincere thank you to Dr. Lesley Henderson for providing me with the distribution information for *N. mexicana* and its hybrids in South Africa. Many thanks are given to all of the people and organisations that had sent me dehydrated plant material for my molecular experiments. I would like to especially thank Dr. Julie Nachtrieb at the Lewisville Aquatic Ecosystem Research Facility in the USA as well as Dr. Michael Netherland from the US Army Engineer Research and Development Center and Mr. Dean Jones from the Center for Aquatic and Invasive Plants at the University of Florida for sending me dehydrated plant material for my molecular experiments. Thank you to the National Research Foundation and the Working for Water organisations for funding this study.

Last but not least I want to thank my parents and sisters' for their continuous love and support throughout my journey of becoming a scientist. To my dear grandparents the love, support and encouragement that you show to me is eternal and therefore this thesis is for you.

This Thesis Is Especially Dedicated To My

Grandparents

Mr Perumal Naidu and Mrs Mariamma Naidu

&

Mr Subba Reddy and Mrs Ellamma Reddy

Chapter 1: General introduction

1.1) Introduction

Biogeographic research is the key to understanding exotic plant invasions (Hierro et al., 2005). The current distribution of a species is linked to its past and present interactions with the biotic and abiotic elements in its environment (Kreft and Jets 2010). Species have been introduced and distributed between many regions around the globe since as far back as the 16th century (Crosby, 1986) and in recent years, increased international travel and trade, as well as globalisation, continue to contribute significantly to species introductions and distributions (Kolar and Lodge, 2000; Catford et al., 2009). Many of these introduced non–indigenous species go on to establish and spread in areas of the non–native environment, often becoming invasive (Kolar and Lodge, 2000; Catford et al., 2009).

1.1.1) Invasive Alien Plants (IAPs)

An Invasive Alien Plant (IAP) can be defined as a species which has been introduced, and subsequently spread, proliferated and established a population outside of its indigenous geographical range (Richardson et al., 2000). IAPs are regarded as one of the biggest threats to biodiversity due to their negative contribution to the loss of ecosystem functioning (Le Maitre et al., 2000; Robertson et al., 2003; Van Wilgen and de Lange, 2011). IAPs have significant economic impacts in many countries around the world. For instance in Australia, the estimated annual value of ecosystem services to manage invasive trees was ~783 million AUD (ZAR8.3 billion) for the year 2010 (de Lange and van Wilgen, 2010). In the British Isles, the damage caused by IAPs and the costs associated with their control range from £200 million (ZAR 3.7 billion) to £300 million (ZAR 5.6 billion) per annum (Williamson, 2002). Damage and control of IAPs in the USA were estimated at $33 billion per annum (ZAR 441 billion per annum) in the year 2002 (Pimentel et al., 2002).

In South Africa, the introduction of invasive alien plants began in the 1600s when European ships that were sailing to and from Indonesia, used the Cape of Good Hope as a refurbishing stop (Moran et al., 2005). This led to non–indigenous plants being introduced from all over the world, particularly from the Americas, Australia and Europe (Moran et al., 2005). Out of the estimated 8000 non–indigenous plant species that were introduced into South Africa, 379 have currently been declared as invasive plants (DEA, 2016). The impacts of IAPs to ecosystem functioning and biodiversity in South Africa has been more devastating

when compared to most other countries around the world which have also been affected by IAPs though the reasons for this are unclear (Moran et al., 2005, DEA, 2016). Ecosystem services in South Africa are estimated at a value of R152 billion per annum, of which R6.5 billion is allocated to the management and control of IAPs (de Lange and van Wilgen, 2010).

1.1.2) Aquatic IAPs in South Africa

Of all ecosystems in South Africa, the country's freshwater ecosystems are some of its most vulnerable (Coetzee et al., 2011b; Moorhouse and MacDonald, 2015). Factors such as urbanization, industrialization, agricultural waste, and increased sewage have contributed to the nutrient enrichment of freshwater ecosystems, and this in turn has led to an increased invasibility by alien aquatic plants in South Africa which take advantage of the eutrophic waters (Oberholster and Ashton 2008; Moran et al., 2013; Ray and Hill, 2013). Aquatic IAPs are associated with a cascade of negative effects on aquatic ecosystem functioning and the quality of life for humans that rely on the services provided by the ecosystem. They form dense mats on the water surface that alter the ecosystem by impeding waterways and preventing the use of boats for commercial fish harvesting and recreational activities (Mitchell, 1974; Howard and Harley, 1998). The mats prevent atmospheric oxygen from diffusing into the water and block out sunlight from reaching any submerged plant species which aid in oxygenating the water column (Mitchell, 1974; Howard and Harley, 1998). This in turn causes anoxic water conditions which subsequently impacts negatively on aquatic food webs and results in fish kill. The resulting decline in fish negatively affects the livelihoods of fishermen, as well as communities whose staple diet is fish (Seshavatharam, 1990; Howard and Harley, 1998). Furthermore, these mats negatively affect human well–being by housing breeding sites for disease vectoring mosquitoes (Mitchell, 1974; Cuda, 2014a).

Floating aquatic weeds form a significant group of aquatic invasive species present in South Africa and include: *Eichhornia crassipes* (C. Mart.) Solms (Pontederiaceae), *Pistia stratiotes* L. (Araceae), *Azolla filiculoides* Lam. (Salviniaceae), and *Salvinia molesta* D.S.Mitch. (Salviniaceae). These four floating aquatic weeds as well as the rooted aquatic weed *Myriophyllum aquaticum* (Vell.) Verdc (Haloragaceae), have long been referred to as the "Big Bad Five" water weeds of South Africa due to their negative impacts on freshwater biodiversity (Hill and Coetzee, 2017). *Eichhornia crassipes* is one of many IAPs which was introduced into the country through the ornamental and/or the aquarium plant trade (Martin and Coetzee, 2011). More recently, access to the internet has contributed significantly to the

global trade and sales of these IAPs, especially the aquatic IAPs and has led to subsequent invasions worldwide, including South Africa (Martin and Coetzee, 2011). Other aquatic IAPs introduced into the country via this route probably include: *Hydrocleys nymphoides* Buchenau (Alismataceae), *Camboba caroliniana* Gray (Cambombaceae), *Hydrilla verticillata* (L.f.) Royle (Hydrocharitaceae) and *Nymphaea mexicana* Zuccarinni. (Nymphaeaceae), the subject of this thesis (Martin and Coetzee, 2011, Hill and Coetzee, 2017).

The Mexican waterlily, *Nymphaea mexicana* is one of many non–indigenous aquatic plant species that have benefitted from the open, nutrient rich waters of South Africa after four of the "Big Bad Five" aquatic weeds have been brought under control (Coetzee et al., 2011b). The species has become problematic in South Africa and is currently listed as a NEM:BA category 1b invasive and therefore legally requires management (NEM:BA, 2004). Only manual and chemical control options are currently available for its management, but biological control using host specific natural enemies is vital for its long term control. To date, however no biological control programme has been implemented against this species anywhere in the world and little is known about the natural enemies associated with it. This study serves as a basis for the implementation of a management programme for *N. mexicana* in the future through the investigation of its invasion ecology, including population genetics and breeding system studies.

1.2) Managing aquatic IAPs in SA

Effective management of aquatic invasive plants in South Africa has historically comprised of four management options: mechanical control, chemical control, biological control and integrated pest management (IPM) (van Wilgen and de Lange, 2011). Each of these control methods is discussed below.

1.2.1) Mechanical control

Mechanical control involves the physical removal of weeds from a waterbody by manual cleaning, using water weed cutters and harvesters, dredging, mowing, netting and barriers such as floating booms (Robson, 1974; Sankaran, 1990; Lancar and Krake, 2002). In man–made systems, weeds can also be removed manually by eco–physiological alterations of the waterbody, such as the manipulation of water levels (Robson, 1974; Sankaran, 1990; Lancar and Krake, 2002). This method is especially effective for the control of submerged weeds in man–made water systems and involves removing water from the waterbody in order to expose the sediment which allows it to dry out, subsequently leading to submerged weeds

dying after which they can then be removed manually (Sankaran, 1990; Lancar and Krake, 2002). However, this method is limited to flowing water systems such as irrigation canals and drainage ditches and not suitable for natural water bodies (Lancar and Krake, 2002). This method may also encourage sexual reproduction and/or production of vegetative propagules; so prompt removal of the weeds and thorough drying of the sediment is required for this method to be effective (Sankaran, 1990).

In natural water systems, removing aquatic weeds by hand, or using machinery like waterweed cutters and harvesters are the most effective method of mechanical control (Robson, 1974; Sankaran, 1990; Lancar and Krake, 2002). Mechanical harvesting can control free–floating weeds such as *E. crassipes*, *P. stratiotes* and *A. filiculoides* (Laranjeira and Nadais, 2008; Hussner et al., 2017). Hand weeding is a more species – specific method and has been widely used for the eradication of new infestations and the reduction of large population of aquatic IAPs when mechanical harvesting has proven to be impossible (Bailey and Calhoun, 2008; Hussner et al., 2017). This method has been used to successfully eradicate early infestations of *Ludwigia grandiflora* (Michaux) Greuter and Burdet (Onagraceae) in the Euro–Mediterranean region (EPPO, 2011). However, limitations of this method are that it is labour and cost intensive and the likelihood of eradication can be limited by factors such as the reproductive capability of an aquatic plant and the skill of the hand – weeding technique used by the SCUBA diver for submerged aquatic plants (Clayton, 1996).

Suction dredging and water jetting (hydro–venturi) have also been used as a mechanical control measure for submerged aquatic weeds. Suction dredging is also carried out by a SCUBA diver and is a highly species – specific technique that has been used in North America to remove plants such as *Myriophyllum spicatum* L. (Haloragaceae) from the waterbody with the roots system, which limits the spread as well as regrowth of the plant (Boylen et al., 1996; Hussner et al; 2017). Hydro–venturi is a relatively new mechanical removal method and is conducted by 'washing' plants and their root systems out of the sediment using water jets. The plants then float to the surface and can be manually removed (Hussner et al., 2017). This method has been used to successfully control *C. caroliniana* and *Myriophyllum heterophyllum* Michx. (Haloragaceae) in parts of Europe (van Valkenburg, 2011). However, limitations of both the suction dredging and hydro–venturi techniques are that they work best in clay, sand, peat and soft organic sediments and can cause water turbidity (Hussner et al., 2017). A summary of the positive and negative effects of mechanical control is given in Table 1.1.

Table 1. 1: Positive and negative effects of mechanical control for aquatic weeds (Lancar and Krake, 2002; Haller, 2014).

Positive	Negative
1) Mechanical control is less harmful to the environment than chemical control. Public concerns regarding the use of herbicides are still widespread despite the extensive research that has been conducted on herbicides as well as the improved application, use and registration requirements enforced by regulatory authorities in certain countries including South Africa. Plants also do not decompose in the waterbody, so the oxygen content of the water is not affected. 2) Target and site specific– plants are only removed where the harvester operates. Whilst herbicides may move into neighbouring waterbodies through adjoining streams or water channels and cause non–target effects. 3) Immediate results– there is no waiting period for plants to decompose as they are immediately removed from the waterbody and the water can also be used immediately following treatment unlike with herbicides in which a waiting period exists between the period of application and the period it takes	1) Not always a cost–effective approach– demand for aquatic weed harvesters is limited, hence the equipment is often custom made and expensive. Other factors contributing to cost include: size of the area requiring removal, size of the harvester, distance to the disposal site and the density of the weeds being harvested. 2) Not all rivers, dams and ponds are suitable for mechanical harvesting. Harvesters require access into the waterbody and if the banks of the dam or pond are too high the harvester may not be able to get into the waterbody. 3) Aquatic weeds may regrow from rootstock– harvesters do not remove submersed plants all the way down to the rootstock. Similar in application to the mowing of a lawn, the roots of the clipped plants remain intact in the sediment and regrowth may begin soon after the harvesting. 4) Thus not always a useful long–term management strategy, due to weeds fast regeneration rates and ability to

for the herbicide to kill the plant and the subsequent utilization of water from that system. 4) Removed weeds can be utilized as feed, manure, energy source, etc. 5) Mechanical control can be implemented in any localised waterbody.	propagate from fragments left behind in the waterbody 5) Harvesters are not selective and in addition to removing water weeds also remove native vegetation as well as aquatic organisms such as fish, crayfish, frogs etc. 6) Lack of ownership of waterbodies and approval from designated authorities create problems in implementing control.

1.2.2) Chemical control

Chemical control of aquatic weeds uses registered herbicides and algaecides (Lancar and Krake, 2002; Netherland, 2014; Hussner et al., 2017), and is sometimes easier and more cost effective than mechanical control depending on the scale of the operation (Lancar and Krake, 2002; Netherland, 2014). There are, however, limitations when it comes to chemical control; 1) there are specifications on the herbicides suited to different aquatic environments, 2) the herbicide should have a high degree of phytotoxicity in order to kill the weed rapidly, and 3) after application, the herbicide must dissipate or degrade immediately from the waterbody (Lancar and Krake, 2002).

Herbicides may be contact or systemic and either broad spectrum (non–selective) in which numerous plants species are targeted, or host–specific (selective), in which only the target weed is killed (Netherland, 2014). Contact herbicides work fast and in most cases, kill the weed rapidly, whereas systemic herbicides are slower, killing over days or weeks (Netherland, 2014). Contact herbicides are most effective for the control of free–floating plants, whereas systemic herbicides are translocated within the plant to the roots and rhizomes, eventually causing death of the weed and thus most useful for the control of emergent weeds which are rooted in the substrate (Netherland, 2014). The most commonly used herbicides usually include active ingredients such as dimethyl amine salt (2,4–D), copper, diquat, carfentrazone, flumioxazin, endothall, fluridone and glyphosate (Sankaran,

1990; Netherland, 2014; Hussner et al., 2017). Although most of these herbicides are licensed and deemed safe to use in aquatic ecosystems in USA, there are none registered for use in Europe and only a few registered for use in South Africa (Netherland, 2014; Hussner et al., 2017). The misuse of herbicides could be detrimental to the aquatic life present in that ecosystem (Netherland, 2014; Hussner et al., 2017). Overreliance on chemical control has also proved to have its shortfalls. Herbicides used to control a specific weed usually have a single mode of action and repeated use of the same herbicide can eventually lead to the weed becoming resistant to the treatment (Netherland, 2014; Hussner et al., 2017). For example, at the lakes in Kissimmee in Florida, *H. verticillata* has become resistant to the herbicide fluridone (Netherland, 2014; Netherland and Jones, 2015; Hussner et al., 2017) and the hybrid between *M. spicatum* and *Myriophyllum sibiricum* Kom. (Haloragaceae) from lakes in the Menominee River watershed in Michigan's Upper Peninsula in USA, has also been reported to be resistant to 2,4–D (LaRue et al., 2013). In such cases, biological control is the best option for control or eradication of an invasive plant, particularly if chemical and mechanical control have failed or are financially and/or logistically unsustainable (Van Wilgen et al., 2013).

1.2.3) Weed Biological Control

Weed biological control involves the collection and identification of host–specific natural enemies from a weed's country of origin. These agents are subsequently released onto the weed in the introduced range after a series of host specificity tests (Moran et al., 2013; Van Wilgen et al., 2013). When a non–indigenous plant species becomes invasive, it may be due to a lack of natural enemies such as pathogens and/or herbivores, a phenomenon referred to as enemy release (Williams, 1954; Colautti et al., 2004). Biological control introduces these natural enemies into the non–native environment and/or inundates small existing natural enemy populations in the non–native environment. In addition, these natural enemies of the weed, the majority of which are insects and in some instances mites or pathogens, are then put through a series of host–specificity tests (Moran et al., 2013; Van Wilgen et al., 2013). The outcome of these highly conservative tests will establish if the biological control agent is suitable for release in the country where the alien plants are invasive and problematic (Van Wilgen et al., 2013).

The different types of biological control include; classical, augmentative, conservation (Batra, 1981) and broad–spectrum biological control (Briese, 2000). Classical biological control stems from the enemy release hypothesis (ERH) and involves the use of

introduced host–specific agents (insects, mites, and/or plant pathogenic microbes) that reduce the fitness, fecundity and subsequent vigour, of the problem weed (Gopal et al., 1990; Keane and Crawley, 2002; Van Wilgen and de Lange, 2011). Augmentative biological control includes the mass propagation and periodic release of exotic or native natural enemies onto a target weed (Batra, 1981; Briese, 2000), whilst conservation biological control is where existing natural enemy populations are managed, enhanced and conserved in order to maintain low host weed populations (Batra, 1981; Briese, 2000). Finally, broad spectrum biological control is the management of a range of unwanted vegetation using polyphagous herbivores (Briese, 2000).

Biological control is a much more cost–effective method than mechanical and chemical control (Van Wilgen et al., 2013). It is also a more environmentally friendly, self–sustaining method and there are no non–target effects and chemicals involved (Gassmann et al., 2006). The biological control agents disrupt the competitive balance between the native and invasive plants (in favour of native plant species) by feeding on and ultimately reducing the invasive potential of the plant species (Van et al., 1998). However, for certain IAPs like *E. crassipes*, biological control alone is not enough to effectively manage the species (van Wyk and van Wilgen, 2002; Jones, 2009). In these cases, an integration of two or more control methods is often needed in order to achieve long–term management (Cardina et al., 1999).

1.2.4) Integrated Pest Management (IPM)

Integrated Pest Management (IPM) is rooted in the concept of integrated control and was initially developed as a means to reduce the use of insecticides and later herbicides (Thill et al., 1991; Cardina et al., 1999; Buhler et al., 2000, Buhler, 2002). IPM can simply be defined as a control method that integrates mechanical, chemical and biological control to provide persistent pest control (Thill et al., 1991). IPM has been an effective method for the control of insect pests and is a relatively new approach when it comes to weeds (Cardina et al., 1999; Buhler et al., 2000). IPM has seen good return on investment and has thus proved to be cost efficient when compared to a single management method (Thill et al., 1991; Cardina et al., 1999; Buhler et al., 2000), as in the case of *Euphorbia esula* L. (Euphorbiaceae) (leafy spurge), a noxious weed which affected grazing lands in the U.S.A. and Canada in the mid–1920s (Lym, 2005). Around 1970, biological control agents were released to control populations of *E. esula*, and soon after, an integrated approach was

adapted, using herbicides in addition to the agents and long – term management of the species was achieved (Lym, 2005).

South Africa has also seen good return on investment with IPM, integrating biological control and chemical control for the management of water hyacinth on the Nseleni and Mposa rivers in KwaZulu– Natal, which proved to be more cost effective compared to chemical control alone on water hyacinth in Hartbeespoort Dam in Pretoria, and biological control alone at New Year's Dam in the Eastern Cape (van Wyk and van Wilgen, 2002). In the Nseleni river, where IPM was implemented, the return on investment was US$39/ha (ZAR 552/ ha) whilst at New Year's Dam, it was US$44/ha (ZAR 623/ ha), and at Hartbeesport Dam US$209/ha (ZAR 2960/ ha) (van Wyk and van Wilgen, 2002).

1.3) Weed biological control practices in South Africa

The first weed biological control programme in South Africa was initiated against *Opuntia monacantha* Haw. (Cactaceae) in 1913, on which the biological control agent *Dactylopius ceylonicus* Green (Hemiptera: Dactylopidae) was released (Moran et al., 2013; Winston et al., 2014). The successful control of *O. monocantha* encouraged the use of weed biocontrol and in 1933, the first research–based biological control agent, *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae) was released against *Opuntia ficus–indica* (L.) Mill. (Cactaceae) (Klein et al., 2011; Moran et al., 2011). Despite the successful management of *Opuntia* species, weed biocontrol methods only began to increase in popularity in South Africa in the early 1960s (Zimmermann et al., 2004), when it was implemented as a management tool for IAPs such as Australian *Acacia* and *Hakea* species which were introduced into South Africa during the mid to late 1800s and spread widely in the Cape fynbos region and other natural habitats around the country (Zimmermann et al., 2004; Moran et al., 2013).

In 1995, the Working for Water (WfW) programme was initiated by the Department of Water Affairs and Forestry, and formed a part of the Expanded Public Works Programme (Richardson and Van Wilgen, 2004; Zimmerman et al., 2004; Marais and Wannenburgh, 2008). The programme was established to manage IAPs that threatened South African water resources and represents a major investment in the sustainability of the country's natural and semi–natural ecosystems (Richardson et al., 2000; Richardson and Van Wilgen, 2004, Marais and Wannenburgh, 2008). WfW first targeted woody alien plant species, originally a labour intensive enterprise, and included an additional goal of serving as a public works programme

to create job opportunities (Macdonald, 2004). The WfW programme which also included the control of major aquatic weeds such as *E. crassipes*, *P. stratiotes*, *A. filiculoides*, *S. molesta* and *M. aquaticum* has since grown immensely (Coetzee et al., 2011b; Moran et al., 2013). The programme has subsequently seen numerous successes with weed biological control and has reduced the high economic demands of controlling well established, problematic aquatic weeds (Zimmermann et al., 2004). The implementation of biological control on terrestrial and aquatic IAPs in SA saved the economy an estimated ZAR 41.7 billion for the year 2010 (de Lange and van Wilgen, 2010).

1.3.1) Pre–emptive biological control in South Africa

Unfortunately, with the successful control of some floating aquatic weed species, a new suite of aquatic IAPs have made use of the newly accessible resources and have established substantial populations (Charudattan, 2001; Scheffer et al., 2003; Coetzee et al., 2011a; Moran et al., 2013; Bownes, 2014). Submerged IAPs such as *Egeria densa* Planch. (Hydrocharitaceae), *H. verticillata*, *M. spicatum*, as well as rooted floating aquatic plants such as *Nymphoides peltata* (S.G. Gemel) Kuntze (Menyanthaceae), *Hydrocleys nymphoides* (Humb and Bonpl. Ex. Willd.) Buchenau (Alismataceae), and *N. mexicana* have begun invading waters in which major floating aquatic weeds have now been controlled (Coetzee et al., 2011b; Hill and Coetzee, 2017). Thus, there has been a shift in aquatic weed research to include research on new potential invaders, by developing biological control programmes to manage IAPs prior to them becoming problematic (Coetzee et al., 2011a; Olckers, 2004; Hill and Coetzee, 2017). This not only reduces the risk to native environments but it also reduces the economic costs of dealing with an invasive species that has become fully established (Simberloff, 2003b; Olckers, 2004). Limited budgets and a predominant focus on controlling well–established invaders rather than potential ones, means that pre–emptive control is not yet a global practice (Simberloff, 2003a; Olckers, 2004; Hill and Coetzee, 2017). South Africa is at the forefront of biological control, both in terms of management, but also pre–emptive control, and a substantial amount of current research is geared towards newly identified potentially problematic aquatic invaders, including submerged aquatic IAPs (Hill and Coetzee 2017; Zachariades et al. 2017).

Feasibility studies of new invasive targets provide insight into the extent of invasion and whether or not the initiation of a biological control programme as a means of managing the invasion will be worthwhile (Hill and Coetzee, 2017). Feasibility studies on species such as *E. densa*, *Sagittaria platyphylla* (Engelm.) J.G. Sm (Alismataceae) and *Iris pseudacoris* L.

(Iridaceae), have recently been completed and these non–native aquatic plants were found to be a serious threat to aquatic ecosystems in South Africa and hence biological control programmes have been initiated against them (Hill and Coetzee, 2017). Currently, feasibility studies are being conducted on new invasive targets such as *Pontederia cordata* L. (Pontederiaceae), *Nasturtium officinale* W.T. Aiton. (Brassicaceae), *N. peltata, H. nymphoides, Limnobium laevigatum* (Humb and Bonpl. Ex. Willd.) Heine. (Hydrocharitaceae), *Elodea canadensis* Michx. (Hydrocharitaceae), as well as *N. mexicana.*

1.4) *Nymphaea mexicana* (Mexican water lily)

The subject of this study, the Mexican water lily or yellow water lily, *Nymphaea mexicana* (Zuccarini) is native to Mexico and southern parts of the United States of America. The species has numerous synonyms: *Castalia flava* (Leitn. ex A.Gray) Greene, *Castalia mexicana* (Zucc.) J.M.Coult, *Leuconymphaea flava* (Leitn. ex A.Gray) Kuntze, *Leuconymphaea mexicana* (Zucc.) Kuntze, *Nymphaea flava* Leitn. ex A.Gray, *Nymphaea lutea* Treat, *Nymphaea planchonii* Casp. Ex Conard, (GBIF, 2018). It was first introduced into South Africa through the ornamental plant trade, and has since established populations in dams, ponds and rivers around the country. Due to its rapid growth, *N. mexicana* can become weedy at an alarming rate (Capperino and Schneider, 1985), and this in turn negatively affects aquatic ecosystem functioning and biodiversity.

1.4.1) Description

The flowers of *N. mexicana* are yellow (Capperino and Schneider, 1985). Flower petals are either elliptic or sagittate in shape, often with cultivars (a plant variety produced in cultivation by selective breeding) possessing the former, and true *N. mexicana,* the latter (Henderson and Cilliers, 2002). Curtiss (1902), was the first to report two different leaf forms in *N. mexicana*; the common elliptic leaf form, and a less common sagittate leaf form (Fig. 1.1 C and D). Leaves measure roughly between 10– 20 cm in diameter with the upper surface of the blades having a bright green colour with blotches of red that are most prevalent in recently emergent leaves (Henderson and Cilliers; Ricketson, 1995). The under surface of the leaf is a deep red colour or a green colour with reddish blotches, with the primary veins on the underside of the leaf being barely visible (Ricketson, 1995; Henderson and Cilliers, 2002; Cook, 2004). Leaves can become emergent, particularly in shallow water bodies (Cook, 2004). The petioles are thick and fleshy, about 1.5 cm in width and an average length of about 83 cm. The Mexican water lily has the largest seeds of the *Nymphaea* subgenera found

in Mexico, with seeds of roughly 4– 6 mm in length (Figure 1.1 (G iii)) (Bonilla–Barbosa, et al 2000; Cook, 2004). Seed shape and colour are used to taxonomically differentiate between *Nymphaea* subgenera in their native range, which are uniformly covered by large trichomes (Capperino and Schneider, 1985; Bonilla–Barbosa, et al 2000). Young plants may give rise to clones by producing a thick stolon (Fig. 1.1 G(i)), or through fragmentation of banana–like tubers with fleshy rhizomes (Fig. 1.1 A(iii) and F). Rhizomes are erect, usually tuberous, sometimes may be elongate up to 30 cm long and 5 cm thick (Cook 2004). In winter, the specialized roots resemble small bananas that are around 2.5 – 5 cm in length (Cook, 2004).

1.4.2) Reproduction

The sexual reproductive biology of *N. mexicana* is not as well reported as for other *Nymphaea* species, except for a study conducted on the species' reproductive biology by Capperino and Schneider (1985), who found that the process of anthesis in *N. mexicana* is diurnal; with first day flowers functioning as female and second day flowers as male (Capperino and Schneider, 1985, Schneider and Chaney, 1981). Asexual reproduction occurs when the stolons of *N. mexicana* become geotropic around autumn (Ricketson, 1995). The stolon tips become buried in the mud and form a type of brood body (Ricketson, 1995). Young plants may also give rise to clones by producing a thick stolon from which the daughter plant arises. The thick fleshy tubers can also fragment giving rise to clones (Ricketson, 1995).

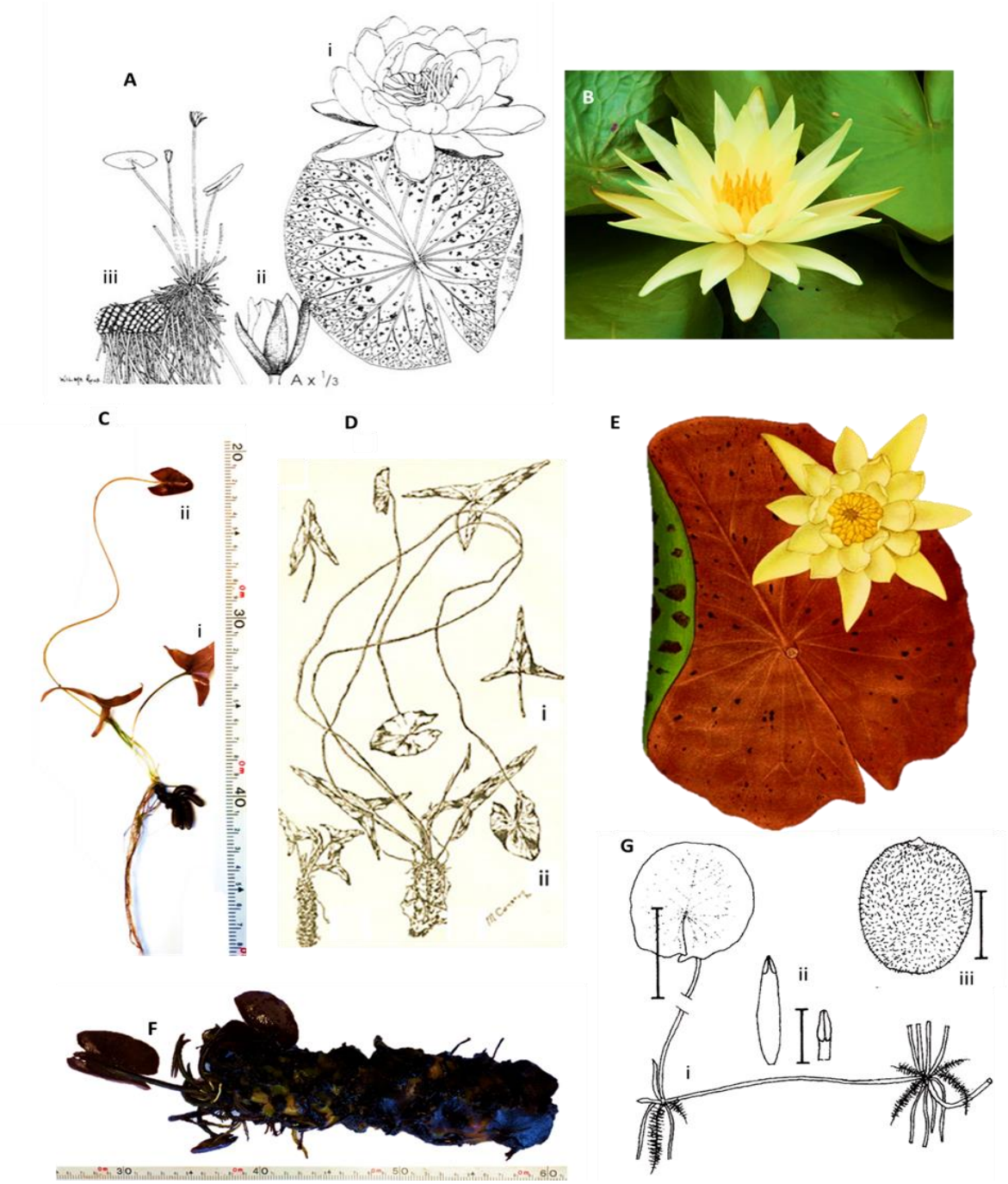


Figure 1. 1: Line diagrams of *Nymphaea mexicana*, depicting two different leaf and petal shapes. (A): (i) Opened flower with elliptic petals and leaf with dark blotches, possibly a hybrid of the pure form (ii) flower bud with sepals; (B) and (E): Opened flowers with sagittate petals, probably the pure form, with (E) depicting characteristic red/purple colouration on underside of leaf; (C) and (D): *N. mexicana* plants displaying 2 leaf forms, (i) sagittate leaf, (ii) elliptic leaf; (F) and (A) (iii): large, thick banana–like tuber with few fleshy rhizomes and newly formed leaves; (G): (i) stolon joining two clonal plants, (ii) outermost and innermost stamens, (iii) seed once the appendage has been shed. (Line drawing (A)

obtained from (Henderson and Cilliers, 2002). Line drawing (D) obtained from (Curtiss, 1902). Diagram (E) obtained from (Conard, 1905). Line drawing (G) obtained from (Cook, 2004). Photograph sources: (B), Anthony King, Modimolle, Limpopo 2011, (C) and (F), Prinavin Naidu, Plettenberg, Kysna, Western Cape 2017).

1.4.3) Distribution

1.4.3.1) Global

The Global Biodiversity Information Facility (GBIF) is an open data research structure funded by governments around the world (GBIF, 2018). The database provides data on the distributions of species worldwide (GBIF, 2018). According to the GBIF (2018), the native distribution of *N. mexicana* is concentrated around the southern parts of the USA and Mexico (Fig. 1.2). Other invaded regions of non–native countries include: Spain, Australia and New Zealand (Fig. 1.2). According to the GBIF database, non– native countries in which *N. mexicana* occurs, but is not widely distributed are Brazil, Sweden and South Africa (GBIF, 2018). Specimens were collected, preserved and recorded in 1975 and 1981 at the same site in São Paulo, Brazil. The GBIF database does not provide a year in which a live specimen was collected in Stockholm, Sweden. GBIF data on the South African distribution of *N. mexicana* only provides records for two localities, one in KwaZulu – Natal recorded in 1998, and another in Modimolle, Limpopo recorded in 2011 (Fig. 1.2). However, as of 2018 from conducted field surveys the species currently occupies a wider distribution in the country than the one recorded on GBIF.

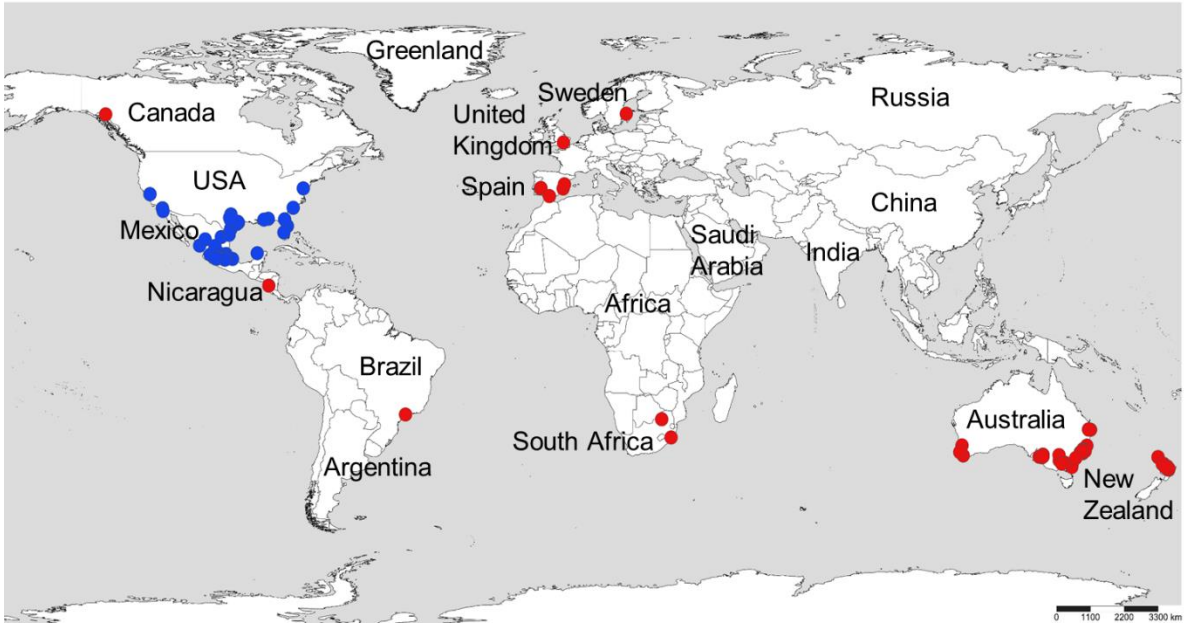


Figure 1. 2: Current worldwide distribution of *Nymphaea mexicana* according to GBIF (2018). Blue dots (●) represent the native range and red dots (●) represent the introduced range. Map created on www.SimpleMappr.com.

1.4.3.2) South Africa

The distribution data of invasive alien plants in South Africa can be found in the Southern African Plant Invaders Atlas (SAPIA) on the Botanical Database of Southern Africa (BODATSA) online website which is administered by South African National Biodiversity Institute (SANBI) (Henderson and Wilson, 2017). BODATSA provides information on the time of introduction, distribution, favoured habitats, and the abundance of more than 500 species of IAPs (Henderson and Wilson, 2017). According to the database, *N. mexicana* in South Africa is mainly distributed around the Western Cape and Gauteng provinces (Fig. 1.3). Wetlands in the Westlake area of Cape Town have a dense infestation of *N. mexicana* amongst other invasive aquatic weeds such as *S. molesta*, *M. aquaticum*, *P. stratiotes* and *A. filiculoides*. In Gauteng, the Vaal River has the highest infestation of Mexican water lily compared to other sites in that region. The Eastern Cape has infestations at the Boardwalk casino and hotel as well as the Kings Beach skate park in Port Elizabeth and an infestation at Radway Green B'nB near Grahamstown. In Limpopo, there are infestations at dams in Modimolle. On the SAPIA database, there has only been one reported sighting of *N. mexicana* in KwaZulu– Natal, at Umhlanga Rocks in 1998 by Carina. J. Cilliers, but upon inspection of the site in 2017, the species was no longer present (pers. obs.). However, photographs taken at Zimbali eco estate in KwaZulu– Natal in 2018 showed the presence of the species. Although most of these records resemble *N. mexicana*, there seemed to be differences in the colour of the flowers as well as the shape of the leaves and the amount of blotching or speckling on the leaves. These differences could be significant in that some of these records may not be *N. mexicana* but hybrids of the species.

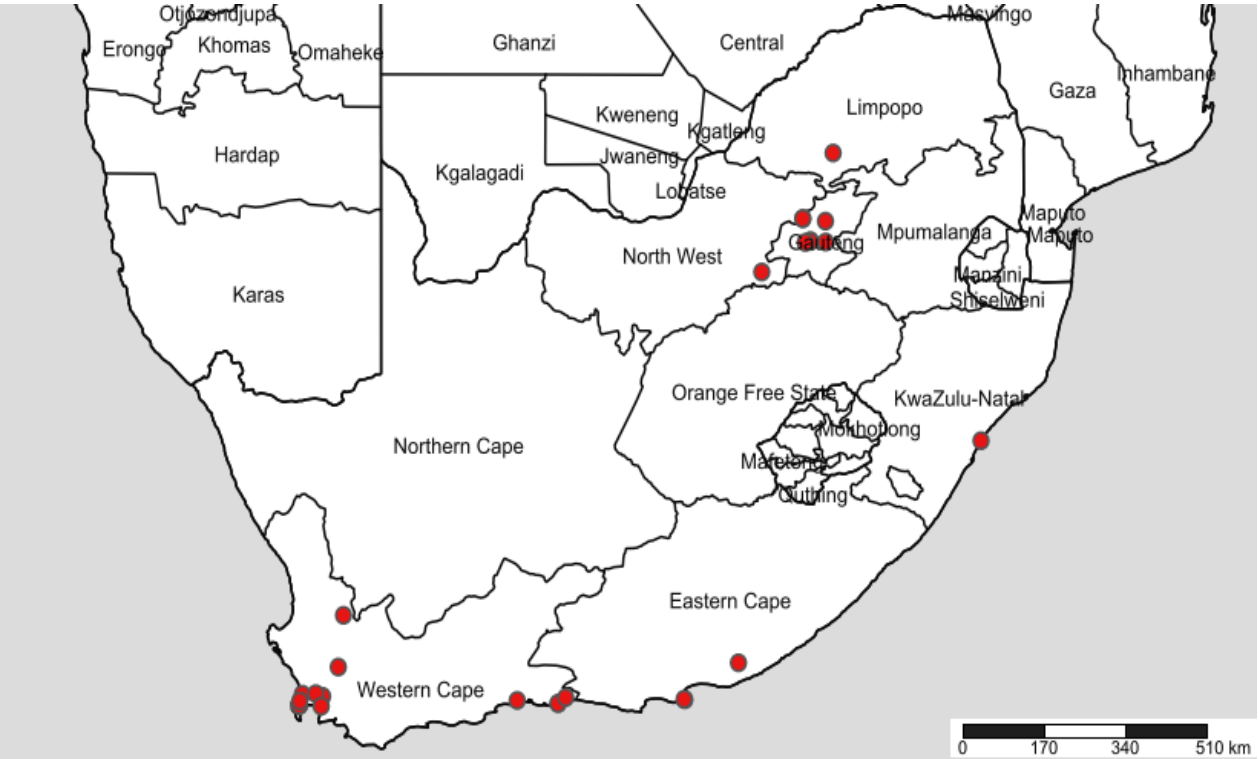


Figure 1. 3: Current distribution of *Nymphaea mexicana* in South Africa from field surveys conducted in 2016–2018. Map created on www.SimpleMappr.com.

1.4.4) *Nymphaea mexicana* hybrids

Natural hybrids of *N. mexicana* with *Nymphaea odorata* Aiton. (Nymphaeaceae) (American white water lily) have been reported in the wild in the southern U.S.A., where the native distributions of these two species overlap (Wiersema and Hellquist, 1997; Woods, 2003). Artificially created hybrids of *N. mexicana*, referred to as cultivars, are also very common in the horticultural trade (Table 1.2). The first reported artificial hybrid of *N. mexicana* according to records on www.victoria–adventure.org was an artificial cross between *N. mexicana* and *N. pygmaea* (Salisb.) W.T. Aiton (white dwarf water lily) (Nymphaeaceae) yielding *N. 'pygmaea helvola'* (Table 1.2). Other common artificial crosses of *N. mexicana* have been with *Nymphaea alba* L. (European white water lily) (Nymphaeaceae) and *Nymphaea rubra* Roxb. ex Andrews (Red water lily) (Nymphaeaceae).

Most of the artificially created *N. mexicana* hybrids have leaves with the characteristic reddish blotching found in the pure *N. mexicana*, but the degree of blotching may differ between cultivars. The flowers of *N. alba* var. *rubra* crossed with *N. mexicana* almost always appear to have a pink to red colouration and this is due to the red hues present

in *N. rubra*. This can be expected since the flowers of *N. rubra* have a deep pink to red colouration. The *N. odorata* x *N. mexicana* cross on the other hand seems to produce white flowers that sometimes have a slight pink hue, whereas the *N. alba* x *N. mexicana* cross produces flowers that are cream to pale yellow in colour. In recent years, creating water lily hybrids have become a hobby for many water lily enthusiasts. There have been numerous books published on the history of artificially created waterlilies, as well as how to create them (Perry, 1989; Slocum et al., 1996; Sacher, 2005; Slocum, 2005; Holmes, 2015). The names of these hybrids can even be registered on some sites such as the International Water lily and Water Gardening Society (IWGS) (www.iwgs.org). According to www.victoria–adventure.org, the only *N. mexicana* hybrid that is currently registered on their website is "Moon Dance," which is a cross between *N. odorata* var. *gigantea* and *N. mexicana* (Table 1.2). The IWGS also has its own journal that publishes newly created water lily hybrids as well as articles on hybridization of waterlilies. Given the ornamental value of waterlilies, they are not regarded as a pest species to many and thus management of these species and cultivars in areas of introduction have taken a backseat.

Table 1. 2: A list of recorded *Nymphaea mexicana* hybrids in the horticultural trade (adapted from http://www.victoria–adventure.org/waterlilies/names/i.htm).

Cultivar name	**Date of origin**	**Parentage**	**Description**	**Picture**
Nymphaea mexicana	1832 Zucc.	Original	Flowers are lemon to bright yellow in colour, with the petals being pointed at the tips. The cup bright yellow. The leaf pads are green with most having bright red blotches/ mottling. The underside of the leaf is also bright red in colour.	

Cape Canaveral	1959	Unknown parentage	A larger variety of the original *N. mexicana* possibly due to a mutation. Flowers are lemon yellow with a yellow cup. Leaf pads are green, it is unknown if purple blotches or speckles are present. In the picture, *N. mexicana* is on the left and *N. mexicana* var. *cape canaveral* is on the right.	
Andreana	1895	*N. alba var. rubra x N. mexicana*	Flowers are brick red in colour, with a shaded yellow cup. Flower petals are shorter than the pure *N. mexicana*. Leaf pads are green with large red blotches.	
Arc en ciel	<1900	*N. odorata var. rosea x N. mexicana*?	Flowers are white in colour with hues of light pink. Petals are long, narrow and pointed at the tips similar to the pure *N. mexicana*. Bright yellow cup present. Leaf pads are green with red blotches.	
Arethusa	1902	*N. alba var. rubra x N. mexicana*?	Flower is crimson in colour and globed shaped in appearance. Petals are short and broad, but pointed at the tips. Leaf pads are green with red blotches.	

Aurora	1895	*N. alba rubra x N. mexicana*	Flowers are peach in colour, with hues of rosy pink and a yellow cup. Petals are short and broad contributing to a globular flower shape. Leaf pads are green with red blotches	
Berit strawn	1993	*N. rembrandt x N. mexicana*	Flowers are small, pinkish–peach in colouration with a yellow cup. The petals are short, broad and rounded at at the tips. Leaf pads are green and have large red blotches.	
Colorado	1994	*N. louise villemarette x N. mexicana*	Flowers are pink or peach in colour with a yellow cup. Flower shape resembles that of the pure *N .mexicana*, with petals pointed at the tips. Leaf pads are green with no visible red blotches.	
Florida sunset	1995	*N. perry's fire opal x N. cape canaveral*	Flowers are cream to amber in colour with pinkish hues and a yellow cup. Petals are numerous, short, broad and pointed at the tips. Green leaf pads have red blotches.	

Fulva	1894	*N. alba rubra x N. mexicana*	Flower is deep pink to red in colour with a yellow cup. Petals are short, broad and pointed at the tips. Green leaf pads have large red blotches.	
Marliacea chromatella	1880	*N. alba x N. mexicana?* Or *N. tuberosa x N. mexicana?*	Flowers are pale yellow with a brighter yellow cup. Petals are short, broad and rounded at the tips. Leaf pads are green with large red blotches.	
Moon dance	1996	*N. odorata var. gigantea x N. mexicana*	Flowers are white in colour with a yellow cup. Petals are long, narrow and pointed at the tips. Leaf pads are green and have red blotches.	
Moorei	1885	*N. alba x N. mexicana*	Flowers are yellowish cream in colour with a bright yellow cup. Petals are short, broad and give the flower a star – shaped appearance. Leaf pads are green and have red blothes.	

Pygmaea helvola	1879	*N. pygmaea x N. mexicana*	Flowers are canary – yellow in colour with a bright yellow cup. Petals are short, broad and pointed at the tips. Flowers are smaller in size compared to the other *N. mexicana* varieties. The leaves are green and have red blotches on the upper surface and brown blotches underneath.	
Seignoureti	1893	*N. alba rubra x N. mexicana*	Flowers are pink in colour with a bright yellow cup. Petals are long, broad and pointed at the tips. The Leaves have red blotches on the surface and are streaked with red–brown colouration underneath.	
Siam full moon	2006	*N. james brydon x N. mexicana*	Flowers are lemon yellow in colour with a yellow cup. Many more petals than the other *N. mexicana* varieties which are short, broad and pointed at the tips. Green leaves with few red blotches.	
Yellow sensation	1991	*N. alba x N. mexicana*	Flowers are a rich yellowish cream colour with a bright yellow cup. Petals are numerous as in the above variety but longer.	

			Leaf pads are green in colour with few red blotches.	

1.4.5) Current management in the most affected countries

Nymphaea mexicana is a significant aquatic weed in Australia, New Zealand, Spain and South Africa. In order to determine how best to manage this invasive species in South Africa, it is important to consolidate and reflect on the control methods (mechanical and chemical) that have been attempted in the past in other affected countries. I will thus reflect on current management practices being carried out in Australia against this weed and compare them to the management practices conducted in South Africa. There is no published data on the management of *N. mexicana* in Spain or New Zealand.

In Australia, the rural water corporation Goulburn–Murray Water (GM–W) manages invasive *N. mexicana* populations by chemical control, no mechanical control has been reported (Dugdale 2012; Dugdale et al., 2013; Hofstra et al., 2013). In 1996, the Goulburn Weir near Nagambie, Victoria in Australia had a massive infestation of *N. mexicana* covering around 200 hectares (GM–W, 2009). GM–W used the registered aquatic herbicide glyphosate (Roundup Biactive) and reduced the infestation to just 30 hectares between 1996 and 2003 (Finlay et al., 2008; GM–W, 2009). However, water quality and aquatic life in the Weir began to decline following the chemical control of the Mexican water lily (Dugdale, 2012), because when glyphosate is sprayed on the emergent leaves, the leaves die, sink and eventually decay, a process in which dissolved oxygen is consumed, resulting in low dissolved oxygen in the waterbody and subsequent fish kills, as well as the alteration of overall water quality (Finlay et al., 2008; GM–W, 2009; Dugdale 2012; Dugdale et al., 2013; Hofstra et al., 2013). This prompted the suspension of the programme in 2004, however three years later, the infestation became problematic once more (GM–W, 2009, Dugdale, 2012; Dugdale et al., 2013). In 2004 when an environmental company, Water Eco Science, conducted a study at the Weir on the effects of glyphosate on water quality, they found no direct impact (Francis and Crapper 2004). A study that tested the rate of dieback of *N. mexicana* leaves and the subsequent oxygen depletion potential found that dissolved oxygen levels were very low under areas in which *N. mexicana* was not sprayed with glyphosate, especially during warm months (Hofstra et al., 2013).

Recently established *N. mexicana* populations in South Africa are currently subjected to both chemical and mechanical control (Phumudzo Ramabulana, Westlake Conservation Centre; pers.comm.). Populations in Emmarentia, Gauteng (26°08'46.3"S 28°00'12.8"E) are currently being controlled by manual removal, with a company contracted to remove the leaves covering the water surface by hand–pulling (Lindelani Nwedo, JHB City Parks; pers.comm.). At Westlake in Cape Town (34°04'54.9"S 18°27'23.4"E), the plants were originally controlled by glyphosate application (Gaertner et al., 2016), as in Australia, but due to public outcry regarding the presumed negative effects of glyphosate on human health, the Westlake conservation unit has now resorted to manual removal of the plants (Phumudzo Ramabulana, Westlake Conservation Centre; pers.comm.). To date, there is no registered herbicide for use on *N. mexicana* in South Africa and mechanical control of the species is labour intensive and costly because the leaves die back in winter, which frees up the infested waterbody temporarily, but as soon as the temperature rises in spring, the tuberous rootstocks sprout new leaves (Fig. 1.4). There is no published data on the cost to control *N. mexicana* in South Africa, but similar to the control of other aquatic invasive species in SA, biological control offers a more environmentally friendly, cost effective, self–sustaining method of control. Therefore, the prospects for biological control need to be examined for this species, since no biological control programme exists for *N. mexicana* worldwide.

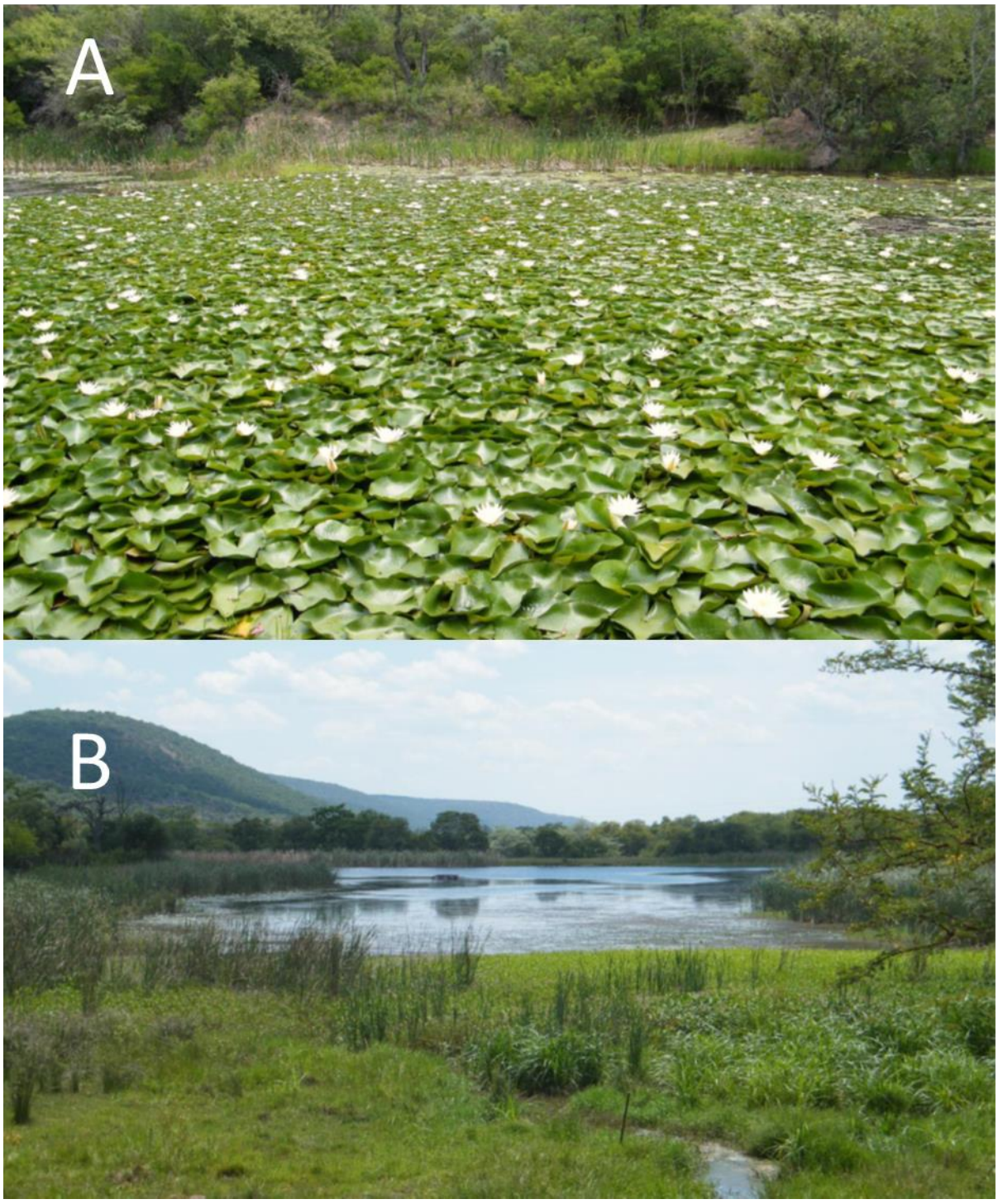


Figure 1. 4: (A) dam in Modimolle, Limpopo, infested with *Nymphaea mexicana*. (B) The species dies back in winter, but the tubers and stolons are still alive under the water and will sprout new leaves when the temperatures warm up again. Photographs by Anthony King, ARC-PPRI 2011.

1.5) Initiating a biological control programme for new IAPs

In order for a biological control programme to be initiated against an IAP, assessments and surveys need to be conducted to first understand the history, biology and ecology of the invasive weed (Morin et al., 2009; Hill and Coetzee, 2017). An evaluation of the weed's ability to rapidly escalate into a serious problem, as well as any prior attempts of mechanical and/or chemical management need to be documented (Simberloff, 2003b; Moran et al., 2011). The entire process involved is often referred to as the biological control "pipeline" (Fig. 1.5) (Cuda, 2014b), briefly described below.

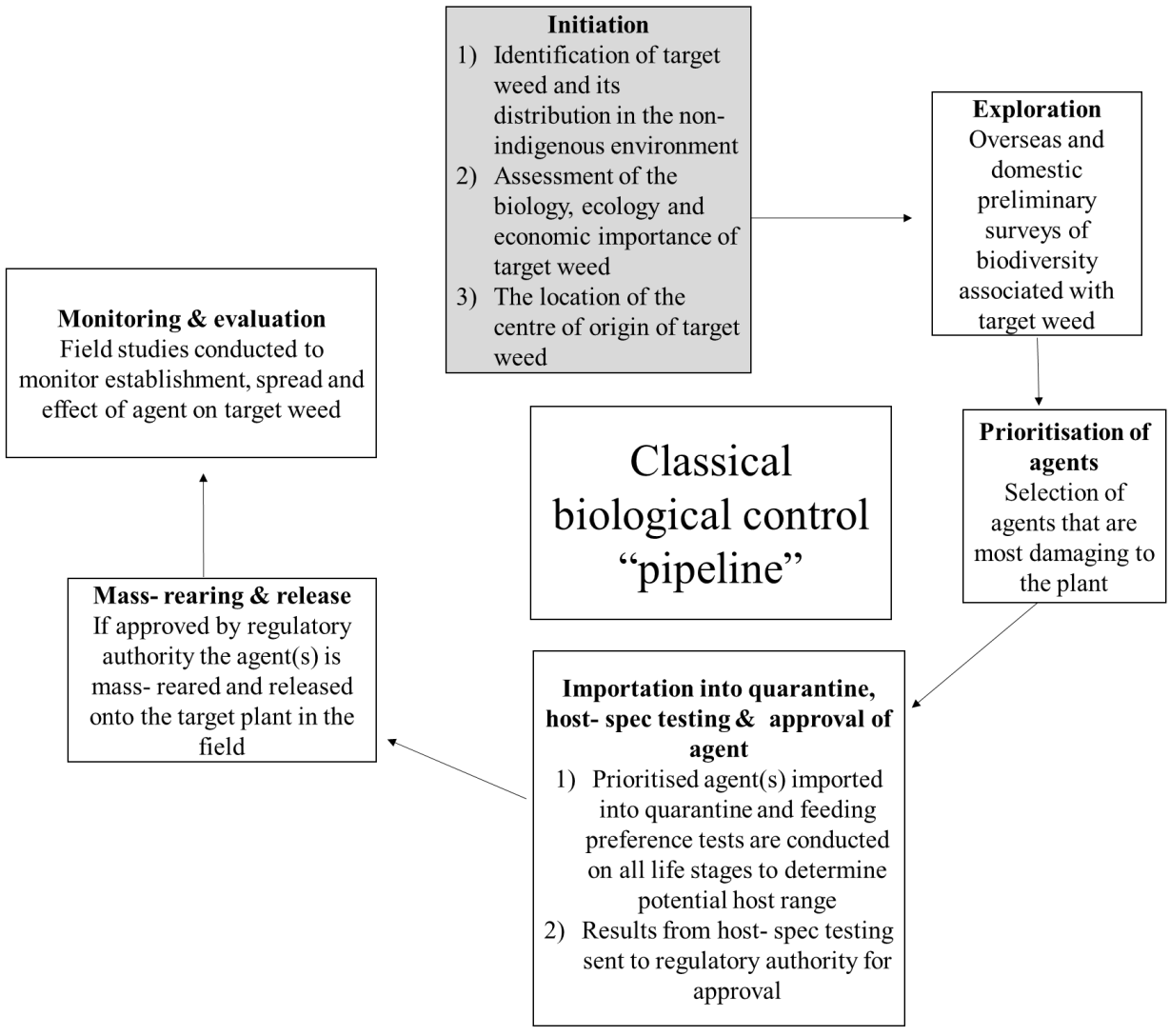


Figure 1. 5: Biological control "pipeline:" Steps involved in initiating a biological control programme for a new invasive alien plant. Modified from Bennett (1977); Cuda (2014b); and van Klinken and Raghu (2006). The process shaded in grey forms a part of this research.

1.5.1) Initiation

In order to determine that an alien plant is a threat that requires some form of management, a procedure of pre–border and post–border weed risk assessment is carried out. There are three broad approaches used in both pre–border and post– border weed risk assessment, namely: 1) quantitative statistical models, 2) semi– quantitative scoring and 3) qualitative expert assessment (Hulme, 2012). A Weed Risk Assessment (AWRA) model developed by Pheloung et al., (1999) for Australia and later adapted for New Zealand has gained popularity over the past decade, for use as a border risk assessment tool.

South Africa does not use the Aquatic Weed Risk Assessment Model to evaluate the invasiveness of NIS in the country. However, there are other strategic approaches for the detection of IAPs which include, but are not limited to: site– specific surveys, species– specific surveys and random surveys conducted by the South African National Biodiversity Institute's Invasive Species Programme (SANBI ISP) (Wilson et al., 2013). The unit, initially called the Early Detection and Rapid Response (EDRR) unit, was developed to: (1) detect and document any new invasions, (2) provide reliable and transparent post– border risk assessments and (3) provide the cross– institutional coordination needed to successfully implement national eradication plans (Wilson et al., 2013). The name change from EDRR to SANBI ISP was brought about by the need to reinforce a management approach that focused on eradication and not exclusively any stage of the invasion process at any spatial scale (Wilson et al., 2013). SANBI ISP have extensively used historical records in the SAPIA database to detect potential invaders, as well as site– specific and species– specific surveys. SANBI ISP has also based employees around the country with a mandate of being able to develop professional networks of people identifying potential invaders and generating knowledge about invasions with the broader public (Figure 1.5) (Wilson et al., 2013). Once a potential invasive alien plant species has been detected, the follow up process is to evaluate the species in detail (biology and centre of origin), in order to make a decision on whether or not, regulation and management is required and if so, an appropriate approach to manage the species is developed (Henderson, 2011; Wilson et al., 2013).

The biology of a potential IAP is assessed by examining the species life history traits and in particular the IAP's mode of reproduction (Barrett, 2011). Breeding system experiments of the IAP can help determine if the primary mode of reproduction is sexual or asexual or a combination of both (Ward et al., 2012). Modes of reproduction are influential in

governing evolutionary response to environmental change (Barrett, 2011; Ward et al., 2012). During reproduction, genetic material is transferred from both parents to the offspring and this genetic transmission between generations of plants will subsequently affect the genetic variation within and among plant populations (Barrett, 2011). The vast diversity in a plant's mode of reproduction therefore has a direct effect on the invasion process in terms of biogeography, demography, and opportunities for the evolution of local adaptation (Barrett, 2011; Ward et al., 2012).

As soon as the target weed has been identified, the next step is usually to identify the origin of the target weed, i.e. the weeds native country (Harley and Forno, 1992). Plant material is obtained from the suspected country of origin and the DNA is extracted (Nissen et al., 1995). Molecular techniques such as amplified fragment length polymorphism (AFLP) are then used to confirm the centre of origin of the target weed (Nissen et al., 1995). The amount and distribution of genetic diversity of an invasive weed can also be assessed using AFLPs (Dodet et al., 2007). Molecular techniques have thus far been applied to successfully elucidate the origin and population structure of numerous weedy species in South Africa such as *Pereskia aculeata* Miller. (Cactaceae) (Paterson et al., 2009), *Chromolaena odorata* (L.) R.M. King and H. Robinson (Asteraceae) (Paterson and Zachariades, 2013), *Campuloclinium macrocephalum* (Less.) DC. (Asteraceae) (Gitonga et al., 2015) and *S. platyphylla* (Kwong et al., 2017).

1.6) Thesis outlines and aims

The principle aim of this research was to investigate the ecology of *N. mexicana* in South Africa, in order to begin an assessment of the feasibility of managing populations of this weed using classical biological control.

Chapter two assessed the genetic variability between and within *N. mexicana* populations in South Africa as well as the genetic similarity between the native (USA and Mexico) and the invasive range in South Africa. Genetic studies are a useful tool in invasion biology because they provide us with knowledge about the centre of origin of an invasive species as well as insight into whether or not the species is reproducing sexually or asexually.

Chapter three investigated the mode of reproduction and the reproductive fitness of *N. mexicana* by assessing the breeding system of this species. It is important to study the modes of reproduction of an invasive plant, because the mode in which an IAP reproduces can give us insight into its evolutionary response to environmental change.

Chapter four provides a general discussion and conclusion of the study as well as recommendations for further studies of *N. mexicana* and the future control of the species in South African aquatic ecosystems.

Chapter 2: Investigating the genetic structure of invasive populations of *Nymphaea mexicana* in South Africa

2.1) Introduction

Population genetic studies in invasion biology require the use of genetic techniques for accurate taxonomic identification, as well as insight into the genetic structure of an invasive species, prior to the initiation of an appropriate and effective management programme (Mueller and Wolfenbarger, 1999; Dodet et al., 2008; Gaskin et al., 2011). Advances in DNA sequencing and PCR have contributed significantly to the formation of molecular techniques which can be used to evaluate genetic resources, and identify molecular markers for the use in breeding programmes (Jones et al., 1997) and recently, in invasion biology (Doorduin et al., 2010). The amount and distribution of genetic diversity of an invasive weed can thus be assessed using molecular markers such as amplified fragment length polymorphisms (AFLP) (Dodet et al., 2008; Gaskin et al., 2011). AFLP markers are dominant markers that are free of artifacts which creates prospects to resolve genetic differences for parentage analysis, mating frequency as well as individual identification of an organism at the DNA fingerprinting level (Vos et al., 1995; Mueller and Wolfenbarger, 1999; Koopman et al., 2008). AFLPs have been used to successfully elucidate the population structures for some weeds that have been targeted for biological control, e.g. *Campuloclinium macrocephalum* (Less.) DC (Asteraceae) (pompom weed) (Gitonga et al., 2014), *S. platyphylla* (Kwong et al., 2017), and *Ambrosia artemisiifolia* L. (Asteraceae) (common ragweed) (Kropf et al., 2018).

The mechanisms driving the population structure of a species and its local adaptations to new environments forms a pivotal component in evolutionary biology (Gaskin et al., 2011). Numerous non-adaptive processes can have an influence on quantitative and molecular variation between and among populations (Chun et al., 2009). Mechanisms such as genetic drift, founder effects, gene flow, mutations, bottlenecks, hybridization and even a mixture of these can contribute to the phenotypical variation within and between populations (Chun et al., 2009; Gaskin et al., 2013). This increase or decrease in genetic variation is

dependent on the mechanism (Amsellem et al., 2000). A decrease in genetic variation may be due to founder effects and/or bottlenecks (Amsellem et al., 2000; Chun et al., 2009), whereas an increase in genetic variation of the introduced species may be the result of hybridization, epistatic variation and/or multiple introductions (Doorduin et al., 2010). Studies conducted on the genetic structure of invasive plants thus provide insight in determining if single or multiple genotypes of the invasive weed is present in the invaded range, as well as identifying the origin of the target weed population (Wardill et al., 2005; Paynter et al., 2012). Herbivores or pathogens that demonstrate local adaptations to a plant genotype in its native distribution are more likely to be compatible to similar plant genotypes in the introduced distribution (Kniskern and Rausher, 2001; Goolsby et al., 2006). Hence the determination of a weed's origin has been an important contributing factor to the success of numerous biological control programmes worldwide (Gaskin et al., 2005; Williams et al., 2005; Goolsby et al., 2006; Paterson et al., 2009; Paterson and Zachariades, 2013; Barker et al., 2015; Barker et al., 2017; Sutton et al., 2017).

For example, *Pereskia aculeata* Miller. (Cactaceae) populations in South Africa were found to have originated in the southern region of the plant's native distribution in South America (Paterson et al., 2009). Similarly South African populations of Siam weed, *Chromolaena odorata* (L.) R.M. King and H. Robinson (Asteraceae) were found to have originated in Jamaica or Cuba (Paterson and Zachariades, 2013). *Campuloclinium macrocephalum* populations in South Africa were genetically very similar to Argentine populations in the native distribution (Gitonga et al., 2014), while *Sagittaria platyphylla* populations in South Africa were the most genetically similar to native populations in the USA (Kwong et al., 2017). All of these studies have benefited the search for natural enemies from target populations most closely related to invasive populations. Thus the use of molecular approaches to determine a weeds origin and population structure are becoming increasingly useful, especially for new plant introductions such as *Nymphaea mexicana.*

Nymphaea is the most phenotypically diverse genus in the order Nymphaeales, with 45– 50 species distributed worldwide (Schneider and Williamson, 1993; Borsch et al., 2007). This phenotypic diversity is partly due to high levels of interspecific polymorphisms which are expressed in leaf and flower morphologies (Heslop– Harrison, 1955; Volkova and Shipunov, 2007; Poczai et al., 2011). The basic haploid chromosome number of the genus is n = 14 (Gupta 1980; Poczai et al., 2011). However species within the genus may be polyploid, with diploid, tetraploid and hexaploid individuals being reported among taxa

(Gupta, 1978; Poczai et al., 2011). Cytological intraspecific variation has also been reported to occur in some taxa, where the chromosome number varies (Heslop– Harrison, 1955; Bolkhovskikh et al., 1969; Gupta, 1978; Lavania, 2002). This was observed for *N. rubra* which had chromosome numbers varying from 42 to 112 (Lavania, 2002), and also for *N. alba* in which individuals had chromosome numbers from 48 to 160 (Heslop– Harrison, 1955; Bolkhovskikh et al., 1969; Gupta, 1978). Although the morphological variation of the genus as a whole has been well documented (Caspary, 1865; Conard, 1905; Radics, 1967; Jones and Clarke, 1981; Muntendam et al., 1996; Volkova and Shipunov, 2007), the genetic diversity, genetic relatedness and the status of hybrids are lacking, with studies having only been conducted on a few species (Woods, 2003; Woods et al., 2005; Lohne et al., 2008a, Lohne at al., 2008b; Dkhar, 2010; Dkhar, 2011; Chaveerach et al., 2011; Poczai et al., 2011; Dkhar, 2013; Borsch et al., 2014; Guruge et al., 2017).

The first artificial *Nymphaea* hybrid was reported in 1852 by French botanist Jules Emile Planchon at the van Houtte nursery in Belgium, and was created by Eduard Ortgies, by crossing *N. rubra* with *N. dentata* (now a synonym of *Nymphaea. lotus* L. (Nymphaeaceae)) yielding *N. ortgiesiano–rubra* (Planchon, 1852; Les et al., 2004). This subsequently led to artificial hybridization between numerous *Nymphaea* species in the eighteenth and nineteenth century (Nierbauer et al., 2014). Around the late 1800s, French horticulturist, Joseph Bory Latour–Marliac began hybridizing waterlilies at his garden in Le Temple–sur–Lot in France (Dana et al., 2017). He coined his hybrids – *Nymphaea* "*marliacea*," or commonly, the Marliac hybrids (Henderson and Cilliers, 2002; Dana et al., 2017). By 1899, Latour– Marliac had created 34 hybrids, which he called "quite distinct varieties," and had named 26 of these hybrids at the time (Latour– Marliac, 1899). The Marliac hybrids however only became sought after globally, once the French painter Oscar–Claude Monet began painting them, between 1840– 1926 (De Socarraz– Novoa, 2012). Monet had seen the Marliac hybrids at the 1889 Exposition Universelle in Paris, and subsequently formed a close friendship with Latour– Marliac, who over the years sent him numerous hybrids for his pond at his home in Giverny (De Socarraz– Novoa, 2012). Monet's paintings of the Marliac hybrids increased their popularity and led to Latour–Marliac shipping of his hybrids to numerous countries around the globe (De Socarraz– Novoa, 2012; Dana et al., 2017). Although Latour– Marliac had stated that his hybrids were "quite distinct varieties," their similarity in physical appearance make it difficult to distinguish between the hybrids by phenotype alone (Dana et al., 2017).

The primary morphological characteristic of the species in this study, *N. mexicana* is its yellow flower colour which distinguishes it from other *Nymphaea* species (Capperino and Schneider, 1985; Cook, 2004). However, *N. mexicana* has been found to produce natural hybrids with *N. odorata*, (American white water lily or fragrant water lily) which also possesses a yellow coloured flower, while many of the artificially produced Marliac hybrids have flowers that possess hues of yellow (Latour– Marliac, 1899; Ward, 1977; Wiersema, 1988; Woods et al., 2005). Artificial *Nymphaea* hybrids are cultivated and sold as ornamentals by plant nurseries around the world. Currently *Nymphaea* hybrids are being documented by Kit and Ben Knotts on their online website (www.victoria–adventure.org), as well as the online website of the International Water lily and Water Gardening Society (www.iwgs.org).

In South Africa, there have been reports and records of suspected hybrids of *N. mexicana*, but genetic studies assessing the population structure of *N. mexicana* in South Africa are lacking. The first reported hybrid of *N. mexicana* was by Anna Amelia Obermeyer– Mauve in 1968, on the banks of the Vaal river in Sasolburg (26°44'56.0"S 27°49'25.4"E) (Mauve, 1968). The flowers were described as pale yellow in colour and the plant had large leaves with deep red blotches (Mauve, 1968; Henderson and Cilliers, 2002). Photographs taken at the same site, many years later, and published by Henderson and Cilliers (2002), clearly show differences in morphology between different *N. mexicana* populations which occured in the Vaal River in South Africa. One of the populations looked very much like *N.* var. *marliacea chromatella*, whilst the other resembled the "pure" *N. mexicana* (Henderson and Cilliers, 2002). In this case, differentiation was possible based on the phenotypes. However, it is often difficult to distinguish *Nymphaea* species and hybrids based on morphological differences in areas where distribution ranges of two or more of the species overlap (Dkhar, 2011; Dabrowski et al., 2015; Woods et al., 2005). This may be particularly true when it comes to differentiating between the Marliac hybrids, as Latour– Marliac himself said that there could be "inextricable confusion" between the hybrids if they were planted in the same waterbody (Latour– Marliac, 1899). Therefore, the only way to clearly distinguish between *N. mexicana* and hybrids of the species is to use molecular techniques to assess their genotypes (Dkhar, 2011).

This study used AFLP molecular markers to compare relative levels of genetic diversity and assess the population genetic structures between native USA and introduced South African populations of *N. mexicana* to evaluate the prospects of successful biological

control for this invasive species. The aims of this study were to: (1) investigate the genetic diversity of *N. mexicana* populations in the native range in southern USA and compare that to the diversity of populations in the introduced range in South Africa. This will help to determine the likelihood of finding pre–adapted biological control agents to attack invasive genotypes; (2) determine if there are single or multiple genotypes of *N. mexicana* present in South Africa, which will aid in determining weed– agent compatibility between all populations in South Africa; and (3) to compare the phenology of the plant populations in the invaded range in South Africa to its genealogy so that hybrids and species can be identified.

2.2) Material and methods

2.2.1) Sampling

Nymphaea mexicana samples were collected from around South Africa from both natural (rivers, dams and ponds) and artificial (ponds) aquatic ecosystems. In order to obtain the widest range of genetic variation and differentiation, sites covering the widest geographic distribution of the introduced range in South Africa were sampled (Table 2.1, Fig. 2.1). Samples from certain parts of the USA (native range) were also sourced (Table 2.1; Fig. 2.2). At each site, healthy, whole leaves were collected at a distance of at least 5 meters apart from each other to avoid the resampling of clones, as well as to sample the entire range of genetic variation (Volkova, 2010). There were 15 leaf samples collected from each site in the invaded range. Leaf samples were also collected from two sites in the native range in southern USA and couriered to the Life Science Building at Rhodes University. Eight samples were collected from Lewisville, Texas, and 20 samples were collected from Kissimmee, Florida. Each leaf sample was rinsed with freshwater and wiped dry with paper towels to remove any extraneous material and/or epiphytes, as well as any excess moisture. Each leaf sample was then individually wrapped in paper towel and placed into sealed bags containing silica gel. If flowers were available at the time of leaf collection, they were photographed in order to compare the plants phenotype to its genotype on completion of the genetic studies.

2.2.2) Plant DNA extraction

DNA extractions were carried out on four dry leaf samples from each locality in South Africa, with the exception of samples from Westlake, where DNA was extracted from 12 leaf samples due to the sample area being larger and the waterbodies being more geographically widespread. For the native range, DNA was extracted from all 8 leaf samples from Lewisville, Texas and only 10 out of the 20 leaf samples from Kissimmee, Florida.

Total genomic DNA from dry leaf tissue (30–40 mg) was extracted using the QIAGEN Mini Plant Extraction kits (QIAGEN Inc.). Leaf tissue from individual plants was ground under liquid nitrogen in a mortar and pestle, and then the QIAGEN protocol was followed (Qiagen, 2006). DNA quality and concentration was measured using a NanoDrop™ 2000 spectrophotometer (Thermo scientific™). These extractions were then stored at –20 ºC until required.

2.2.3) Amplified Fragment Length Polymorphisms (AFLPs)

2.2.3.1) Restriction– Ligation

AFLP protocols followed Blignaut et al., (2013) with minor modifications as described below. Genomic DNA (200 ng) was digested with restrictive enzymes 1U PstI and 1U MseI (New England Biolabs) for 15 mins at 37 ° C in a 10 µL volume. Ligation was then performed in a 30 µL reaction containing 10 µL of digested genomic DNA product, 1.0 µL T4 DNA ligase buffer, 0.2 µL T4 DNA ligase, 1.0 µL PEG 0.5X BSA, 1.0 µL MseI adaptor, 1.0 µL PstI adapter and and 5.8 µL of ddH_2O. Following ligation the digestion–ligation reaction mixes were diluted 1:5 with ddH_2O and used as a template for the pre–selective PCR.

Step 1: Template preparation

The standard master mix recipe included: 0.5 µl *Mse*I restriction enzyme (ThermoFisher Scientific Inc), 0. 25 µl *Eco*RI restriction enzyme (ThermoFisher Scientific Inc), 3.0 µl cutsmart buffer (New England, BioLabs inc, 10 x concentration) and 22.25 µl ddH_2O. The above mentioned quantities for each ingredient was multiplied by the number of samples plus two blanks for every 8 samples (e.g. if there are 8 samples, each ingredient quantity was multiplied by 10). Thereafter 26 µl of the master mix was added, followed by 4 µl of DNA to each PCR tube. The PCR tubes were spun down using a mini centrifuge (PRISM™ mini, Labnet International, Inc.) The tubes were then flicked by hand and spun down once more in order to get all the constituents to mix together. The PCR tubes were then loaded into the PCR machine (BIO– RAD, T100™ Thermal Cycler) and incubated for 15 minutes at 37^0C.

Step 2: Ligation

The ligation master mix recipe was made by using: 0.2 µl T4 ligase (Integrated DNA Technologies Inc.), 1.0 µl T4 ligase buffer (Integrated DNA Technologies Inc.), 1.0 µl *Eco*RI adapter, 1.0 µl *Mse*I adapter, 1.0 µl PEG (Integrated DNA Technologies Inc.) and 5.8 µl

ddH_2O. The PCR tubes were then loaded into the PCR machine (BIO– RAD, T100™ Thermal Cycler) and the PCR was run as follows: Ligation for 60 minutes at 22 ^{0}C, then 10 minutes at 65 ^{0}C and a final hold at 4 ^{0}C. Once the PCR ligation step was completed the samples were diluted with ddH_2O in a 1: 5 ratio in 1.5 ml Eppendorf tubes.

2.2.3.2) Pre–selective amplification

Pre–selective amplification was carried out using *EcoRI+A* and *MseI+C* primers. Each 15 µL pre–selective PCR contained: 2.5 µL of the diluted digestion–ligation reaction mix, 1.5 µl *MseI+C*, 1.5 µl *EcoRI+A*, 7.5 µl Promega Taq master mix (Madison, WI, USA), and 2.0 µl ddH_2O. Pre–selective PCR amplification was carried out as follows: initial denaturation at 95 °C for 2 min and then 30 cycles of 95 °C for 30 s, 56 °C for 30 s, 72 °C for 1 min, followed by a final extension of 72 °C for 5 min.

Successful amplification was confirmed by running 5 µl of the PCR product on a 1% ethidium bromide (EtBr) agarose gel, which aided in visualizing a smear in the range of 100–500 base pairs. Following successful amplification the pre–selective PCR products were diluted 1: 19 with ddH_2O and used for selective PCR amplification.

2.2.3.3) Selective amplification

Selective amplification was completed using three primer combinations (*MseI–CTA/ EcoRI–AGT, MseI–CGT/ EcoRI–ACA, MseI–CCA/ EcoRI–ACA*), which showed high polymorphism and repeatability (Volkova et al., 2010). The *EcoRI+3* were fluorescently labelled and the *MseI+3* were unlabelled primers in order to visualize them on an ABI 3130xl Genetic Analyzer (Applied Biosystems Inc.) using an ABI GS600LIZ internal ladder. Each 20 µl selective amplification PCR contained: 5.0 µl of the diluted pre–selective PCR product 0.25 µl of each primer (*Mse*I+NNN and *Eco*RI+NNN), 7.5 µl Promega Taq master mix (Madison, WI, USA), and 7.0 µl ddH_2O. Selective PCR amplification was carried out as above. Banding patterns generated by the labelled primers were visualized using capillary electrophoresis on an ABI 3130 genetic analyser at Stellenbosch University, Stellenbosch, South Africa.

2.2.3.4) AFLP scoring

Electropherograms were analysed using GeneMarker® version 2.7.0 (SoftGenetics LLC.). The filtering parameters for scoring in GeneMarker were set as follows: minimum bin width of 1.0 base pairs (bp), maximum bin width of 1.5 bp, scoring ranges from 50 bp to 800 bp, low frequency threshold of 200 fluorescence units and reproducibility at 80%. These

filtering parameters are considered as conservative and were employed to minimise genotyping errors (Whitlock et al., 2008). The resulting binary matrix was imported and consolidated into Microsoft® Excel. The matrix was then converted to a pairwise similarity matrix in the statistical software PAST®: paleontological statistics package ver. 3.0 (Hammer and Harper, 2001), applying the Jaccard's transformation for binary genetic data as it excludes shared absence as a genetic character (Sokal, 1963).

2.2.3.5) AFLP genetic clustering analyses

The consolidated binary matrix was used to create a covariance matrix, visualised as a principal component analysis (PCA) plot in PAST. The genetic structure of the invasive and native populations of *N. mexicana* were determined by analysing the AFLP binary dataset using the software programme STRUCTURE ver. 2.3.4 (Pritchard et al., 2000). STRUCTURE uses a Bayesian– clustering algorithm to assign individuals to genetic clusters (*K*) according to their genotypic similarities (Pritchard et al., 2000). The probability of assignment (*Q*) of each sample for $K = 1$ through $K = 6$ was estimated by ten independent runs of 250 000 Monte Carlo Markov Chain (MCMC) iterations, following a burn– in period of 100 000 iterations. Each genetic cluster on the STRUCTURE output graph was assumed to contain haploid alleles (Oliveira et al., 2010). The analysis was performed using the population admixture model, as this has been demonstrated as the most efficient model to study intra–specific variation (Falush et al., 2003). Each sample which was assigned a *Q*– value ≥ 0.80 to any genetic cluster was considered to be a "pure" individual belonging to that particular cluster, while any sample assigned a *Q*– value ≤ 0.80 to any genetic cluster were considered to be of hybrid origin (Andersen and Mills, 2016). The optimal *K*– value (i.e., the number of natural clusters that samples were partitioned into) was determined using STRUCTURE HARVESTER ver. 0.6.94 (Evanno et al., 2005; Earl, 2012). STRUCTURE runs were summarized and graphical outputs obtained using the CLUMPAK software (Kopelman et al., 2015).

A phylogenetic network was constructed using SplitsTree4 (ver. 4.14.4) with 1000 bootstrap replications for support of nodes (Huson and Bryant, 2006). Unlike the traditional phylogenetic analyses such as NJ, MP and Bayesian analyses, the network analyses takes intra–specific and population level phenomenon such as recombination into account (Posada and Crandall, 2001).

2.2.3.6) AFLP genetic distance and diversity analyses

The genetic variation between and among samples in the native range, invasive range and both ranges combined were determined by performing an Analysis of Molecular Variance (AMOVA) using the GENAlex ver. 6 software package (implemented in Microsoft® Excel), (Peakall and Smouse, 2006), employing 999 permutations of the analysis. Genetic variation between and among the two clusters (Group A and Group B) from the PCA analysis were also examined. The population estimator PhiPT (Φ_{PT}), which is an analogous statistic of F_{st}, was calculated from the amongst population variability observed in the AMOVA analysis, which is a measure of population differentiation for binary data (Timm et al., 2010). Gene flow via the number of migrants (Nm) was also calculated in GENalex by using PhiPT (Nm = [(1/PhiPT-1]/4), R_{st} and the private alleles method (Bartin and Slatkin, 1986).

This pairwise similarity index generated by PAST was then used to compare the levels of genetic variation in Statistica® ver. 13.2 (StatSoft) using all within population pairwise values as data. Significant differences between populations were tested by conducting a two–sample t–test (native vs invasive) and a one – way ANOVA was run to compare significant differences between the average genetic distances of *N. mexicana* populations in the native and invaded regions. A Tukey's HSD *post hoc* analysis was also done to test for differences in genetic diversity between regions in the native and invaded ranges.

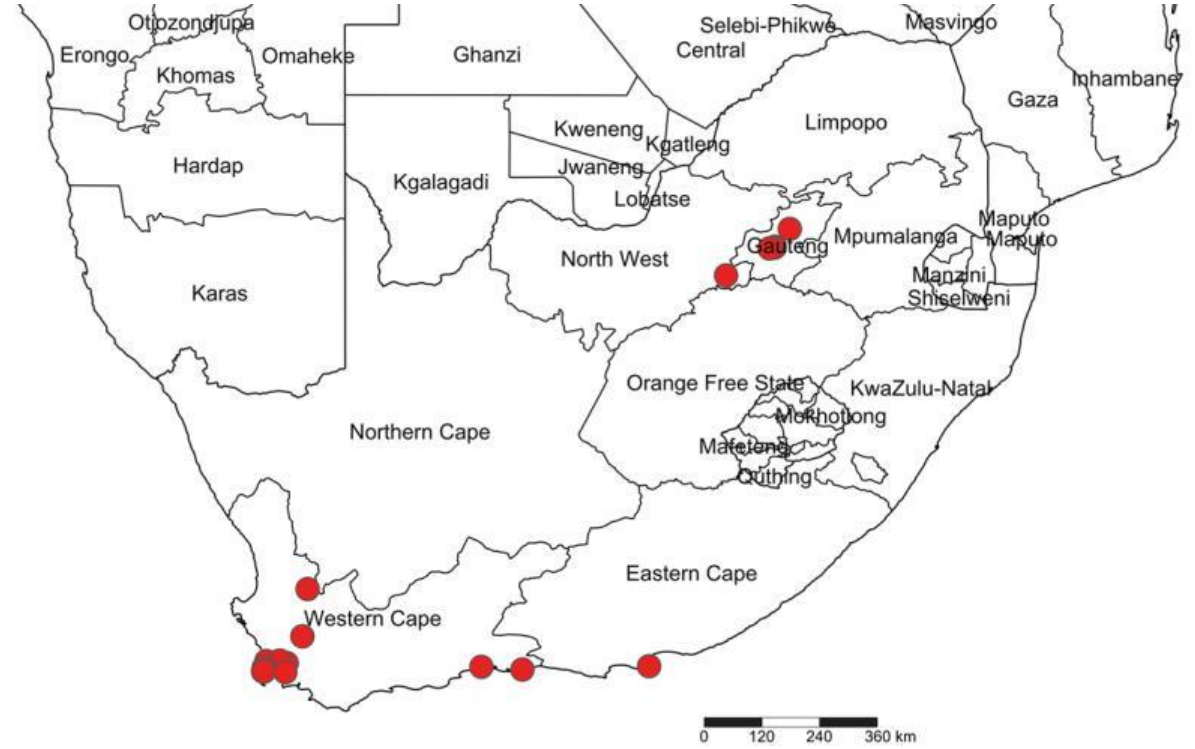


Figure 2. 1: Map of South Africa with red circles indicating the sites from which suspected *Nymphaea mexicana* samples were collected for AFLP analysis.

Figure 2. 2: Map showing States in the southern USA from which *Nymphaea mexicana* samples were collected for AFLP analysis.

Table 2. 1: GPS coordinates of sites in South Africa and southern USA from which *Nymphaea mexicana* samples were collected for AFLP analyses. The same colour codes are used throughout the results section.

Province	**Locality**	**Geographic Coordinates**	**No of samples**	**Sample code**	**Colour code**
South Africa					
Western Cape	Muizenberg, Westlake	34°04'54.9"S 18°27'23.4"E	12	WL	●
Western Cape	Neil Ellis Stellenbosch	33°55'29.6"S 18°53'28.4"E	4	NE	●
Western Cape	Century City	33°53'16.2"S 18°30'46.0"E	4	CC	●
Western Cape	Bellevue wine estate, Stellenbosch	33°52'44.7"S 18°45'46.7"E	4	BW	●
Western Cape	Maynardville Wynberg	34°00'19.1"S 18°27'51.5"E	4	M	●
Western Cape	George	33°59'40.3"S 22°31'34.2"E	4	G	●
Western Cape	Dam 1, Plettenberg, Knysna	34°02'41.2"S 23°17'30.7"E	4	K	●
Western Cape	Dam 2, Plettenberg, Knysna	34°02'45.0"S 23°17'16.5"E	4	KP	●
Western Cape	Yellowwood dam, Somerset West	34°05'38.7"S 18°51'54.2"E	4	SW	●
Western Cape	Kluitjieskraal	33°25'30.3"S 19°11'01.5"E	4	KK	●
Western Cape	Cottage dam farm, KromRivier	32°32'30.1"S 19°16'52.0"E	4	KR	●

Eastern Cape	Boardwalk, Port Elizabeth	33°58'58.9"S 25°39'26.8"E	4	PE	●
Gauteng	Benoni	26°10'13.7"S 28°17'20.4"E	4	B	●
Gauteng	Morelata Park, Pretoria	25°48'50.2"S 28°17'05.2"E	4	P	●
Gauteng	Louw Geldenhuys drive, Emmarentia, Randburg	26°08'46.3"S 28°00'12.8"E	4	E	●
Gauteng	Florida lake	26°10'41.8"S 27°54'23.1"E	4	FL	●
North West	North West University, Potchefstroom	26°40'56.2"S 27°05'42.0"E	4	NW	●
Southern USA					
Texas	Lewisville Aquatic Ecosystem Research Facility	33°03'08.8"N 96°56'14.1"W	8	LEW	●
Florida	Lake Kissimmee	27°57'54.3"N 81°19'39.9"W	10	KIS	●

2.3) Results

2.3.1) STRUCTURE analysis

The Evanno method for optimal *K* selection provided statistical support for six distinct genetic clusters when simulations were run using the full dataset (native and invaded occurrence localities), but *K* = 2 received the most support as the optimal *K*– value by STRUCTURE HARVESTER. The limitations of selecting an optimal *K*– value without taking the study organisms biology and history into consideration has been highlighted by numerous authors (Gilbert et al., 2012). Therefore the STRUCTURE output for both *K* = 2 and *K* = 3 are presented in this study (Figs. 2.3a and 2.3b). Regardless of which *K*–value was

selected, *N. mexicana* individuals from Westlake (WL) in the Western Cape Province, South Africa, were assigned to a genetic cluster along with individuals from the native range in Kissimmee (KIS) and Lewisville (LEW), southern USA, with red bars (■) for $K = 2$ and blue bars (■) for $K = 3$ (Figs. 2.3a and 2.3b). A high proportion of individuals from these 3 sites also had assignment probabilities of $(Q) \geq 0.80$, as can be seen on the graph, indicating a strong population structure within this genetic cluster for both $K = 2$ and $K = 3$ (Fig.2.3) (Pritchard et al., 2007; Andersen and Mills, 2016).

In contrast, the population structure for samples from South Africa excluding Westlake (WL), were more dependent on the selection of K (Figs. 2.3a and 2.3b). For $K = 2$, there was no genetic differentiation between the samples from South Africa, with all these individuals being more or less predominantly green in colour (■) on the graph (Figure 2.3a). However for $K = 3$, an additional genetic cluster was observed, with individuals from Kluitjieskraal (KK), Kromrivier (KR) and Emmarentia (E) clustered together in red (■) and all other individuals obtained throughout South Africa excluding Westlake (WL) clustered together in green (■) (Fig. 2.3b). The Kluitjieskraal (KK), Kromrivier (KR) and Emmarentia (E) cluster showed high population structure for $K = 3$, with the bars on the graph being predominantly red in colour (■) for individuals from these sites (Fig 2.3b). Individuals obtained from Century city (CC), Florida lake (FL), George (G), Pretoria (P), Somerset West (SW), Port Elizabeth (PE) and one of the Knysna (K) dams, also showed high population structure within the $K= 3$ cluster, with the bars on the graph for these individuals being predominantly green in colour (■). In contrast Neil Ellis (NE), North West Province (NW), Bellevue (BW) and the second dam in Knysna (KP), had moderate population structure, with bars for these individuals having some degree of all three colours (green, blue and/ or red, (■)(■)(■)) for $K= 3$ (Fig 2.3b).

2.3.2) Principal component analysis (PCA)

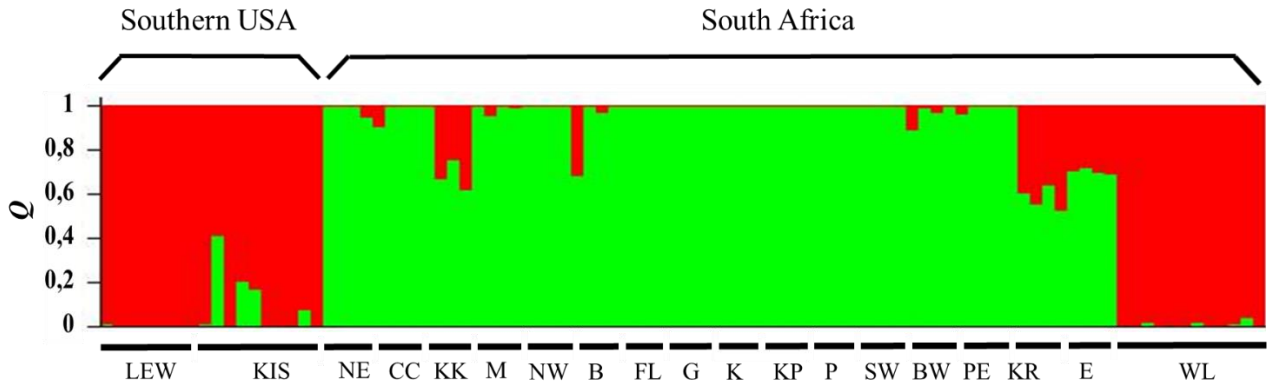
Similar to the STRUCTURE analysis, the PCA grouped *N. mexicana* samples from Westlake (WL), in the Western Cape province of South Africa with plants from the native distribution in southern USA originating from Lewisville (LEW) and Kissimmee (KIS) (Fig. 2.4 – Group A). In this grouping, there was little evidence of phylogeographic structure between the samples (Fig. 2.4 – Group A). All other samples from the invaded range in South Africa, excluding Westlake (WL) grouped together in Group B. Within Group B, there was a relatively large genetic distance between samples obtained from Century city (CC), Florida Lake (FL), George (G), Pretoria (P), Somerset West (SW), Port Elizabeth (PE), one of the

Knysna (K) dams and the samples obtained from KluitjiesKraal (KK), Kromrivier (KR), and Emmarentia (E) (Fig. 2.4 – Group B). There was also a large genetic distance between samples obtained from Neil Ellis (NE), North West Province (NW), Bellevue (BW) and the second dam in Knysna (KP) and the samples obtained from KluitjiesKraal (KK), Kromrivier (KR) and Emmarentia (E) (Fig. 2.4 – Group B).

2.3.3) SplitsTree analysis

The phylogenetic network once again grouped samples from southern USA in the native range with samples from Westlake (WL) in the invaded range. Within this grouping, there was once again little phylogenetic structure related to geographic region, similar to the PCA analysis, indicating that the samples in this group were closely related and that in the recent past, gene flow between these individuals may have occurred (Fig 2.5). The remaining samples that were collected in South Africa excluding Westlake (WL), also grouped together once again, as in the PCA and STRUCTURE analyses (Figs. 2.3, 2.4 and 2.5). However samples from KluitjiesKraal (KK), Kromrivier (KR) and Emmarentia (E), formed a sister clade in Group B (Fig 2.5). The splitting of samples in Group A from samples in Group B was also supported by a bootstrap value of 98.2% for the Bayesian analysis.

a) ***K* = 2**



b) ***K* = 3**

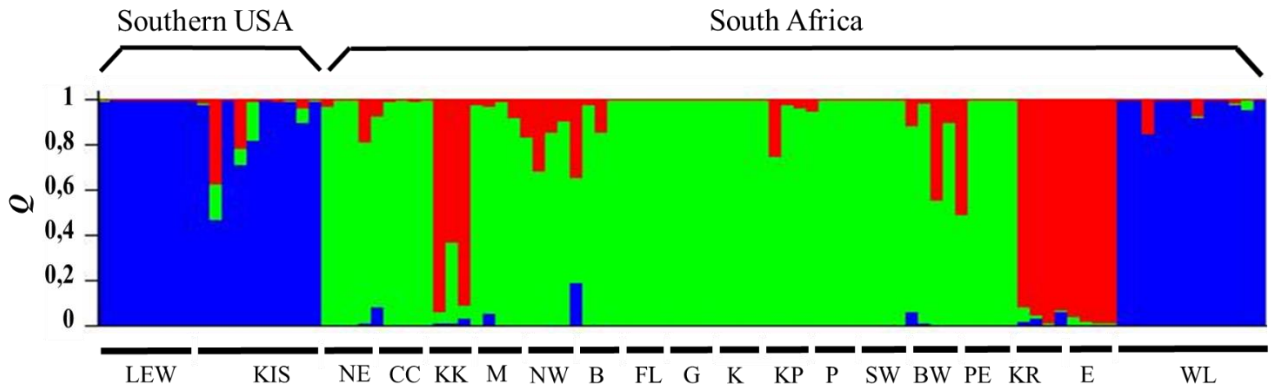


Figure 2. 3: Probability of assignment of individuals of *Nymphaea mexicana* to genetic clusters (*K*) based on the analysis of Amplified fragment length polymorphisms (AFLP) data. Each column represents the probability of assignment of an individual (*Q*) to each of, (3a) two genetic clusters or (3b) three genetic clusters, using the Bayesian– clustering algorithm in STRUCTURE. Individuals are grouped by the geographic locality from which they were collected. n = 8 for Lewisville, n = 10 for Kissimmee, n = 12 for Westlake and n = 4 for all other samples. Sample code names are presented in Table 2.1.

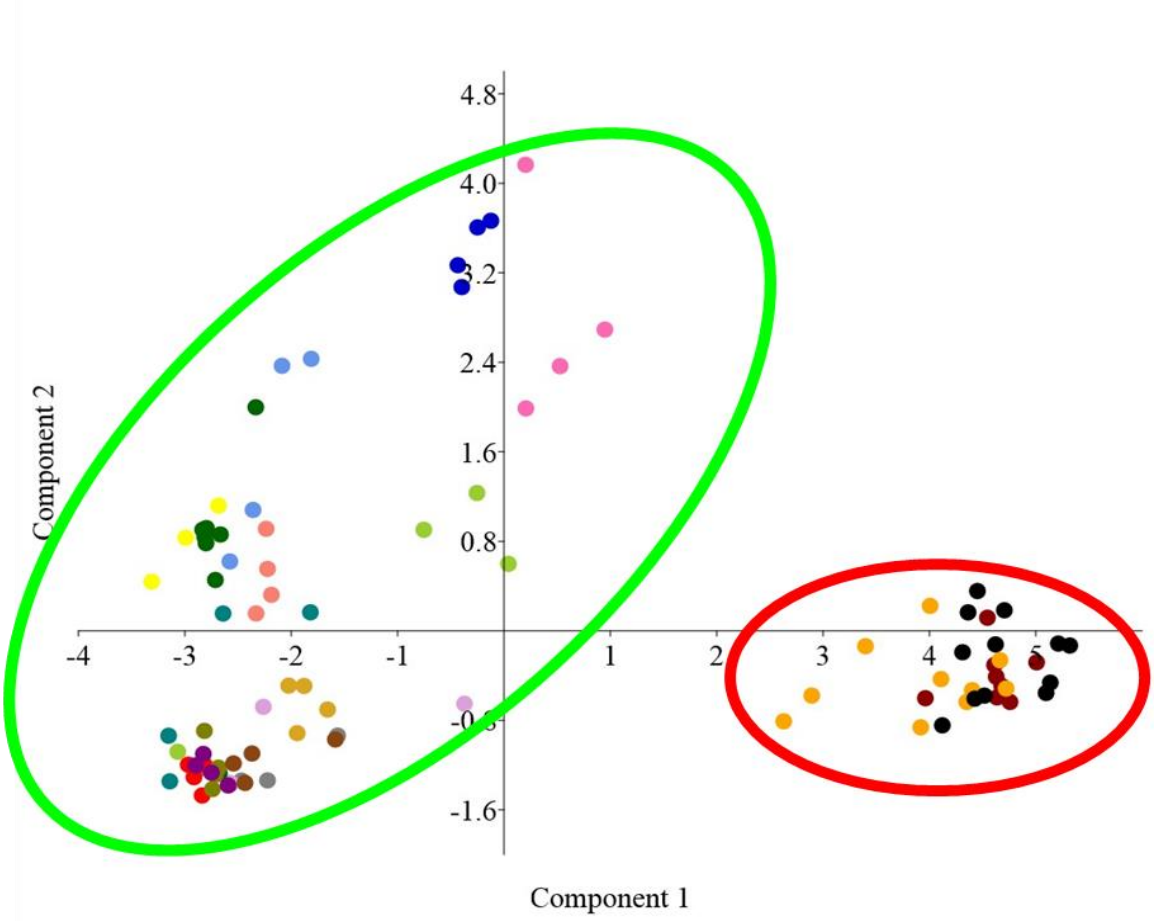


Figure 2. 4: Principal component analysis (PCA) demonstrating two distinct genetic groups of *Nymphaea mexicana* in the native and introduced range, based on the AFLP–PCR dataset. Group A (▮) clustered plants from the native range in southern USA (Kissimmee and Lewisville) and the invaded range in Westlake in the Western Cape, South Africa. Group B (▮) clustered plants from the introduced range in South Africa in areas of the Western Cape, Eastern Cape, Gauteng, and North West provinces. Sample colour codes on the PCA graph are presented in Table 2.1.

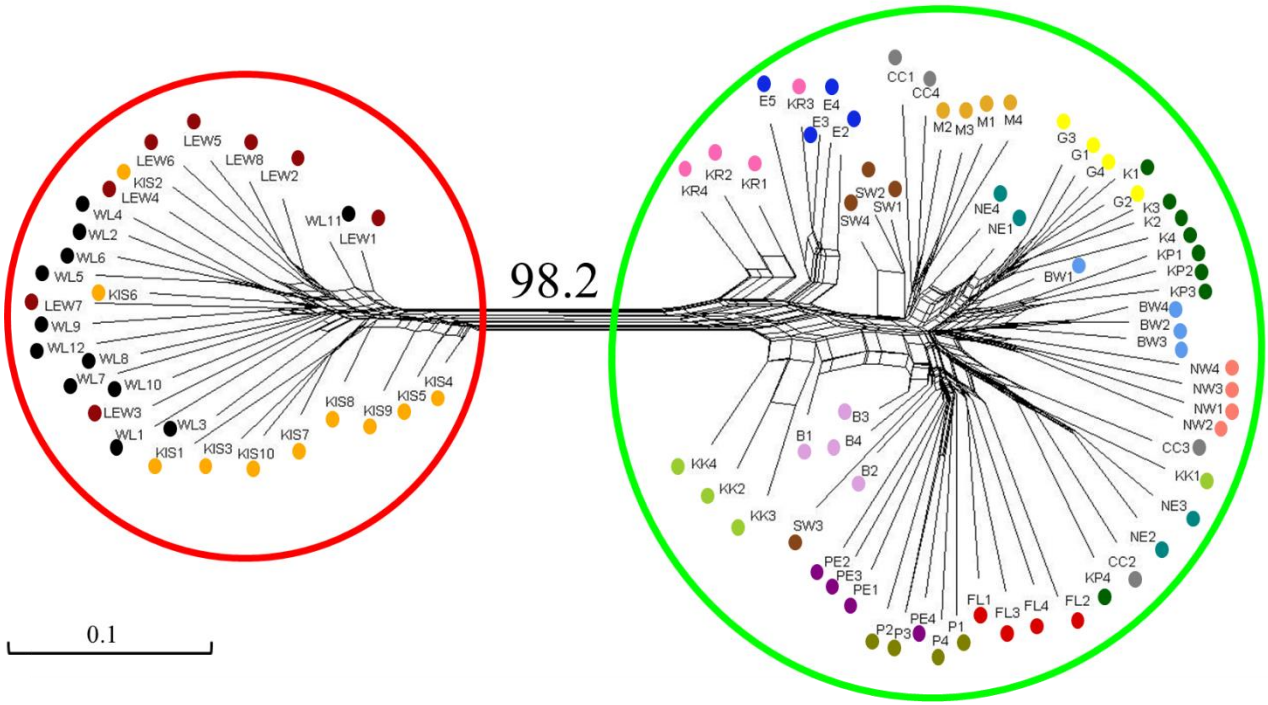


Figure 2. 5: A phylogenetic network constructed using SplitsTree4 with 1000 bootstrap replications for support of nodes. The splitting of samples from the native range in Lewisville and Kissimmee in southern USA (■) and samples from Westlake in South Africa (■) from all other samples in the invaded range in South Africa (■), was supported by a bootstrap value of 98.2%. Sample and colour codes are presented in Table 2.1.

2.3.4) AFLP genetic distance and diversity analysis for the invaded and native range

An AMOVA was performed to test significant differences between areas in the invaded range (SA) and areas in the native range (USA) as well as between areas in the invaded range (SA). For the native range, the AMOVA indicated that although the majority of the genetic variability (82%, P = 0.001) of *N. mexicana* can be attributed to within species variation, a significant amount of genetic variability was attributed to amongst species variation (18%, P = 0.001), with an estimated gene flow (Nm) of 1,102, indicating gene flow between populations in the native range, since Nm > 1 (Table 2.2). In contrast there was a significantly higher amount of genetic variability for within species variation (45%, P = 0.001) than amongst species variation (55%, P = 0.001), with an estimated gene flow (Nm) of 0,201 for the invaded range in South Africa, indicating restricted levels of gene flow, since Nm < 1 (Table 2.2). There was also strong support for population differentiation between *N. mexicana* populations in the

native range (southern USA) and the invaded range (SA), (ϕ_{PT} = 0.585, Nm = 0,178, *P* = 0.001), (Table 2.2).

2.3.5) AFLP genetic distance and diversity analysis based on the groups in the PCA analysis.

Another AMOVA was performed to test significant differences between Groups A (Lewisville and Kissimmee in the native range and Westlake in the invaded range) and B (all samples from SA excluding Westlake) from the PCA analysis (Fig, 2.4). Results indicated a significantly higher percentage of genetic variability (83%, *P* = 0.001) for within species variation compared to amongst species variation (17%, *P* = 0.001), with an estimated gene flow (Nm) of 1,193 for the three populations, indicating that gene flow has occurred between these populations in the recent past (Westlake, Lewisville and Kissimmee) that clustered into Group A (Table 2.3). This result is similar to the result obtained from the AMOVA performed on the native range populations (Lewisville and Kissimmee) (Table 2.2), suggesting that the population from Westlake in the invaded range in South Africa is akin to the populations from the native range in the southern USA. The percentage of genetic variability for within–species variation for populations that clustered in Group B was also higher (59%, *P* = 0.001) than the genetic variability for amongst species variation (41%, *P* = 0.001), with an estimated gene flow (Nm) of 0,363. This is indicative that although populations from the invaded range here in South Africa clustered together in Group A, these may be subspecies or hybrids of *N. mexicana,* due to the relatively low level of gene flow (Nm) as was observed from the STRUCTURE analysis (Figure 2.3).

The one–way ANOVA indicated significant differences between the average genetic distances of *N. mexicana* populations in native and invaded regions ($F_{18, 244}$ = 19.950, *P* = 0.001) (Table 2.4 and Table 2.5). The average genetic distance between native (0.335 ± 0.104) and invasive (0.480 ± 0.216) plants was also significantly different (*t* = 5.80, df = 260, *P* = 0.001) (Figure 2.6).

Table 2. 2: Analysis of Molecular Variance (AMOVA) comparing the genetic variation between and among populations of *Nymphaea mexicana* in the native range (southern USA) and the invaded range (South Africa).

Source of variation	**df**	**Sum of squares**	**Mean square**	**Variance**	**% variance**

Native and invaded					
Among Populations	16	2016.574	118.622	19.671	58%
Within Populations	77	1104.519	13.981	13.981	42%
Total	93	3121.093		33.652	100%
P - value	0.001				
PhiPT	0.585				
Number of migrants (Nm)	0.178				
Invaded					
Among Populations	13	1396.615	93.108	16.577	55%
Within Populations	62	825.167	13.309	13.309	45%
Total	75	2221.782		29.887	100%
P- value	0.001				
PhiPT	0.555				
Number of migrants (Nm)	0.201				
Native					
Among Populations	1	50.964	50.964	3.728	18%
Within Populations	16	279.352	16.432	16.432	82%
Total	17	330.316		20.160	100%
P - value	0.001				
PhiPT	0.185	0.001			
Number of migrants (Nm)	1.102				

Table 2. 3: Analysis of Molecular Variance (AMOVA) comparing the genetic variation between and among populations of *Nymphaea mexicana* that grouped together in the Principal component analysis (PCA) (Figure 2.4).

Source of variation	**df**	**Sum of squares**	**Mean square**	**Variance**	**% variance**
Group A cluster from PCA					
Among Pops	2	98.634	49.317	3.296	17%
Within Pops	27	440.269	15.724	15.724	83%
Total	29	538.903		19.019	100%
P - value	0.001				
PhiPT	0.173				
Number of migrants (Nm)	1.193				
Group B cluster from PCA					
Among Pops	13	731.871	52.277	8.960	41%
Within Pops	50	664.250	13.025	13.025	59%
Total	63	1396.121		21.984	100%
P - value	0.001				
PhiPT	0.408				
Number of migrants (Nm)	0.363				

Table 2. 4: A one– way analysis of variance (ANOVA) comparing the mean genetic variability of *Nymphaea mexicana* between regions in South Africa (invaded range) and Southern USA (native range).

	Sum of squares	**df**	**Mean square**	**F**	***p***
Intercept	32.12483	1	32.12483	1801.453	0.001
Regions	6.04788	18	0.35576	19.950	0.001
Error	4.35119	244	0.01783		

Table 2. 5: A Tukey HSD *post hoc* test examining differences in genetic diversity between regions in South Africa and Southern USA. alpha = 0.05, MS = .01783, df = 244. Sample code abbreviations are those presented in Table 2.1.

Regions	Mean Distances	Group							
		A	B	C	D	E	F	G	H
TEX	0,260047	****							
P	0,283755	****	****	****					
WL	0,303383	****	****						
G	0,323542	****	****	****					
NW	0,334050	****	****	****					
SW	0,341333	****	****	****			****		
KIS	0,373771		****	****					
PE	0,395637	****	****	****	****		****		
M	0,414803	****	****	****	****	****	****		
FL	0,457225	****	****	****	****	****	****		
KR	0,483330		****	****	****	****	****	****	
BW	0,506912			****	****	****	****	****	
E	0,586270				****	****		****	
K	0,605893					****		****	****
B	0,607758				****	****	****	****	****
CC	0,733448							****	****
NS	0,802277								****
KK	0,808847								****

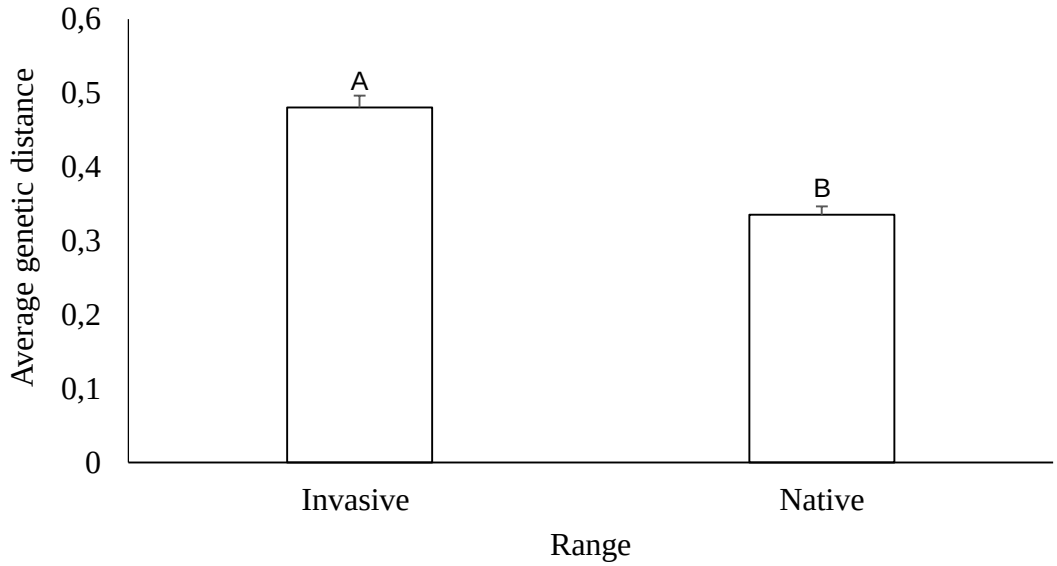
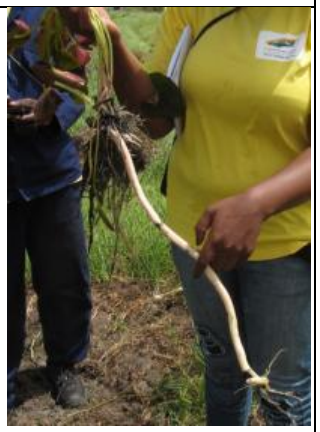


Figure 2. 6: Comparison of the mean genetic variability of *Nymphaea mexicana* between the native range (southern USA) and the invasive range (South Africa). df = 260, $t = 5.80$, $P < 0.001$.

2.3.6) Morphological description and photographs of Nymphaea mexicana and N. mexicana hybrids in SA

Similar to the genetic differences between *N. mexicana* populations in South Africa (Figs. 2.3, 2.4, 2.5), there were also differences in the phenotypes of the populations that were sampled for the molecular analyses in this study. Populations at Westlake in the Western Cape Province had flowers that were small and bright yellow in colour with pinkish sepals and leaves that had deep red blotches with serrated edges (Table 2.6). This plant resembled the "pure" *N. mexicana* in the native range in southern USA. In contrast, the flowers of populations at a site in Emmarentia, Gauteng were medium in size, salmon pink in colour and showy with leaves that had no serrations on the edges (Table 2.6). Plants from a site in the North West Province produced flowers that were also medium in size but lemon in colour with petals that were rounded at the tips and leaves that had much larger red blotches with no serrated edges (Table 2.6). At a site in Knysna in the Western Cape Province, the plants also had medium sized, lemon coloured flowers but these flowers had petals that were lanceolate in shape and sepals that were pink in colour similar to the "pure" *N. mexicana* (Table 2.6). Populations at Port Elizabeth Boardwalk in the Eastern Cape Province had the largest flowers of all the populations that were sampled for molecular analysis in this study. These flowers were also lemon in colour with long petals which were lanceolate in shape (Table 2.6).

Table 2. 6: Morphological descriptions with accompanying photographs of pure *Nymphaea mexicana* and some *N. mexicana* hybrids that were included in the genetic analyses.

Site	**Flower description**	**Flower photograph**	**Leaf description**	**Leaf photograph**	**Rootstock description**	**Rootstock photograph**
Westlake	Bright yellow in colour. Petals are lanceolate in shape with pointed tips.		Ventral surface of leaves are green in colour with serrated edges and red blotches especially on younger leaves. Dorsal surface of mature leaves is green but red with black dots in immature leaves.		Rhizomes are tuberous with fibrous roots that anchor into the substrate. Stolons are present and aid in clonal reproduction.	
Emmarentia	Light pink in colour. Petals are lanceolate in shape with pointed tips.		Ventral surface of leaves are green in colour with fewer serrations around the leaves. Dorsal surface is red in colour. Some of the older leaves		Rhizomes are tuberous.	

			have lost the red colour and instead have red spots on the dorsal surface.			
North West University	Cream to lemon yellow in colour with ovate petals and rounded tips.		Ventral surface of leaves are green with a large degree of red blotching, even more than the pure *N. mexicana*. Edges are smooth with no serrations. Leaf is more oval in shape. Dorsal surface is deep red in colour.		Rhizomes are tuberous. No stolons.	

Knysna	Medium sized flower. Cream to lemon yellow in colour with lanceolate petals. Sepals pink.		Ventral surface is green in colour. Red blotching is prominent in younger leaves. Dorsal surface is red and leaves have no serrations on edges.		Rhizomes are tuberous. No stolons.	
Port Elizabeth	Large flower. Cream to lemon yellow in colour with lanceolate tips. Sepals green.		Ventral surface is green in colour with blotches in younger leaves. Dorsal surface is red with darker red spots and green in the centre towards the midrib. Serrations are present on the leaf edge but not as prominent as the pure *N. mexicana.*		Rhizomes are tuberous. Stolons are also present similarly to the pure *N. mexicana.*	 

2.4) Discussion

There are many important determinants for the likely success of a biological control agent on its host plant (Chaboudez and Sheppard, 1995). Some of these include: (1) whether the target weed population has undergone any genetic bottleneck in the introduced range (Burdon and Brown 1986; Gaskin et al., 2011); (2) if the biological control agent and the host plant share an evolutionary history, in which case the chances of the agent being host specific as well as effective are greater (Nissen et al., 1995; Roderick and Navajas, 2003; Hufbauer and Roderick, 2005); and (3) whether or not the weed population in the introduced range has novel and cryptic hybrids between closely related taxa, that may create compatibility issues between the biological control agent and the weed (Nissen et al., 1995). AFLP molecular markers were used in this study to address some of these determinants, by comparing the amount of genetic diversity of *N. mexicana* individuals in the native range in southern USA and the introduced range in South Africa. The results indicate that *N. mexicana* populations in the introduced range in South Africa had substantially more genetic diversity and higher levels of genetic differentiation compared to populations in the native range. This high genetic diversity was corroborated by the STRUCTURE, PCA and SplitsTree analyses which indicate at least two genetically distinct forms of *N. mexicana* in the country.

In contrast to this finding, photographs obtained from the field for some of the sample plants used in this study revealed that there are more than two morphological forms. The high genetic diversity of introduced *N. mexicana* found in South Africa is contradictory to most studies on invasive plants, in which a higher genetic diversity is reported for plants in the native range, and a significantly lower genetic diversity in the introduced range (Hofstra et al., 2000; Ye et al., 2003; Kliber and Eckert, 2005; Li et al., 2006; Paterson et al., 2009; Gitonga et al., 2014). For example, *Hydrilla verticillata* L.f. Royle (Hydrocharitaceae) in New Zealand (Hofstra et al., 2000), *Alternathera philoxeroides* (Mart.) Grisb (Amaranthaceae) (Ye et al., 2003) and *Eichhornia crassipes* (Mart.) Solms (Pontederiaceae) (Li et al., 2006) in China, *Campuloclinium macrocephalum* (Gitonga et al., 2014) and *Pereskia aculeata* (Paterson et al., 2009) in South Africa, were all found to exhibit a lower genetic diversity in their introduced ranges than their native ranges. The low genetic diversity of these plant introductions was attributed to; (1) single introductions, (2) founder effects and bottlenecks, and/ or (3) lack of plant sexual reproduction.

In this study, higher genetic diversity was found for *N. mexicana* populations in the introduced range in South Africa than in the native range in southern USA, which can be attributed to; (1) a greater sampling effort in the introduced range in South Africa than in the native range in southern USA, (2) multiple introductions of *N. mexicana* into South Africa and/ or (3) multiple forms/ hybrids of *N. mexicana* in South Africa. This study is not the first to suggest that an IAP may have been subject to multiple introductions into an invaded region. For example, a genetic study conducted on the highly successful invasive shrub, *Cytisus scoparius* Link (Fabaceae) (Scotch broom), which is native to Europe, central Asia and western Africa, has invasive populations from Chile with high levels of genetic differentiation from native populations in Europe. This invasive shrub thrives in temperate areas such as western USA, Hawaii, Canada, New Zealand, southeastern Australia, Chile and South Africa. The authors attributed this finding to multiple source population introductions into the invaded range in Chile (Kang et al., 2007). Similarly, a genetic study conducted on the terrestrial invasive, *Spathodea campanulata* Beauv. (Bignoniaceae) showed relatively high levels of genetic variability within invasive Pacific Island populations, compared to native populations in central and eastern Africa (Sutton et al., 2017). *Sagittaria platyphylla*, an aquatic invasive also showed a comparable amount of genetic diversity between native USA and introduced Australian and South African populations, suggesting that founding populations of the weed in the introduced ranges may have been diverse and/ or multiple introductions from different native source populations may have generated genetically mixed populations within invaded ranges (Kwong et al., 2017).

The AMOVA results of this study indicated that native populations of *N. mexicana* had a higher genetic variation within populations than amongst populations as well as relatively higher gene flow within populations. This result may have been influenced by sample size, since fewer sites were sampled in the native range than in the introduced range, and a greater number of individuals from each of the two indigenous sites (n = 10 for Kissimmee, Florida and n = 8 for Lewisville, Texas) in the native range were sampled compared to the introduced range (n = 4 for all samples with the exception of Westlake, n = 12). However the high Nm value in the results section also indicates that gene flow has been occurring between individuals in the population in the native range. *Nymphaea mexicana* has previously been reported to engage in sexual reproduction in the wild in the native range, so outcrossing between individuals within a population is not uncommon (Capperino and Schneider, 1985; Wiersema and Hellquist, 1997). Other species that have been found to have

a higher genetic variation within populations, suggestive of outcrossing are *Phyla canescens* (Kunth) Greene (Verbenaceae) and *Clidemia hirta* (L.) D. Don (Melastomataceae) in Hawaii (DeWalt and Hamrick, 2004; Xu et al., 2010). In contrast, the AMOVA results for the introduced range of *N. mexicana* in South Africa indicated a significantly higher genetic variation among populations than within populations. This was due to the entire invasive population, including Westlake being included in the AMOVA analysis. However when an AMOVA was run based on the groups obtained from the PCA analysis, the within population variance for the invasive group (excluding Westlake) was significantly higher than the variance among populations. The results of this study indicate that only one out of the 17 invasive populations of *N. mexicana* sampled from South Africa is genetically similar to *N. mexicana* individuals that were sampled from native populations in southern USA. AFLPs provided support for native *N. mexicana* individuals from Kissimmee and Texas in southern USA being the most genetically similar to *N. mexicana* individuals from Westlake in South Africa and this was further supported by an Nm value greater than one, indicating that gene flow among these populations may have occurred in the recent past. Herbivores and pathogens collected from *N. mexicana* populations in southern USA are therefore more likely to demonstrate local adaptations to *N. mexicana* plants present at Westlake, and are thus expected to have potential as source populations for biological control agents for the infestation at Westlake.

Individuals from the remaining populations in South Africa excluding Westlake, did not match with individuals from the southern USA populations. Results of the Structure analysis indicated that there are at least two genetically distinct forms of *N. mexicana* present in the country. In contrast to this finding, more than two morphological forms of *N. mexicana* were observed in the field and from the photographs of the flowers and leaves that were taken at some of the sample sites. These forms are possible hybrids of the "pure" *N. mexicana* which were created by the ornamental plant trade and imported into South Africa from multiple sources. Reports of suspected hybrids of the species in South Africa have been documented before, with the first record being at the Vaal river in 1968 (Mauve, 1968; Henderson and Cilliers, 2002). The photographs of *N. mexicana,* which appear in Henderson and Cilliers, (2002), clearly show two very different morphological forms of the species; with one of the forms possessing a bright yellow flower colour and lanceolate petals, as the "pure" *N. mexicana* does, and the other possessing a paler yellow flower colour with more showy petals and ovate at the edges, as in the *N. marliacea chromatella* hybrid. Plants from a pond

in Emmarentia, Ranburg, a dam in Kluitjies Kraal and another dam in Krom Rivier formed a third cluster in the structure analysis for $K = 3$, suggesting a second genetically distinct form from the "pure" *N. mexicana*. The flowers from the population at Emmarentia were salmon pink in colour with lanceolate shaped petals. These flowers were morphologically distinct in shape and/ or colour to both the "pure" *N. mexicana,* as well as the other forms of *N. mexicana* that were sampled in this study. The Emmarentia form could also possibly be another Marliac hybrid, as it clustered more closely to group B in the PCA analysis than to Group A which clustered the "pure" *N .mexicana* together. Unfortunately, no photographs were taken of the flowers at the Kluitjies Kraal and Krom Rivier sites, as they were not present at the time when leaf samples were collected for the molecular analysis. The Western Cape Province had also experienced a severe drought during this study which had caused the dams at these two sites to dry up, making it impossible to obtain photographs of the plants.

However, as noted above, this vast morphological variation of introduced water lily plants in South Africa can be attributed to hybridization. The divergence of the South African populations, excluding Westlake, from the native populations in the USA suggests that intraspecific hybridization between genetically distinct lineages from the native range may have occurred. Gene flow generally does not occur between two distinct water lily species, therefore any hybrids produced through interspecific hybridization are almost always sterile and have decreased viability (Chomchalow and Chansilpa, 2007). In cases where gene flow does occur and a viable hybrid is produced, a genetic bridge is formed between two distinct species and is referred to as introgressive hybridization, and this has been reported to occur for *Nymphaea capensis* Thunb. (Nymphaeaceae) and its hybrids which make up the '*Suthsinobon*' complex (Chomchalow and Chansilpa, 2007). Since introgressive hybridization is a phenomenon that has been observed and reported for other water lily species like *N. capensis*, this may represent another mechanism contributing to the genetic divergence of the South African *N. mexicana* populations. The South African *N. mexicana* populations could be introgressed hybrids formed by artificial crosses between *N. mexicana* and other *Nymphaea* species. We could thus be dealing with an entire *N. mexicana* hybrid complex in the country. There have been studies reporting gene flow between populations of *N. mexicana* and populations of *N. odorata* in the wild where the distributions of these two species overlap (Wiersema and Hellquist, 1997; Woods, 2003). Natural hybrids between these two species are also common in the wild (Wiersema and Hellquist, 1997). Artificial water lily hybrids created by the ornamental plant trade are also very common since the late

1800s (Latour– Marliac, 1899). These hybrids are still imported into the country by plant nurseries around South Africa on a regular basis.

Currently all of these hybrids have become invasive in the country. In order to determine if the forms of *N .mexicana* present in South Africa are the Marliac hybrids created by Latour– Marliac, one would need to assess the genetic structure of all the Marliac hybrids with the closest possible phenotypes to the forms present in South Africa. This may prove to be a costly task since there were at least thirty–four hybrids originally created by Latour– Marliac in the 1800s (Latour– Marliac, 1899). The possibility of these hybrids even occurring in the wild in the native ranges of the parent plants and having natural enemies is limited due to a lack of distribution records for these hybrids. Also, many of these hybrids are created by the horticultural trade and therefore lack natural enemies. This significant divergence between native and invasive populations may limit future prospects of biological control of the *N. mexicana* complex in South Africa, but further genetic analysis of the parentage of these forms, as well as genotype matching to other native forms may increase the prospects of success.

Conclusion

The purpose of this study was to reveal the likelihood of success for the biological control of *N. mexicana* in South Africa based on the degree of genetic variability and divergence between native and invasive populations. Introduced populations of *N. mexicana* throughout South Africa showed differing levels of genetic diversity compared to native populations in southern USA, with the exception of the population at Westlake, which exhibited a similar genotype to its native counterparts. The majority of the genetic diversity of *N. mexicana* populations in South Africa excluding Westlake resided among, rather than within populations. This suggests that intraspecific hybridization may have occurred between different species of *Nymphaea* prior to the invasion process and that multiple introductions of numerous hybrids have been introduced into the country. In addition, the STRUCTURE analysis indicated genotypic similarities for individuals from the native range in USA and individuals from Westlake in South Africa. Thus *N. mexicana* agents sourced from the native Lewisville and Kissimmee populations in southern USA, may prove to be effective against the Westlake population. However, they may not prove to be effective against the other forms/ hybrids of *N. mexicana* in South Africa which the STRUCTURE analysis assigned

different clusters to. In addition, hybrids of *Nymphaea* are usually sterile, so research on the mode of reproduction for these *N. mexicana* hybrids present in South Africa may provide insight into the spread of this invasive hybrid complex around the country and could also aid in predicting the effectiveness of biological control of *N. mexicana* and its multiple forms.

Chapter 3: Investigating the breeding systems and pollinators associated with invasive *Nymphaea mexicana* hybrids in South Africa.

3.1) Introduction

In 1887, Bory Latour–Marliac began communicating with a Mr William Robinson in England regarding hybrid water lily crosses that he had bred through cross pollination between different water lily species (Holmes 2015; Holmes 2016). Caroline Holmes, a garden historian documented the entire correspondence between Latour–Marliac and Robinson in her book, Water lilies: and Bory Latour–Marliac, the genius behind Monet's water lilies (Holmes, 2015). In one of his letters to Robinson, Latour–Marliac admitted to have undertaken a haphazard approach in some of his initial water lily crosses, which subsequently led to confusion of the parentage of his first ever water lily hybrids (Pring, 1934; Holmes 2015). His initial crosses were between white coloured species such as *Nymphaea alba* L. (Nymphaeaceae) from Europe, *N. candida* J. Presl (Nymphaeaceae) from Bohemia in central Europe, *N. odorata* Aiton. (Nymphaeaceae) and *N. tuberosa* (Paine) Wiersema and Hellq. (Nymphaeaceae), from North America as well as a number of other tropical *Nymphaea* species which were blue, red and yellow in colour, including *N. mexicana* (Latour–Marliac, 1899; Perry, 1989; Les and Philbrick, 1993; Holmes 2015; Holmes, 2016). Due to the confusion regarding the parentage of the hybrids, he could not assign an exact botanical parentage and therefore classed all of them as 'Castalia' (Latour–Marliac, 1899). Latour–Marliac considered many of these crosses unstable, since only a few of the hybrids were able to yield viable pollen for further hybridization (Latour–Marliac 1899; Pring, 1934; Holmes, 2016). These first hybrids were also not capable of reproducing asexually through the fragmentation of their rhizomes (Holmes, 2016). However sexual crosses between fertile hybrids with other species which could reproduce asexually through the fragmentation of their rhizomes, led to the creation of 'some remarkably hardy novelties', as described by Latour – Marliac himself, in one of his letters to Robinson (Latour–Marliac, 1899; Holmes, 2015; Holmes, 2016). These 'hardy' hybrid waterlilies were then later made popular by

Monet through his paintings, and Latour – Marliac's hybrids were subsequently transported to water gardens throughout the world (De Socarraz– Novoa, 2012; Holmes, 2015).

The first *Nymphaea* hybrid to be recorded in the wild in South Africa was the cultivar *Nymphaea* var. *marliaceae chromatella*, which has pale yellow flowers, with petals that are ovate in shape at the tips and waxy sepals that are green with deep pink hues (Mauve, 1968); Henderson and Cilliers, 2002). This hybrid was first recorded on the banks of the Vaal River in the Gauteng province (Mauve, 1968). *Nymphaea* var. *marliacea chromatella* as well as the pure *N. mexicana*, subsequently began to spread throughout the country, through human introduction into various provinces (Henderson and Cilliers, 2002), and through the use of amplified fragment length polymorphisms (AFLPs), there is evidence that more than one hybrid of *N. mexicana* currently exists in South Africa (Chapter 2). In recent years, these hybrids could have been introduced into the country by plant nurseries and subsequently escaped into the wild (Henderson and Cilliers, 2002). Due to the nitrogen enriched waters in South Africa and the control of the major floating aquatic weeds in recent years, these hybrids have become opportunistic invaders (Coetzee, et al., 2011a; Hill and Coetzee, 2017). There is currently no knowledge of the breeding systems for any of these *N. mexicana* hybrids in South Africa.

Understanding the reproductive traits of an invasive plant species is an important component in invasion biology, since these traits are tightly linked to the fitness of a plant, and are therefore one of the mechanisms driving the invasion process (Baker, 1965; Roy, 1990; Rejmánek and Richardson, 1996; Burns et al., 2011). The knowledge of the breeding system of an invasive plant can be used to elucidate its mating strategy, and the subsequent quantity and quality of propagules which disperse and spread to new environments (Sans et al., 2004; van Kleunen and Johnson, 2007; Barrett, 2011). This information can be useful in the development of an appropriate management programme against the invasive plant species (Coetzee et al., 2011b; Hill and Coetzee, 2017). Invasive plant species may have a great diversity in terms of their reproductive systems and the ways in which they utilize these systems to produce offspring (Barrett, 2008). They can either engage in sexual reproduction through pollination, or asexual reproduction through the fragmentation of rhizomes and/or stems (Barrett and Hough, 2012, Lande, 1980, Thomson and Barrett, 1981). Some invasive plant species may even engage in a combination of these two reproductive strategies (Barrett and Hough, 2012; Barrett, 2011). Asexual reproduction or apomixis allows plants the freedom to reproduce without having to rely on pollinators (Barrett, 2008; Barrett, 2011).

Asexual reproduction is common amongst invasive plant species and has been reported to occur in all five of the once major aquatic invasive plant species in South Africa; *Eichhornia crassipes, Pistia stratiotes, Salvinia molesta, Azolla filiculoides* and *Myriophyllum aquaticum* (Henderson and Cilliers, 2002; Coetzee et al, 2011b; Hill and Coetzee, 2017) (Table 3.1). However some of these aquatic invasive plants are also capable of sexual reproduction through seeds, which become fertilized through the pollination of their flowers (Henderson and Cilliers, 2002; Coetzee and Hill, 2011) (Table 3.1).

Primary and secondary sexual characteristics contribute to a plants reproductive strategy (sexual or asexual), which in turn, influences the degree of mating that occurs among unrelated individuals (outcrossing) vs within an individual (self– fertilization) (Ellstrand, 1992; Barrett, 2008). Plants that are introduced and which subsequently spread into new environments, are considered to have the reproductive trait of self – compatibility (Willson, 1979; Barrett, 2008). Invasive plants have been found to frequently engage in self – fertilization or "selfing", which occurs when a plant is self – compatible and can mate with itself, and therefore does not depend on the pollen from another plant in the population to complete the fertilization process (Rambuda and Johnson, 2004; van Kleunen and Johnson, 2007). This mating strategy has resulted in the successful colonization and spread of numerous plant species around the globe (Trivers, 1972; Willson, 1979; Thomson and Barrett, 1981). This is in accordance to 'Baker's rule', which predicts that the chances of a self – compatible plant species establishing in a new environment, after a long distance dispersal event would be much higher than that of a self – incompatible plant species, since the former would be able to create a sexually reproducing population from a single individual (Baker, 1974). Some invasive plant species in South Africa have been found to efficiently self – fertilize, including *Solanum mauritianum* Scop. (Solanaceae), *Lantana camara* L. (Verbenaceae) and *Sesbania punicea* (Cav.) Benth. (Fabaceae) (Rambuda and Johnson, 2004). *Lantana camara,* similarly to *N. mexicana,* has many invasive garden varieties that have been introduced into South Africa as ornamentals through the horticultural trade (Cilliers and Nesser, 1991; Vardien et al., 2012). Apart from self – fertilization, the *L. camara* hybrid complex has also been found to engage in outcrossing among individuals of the same cultivar as well as between individuals of different cultivars (Spies, 1984; Spies and du Plessis, 1987). This dual strategy of self and cross-fertilization is common amongst many plant species, however there are also plant species which have evolved mechanisms to completely avoid self – fertilization (Barrett, 2008; Barrett and Hough, 2012). This is due to

the high genetic loads that are associated with self – fertilization, which often results in the expression of deleterious alleles that are harmful to plant populations in the long term (Willson, 1994). Therefore, selfing is a good solution for the short–term, but not for the long term with regards to evolutionary time and the ultimate survival of a plant species under changing conditions (Willson, 1979; Grant, 1995; Barrett and Hough, 2012).

Self – incompatibility systems have evolved numerous times in independent lineages of flowering plants, as a strategy to limit gender interference in flowers and in turn reduce self–fertilization and promote outcrossing (Franklin–Tong and Franklin, 2003; Charlesworth et al., 2005; Kiepiel and Johnson, 2014). Three types of self– incompatibility systems have been documented; the gametophytic self– incompatibility (GSI) system, the sporophytic self–incompatibility (SSI) system and the late acting self–incompatibility (LSI) system (de Nettancourt, 1997; Charlesworth et al., 2005; Kiepiel and Johnson, 2014). Apart from self – incompatibility systems, some plant species have evolved floral polymorphisms as a strategy to limit gender interference, such as heterostyly (Ganders, 1979; Barrett, 2002). All flowers share the same morph on each individual plant in the population and the lengths of the pistils and stamens differ between morphs (Mulcahy, 1975; Barrett, 1988, Barrett, 1998). This intricate system has evolved as a means to disable the occurrence of self– fertilization, thus only flowers that are from different morphs can successfully complete the fertilization process (Barrett, 1998, Barrett, 2002). *Eichhornia crassipes, Pontederia cordata* and *Lythrum salicaria*, invasive aquatic plants in South Africa, all exhibit floral polymorphisms (Barrett, 1977; Henderson and Cilliers, 2002) (Table 3.1). *Eichhornia crassipes,* for example, is tristylous, having three different flower morphs, which prevents the occurrence of pollen being transferred onto the stigma of the same flower and promotes outcrossing (Barrett, 1977; Haynes, 1988).

Outcrossing increases genetic variation of a species and in turn increases the invasive potential of that species (Ellstrand, 1992; Ellstrand and Schierenbeck, 2000). Outcrossing also increases the likelihood of hybridization between species, as observed in the *L. camara* hybrid complex in South Africa (Spies, 1984; Ellstrand, 1992; Rieseberg, 1997; Ellstrand and Schierenbeck, 2000). As mentioned previously, outcrossing in the genus *Nymphaea* is also well documented, with numerous cultivars, having been created through artificial crosses between water lily species from different regions of the world (Latour–Marliac, 1899; Pring, 1934; Perry, 1989). Gene flow occurring naturally in the wild, leading to hybrids between *Nymphaea* species, such as, *N. nouchali* var. *caerulea* (Savigny) Verdc. (Nymphaeaceae)

with *N. micrantha* Guill and Perr. (Nymphaeaceae) in Sri Lanka, as well as *N. odorata* with *N. mexicana,* in the USA, have also been reported (Wiersema and Hellquist, 1997; Woods, 2003; Yakandawala et al., 2017).

Nymphaea species are hermaphroditic, and produce bisexual flowers, which have both male and female sexual organs (Conard, 1905; Wiersema, 1988). However these flowers exhibit dichogamy, in which the female and male sexual organs become receptive at different times (Schneider and Chaney, 1981; van der Velde, 1986; Wiersema, 1988). This flowering period of the plant is referred to as anthesis, and is diurnal as reported by Capperino and Schneider, (1985). Flowers are protogynous and function on the first day as female, with the stigmatic papillae releasing a small quantity of fluid, which attracts pollinators and allows them to crawl onto the stigmatic surface (Capperino and Schneider, 1985). The secretion then aids in loosening the pollen off the insect's body and onto the stigma. On the second day of anthesis, the stigmatic fluid disappears and the anthers begin to dehisce (Capperino and Schneider, 1985, Schneider and Chaney, 1981). Upon completion of anthesis, the peduncle coils, which causes the flower to submerse underwater (Capperino and Schneider, 1985, Schneider and Chaney, 1981). Once the peduncles of the flowers have submerged, fruit development takes place under water for a period of 1– 2 weeks (Capperino and Schneider, 1985; Cook, 2004). Ripened fruit then eventually burst releasing fertilized seeds into the substrate which, over a period of time, begin to germinate (Capperino and Schneider, 1985; Cook, 2004).

This sexual reproductive process is similar to that of many other *Nymphaea* species such as *N. odorata*, *N. alba*, *N. candida* and *N. elegans*, (Schneider and Chaney, 1981; Schneider, 1982; van der Velde, 1986). Therefore, hybridization between each of these species can easily be achieved, since all of these species are protygonous, exhibiting the female phase on the first day and the male phase the day after, and with all sharing the same insect groups as pollinators (Schneider and Chaney, 1981; van der Velde, 1986; Wiersema, 1988). However, some of the reported hybrids for these crosses, have also been found to be sterile, due to high levels of ploidy (Gupta, 1980; Les and Philbrick, 1993). A positive correlation has also been found between polyploidy and invasive plants, suggesting that the genus *Nymphaea,* which exhibits high and differing levels of polyploidy is more than likely to be invasive, similar to *L. camara* (Gupta, 1978; Spies, 1984; Pandit et al., 2011). *Nymphaea mexicana* also engages in asexual reproduction through the fragmentation of its tuberous rhizomes which gives rise to new plants (Henderson and Cilliers, 2002; Cook,

2004), as well as long stolons which run horizontally on the substrate under the water that can give rise to daughter plants (Cook, 2004) (Table 3.1). Both, sexual and asexual reproductive strategies of *N. mexicana*, have contributed significantly to the invasion of this species worldwide (Dugdale et al., 2012; GBIF, 2018).

Although the mode of reproduction of *N. mexicana* has been previously studied and documented (Capperino and Schneider, 1985), no studies have been done on the breeding systems or pollinators associated with invasive *N. mexicana* hybrids that occur in South Africa. Thus understanding the way in which these hybrids reproduce is an important aid in understanding their spread and invasiveness in the country, especially since they are much more widespread in South Africa than the pure *N. mexicana*. Therefore the aims of this study were, (1) to investigate the breeding systems of two of the most widespread invasive *N. mexicana* hybrids in South Africa, in order to determine if these hybrids can reproduce sexually, similarly to the pure *N. mexicana*, and (2) to investigate the pollinators associated with an *N. mexicana* hybrid, in order to compare if the pollinators associated with the invasive *N. mexicana* hybrid are similar to those that were found to be associated with the pure *N. mexicana* in the USA in a study conducted by Capperino and Schneider, 1985. This will aid by providing insight into the groups of insects which could be responsible for gene flow between hybrid *N. mexicana* plants, and in turn contribute to the possible sexual reproduction and creation of new hybrids between existing hybrids in South Africa.

3.2) Materials and methods

3.2.1) Breeding system studies

Study species

Pure *N. mexicana* as well as two hybrids were used for these breeding system experiments. The pure *N. mexicana* was collected from waterbodies at Westlake in the Western Cape Province (Figs. 3.1A, 3.4). One of the hybrids was collected from an invaded pond at North West University (NWU) in the North West Province of South Africa, and had been identified as the cultivar – *Nymphaea* var. *marliaceae chromatella* (Figs. 3.1B,. 3.2A, 3.4). The second hybrid was collected from an invaded dam on a farm in Knysna in the Western Cape Province of South Africa and is an unidentified Marliac hybrid (Figs. 3.1C, 3.2B) that grouped closely to *N.* var. *marliaceae chromatella*, in the PCA and SplitsTree analyses (Chapter 2). Both hybrids as well as the pure *N. mexicana* showed great differences

in their flower morphologies, with hybrid plants from NWU producing flowers with a greater number of petals than those produced by hybrid plants from Knysna and the pure *N. mexicana* (Table 3.2; Figs. 3.1, 3.2). However, the sepal and petal lengths for hybrid plants from Knysna were longer than those of the NWU hybrid as well as the pure *N. mexicana* (Table 3.2). Both hybrids, including the pure *N. mexicana* produce around 50 stamens per flower, however the NWU hybrid was found to have stamens that were greater in length than the Knysna hybrid as well as the pure *N. mexicana* (Table 3.2). Although the leaf morphologies for both hybrids are similar, and can easily be confused with one another, especially without the presence of flowers, they are very different to that of the pure *N. mexicana* (Table 3.3; Fig. 3.3). The pure form is easily distinguishable from the hybrids due to its paler green colour, fewer red blotches and serrations on the outer edges (Table 3.3; Fig. 3.3).

Figure 3. 1: (A) Pure *Nymphaea mexicana*, from Westlake, Western Cape Province. (B) *N. mexicana* Hybrid 1, from North West University (NWU), North West Province, (C) *N.*

mexicana Hybrid 2, from Knysna, Western Cape Province. Images A – C taken by Prinavin Naidu.

Table 3. 1: Flower characters of *Nymphaea mexicana* plants from Westlake and *N. mexicana* hybrid plants from NWU and Knysna in South Africa.

Species	**Flower colour**	**No of Petals**	**Petal length**	**Petal breadth**	**No of Sepals**	**Sepal length**	**Sepal breadth**	**No of Stamens**	**Stamen length**	**Stamen breadth**
N. mexicana from Westlake	bright yellow	23	21 – 47 mm	6 – 13	4	45 mm	13 mm	50	10 – 21 mm	1 – 3 mm
N. mexicana hybrid from NWU	pale yellow	24	23 – 49 mm	10 – 18 mm	4	47 mm	22 mm	50	20 – 26 mm	1 – 5 mm
N. mexicana hybrid from Knysna	pale yellow	20	29 – 53 mm	6 – 19 mm	4	50 mm	21 mm	50	12 – 23 mm	1 – 5 mm

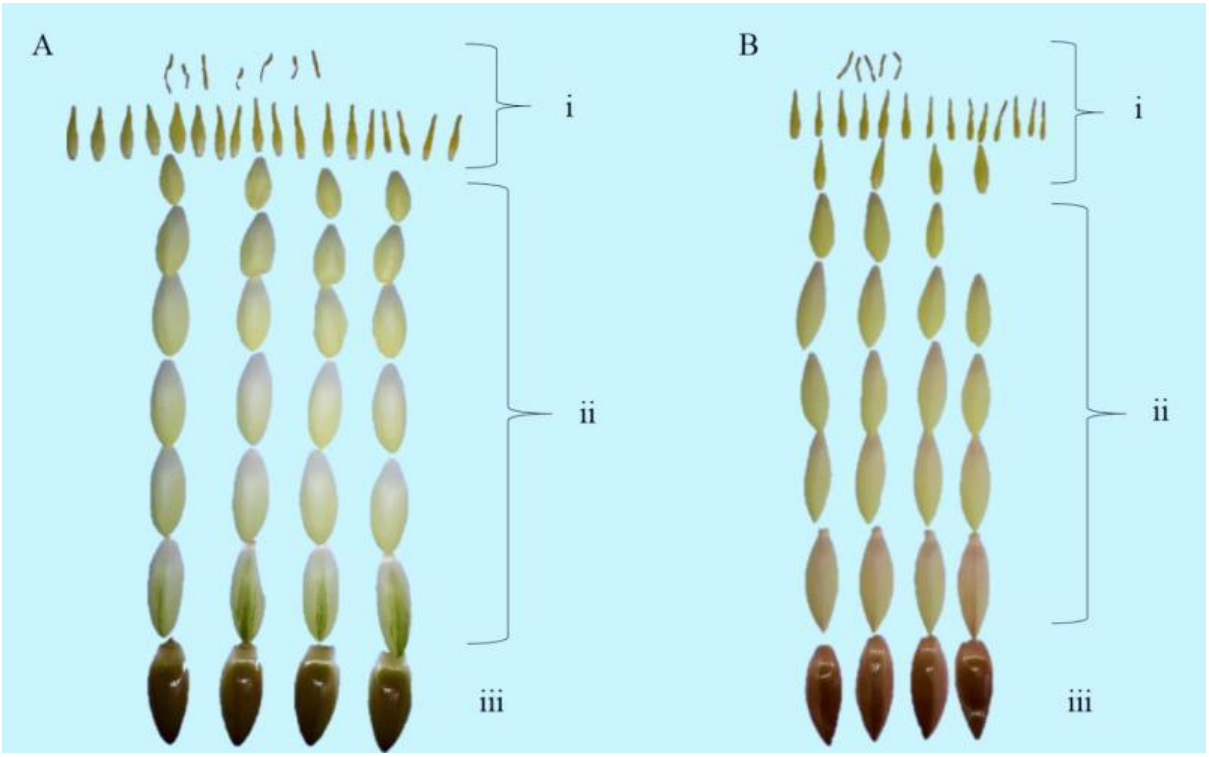


Figure 3. 2: Digital images of two hybrids of *Nymphaea mexicana*. (A) Hybrid from North West Province and (B) Hybrid from Knysna. (i) Anthers, not all are presented in the figure, (ii) Petals, (iii) Sepals. The petals of the North West Province hybrid (A) are wider and more rounded at the tips than the Knysna hybrid (B), which has more narrow and pointed petals.

Table 3. 2: Leaf characters of *Nymphaea mexicana* plants from Westlake and *N. mexicana* hybrid plants from NWU and Knysna in South Africa.

Species	**Leaf shape**	**ventral surface colour**	**Dorsal surface colour**	**Leaf edge**
N. mexicana from Westlake	ovate	green with small bright red streaks, more prominent in younger leaves	bright red with black speckling	inconsistent serrations
N. mexicana hybrid from NWU	circular	green with large dark red streaks, more prominent in younger leaves	deep red with black speckling	smooth
N. mexicana hybrid from Knysna	circular	green with large dark red streaks, also more prominent in younger leaves	deep red with black speckling	smooth

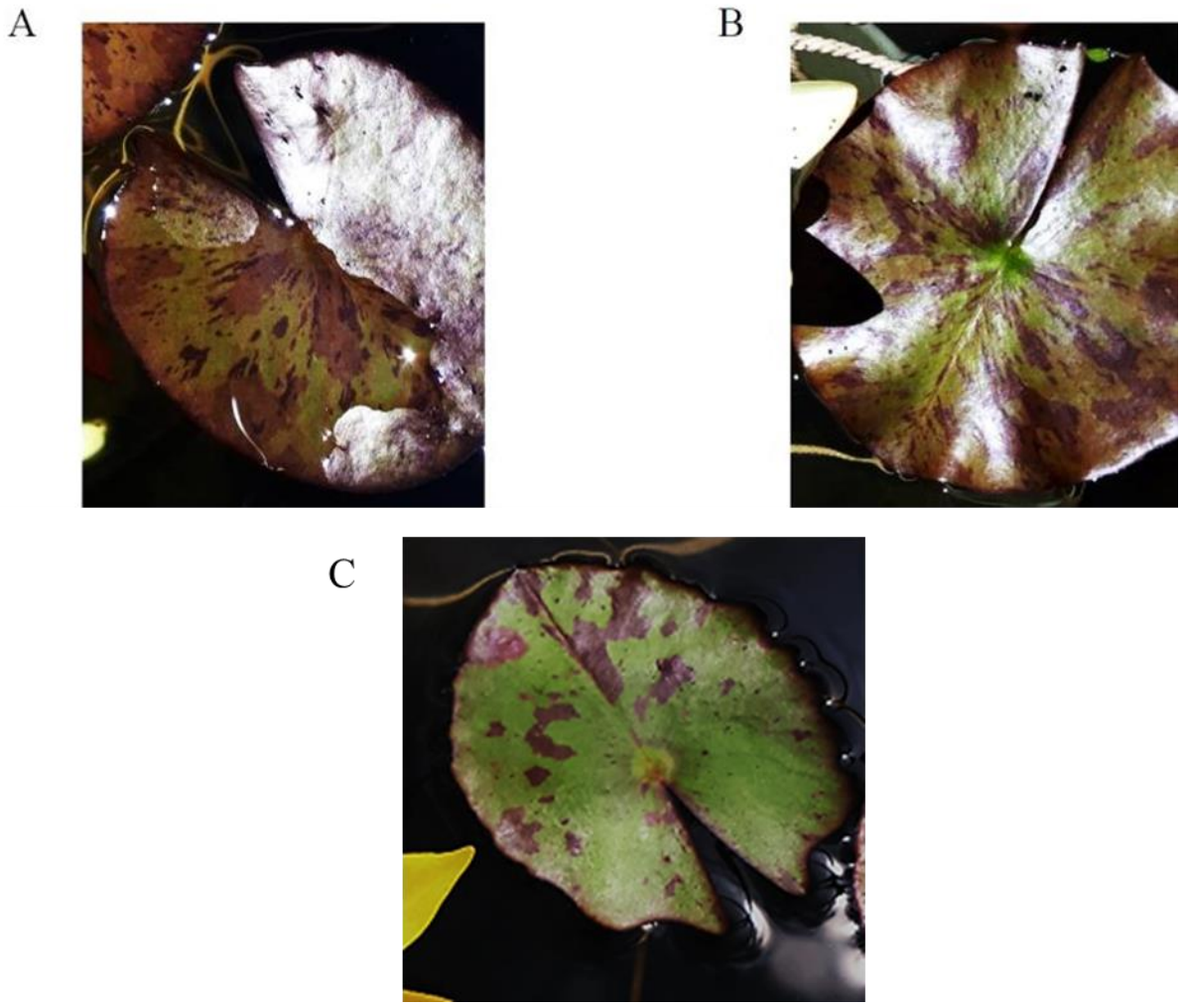


Figure 3. 3: Digital images of leaves from *Nymphaea mexicana* hybrids and the pure *N. mexicana*. (A) Leaf of the Knysna hybrid, (B) leaf of the North West Province hybrid. (C) leaf of the pure *N. mexicana* from Westlake. Images A – C taken by Prinavin Naidu.

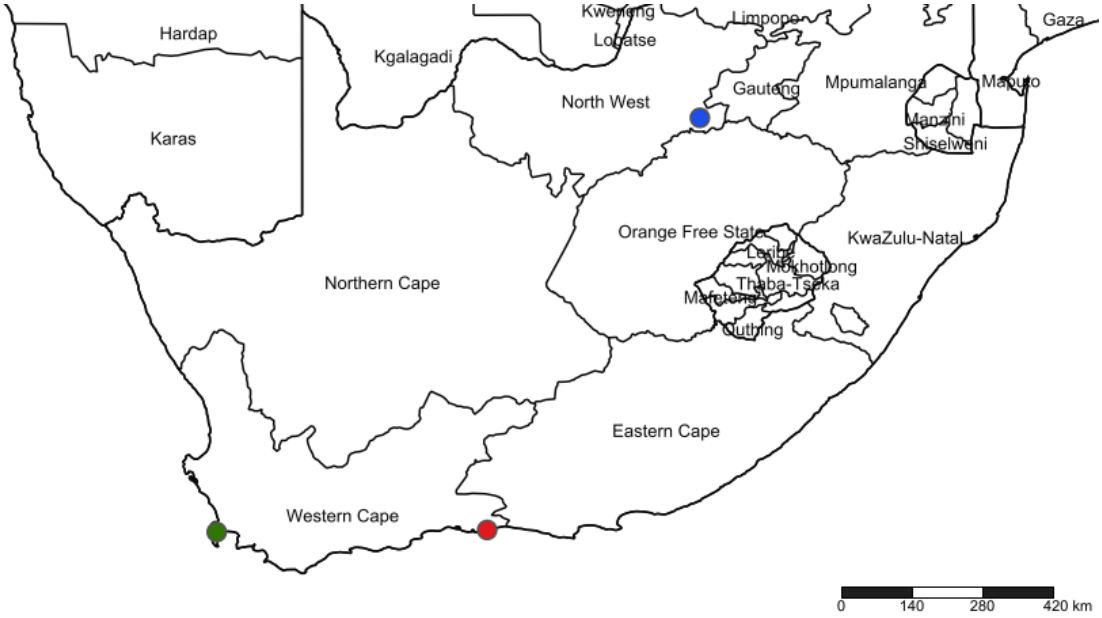


Figure 3. 4: A map of South Africa showing the sites at which plants for the breeding system experiments were collected. The green dot (●) indicates pure *N. mexicana* plants collected from waterbodies at Westlake, in the Western Cape Province. The red dot (●) indicates *N. mexicana* hybrid plants collected from a dam at Knysna, in the Western Cape Province. The blue dot (●) indicates *N. mexicana* hybrid plants collected from a pond at North West University, Potchefstroom, in the North West Province.

Experimental procedure

The experiments were conducted at the Department of Zoology and Entomology at Rhodes University in Grahamstown in the Eastern Cape Province. Hand pollination experiments of concealed flowers were conducted to determine the breeding systems of the three test species (pure *N. mexicana*, *N. mexicana* hybrid 1 from North West University and *N. mexicana* hybrid 2 from Knysna). Plants were potted in sediment sourced from Jameson dam, Grahamstown (33°19'06.8"S 26°26'25.7"E) in plastic flower pots (20 cm x 30 cm) and placed within concrete pools (200 cm x 100 cm; 1672 litres). Umbrella style food covers (43 cm x 43 cm) made of nylon mesh, were used as concealment/ exclusion cages of flowers to prevent pollinators from interacting with the flowers. The food covers were modified to allow for floating on the water surface. Two polystyrene strips (30 cm x 5 cm) were attached onto the base of the food cover at either end to allow floating to occur. Cotton string with stones at the ends were tied onto the base of the food covers and the stones were submerged into the

water to prevent movement of the cages around the pool. Four treatments were conducted on each of the three test species:

1) Treatment 1: Non–concealed and un–manipulated flowers
   Flowers were left open to allow free movement of pollinators and flower visitors.
2) Treatment 2: Concealed and un–manipulated flowers
   Flowers were concealed using a floating exclusion cage. The flowers were un–manipulated to test if autonomous self– pollination would occur.
3) Treatment 3: Concealed and manipulated (geitonogamy/ selfing) flowers
   Pollen from the anthers of a day 2 opened flower were transferred onto the stigma of a day 1 opened flower of the same plant, using a fine paint brush. This treatment was done to test if the plant species would engage in self– pollination.
4) Treatment 4: Concealed and manipulated (outcrossing) flowers
   Pollen from the anthers of a virgin flower were transferred onto the stigma of another virgin flower of a different plant, using a fine paint brush. This was done to test if the plant species would engage in cross–pollination.

A total of twelve concrete pools, located on the Rhodes University campus, were used for the entire experiment. Four concrete pools (1 per treatment) were designated to each of the three test plants. For each treatment, 20 virgin flowers were used and were spread across at least six plants (to ensure sufficient genetic variability). All treatments were nested together within plants and pools so that differences between treatments would not reflect individual plant effects. For pollination, fine – tipped paint brushes were used to collect the pollen off the anthers of male flowers. The paint brush containing the pollen was then dipped into the stigmatic fluid of the female flower. Each pollinated flower per treatment was then tagged and monitored daily for fruit development. Statistica® ver. 13.2 (StatSoft) was used to run a one–way ANOVA test on the raw data collected from each treatment for both plant hybrids.

3.2.2) Pollinator studies

Study species'

An *N. mexicana* hybrid population from a dam at Emmarentia in Randburg, Gauteng Province in South Africa was used for the pollinator experiment (Figure 3.5).

Figure 3. 5: A light pink *N. mexicana* hybrid, in a dam at Emmarentia in Randburg, Gauteng Province.

Experimental procedure and statistical analyses.

Pollinators visiting the flowers of an *N. mexicana* hybrid at Emmarentia were observed for five minute time intervals in the morning and afternoon for a period of three consecutive days. A boat was used to sample flowers across the entire dam, which had a surface area of 246,63 meters, with a 75% cover of the *N. mexicana* hybrid as the predominant vegetation in the dam. The duration of visitation for both periods of the day were recorded in random patches of plants with flowers, spread across the entire dam for 5 minute intervals using a voice recorder. The number of approaches and landings made by different insect groups on flowers of the test species used in this study was recorded. The above procedure was conducted for male as well as female flowers. Generalized linear models (GLMs) were run on the data as well as likelihood ratio tests (LRTs) and chi square tests, in RStudio desktop (RStudio inc.) to test for significant differences between pollinators and the time of day.

3.3) Results

3.3.1) Breeding systems

Nymphaea mexicana hybrids from North West University (NWU), Potchefstroom, in the North West Province and Plettenburg, Knysna in the Western Cape Province did not

produce fruit across all treatments (Fig. 3.7). The ovaries of these hybrids would submerge underwater and degrade two days after pollination before forming fruit. However the average number of unfertilized seeds produced by plants from NWU was significantly higher than that of the number of seeds produced by plants from Knysna ($F_{(6, 72)} = 1.5173$, $P < 0,001$) (Fig. 3.6). Plants from NWU produced the greatest number of seeds for the concealed selfing treatment, whilst plants from Knysna produced the greatest number of seeds for the open un–manipulated treatment. However, this finding is inconsequential, since as mentioned above, none of the treatments for plants from both sites developed into fruit. The same treatments were to be carried out on pure *N. mexicana* plants obtained from Westlake in the Western Cape Province, South Africa, however the plants did not produce enough flowers for all of the treatments to be conducted and is therefore not represented in Figure 3.6. Although a few flowers that were cross pollinated developed into fruit (Fig 3.8 (Bi and Bii)), indicating that the pure *N. mexicana* present in South Africa does engage in sexual reproduction, unlike its hybrids (Fig 3.8 A).

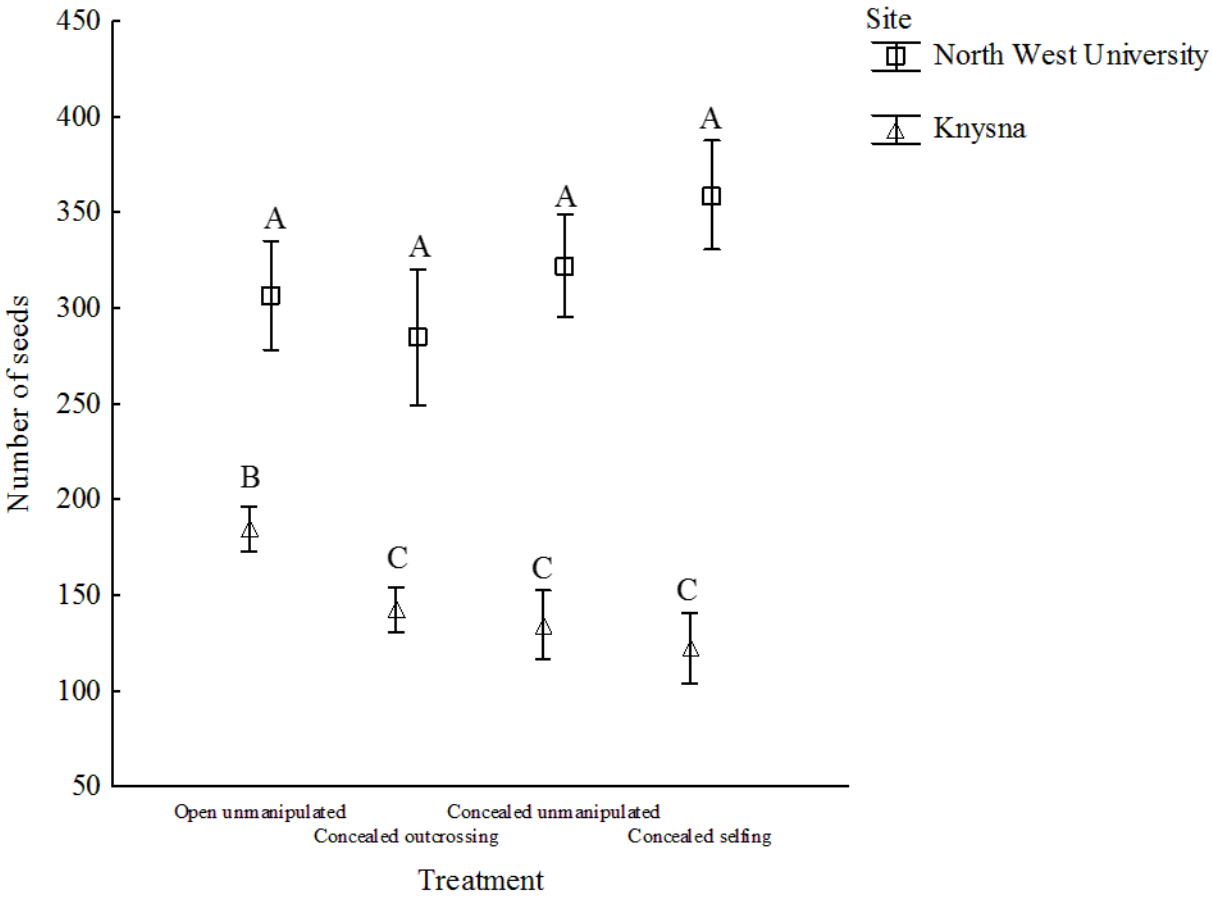


Figure 3. 6: Mean number of seeds (±S.E.) produced per ovary for each treatment for two *Nymphaea mexicana* cultivars collected from North West University, and Knysna. Error bars represent standard error. Letters indicate significant differences, $P < 0.001$; One – way ANOVA.

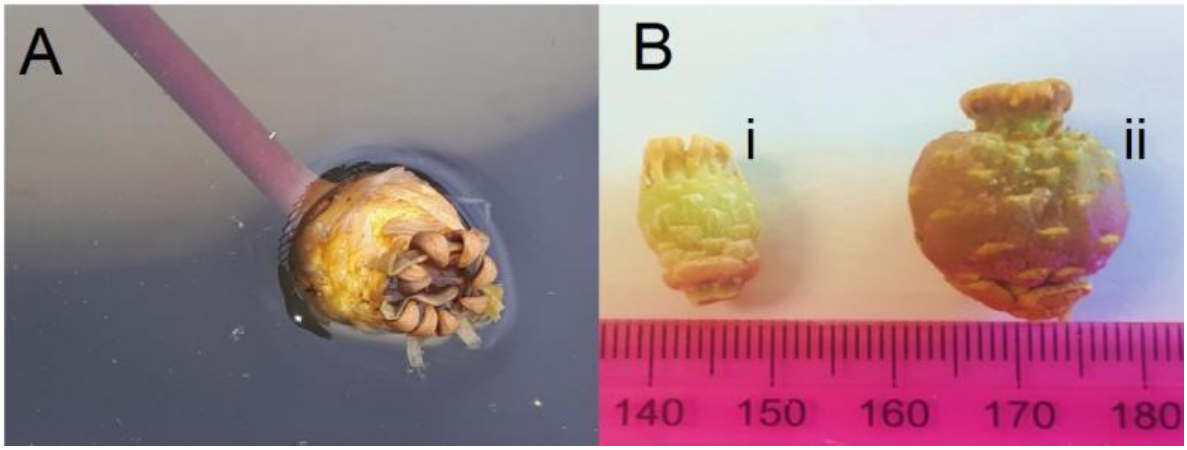


Figure 3. 7: (A) A pollinated ovary of *Nymphaea* var. *marliaceae chromatella*; the ovary would submerge underwater after pollination and after two days degrade, without forming into a fruit. (Bi) An unpollinated ovary of the pure *N. mexicana*, (Bii) a pollinated ovary of the pure *N. mexicana*, developing into a fruit after being cross pollinated.

3.3.2) Pollination studies

The time taken for insect groups to probe male and female flowers of a pink *N. mexicana* hybrid, present in a dam at Emmarentia, Randburg, Gauteng, was recorded for two periods (morning and afternoon) in a day. Significant differences were found between flowers that were pollinated in the morning versus flowers pollinated in the afternoon (LRT, $\chi^2 = 646{,}07$, df = 1, $P < 0{,}001$). The average probe time for both male and female flowers was significantly higher for time periods recorded in the morning than those recorded in the afternoon (Fig 3.8). There were also significant differences between the number of pollinator visits to male flowers versus female flowers (LRT, $\chi^2 = 1594{,}09$, df = 1, $P < 0{,}001$). On average, male flowers were visited more than female flowers regardless of the time of day (Fig. 3.8). A significant interaction between the sex of the flower and the time of day was also observed (LRT, $\chi^2 = 189.77$, df = 1, $P < 0{,}001$).

Insect groups that visited the flowers of this *N. mexicana* hybrid, included, honeybees, *Apis mellifera scutellata*, Lepeletier, 1836. (Apidae), halictids, *Halictus spp.*, Thomson, 1869. (Halictidae), scarab beetles, Latreille, 1802. (Scarabaeidae) and syrphid flies Latreille, 1802. (Syrphidae) (Fig. 3.9). There were significant differences observed between the insect groups that probed both male and female flowers (LRT, χ^2 = 2980,93, df = 3, $P < 0,001$). On average, honeybees probed both male and female flowers more than any of the other three insect groups (Figs. 3.9, 3.10). Flies probed male flowers the least, compared to the other three insect groups, regardless of the time of day (Fig. 3.9) Beetles were the only insect group which did not probe any female flower regardless of the time of day (Fig. 3.9). There was also a significant interaction observed, between the time of day, sex of flower and the insect group (LRT, χ^2 = 328,33, df = 10, $P < 0,001$). On average, male flowers were approached and probed more than female flowers, regardless of the time of day and the insect group (Fig. 3.9).

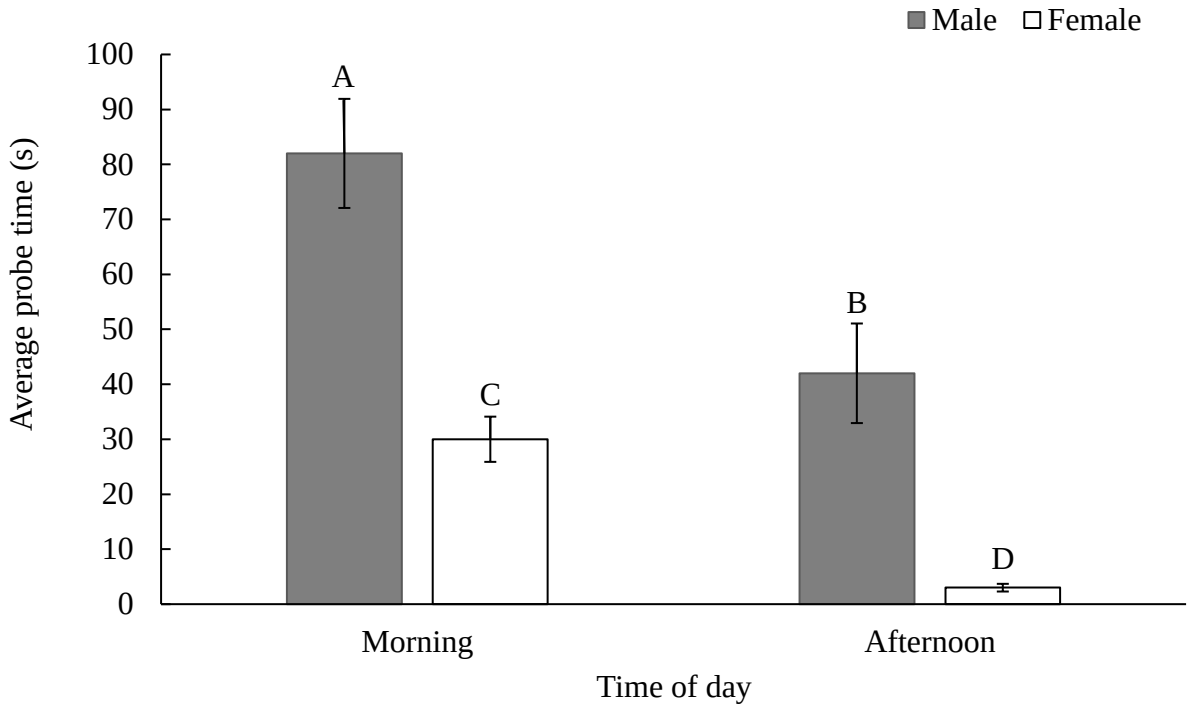


Figure 3. 8: The average time taken for all pollinators to probe the anthers of male and female *Nymphaea mexicana* hybrid flowers at Emmarentia dam, Randburg, Gauteng Province, for different parts of the day (morning and afternoon). Letters indicate significant differences, $P < 0.001$; General Linear Model (GLM).

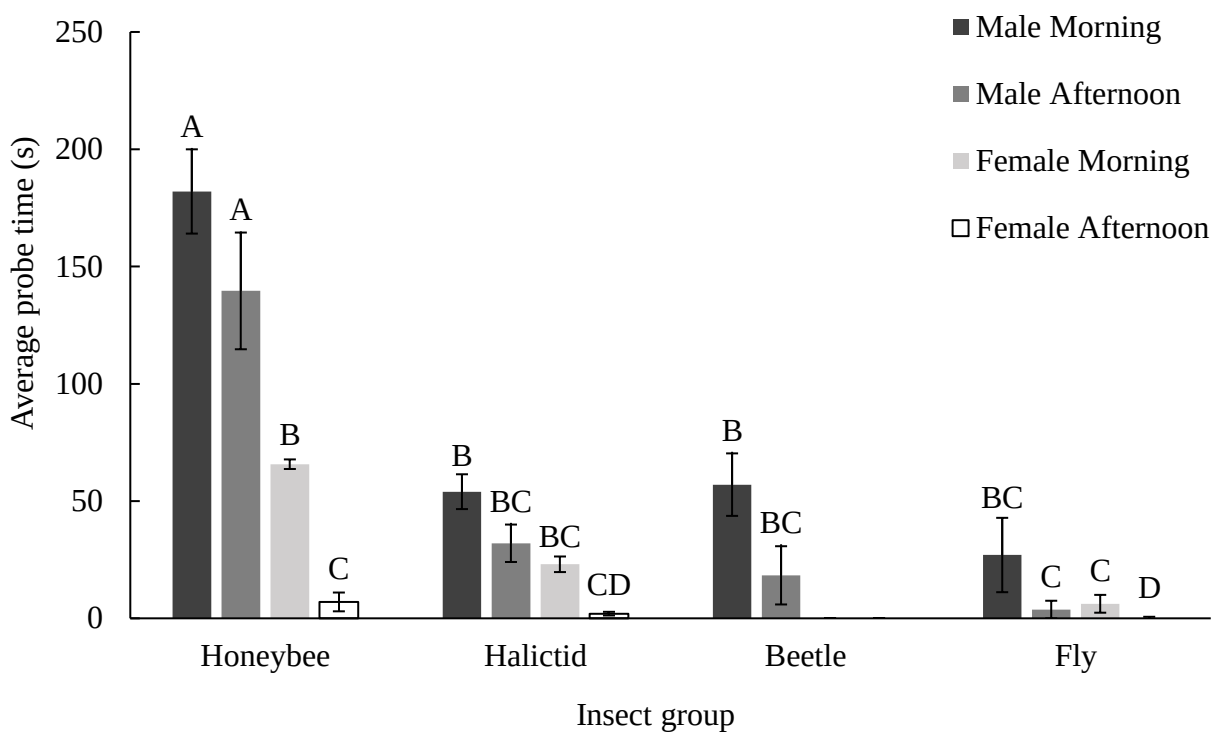
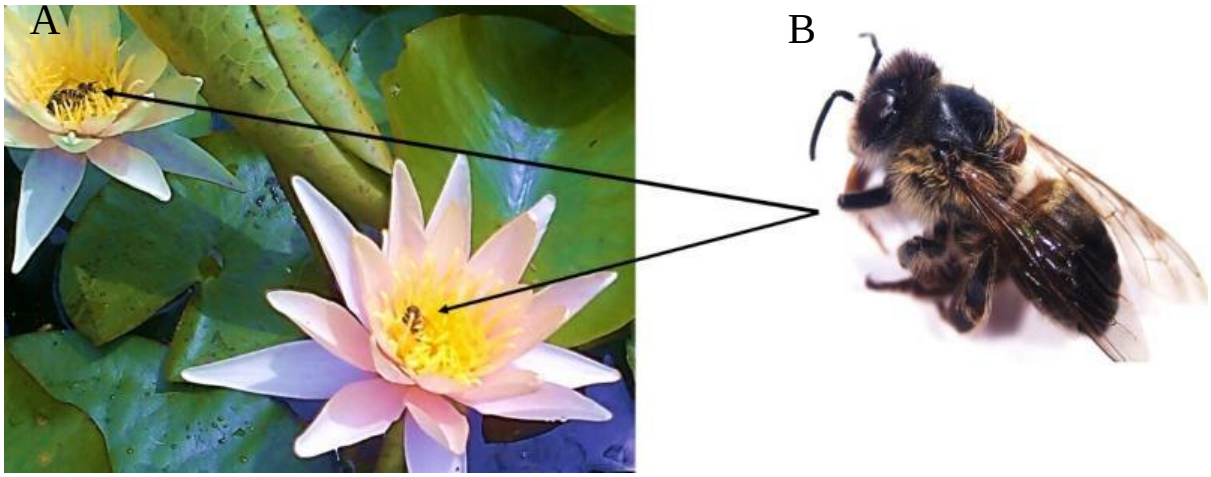


Figure 3. 9: The average time taken by each insect group to probe the anthers of male and female *Nymphaea mexicana* hybrid flowers at Emmarentia dam, Randburg, Gauteng Province, for morning and afternoon. Letters indicate significant differences, $P < 0.001$; General Linear Model (GLM).



.

Figure 3. 10: Digital images, showing honeybees pollinating a pink *Nymphaea mexicana* hybrid in a dam at Emmarentia, Randburg, Gauteng Province. (A) Honeybees probing the

flowers of *N. mexicana* hybrids for pollen. (B) A Leica microscope image of a dead honeybee specimen collected from one of the pink hybrid flowers in a dam at Emmarentia.

3.4) Discussion

Breeding system experiments

The breeding systems of *N. mexicana* hybrids present in South Africa have not been examined prior to this study. This study thus served to provide insight into the mode of reproduction for two widespread *N. mexicana* hybrids in the country. From field observations, both hybrids used in this experiment were able to reproduce asexually through the fragmentation of their rhizomes (pers. obs.). This is similar to the asexual reproduction of the pure *N. mexicana* (Henderson and Cilliers, 2002; Cook, 2004). However unlike the pure *N. mexicana* which reproduces sexually through seed set in its native range in USA, no records of sexual reproduction for any of the *N. mexicana* hybrids in South Africa existed.

Results from the breeding system experiments for both the Knysna and North West University hybrids revealed that both plants were sterile across all four pollination treatments (self–pollination, cross–pollination, concealed un–manipulated and opened un–manipulated). Neither hybrid produced fruit, with pollinated ovaries submerging underwater and degrading after two days of pollination. While ovaries of the pure *N. mexicana* plants did develop into fruit after the cross–pollination treatment, only a limited number of flowers were produced by the pure *N. mexicana* plants, which made it difficult to complete all treatments for the species. In contrast, the two hybrids produced large numbers of flowers, but were unable to form fruit. The difference in flower production between the pure *N. mexicana* and the hybrids of the species could be due to the hybrids being artificial garden varieties, which were selectively bred to produce large numbers of flowers for aesthetic value (Pring, 1934; Perry, 1989).

Sterile *Nymphaea* hybrids have been reported before, with many water lily breeders and enthusiasts failing to obtain viable seed from intersubgeneric (between subgenera) as well as interspecific (between species) water lily crosses (Pring, 1934; Perry, 1989; Gupta, 1978; Strawn, 2017). A recently discovered *Nymphaea* hybrid; *Nymphaea* x *erangae;* which is a natural cross between *N. nouchali* var. *caerulea* and *N. micrantha*, in the wild in Sri Lanka, was also reported to be sterile (Yakandawala et al., 2017). In addition, cytological studies conducted on eight Indian wild and 11 American cultivated *Nymphaea* species

revealed abnormalities during meiosis, such as desynapsis, chromosome association and segregational irregularities, which are indicative of hybrids that are pollen and/ or ovule sterile (Stebbins, 1971; Gupta, 1978; Wani and Bhat, 2017). There have also been reports stating that only a few of Latour – Marliac's *N. mexicana* hybrids produce pollen that are pod fertile, with a great number producing pollen that cannot fertilize female flowers (Les et al., 1993; Strawn, 2016; Strawn, 2017). The only Marliac hybrids that have been reported to be pod fertile are 'Arc–en–Ciel', 'Sultan', 'Virginalis' and 'Escarboucle'. However, although 'Escarboucle' is pod fertile, it was found to be pollen infertile (Strawn, 2016; Strawn, 2017). Strawn (2017) associates the use of the term 'Marliac hybrid' as an indication of plants that are sterile, since numerous breeders have not been able to achieve fertile seeds from their marliac hybrid waterlilies. Hybrids that were not produced by Latour – Marliac, but by the horticultural trade, such as 'Hollandia', 'Perry's Pink', 'Peter Slocum', 'Pink Opal', 'Pink Sensation' and 'Rosy Morn', to name a few, have all been reported to be pod and pollen fertile, yielding viable seeds (Strawn, 2016; Strawn, 2017). Intersubgeneric crosses between different water lily subgenera have also been reported to have low germination and high seedling mortality rates, which could be the result of the unequal pairing of parent chromosomes during meiosis (Les et al., 2004; Songpanich and Hongtrakul, 2010).

Nymphaea hybrid species with differing chromosome numbers have been reported previously and were found to be diploid, triploid, tetraploid and hexaploid, with some hybrids even exhibiting a combination of ploidy within the same meiotic tissue (Gupta, 1978, Gupta, 1980). A global review on the ploidy levels amongst invasive, rare and localized plants, illustrated a positive correlation between polyploidy and invasiveness (Pandit et al., 2011). Invasive plants that are polyploid potentially exhibit traits such as higher phenotypic plasticity and higher genetic diversity, which in turn allows them to be more tolerant of stress and changing environmental conditions contributing to their invasiveness (te Beest et al., 2011). The *N. mexicana* hybrid complex in South Africa has been found to possess high genetic diversity as well as phenotypic plasticity (Chapter 2). Another invasive species complex in South Africa that shares these attributes is the *L. camara* hybrid complex, which similarly to *Nymphaea* species, has been found to possess extreme ploidal diversity; from diploid, triploid, tetraploid, pentaploid and hexaploid cultivars being reported in the country (Spies, 1984; Spies and du Plessis, 1987). Polyploidy has also been linked to sterility in organisms, due to unequal chromosome numbers, which leads to the formation of unviable gametes during meiosis (Ramsey and Schemske, 1998; Parisod et al., 2010).

Sterility of invasive aquatic plants that have high and differing levels of ploidy have been reported previously, as in *Butomus umbellatus* L. (Butomaceae) (Eckert, 2002; Lui et al., 2005). In North America, diploid populations of *B. umbellatus* were found to be fertile and produced a large number of viable seeds, however populations that were triploid, were sterile and thus incapable of sexual reproduction (Krahulcova´ and Jarolimova´1993; Eckert et al., 2000; Lui et al., 2005). This could be the reason that a great number of hybrids in the genus *Nymphaea* have also been reported to be sterile by water lily breeders around the world, since they possess differing levels of ploidy (Gupta, 1978; Strawn, 2017). The South African *N. mexicana* hybrids could thus have *N. odorata, N. alba, N. tuberosa* or *N. candida,* or even a hybrid between these species, as one of their parents (either pod or pollen) with *N. mexicana* (either pod or pollen) as the other, especially since these species were reported to have been used by Latour–Marliac in his initial crosses (Holmes 2015; Holmes; 2016). *Nymphaea mexicana* and *N. odorata* have also been reported to hybridize in the wild (Wiersema and Hellquist, 1997; Woods, 2003; Woods et al., 2005). However, unlike *B. umbellatus,* and a number of *Nymphaea* species, in which polyploidy may have resulted in sterility, polyploid individuals of the *L. camara* species hybrid complex in South Africa were found to successfully hybridize with one another (Spies and du Plessis, 1987). The pollen from these garden varieties of *L. camara* were fertile and capable of producing viable seeds (Spies and du Plessis, 1987). Although this study looked at outcrossing within the same hybrid, it did not test outcrossing between different hybrids and whether viable seeds can be produced between different *N. mexicana* hybrids in South Africa, which would be a cause for concern.

Nevertheless, regardless of the inability of the *N. mexicana* hybrid complex to reproduce sexually, the hybrids are more than capable of asexual reproduction and can regenerate new plants from fragmented rhizomes (pers. obs.). This mode of reproduction alone can successfully contribute to the invasion of this species into newly introduced waterbodies in South Africa. More especially, since, two of South Africa's worst aquatic invaders, *M. aquaticum* and *S. molesta* have spread throughout and invaded new regions of the country by engaging in asexual reproduction alone (Coetzee et al., 2011a; Coetzee et al., 2011b). *Salvinia molesta* is sterile and, although, *M. aquaticum* is not sterile, there are only female plants present in the country, rendering the species incapable of reproducing sexually (Henderson and Cilliers, 2002; Hill and Coetzee, 2017). There have also been reports of certain *Nymphaea* species being able to engage in vivipary, a process whereby an individual plant can develop epiphyllous plantlets on its lamina, which can detach from the lamina and

float away, eventually establishing into new independent plants (Yakandwala et al., 2017). However, this reproductive process for *Nymphaea* species is regarded as rare and has thus far only been observed to occur in three pure species and one natural hybrid species; *N. micrantha, N. lasiophylla* Mart. and Zucc. (Nymphaeaceae), *N. prolifera* Wiersema. (Nymphaeaceae) and the hybrid *Nymphaea x erangae* (Yakandwala et al., 2017). No epiphyllous plantlets were observed on any of the *Nymphaea* hybrids that were used in this study, so vivipary in South Africa is unlikley. Nevertheless, the *N. mexicana* hybrid complex is potentially capable of reaching the invasive potential of both *S. molesta* and *M. aquaticum* in South Africa and successfully invading new regions of the country, solely through asexual reproduction.

Future cytological studies of the *N. mexicana* hybrid complex in South Africa would be useful in providing further confirmation of the sterility of this invasive complex. In this study, outcrossing between different *N. mexicana* hybrid species was not tested and therefore the possibility of viable seeds being produced from such crosses was not revealed. Thus studies involving the outcrossing between different *N. mexicana* hybrids needs to be conducted. If outcrossed individuals between hybrid species are capable of producing viable seeds, then new hybrids could be created in the wild in South Africa, especially at sites which have more than one infestation of an *N. mexicana* hybrid, which have been observed in the field (pers. obs.). This scenario would then be similar to that of the *L. camara* hybrid complex in South Africa and would be especially challenging in terms of biological control, since, novel and cryptic hybrids between closely related taxa, may create compatibility issues between the biological control agent and the weed (Nissen et al., 1995).

Pollinator studies

In concordance with the breeding system studies of the *N. mexicana* hybrids in South Africa, the pollinators associated with these hybrids have also not been reported or studied before. This study was thus useful in providing insight into the groups of insects which could be responsible for gene flow between hybrid *N. mexicana* plants, and in turn contribute to the possible sexual reproduction and creation of new hybrids between existing hybrids in South Africa. In this study, four insect groups visited and probed the anthers of male flowers and the stigmatic cups of female flowers for nectar, of the *N. mexicana* hybrid. These pollinators included honeybees, halictids, beetles and flies. The predominant flower visitor group in this

study was the honeybees. Honeybees have also been observed to forage on other *Nymphaea* species, such as *N. odorata, N. alba, N. candida* and even the pure *N. mexicana* (Capperino and Schneider, 1985; Schneider and Chaney, 1981; van der Velde, 1986).

Male flowers were visited and probed more frequently than female flowers, by all four insect groups and this could be due to insect foraging preference of pollen over nectar. Fewer visits to female flowers could also have been due to lower quantities of stigmatic fluid being produced and thus not being as rewarding as the male flowers which produce much greater volumes of pollen. The frequency of visits to either sex decreased significantly between the morning and afternoon, with fewer visits by all insect groups towards the afternoon compared to a greater number of visits in the morning. This has also been reported to occur for the pure *N. mexicana,* in which a higher pollinator visitation had occurred during the morning part of the day, peaking around noon, and then decreasing in the afternoon (Capperino and Schneider, 1985). This could be due to both male and female flowers having a decreased reward towards the afternoon, since most of the reward had been foraged by visitors in the morning. Honeybees were the predominant visitors of both male and female flowers for both the morning and afternoon parts. These floral visitors have also been reported to forage on the pure *N. mexicana* and *N. odorata* (Schneider and Chaney, 1981; Capperino and Schneider, 1985). Beetles were the only insect group that did not visit female flowers for both the morning and afternoon observations. This could simply be due to beetles having a preference for pollen over nectar. Beetles visiting flowers of the pure *N. mexicana* in the native range in southern USA, were recorded spending longer periods of time foraging on male flowers for pollen (Capperino and Schneider, 1985). The groups of pollinators observed in this study are not particular to the *N. mexicana* hybrids or pure *N. mexicana,* since they have all been observed and documented for many other *Nymphaea* species (Schneider and Chaney, 1981; Schneider, 1982; van der Velde, 1986; Wiersema, 1988). Thus, these results can be extrapolated to the entire *N. mexicana* hybrid complex in South Africa and not just the hybrid species that was used as a test plant in this study.

Although the breeding system experiments of this study tested for pollination among each individual hybrid and subsequently revealed them to be sterile, it did not test for pollination between different hybrids. The fact that these hybrids have potential pollinators in South Africa creates a cause for concern, since future outcrossing between different *N. mexicana* hybrids in South Africa could be achieved if the pollen transferred between the hybrids is fertile and the ova to which that pollen is transferred is receptive of that pollen. In

this instance, in areas of overlap, hybrids of the existing *N. mexicana* hybrids could be produced in South Africa and, as mentioned above, this would pose a challenge in terms of management of the species.

Conclusion

The purpose of this study was to reveal the breeding systems and pollinators associated with some of the invasive *N. mexicana* hybrids present in South Africa. Two widespread hybrids of *N. mexicana*, in South Africa were found to be self–incompatible, but also cannot produce viable seeds through cross-pollination. However, this study only tested for cross–pollination within the same hybrid and not between different hybrids, therefore the possibility of outcrossing between different hybrids cannot be ruled out. Thus, the main mode of reproduction for both these hybrids is asexual, through the fragmentation of their tuberous rhizomes. Four insect groups that could contribute to the sexual reproduction of these hybrids of *N. Mexicana* were also found.

In the future, cytological studies should be conducted on the meiotic tissue of the entire *N. mexicana* hybrid complex in South Africa, to elucidate if all of the hybrids are sterile. While pure *M. mexicana* in South Africa reproduces both asexually and sexually, hybrids are restricted to vegetative reproduction unless interbreeding studies between the different hybrids indicate that cross hybrid pollination is viable. The lack of sexual reproduction does not exclude the hybrids from the possibility of becoming very serious invasive species, since many of the worst aquatic weeds rely primarily on vegetative reproduction. For the purpose of biological control, this excludes agents that primarily damage the sexually reproductive organs of the plant such as flowers, fruits or seeds, as these agents would only target a small proportion of the problem plants in the country.

Chapter 4: General discussion

4.1) Introduction

In South Africa, there are only two native water lily species; *N. nouchali* and *N. lotus*, with the former also having two wild varieties; *N. nouchali* Burm. f. var. *caerulea* (Sav.) Verdc. and *N. nouchali* Burm.f. var. *zanzibariensis* (Casp.) Verdc. (Fig. 4.1 A and B) (Cook, 2004; BODATSA, 2018). These native water lily species are a natural component of the aquatic flora in South Africa and therefore do not have a negative impact on our natural water systems in the country (Fig. 4.1 C). Unlike the native waterlilies, the introduced water lily species, and in particular *N. mexicana* and its hybrids, negatively impact South Africa's aquatic ecosystems (Hill and Coetzee, 2017). The hybrids are more widespread in the country than the pure form of *N. mexicana*, forming dense mats with their large overlapping leaves which limit recreational activities and prevent sunlight from filtering through the water column (Fig. 4.2) (Chapter 2).

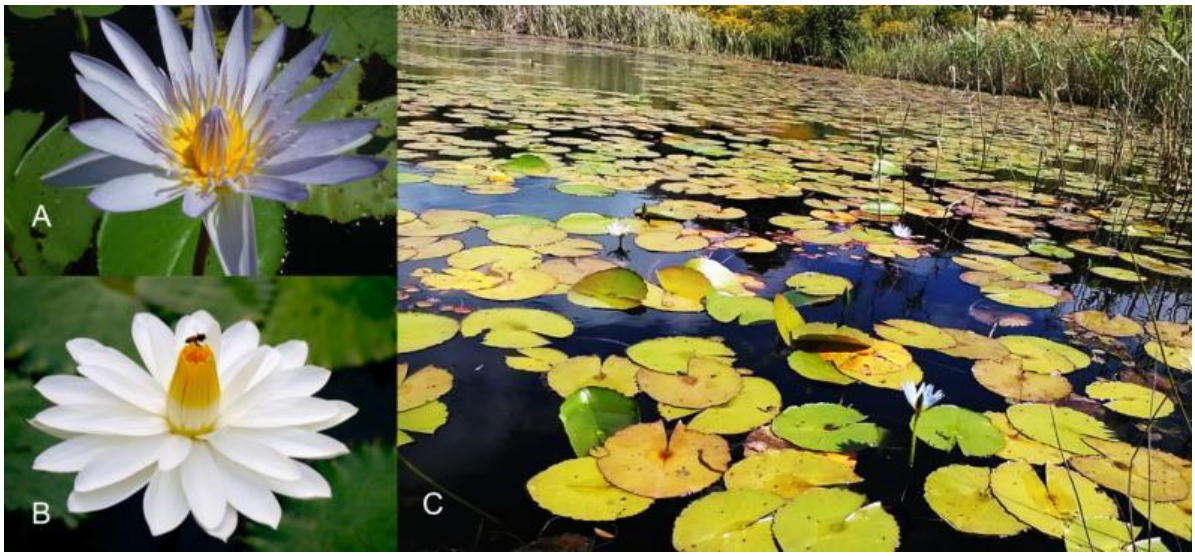


Figure 4. 1: Native *Nymphaea* species in South Africa. (A) *Nymphaea nouchali* (blue water lily), which also has two natural varieties; *N. nouchali* var. *caerulea* and *N. nouchali* var. *zanzibariensis*, in the wild in South Africa. (B) *Nymphaea lotus* (white Egyptian water lily). (C) *N. nouchali* and *N. lotus plants* growing in a dam at George, Western Cape Province, South Africa; NB: the surface of the dam is still visible. Image A and B were taken from google images. Image C was taken by Prinavin Naidu.

Figure 4. 2: *Nymphaea mexicana* hybrid infestations across South Africa; (A) Knysna, Western Cape, (B) Modimolle, Limpopo, (C) Port Elizabeth, Eastern Cape, (D) Emmarentia, Gauteng, (E) George, Western Cape. NB: the surface of the waterbodies are not visible due to large overlapping leaves. Images A, C, D and E were taken by Prinavin Naidu. Image B was taken by Anthony King, 2011.

The main focus of this thesis was to determine the genetic diversity and aspects of the invasion ecology of *N. mexicana* in South Africa because it has not been targeted for biological control elsewhere in the world. The development and realization of this study thus aimed to provide a baseline for the future management of this aquatic invasive species, using biological control. Molecular studies, using amplified fragment length polymorphisms (AFLPs) determined the molecular variation between plants from infested sites around the country and plants from native sites in the USA. It was revealed that more than one genotype of *N. mexicana* occurs in South Africa (Chapter 2). Only one infestation in South Africa which is present in waterbodies at Westlake in the Western Cape Province, matched with native *N. mexicana* plants in southern USA. The ancestry of the remaining invasive water lily populations in South Africa was unknown prior to this study. However, digital images acquired from the infested sites in South Africa linked the morphology of some of these invasive water lily species to hybrids that were created by Latour– Marliac (Chapter 2). A

great number of Latour– Marliac's hybrids have been reported to have *N. mexicana* as either a pod (female) or pollen (male) parent (Perry, 1989; Les and Philbrick, 1993; Holmes 2015). The molecular results of this thesis also revealed a relatively low genetic distance between two widespread Marliac hybrids in South Africa; *N.* var. *marliaceae chromatella* and *N.* var. *cape canaveral* (Chapter 2). Plants from a number of the sampled sites in South Africa for this study, matched closely to either one of these two hybrid species, with both having confirmed *N. mexicana* in their ancestry (Holmes, 2015; Strawn, 2017). We can thus infer that an invasive *N. mexicana* hybrid complex is present in the country. The introductory pathway of *N. mexicana* and its hybrids into South Africa is primarily through the importation of these species by local garden nurseries, which subsequently sell them to the South African public (Henderson and Cilliers, 2002).

The mode of reproduction for some of these forms of *N. mexicana* was therefore also assessed to understand the proliferation and subsequent spread of this invasive water lily hybrid complex around the country (Chapter 3).The breeding system studies for two of the invasive *N. mexicana* hybrids in South Africa, revealed that the plants are sterile (Chapter 3). Pollinators associated with one of these hybrids were found to be similar to those associated with the pure *N. mexicana* in the native range in southern USA (Capperino and Schneider, 1985). However, although these hybrids in South Africa produce a large amount of pollen and have pollinators, they cannot successfully fertilize ova, which may be due to their high and differing levels of ploidy, which has ultimately rendered them infertile. However regardless of the hybrids not being able to reproduce sexually, they can successfully engage in asexual reproduction through the fragmentation of their tuberous rhizomes. The rapid regeneration of new plants from these fragmented rhizomes have posed a challenge for the management of *N. mexicana* and its hybrids in invaded countries, such as Australia, New Zealand and now South Africa (Dugdale 2012; Dugdale et al., 2013; Hofstra et al., 2013). Here I discuss the impact of hybridization and clonality on biological control, followed by the implications for managing the *N. mexicana* hybrid complex in South Africa based on my findings. I also suggest future research and recommendations for the management of the *N. mexicana* hybrid complex in South Africa.

4.2) The impact of hybridization and clonality of invasive plants on biological control

The lack of specialist herbivores for the *N. mexicana* hybrid complex in South Africa, provides compelling evidence for the introduction of various genotypes of this species into the country. Enemy escape is common for invasive plants, with comparisons between native and introduced ranges illustrating significantly lower enemy loads in introduced ranges than in native ranges (Wolfe, 2002; Vila´ et al., 2005; Liu and Stiling, 2006). A factor which has a profound effect on the enemy loads of introduced species is hybridization, which can alter a plant species interactions with its natural enemies (Whitham, 1989; Fritz et al., 1999). An invasive species which has several forms present in an introduced range may be difficult to manage using biological control, due to natural enemies showing a preference towards some forms whilst barely being able to survive on other forms, as in the case of *L. camara* (Cilliers and Neser, 1991). Hybrids may also tend to be more, less or equally resistant to enemy attack than their parental species (Hjältén, 1998; Fritz et al., 1994, 1999, 2001). For instance in the genus *Myriophyllum,* invasive hybrids between *M. spicatum* and *M. simbricum,* as well as between *M. heterophyllum* and *M. pinnatum,* have been reported in North America (Moody and Les, 2002). *Myriophyllum spicatum* is currently under control by the weevil, *Euhyrchiopsis lecontei* Dietz (Coleoptera: Curculionidae), which is found on native *Myriophyllum* species (Cilliers, 1999; Winston et al., 2014). Adult *E. lecontei* feed on the stems and leaves of *M. spicatum,* whilst the larvae feed on the internal stem tissue (Alwin and Cheruvelil, 2009). *Euhyrchiopsis lecontei* has a higher preference for the invasive *M. spicatum* over the native *M. simbricum,* and an intermediate preference for hybrids between the two species (Hairston and Johnson, 2001; Roley and Newman, 2006). In contrast, paired feeding trials of the specialist *Larinus minutus* Gyllenhal (Coleoptera: Curculionidae), which feeds on *Centaurea diffusa* Lam., Encycl. Méth. Bot., 1: 675–676, 1785 (Asteraceae) and *C. maculosa* Lam. (Asteraceae), demonstrated a preference for newly created artificial hybrids between the two species over the pure *C. diffusa.* This has also been observed for cottonwood trees, which demonstrated higher enemy loads on hybrid trees than trees of either pure parents (Whitman, 1989). Hybridization may therefore contribute a positive or negative interaction between the biological control agent and the target weed. In addition clonal reproduction and sterility also play a role in the biological control of an invasive plant species.

In South Africa, aggressive aquatic plant invaders such as *E. crassipes, P. stratiotes, A. filiculoides, M. aquaticum* and *S. molesta* can all reproduce clonally. Clonal reproduction lowers the fitness as well as increases inbreeding depression in plant populations (Willson,

1994; Anderson, 1994). This makes a plant population more susceptible to attack from natural enemies, and in turn has contributed to the success in the biological control of the above mentioned aquatic weeds (Coetzee et al., 2011b). Sterility of an invasive plant population also plays a role in the success of biological control. *Salvinia molesta* and *M. aquaticum* are both sterile in South Africa and solely engage in clonal reproduction, which has led to a low genetic variation within populations of these two species in invaded regions around the country (Hill and Coetzee, 2017). Biological control agents have been found to be highly successful on several invasive clonal plants with low genetic variation (Burdon and Marshall, 1981). Currently both *M. aquaticum* and *S. molesta* have biological control agents released against them and are successfully controlled at numerous invaded regions around South Africa (Coetzee et al., 2011a; Coetzee at al., 2011b). Similarly, if the entire *N. mexicana* hybrid complex in South Africa is found to be sterile, then the chances for successful control by host – specific natural enemies of these hybrids are high.

4.3) The implications of managing the *Nymphaea mexicana* hybrid complex in South Africa.

Mechanical control of *N. mexicana* has been implemented in many regions of the country, with the main method consisting of manual 'hand–pulling', in which the petioles with leaves are pulled off the rhizome (Lindelani Nwedo, JHB City Parks and Phumudzo Ramabulana, Westlake Conservation Centre, pers. comm.). However, this method is only effective during winter, when the plants are overwintering, and not as effective in summer due to the plants ability to regenerate new stems and leaves at a rapid rate (Dugdale 2012; Dugdale et al., 2013; Hofstra et al., 2013). Chemical control was also initiated as a management option at an infested site at Westlake in the Western Cape Province. The herbicide glyphosate was used to control the infestation at this site, however application was stopped when locals boycotted the use of the chemical in the water, due to health concerns (Phumudzo Ramabulana, Westlake Conservation Centre, pers. comm.). Thus the next step in the management of *N. mexicana* and its hybrid complex in South Africa is biological control, a cost– effective, sustainable, environmentally friendly and long – term management method.

In South Africa, waterbodies are infested with either a single form of *N. mexicana* or with multiple forms of the species. For instance, waterbodies at (1) Emmarentia, Randburg, Gauteng, (2) the Vaal River, Gauteng, and (3) Boardwalk, Port Elizabeth, Eastern Cape, are infested with more than one genotype of *N. mexicana*. At these sites, the prospects of biological control may be challenging, especially if the natural enemies released are more

host specific to one form over another. If all forms are not controlled simultaneously, the forms that are removed by their host–specific natural enemies may be replaced by the forms which are 'resistant' to those enemies or which do not have natural enemies of their own, as reported to occur in the *L. camara* hybrid complex in South Africa (Cilliers and Nesser, 1991). The major difference between terrestrial invasive species like *L. camara* and aquatic invasive species like *N. mexicana* is that terrestrial invasive species are not confined to a particular habitat, and can spread more rapidly across landscapes, leading to a greater management challenge. However aquatic invasive species such as *N. mexicana* and its associated hybrids in South Africa are confined to the dam or pond in which they occur. The only way in which *N. mexicana* hybrids can spread into new systems, especially for those in enclosed water systems, is through human introduction of their rhizomes into new dams or ponds, since these plants are sterile and cannot produce viable seeds (Chapter 3). Thus one of the main invasive pathways of the *N. mexicana* hybrid complex in South Africa is anthropogenic, through garden nurseries, which import numerous cultivars of waterlilies and also through the sharing of these plants and tubers among the South African population (Henderson and Cilliers, 2002; pers.obs.).

4.4) Future research and recommendations.

Although this study provided a baseline for determining if one or multiple forms of *N. mexicana* occurs in South Africa, molecular analyses of plants from newly invaded waterbodies in the country which were not included in this study should be conducted. This will aid in providing an 'invasive water lily genetic database', which would be a useful resource in the future management of this species and its multiple forms, especially if biological control is implemented. By doing this, one would be able to identify if any new hybrids or even non–related invasive water lily species have been introduced into the country. This will be a useful step in keeping track of this invasive water lily hybrid complex, especially since only the pure *N. mexicana* has a NEM:BA category 1b legislation in place. Therefore, it is currently not illegal to plant or trade any hybrids associated with *N. mexicana*, and this subsequently contributes to the spread of the multiple forms of this species into the country. Thus apart from developing a management programme for this species, appropriate NEM:BA legislation also needs to be put into place in order to prevent the anthropogenic distribution and spread of the *N. mexicana* hybrid complex in South Africa from continuing (SAPIA, 2018).

In addition, future research needs to be conducted on natural enemies, which feed on the tubers of *N. mexicana* and its hybrids in South Africa since fragments of these tubers contribute to the invasion success of this hybrid complex in South Africa. The *N. mexicana* hybrid complex, including the pure form, all produce hard tubers (pers. obs.). The hybrids have adopted this trait from their parents, since *N. mexicana*, *N. odorata* as well as *N. alba*, all produce hard tubers with shoots arising along numerous points of the tuber and not from a single point (Fig. 4.4 C, D, H). In addition, the pure *N. mexicana* also produces long fleshy stolons, which give rise to daughter plants (Fig. 4.4 G). In contrast, the native water lily species in South Africa, *N. lotus* and *N. nouchali*, as well as the two wild varieties of the latter, *N. nouchali* var. *caerulea* and *N. nouchali* var. *zanzibariensis*, do not produce these hard tubers, and instead produce fleshy bulbs (Cook, 2004). Fragments of the bulbs from these native forms are unable to regenerate new plants, in comparison to the invasive *N. mexicana* hybrid complex (pers. obs.). When the bulbs are fragmented, they begin to decompose after a few days (pers. obs.). Both *N. lotus* and *N. nouchali* generate all their shoots from the top of the bulb, with a network of long, spongy roots, growing around the bulb (Fig. 4.4. A, B, E, F). The difference in the root systems between the native and introduced waterlilies in South Africa could increase the prospects of biological control for the invasive *N. mexicana* hybrid complex in the country, especially if natural enemies with a preference for feeding on the hard tubers of the *N. mexicana* hybrids, rather than the fleshy bulbs of the native *N. lotus* and *N. nouchali,* are found.

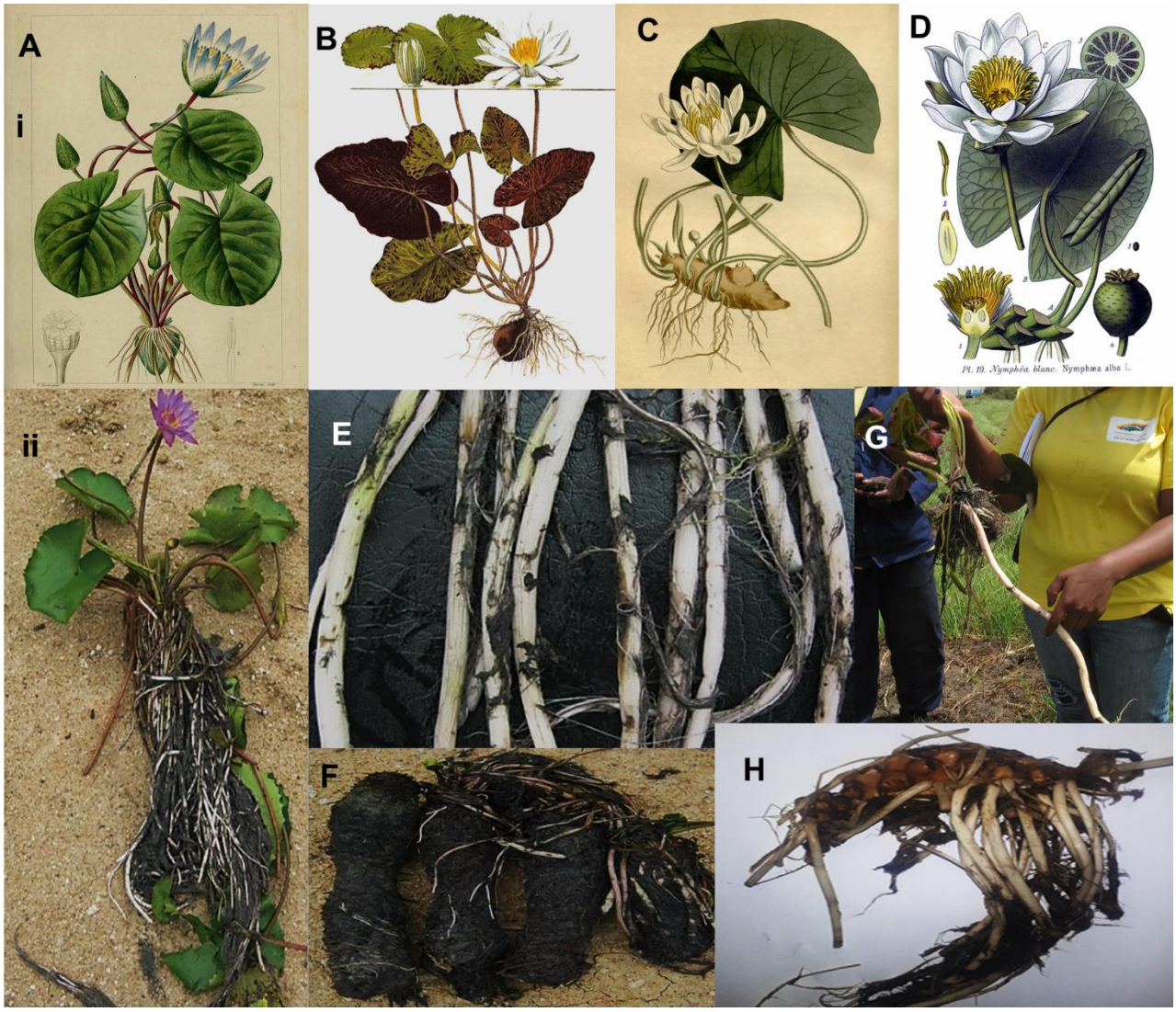


Figure 4. 3: (Ai) *Nymphaea nouchali* diagram depicting the central crown, at the top of a bulb from which all shoots arise, (Aii) live specimen of *N. nouchali* depicting long fibrous roots surrounding the bulb, (B) *N. lotus* diagram depicting the soft fleshy bulb, similarly to *N. nouchali* (C) *N. odorata* with hard tubers (D) *N. alba* with hard tubers (E) fibrous roots of *N. nouchali*, also found in *N. mexicana* (F) *N. nouchali* fleshy bulbs, (G) *N. mexicana* stolon, (H) hard fibrous tuber of an *N. mexicana* hybrid, with fibrous roots arising from many sections along the tuber. Images Ai – D were sourced of google images. Images Aii, E and F were taken from (Yakandawala et al., 2017). Image D was taken by the Westlake Conservation Centre. Image H was taken by Prinavin Naidu.

4.5) Conclusions

Nymphaea mexicana is an invasive water lily in South Africa and has multiple hybrids that are also invasive. Together they form an invasive hybrid water lily complex in the country, similarly to that of *L. camara*. In contrast to the pure *N. mexicana,* the hybrid genotypes are sterile and cannot reproduce sexually, but they do engage in asexual reproduction through the fragmentation of their tuberous rhizomes which can regenerate new plants. Mechanical and chemical control of *N. mexicana* and its multiple genotypes have been applied but have not been efficient due to the fast regeneration of shoots, especially in summer. Therefore, these two management options are not long–term solutions and will also be costly due to the widespread occurrence of the hybrids in South Africa. Thus the only cost–effective, environmentally friendly, self – sustainable and long–term management option is biological control. Although there are limitations associated with implementing a biological control programme for an invasive species with a hybrid complex, it has been done before in South Africa, as in the case of *L. camara.* Future research on host – specific natural enemies, which feed on the hard regenerative tubers of the invasive waterlilies, and not the soft fleshy non – regenerative bulbs of the native waterlilies in South Africa needs to be conducted.

References

ALWIN, T. & MATT, F. 2009. Biological control of Eurasian watermilfoil: A review of the native watermilfoil weevil. *Extension Bulletin WQ*, 61, 1-8.

AMSELLEM, L., NOYER, J., LE BOURGEOIS, T. & HOSSAERT-MCKEY, M. 2000. Comparison of genetic diversity of the invasive weed *Rubus alceifolius* Poir. (Rosaceae) in its native range and in areas of introduction, using amplified fragment length polymorphism (AFLP) markers. *Molecular Ecology*, 9, 443-455.

ANDERSEN, J. C. & MILLS, N. J. 2016. Geographic origins and post-introduction hybridization between strains of *Trioxys pallidus* introduced to western North America for the biological control of walnut and filbert aphids. *Biological Control*, 103, 218-229.

BAILEY, J. E. & CALHOUN, A. 2008. Comparison of three physical management techniques for controlling variable-leaf milfoil in Maine lakes. *Journal of Aquatic Plant Management*, 46, 163-167.

BAKER, H. G. 1965. Characteristics and modes of origin of weeds. In: *The Genetics of Colonizing Species.* Academic Press, New York. Pp. 147–172.

BAKER, H. G. 1974. The evolution of weeds. *Annual Review of Ecology and Systematics*, 5, 1-24.

BARKER, B. S., ANDONIAN, K., SWOPE, S. M., LUSTER, D. G. & DLUGOSCH, K. M. 2017. Population genomic analyses reveal a history of range expansion and trait evolution across the native and invaded range of yellow starthistle (*Centaurea solstitialis*). *Molecular Ecology*, 26, 1131-1147.

BARKER, N. P., PATERSON, I. & HOWIS, S. 2015. "Barcoding" and ISSR data illuminate a problematic infraspecific taxonomy for *Chrysanthemoides monilifera* (Calenduleae; Asteraceae): Lessons for biocontrol of a noxious weed. *Biochemical Systematics and Ecology*, 61, 541-551.

BARRETT, S. C. 1977. Tristyly in *Eichhornia crassipes* (Mart.) Solms (Water hyacinth). *Biotropica*, 9, 230-238.

BARRETT, S. C. 1988. Evolution of breeding systems in *Eichhornia* (Pontederiaceae): a review. *Annals of the Missouri Botanical Garden*, 75, 741-760.

BARRETT, S. C. 1998. The evolution of mating strategies in flowering plants. *Trends in Plant Science*, 3, 335-341.

BARRETT, S. C. 2002. Evolution of sex: the evolution of plant sexual diversity. *Nature Reviews Genetics*, 3, 274-284.

BARRETT, S. C. 2011. Why reproductive systems matter for the invasion biology of plants. In: Richardson D. M. (ed). *Fifty Years of Invasion Ecology: The Legacy of Charles Elton*, 1st edition. Blackwell, Oxford. Pp. 195-210.

BARRETT, S. C. & HOUGH, J. 2012. Sexual dimorphism in flowering plants. *Journal of Experimental Botany*, 64, 67-82.

BARRETT, S. C., COLAUTTI, R. I. & ECKERT, C. G. 2008. Plant reproductive systems and evolution during biological invasion. *Molecular Ecology*, 17, 373-383.

BARTON, N.H. and SLATKIN, M., 1986. A quasi-equilibrium theory of the distribution of rare alleles in a subdivided population. *Heredity*, 56, 409-415.

BATRA, S. W. 1981. Biological control of weeds: Principles and prospects. In: Papvizas, G.C. (ed.), *Biological Control in Crop Production.* Granada Publishing, London. Pp. 45-59.

BLIGNAUT, M., ELLIS, A. G. & LE ROUX, J. J. 2013. Towards a transferable and cost-effective plant AFLP protocol. *PloS one*, 8, e61704.

BODATSA. 2018. Southern African plant names and floristic details from the South African National Biodiversity Institute [Online]. Available: http://newposa.sanbi.org/ [Accessed 22-08-2018].

BOLKHOVSKIKH, Z., GRIP, V., ZAKHAR, E. & MATVEEVA, T. 1969. *Chromosome Numbers of Flowering Plants.* Nauka, Leningrad. Pp. 526-559.

BONILLA-BARBOSA, J., NOVELO, A., OROZCO, Y. H. & MÁRQUEZ-GUZMÁN, J. 2000. Comparative seed morphology of Mexican *Nymphaea* species. *Aquatic Botany*, 68, 189-204.

BORSCH, T., WIERSEMA, J. H., HELLQUIST, C. B., LÖHNE, C. & GOVERS, K. 2014. Speciation in North American water lilies: evidence for the hybrid origin of the newly discovered Canadian endemic *Nymphaea loriana sp.* nov. (Nymphaeaceae) in a past contact zone. *Botany*, 92, 867-882.

BORSCH, T., HILU, K. W., WIERSEMA, J. H., LÖHNE, C., BARTHLOTT, W. & WILDE, V. 2007. Phylogeny of *Nymphaea* (Nymphaeaceae): evidence from substitutions and microstructural changes in the chloroplast trnT-trnF region. *International Journal of Plant Sciences*, 168, 639-671.

BOWNES, A. 2014. Suitability of a leaf-mining fly, *Hydrellia sp*., for biological control of the invasive aquatic weed, *Hydrilla verticillata* in South Africa. *BioControl*, 59, 771-780.

BOYLEN, C. W., EICHLER, L. W. & SUTHERLAND, J. W. 1996. Physical control of Eurasian watermilfoil in an oligotrophic lake. *Hydrobiologia*, 340, 213-218.

BRIESE, D. 2000. Classical biological control. In: Sindel, B.M. (ed.), *Australian Weed Management Systems*. Richardson, Melbourne. Pp. 161-192.

BUHLER, D. D. 2002. 50th Anniversary—Invited Article: Challenges and opportunities for integrated weed management. *Weed Science*, 50, 273-280.

BUHLER, D. D., LIEBMAN, M. & OBRYCKI, J. J. 2000. Theoretical and practical challenges to an IPM approach to weed management. *Weed Science*, 48, 274-280.

BURDON, J. & BROWN, A. 1986. Population genetics of *Echium plantagineum* L. target weed for biological control. *Australian Journal of Biological Sciences*, 39, 369-378.

BURNS, J. H., ASHMAN, T.-L., STEETS, J. A., HARMON-THREATT, A. & KNIGHT, T. M. 2011. A phylogenetically controlled analysis of the roles of reproductive traits in plant invasions. *Oecologia*, 166, 1009-1017.

CANAVAN, K., PATERSON, I. & HILL, M. 2014. The herbivorous arthropods associated with the invasive alien plant, *Arundo donax*, and the native analogous plant, *Phragmites australis*, in the Free State Province, South Africa. *African Entomology*, 22, 454-459.

CAPPERINO, M. & SCHNEIDER, E. 1985. Floral biology of *Nymphaea mexicana* Zucc.(Nymphaeaceae). *Aquatic Botany*, 23, 83-93.

CARDINA, J., WEBSTER, T., HERMS, C., REGNIER, E. & BUHLER, D. 1999. Development of weed IPM: levels of integration for weed management. *Journal of Crop Production*, 2, 239-267.

CASPARY, R. 1865. Nymphaeaceae. *Annals Museum Botany Lugduno-Bavatum*, 2, 241-253.

CATFORD, J. A., JANSSON, R. & NILSSON, C. 2009. Reducing redundancy in invasion ecology by integrating hypotheses into a single theoretical framework. *Diversity and Distributions*, 15, 22-40.

CHABOUDEZ, P. & SHEPPARD, A. W. 1995. Are particular weeds more amenable to biological control? A reanalysis of mode of reproduction and life history. In: Delfosse, E. S. and Scott, R. R. (eds.), *VIII International Symposium on Biological Control of Weeds*. DSIR/CSIRO, Melbourne, Lincoln University, Canterbury. Pp. 95-102.

CHARLESWORTH, D., VEKEMANS, X., CASTRIC, V. & GLÉMIN, S. 2005. Plant self-incompatibility systems: a molecular evolutionary perspective. *New Phytologist*, 168, 61-69.

CHARUDATTAN, R. 2001. Are we on top of aquatic weeds? Weed problems, control options, and challenges. In: *International Symposium on the World's Worst Weeds*. November 12, 2001, British Crop Protection Council, Brighton, United Kingdom. Pp. 43-68.

CHAVEERACH, A., TANEE, T. & SUDMOON, R. 2011. Molecular identification and barcodes for the genus *Nymphaea*. *Acta Biologica Hungarica*, 62, 328-340.

CHOMCHALOW, N. & CHANSILP, N. 2007. The role of the 'Suthasinobon' water lily complex in introgressive hybridization. *Assumption University Journal*, 11, 67-76.

CHUN, Y. J., NASON, J. D. & MOLONEY, K. A. 2009. Comparison of quantitative and molecular genetic variation of native vs. invasive populations of purple loosestrife (*Lythrum salicaria* L., Lythraceae). *Molecular Ecology*, 18, 3020-3035.

CILLIERS, C. 1999. Biological control of parrot's feather, *Myriophyllum aquaticum* (Vell.) Verdc. (Haloragaceae), in South Africa. In: Olckers, T. and Hill, M.P. (eds.), *Biological control of Weeds in South Africa (1990–1998)*. African Entomology Memoir 1, 13–118.

CILLIERS, C. & NESER, S. 1991. Biological control of *Lantana camara* (Verbenaceae) in South Africa. *Agriculture, Ecosystems & Environment*, 37, 57-75.

CLAYTON, J. S. 1996. Aquatic weeds and their control in New Zealand lakes. *Lake and Reservoir Management*, 12, 477-486.

COETZEE, J., MARTIN, G. & BOWNES, A. 2011a. Prospects for the biological control of submerged macrophytes in South Africa. *African Entomology*, 19, 469-487.

COETZEE, J., HILL, M., BYRNE, M. & BOWNES, A. 2011b. A review of the biological control programmes on *Eichhornia crassipes* (C. Mart.) Solms (Pontederiaceae), *Salvinia molesta* DS Mitch. (Salviniaceae), *Pistia stratiotes* L. (Araceae), *Myriophyllum aquaticum* (Vell.) Verdc. (Haloragaceae) and *Azolla filiculoides* Lam. (Azollaceae) in South Africa. *African Entomology*, 19, 451-468.

COLAUTTI, R. I., RICCIARDI, A., GRIGOROVICH, I. A. & MACISAAC, H. J. 2004. Is invasion success explained by the enemy release hypothesis? *Ecology Letters*, 7, 721-733.

CONARD, H. S. 1905. *The Waterlilies: A Monograph of the Genus Nymphaea*. The Carnegie Institution of Washington, Washington.

COOK, C. D. K. 2004. *Aquatic and Wetland Plants of Southern Africa*, Leiden, The Netherlands, Backhuys Publishers.

CROSBY, A. W. 1986. *Ecological Imperialism: The Biological Expansion of Europe, 900-1900*. Cambridge University Press.

CUDA, J. 2014a. Chapter 5: Aquatic plants, mosquitoes and public health. In: Gettys, L.A., Haller, W.T. and Bellaud, M. (eds.), *Biology and Control of Aquatic Plants: A Best Management Practices Handbook*. Aquatic Ecosystem Restoration Foundation, Marietta GA, 210. Pp. 31-34.

CUDA, J. 2014b. Chapter 8: Introduction to Biological Control of Aquatic Weeds. In: Gettys, L.A., Haller, W.T. and Bellaud, M. (eds.), *Biology and Control of Aquatic Plants: A Best Management Practices Handbook*. Aquatic Ecosystem Restoration Foundation, Marietta GA, 210. Pp. 51-65.

CURTISS, A. 1902. The yellow water lily of Florida. *The Plant World*, 5, 106-109.

DANA, E. D., VERLOOVE, F., ORTIZ, D. G. & LUIS, J. 2017. First record of *Nymphaea* × *marliacea* Lat.-Marl. 'Rosea' in the Iberian Peninsula: identification based on morphological features and molecular techniques. *Bouteloua*, 28, 132-139.

DE LANGE, W. J. & VAN WILGEN, B. W. 2010. An economic assessment of the contribution of biological control to the management of invasive alien plants and to the protection of ecosystem services in South Africa. *Biological Invasions*, 12, 4113-4124.

DE NETTANCOURT, D. 1997. Incompatibility in angiosperms. *Sexual Plant Reproduction*, 10, 185-199.

DE SOCARRAZ-NOVOA, L. 2012. The Politics of Landscape: Claude Monet and his Water Lilies. *Saint Louis University, Madrid Campus,* 1-25.

DEA 2016. National Environmental Management: Biodiversity Act, 2004 (ACT NO. 10 OF 2004) - Alien and invasive species lists, 2016. *Staatskoerant No. 400166,* 31-104.

DEWALT, S. J. & HAMRICK, J. L. 2004. Genetic variation of introduced Hawaiian and native Costa Rican populations of an invasive tropical shrub, *Clidemia hirta* (Melastomataceae). *American Journal of Botany,* 91, 1155-1163.

DKHAR, J., KUMARIA, S., RAO, S. R. & TANDON, P. 2010. Molecular phylogenetics and taxonomic reassessment of four Indian representatives of the genus *Nymphaea*. *Aquatic Botany,* 93, 135-139.

DKHAR, J., KUMARIA, S., RAO, S. R. & TANDON, P. 2013. New insights into character evolution, hybridization and diversity of Indian *Nymphaea* (Nymphaeaceae): evidence from molecular and morphological data. *Systematics and Biodiversity,* 11, 77-86.

DKHAR, J., KUMARIA, S. & TANDON, P. 2011. *Nymphaea alba* var. *rubra* is a hybrid of *N. alba* and *N. odorata* as evidenced by molecular analysis. *Annales Botanici Fennici,* 48 317-324.

DODET, M., PETIT, R. & GASQUEZ, J. 2008. Local spread of the invasive *Cyperus esculentus* (Cyperaceae) inferred using molecular genetic markers. *Weed Research,* 48, 19-27.

DOORDUIN, L. J., VAN DEN HOF, K., VRIELING, K. & JOSHI, J. 2010. The lack of genetic bottleneck in invasive Tansy ragwort populations suggests multiple source populations. *Basic and Applied Ecology,* 11, 244-250.

DUGDALE, T. M. 2012. *Strategies to Minimise Oxygen Depletion Associated with Glyphosate Control of Mexican Water Lily: amended May 2012*. Department of Primary Industries - Weed Sciences, 1 Spring Street, Melbourne 3000. Pp. 1-31.

DUGDALE, T. M., HUNT, T. D., CLEMENTS, D. & BUTLER, K. 2012. Potential new herbicides for submerged aquatic weeds in Victoria. In: Weed Science Society of Victoria Inc. (eds.), *Developing solutions to evolving weed problems. 18th Australasian Weeds Conference,* Melbourne, Victoria, Australia, 8-11 October 2012. 41-44.

EARL, D. A. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, 4, 359-361.

ECKERT, C. G. 2002. The loss of sex in clonal plants. In: Stuefer, J.F., Erschbamer, B., Huber, H., Suzuki, J. (eds.), *Ecology and Evolutionary Biology of Clonal Plants*, Obergurgl, Austria, 20–25 August 2000. Springer. Pp. 79-298.

ECKERT, C. G., MASSONNET, B. & THOMAS, J. J. 2000. Variation in sexual and clonal reproduction among introduced populations of flowering rush, *Butomus umbellatus* (Butomaceae). *Canadian Journal of Botany*, 78, 437-446.

ELLSTRAND, N. C. 1992. Gene flow by pollen: implications for plant conservation genetics. *Oikos*, 63, 77-86.

ELLSTRAND, N. C. & SCHIERENBECK, K. A. 2000. Hybridization as a stimulus for the evolution of invasiveness in plants? *Proceedings of the National Academy of Sciences*, 97, 7043-7050.

EPPO 2011. *Ludwigia grandiflora* and *L. peploides* Onagraceae–Water primroses. *EPPO Bulletin*, 41, 414-418.

EVANNO, G., REGNAUT, S. & GOUDET, J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology*, 14, 2611-2620.

FALUSH, D., STEPHENS, M. & PRITCHARD, J. K. 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics*, 164, 1567-1587.

FINLAY, M., GRAESSER, A. & SIMPSON, E. 2008. Risk Management Plan—Management of nuisance aquatic vegetation in Goulburn Weir. *Goulburn-Murray Water Document*, 2390162, 1-23.

FRANCIS, N. & CRAPPER, H. 2004. *Weed spraying in the Goulburn Weir Pool: Summary of weed management activities in the Goulburn Weir Pool*. Sinclair Knights Merc Rep. Pp. 1-24.

FRANKLIN-TONG, N. V. & FRANKLIN, F. C. H. 2003. Gametophytic self-incompatibility inhibits pollen tube growth using different mechanisms. *Trends in Plant Science*, 8, 598-605.

FRITZ, R. S. 1999. Resistance of hybrid plants to herbivores: genes, environment, or both? *Ecology*, 80, 382-391.

FRITZ, R., NICHOLS-ORIANS, C. & BRUNSFELD, S. 1994. Interspecific hybridization of plants and resistance to herbivores: hypotheses, genetics, and variable responses in a diverse herbivore community. *Oecologia*, 97, 106-117.

FRITZ, R. S., HOCHWENDER, C. G., LEWKIEWICZ, D. A., BOTHWELL, S. & ORIANS, C. M. 2001. Seedling herbivory by slugs in a willow hybrid system: developmental changes in damage, chemical defense, and plant performance. *Oecologia*, 129, 87-97.

G-MW- GOULBURN-MURRAY WATER 2009. Management of yellow water lily in the Goulburn River Weir Pool. Fact Sheet, February. www.g-mwater.com.au. [Accessed 02-06-2017].

GAERTNER, M., LARSON, B. M., IRLICH, U. M., HOLMES, P. M., STAFFORD, L., VAN WILGEN, B. W. & RICHARDSON, D. M. 2016. Managing invasive species in cities: A framework from Cape Town, South Africa. *Landscape and Urban Planning*, 151, 1-9.

GANDERS, F. R. 1979. The biology of heterostyly. *New Zealand Journal of Botany*, 17, 607-635.

GASKIN, J. F., ZHANG, D. Y. & BON, M. C. 2005. Invasion of *Lepidium draba* (Brassicaceae) in the western United States: distributions and origins of chloroplast DNA haplotypes. *Molecular Ecology*, 14, 2331-2341.

GASKIN, J. F., SCHWARZLÄNDER, M., HINZ, H. L., WILLIAMS III, L., GERBER, E., RECTOR, B. G. & ZHANG, D. 2013. Genetic identity and diversity of perennial pepperweed (*Lepidium latifolium*) in its native and invaded ranges. *Invasive Plant Science and Management*, 6, 268-280.

GASKIN, J. F., BON, M.-C., COCK, M. J., CRISTOFARO, M., DE BIASE, A., DE CLERCK-FLOATE, R., ELLISON, C. A., HINZ, H. L., HUFBAUER, R. A. & JULIEN, M. H. 2011. Applying molecular-based approaches to classical biological control of weeds. *Biological Control*, 58, 1-21.

GASSMANN, A., COCK, M. J., SHAW, R. & EVANS, H. C. 2006. The potential for biological control of invasive alien aquatic weeds in Europe: a review. In: Caffrey, J.M.,

Dutartre, A., Haury, J., Murphy, K.M., Wade, P.M. (eds.), *Macrophytes in Aquatic Ecosystems: From Biology to Management*. Springer,Netherlands. Pp. 217-222.

GBIF 2018. GBIF Secretariat. Checklist Dataset https://www.gbif.org/species/2882420 [Accessed month 05-06-2018].

GILBERT, K. J., ANDREW, R. L., BOCK, D. G., FRANKLIN, M. T., KANE, N. C., MOORE, J. S., MOYERS, B. T., RENAUT, S., RENNISON, D. J. & VEEN, T. 2012. Recommendations for utilizing and reporting population genetic analyses: the reproducibility of genetic clustering using the program STRUCTURE. *Molecular Ecology*, 21, 4925-4930.

GITONGA, L., CRON, G., MCCONNACHIE, A. & BYRNE, M. 2015. Genetic variation of the invasive *Campuloclinium macrocephalum*, Asteraceae in South Africa, inferred from molecular markers. *Weed Research*, 55, 51-61.

GOOLSBY, J. A., DE BARRO, P. J., MAKINSON, J. R., PEMBERTON, R. W., HARTLEY, D. M. & FROHLICH, D. R. 2006. Matching the origin of an invasive weed for selection of a herbivore haplotype for a biological control programme. *Molecular Ecology*, 15, 287-297.

GOPAL, B. & SHARMA, K. 1990. Ecology of plant populations I: Growth. In: Gopal, B. (ed.), *Ecology and Management of Aquatic Vegetation in the Indian Subcontinent*. Springer, Netherlands. Pp. 79-106.

GRANT, V. 1995. Sexual selection in plants: pros and cons. *Proceedings of the National Academy of Sciences*, 92, 1247-1250.

GUPTA, P. 1978. Cytogenetics of aquatic ornamentals. II. Cytology of *Nymphaeas*. *Cytologia*, 43, 477-484.

GUPTA, P. 1980. Cytogenetics of aquatic ornamentals. VI. Evolutionary trends and relationships in the genus *Nymphaea*. *Cytologia*, 45, 307-314.

GURUGE, S., YAKANDAWALA, D. & YAKANDAWALA, K. 2017. A taxonomic synopsis of *Nymphaea nouchali* Burm. f. and infraspecific taxa. *Journal of the National Science Foundation of Sri Lanka*, 45, 307-318.

HAIRSTON JR, N., JOHNSON, R. & BELINSKY, K. 2001. *Monitoring and Evaluating the Impacts of Herbivorous Insects on Eurasian Watermilfoil.* Report to New York State

Department of Environmental Conservation, Division of Fish and Wildlife. Cornell University, Ithaca, New York.

HALLER, W. 2014. Chapter 7: Mechanical control of aquatic weeds. In: Gettys, L.A., Haller, W.T. and Bellaud, M. (eds.), *Biology and Control of Aquatic Plants: A Best Management Practices Handbook*. Aquatic Ecosystem Restoration Foundation, Marietta GA, 210. 43-50.

HAMMER, Ø., HARPER, D. & RYAN, P. 2001. PAST-palaeontological statistics, ver. 1.89. Palaeontol electron, 4, 1-9.

HARLEY, K. L. S. & FORNO, I. W. 1992. *Biological Control of Weeds: A Handbook for Practitioners and Students*, Inkata Press, Australia.

HAYNES, R. R. 1988. Reproductive biology of selected aquatic plants. *Annals of the Missouri Botanical Garden*, 805-810.

HENDERSON, L. 2011. Mapping of invasive alien plants: the contribution of the Southern African Plant Invaders Atlas (SAPIA) to biological weed control. *African Entomology*, 19, 498-503.

HENDERSON, L. & CILLIERS, C. 2002. *Invasive Aquatic Plants – A Guide to the Identification of the Most Important and Potentially Dangerous Invasive Aquatic and Wetland Plants in South Africa*. PPRI Handbook No. 16. Agricultural Research Council, Pretoria.

HENDERSON, L. & WILSON, J. R. 2017. Changes in the composition and distribution of alien plants in South Africa: An update from the Southern African Plant Invaders Atlas. *Bothalia-African Biodiversity & Conservation*, 47, 1-26.

HESLOP-HARRISON, Y. 1955. *Nymphaea* L. *Journal of Ecology*, 43, 719-734.

HIERRO, J. L., MARON, J. L. & CALLAWAY, R. M. 2005. A biogeographical approach to plant invasions: the importance of studying exotics in their introduced and native range. *Journal of Ecology*, 93, 5-15.

HILL, M. P. & COETZEE, J. 2017. The biological control of aquatic weeds in South Africa: Current status and future challenges. *Bothalia-African Biodiversity & Conservation*, 47, 1-12.

HJÄLTÉN, J. 1997. Willow hybrids and herbivory: a test of hypotheses of phytophage response to hybrid plants using the generalist leaf-feeder *Lochmaea caprea* (Chrysomelidae). *Oecologia*, 109, 571-574.

HOFSTRA, D., CLAYTON, J., GREEN, J. & ADAM, K. 2000. RAPD profiling and isozyme analysis of New Zealand *Hydrilla verticillata*. *Aquatic Botany*, 66, 153-166.

HOFSTRA, D., CHAMPION, P., DUGDALE, T., FRIDMAN, M., BAKER, R. & FINLAY, M. 2013. Comparison of use rates and treatment timing with glyphosate to control Mexican water lily. *Journal of Aquatic Plant Management*, 51, 69-76.

HOLMES, C. 2015. *Water Lilies: and Bory Latour-Marliac, the Genius Behind Monet's Water Lilies*. Antique Collectors Club Dist, Woodbridge, Suffolk, United Kingdom.

HOLMES, C. 2016. Hybrid history- and you thought intersubgeneric crosses were new? *International Water lily and Water Gardening Society Water Garden Journal*, 31, 12-13.

HOWARD, G. & HARLEY, K. 1997. How do floating aquatic weeds affect wetland conservation and development? How can these effects be minimised? *Wetlands Ecology and Management*, 5, 215-225.

HUFBAUER, R. A. & RODERICK, G. K. 2005. Microevolution in biological control: mechanisms, patterns, and processes. *Biological Control*, 35, 227-239.

HULME, P. E. 2012. Weed risk assessment: a way forward or a waste of time? *Journal of Applied Ecology*, 49, 10-19.

HUSON, D. H. & BRYANT, D. 2005. Application of phylogenetic networks in evolutionary studies. *Molecular Biology and Evolution*, 23, 254-267.

HUSSNER, A., STIERS, I., VERHOFSTAD, M., BAKKER, E., GRUTTERS, B., HAURY, J., VAN VALKENBURG, J., BRUNDU, G., NEWMAN, J. & CLAYTON, J. 2017. Management and control methods of invasive alien freshwater aquatic plants: a review. *Aquatic Botany*, 136, 112-137.

IWGS 2018. http://iwgs.org/ [Accessed month 10-02-2018].

JONES, M. R. & CLARKE, G. 1981. Nymphaeaceae. *Review of Palaeobotany and Palynology*, 33, 57-67.

JONES, R. W. 2009. The impact on biodiversity, and integrated control, of water hyacinth, *Eichhornia crassipes* (Martius) Solms-Laubach (Pontederiaceae) on the Lake Nsezi-Nseleni River System. Dissertation Master of Science. Rhodes University.

JONES, C., EDWARDS, K., CASTAGLIONE, S., WINFIELD, M., SALA, F., VAN DE WIEL, C., BREDEMEIJER, G., VOSMAN, B., MATTHES, M. & DALY, A. 1997. Reproducibility testing of RAPD, AFLP and SSR markers in plants by a network of European laboratories. *Molecular Breeding*, 3, 381-390.

KANG, M., BUCKLEY, Y. M. & LOWE, A. J. 2007. Testing the role of genetic factors across multiple independent invasions of the shrub Scotch broom (*Cytisus scoparius*). *Molecular Ecology*, 16, 4662-4673.

KEANE, R. M. & CRAWLEY, M. J. 2002. Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology & Evolution*, 17, 164-170.

KIEPIEL, I. & JOHNSON, S. D. 2014. Breeding systems in *Clivia* (Amaryllidaceae): late-acting self-incompatibility and its functional consequences. *Botanical journal of the Linnean Society*, 175, 155-168.

KLEIN, H., ZACHARIADES, C., HILL, M. & ZIMMERMANN, H. 2011. Regulation and risk assessment for importations and releases of biological control agents against invasive alien plants in South Africa. *African Entomology*, 19, 488-497.

KLIBER, A. & ECKERT, C. G. 2005. Interaction between founder effect and selection during biological invasion in an aquatic plant. *Evolution*, 59, 1900-1913.

KNISKERN, J. & RAUSHER, M. D. 2001. Two modes of host–enemy coevolution. *Population Ecology*, 43, 3-14.

KNOTTS, K. & KNOTTS, B. 2018. http://www.victoria-adventure.org/waterlilies/waterlilies_index.html accessed via victoria-adventure.org [Accessed month 10-02-2018].

KOLAR, C. S. & LODGE, D. M. 2000. Freshwater nonindigenous species: interactions with other global changes. In: Mooney, H.A. and Hobbs, R.A. (eds.), *Invasive Species in a Changing World*. Island Press, Washington, DC. Pp. 3-30.

KOPELMAN, N. M., MAYZEL, J., JAKOBSSON, M., ROSENBERG, N. A. & MAYROSE, I. 2015. Clumpak: a program for identifying clustering modes and packaging population structure inferences across K. *Molecular Ecology Resources*, 15, 1179-1191.

KRAHULCOVÁ, A. & JAROLÍMOVÁ, V. 1993. Ecology of two cytotypes of *Butomus umbellatus* I. Karyology and breeding behaviour. *Folia Geobotanica et Phytotaxonomica*, 28, 385-411

KREFT, H. & JETZ, W. 2010. A framework for delineating biogeographical regions based on species distributions. *Journal of Biogeography*, 37, 2029-2053.

KROPF, M., HUPPENBERGER, A. & KARRER, G. 2018. Genetic structuring and diversity patterns along rivers–local invasion history of *Ambrosia artemisiifolia* (Asteraceae) along the Danube River in Vienna (Austria) shows non-linear pattern. *Weed Research*, 58, 131-140.

KWONG, R. M., BROADHURST, L. M., KEENER, B. R., COETZEE, J. A., KNERR, N. & MARTIN, G. D. 2017. Genetic analysis of native and introduced populations of the aquatic weed *Sagittaria platyphylla*–implications for biological control in Australia and South Africa. *Biological Control*, 112, 10-19.

LANCAR, L. & KRAKE, K. 2002. *Aquatic Weeds and their Management*. International Commission on Irrigation and Drainage.

LANDE, R. 1980. Sexual dimorphism, sexual selection, and adaptation in polygenic characters. *Evolution*, 34, 292-305.

LARANJEIRA, C. & NADAIS, G. 2008. *Eichhornia crassipes* control in the largest Portuguese natural freshwater lagoon 1. *EPPO Bulletin*, 38, 487-495.

LARUE, E. A., ZUELLIG, M. P., NETHERLAND, M. D., HEILMAN, M. A. & THUM, R. A. 2013. Hybrid watermilfoil lineages are more invasive and less sensitive to a commonly used herbicide than their exotic parent (Eurasian watermilfoil). *Evolutionary Applications*, 6, 462-471.

LATOUR–MARLIAC , J. 1899. Hardy hybrid water lilies. *The Garden*, 18, 1-4.

LAVANIA, U. 2002. Chromosome diversity in population: Defining conservation units and their micro-identification through genomic in situ painting. Current Science, 83, 124-127.

LE MAITRE, D. C., VERSFELD, D. & CHAPMAN, R. 2000. Impact of invading alien plants on surface water resources in South Africa: A preliminary assessment. *Water SA,* 26, 397-408.

LES, D. & PHILBRICK, C. 1993. Studies of hybridization and chromosome number variation in aquatic angiosperms: evolutionary implications. *Aquatic Botany,* 44, 181-228.

LES, D., MOODY, M. L., DORAN, A. S. & PHILLIPS, W. E. 2004. A genetically confirmed intersubgeneric hybrid in *Nymphaea* L.(Nymphaeaceae Salisb.). *Horticultural Science,* 39, 219-222.

LI, W., WANG, B. & WANG, J. 2006. Lack of genetic variation of an invasive clonal plant *Eichhornia crassipes* in China revealed by RAPD and ISSR markers. *Aquatic Botany*, 84, 176-180.

LIU, H. & STILING, P. 2006. Testing the enemy release hypothesis: a review and meta-analysis. *Biological Invasions*, 8, 1535-1545.

LÖHNE, C., BORSCH, T., JACOBS, S. W., HELLQUIST, C. B. & WIERSEMA, J. H. 2008a. Nuclear and plastid DNA sequences reveal complex reticulate patterns in Australian water-lilies (*Nymphaea* subgenus *Anecphya*, Nymphaeaceae). *Australian Systematic Botany*, 21, 229-250.

LÖHNE, C., YOO, M.-J., BORSCH, T., WIERSEMA, J., WILDE, V., BELL, C. D., BARTHLOTT, W., SOLTIS, D. E. & SOLTIS, P. S. 2008b. Biogeography of Nymphaeales: extant patterns and historical events. *Taxon,* 57, 1123-1123.

LUI, K., THOMPSON, F. L. & ECKERT, C. G. 2005. Causes and consequences of extreme variation in reproductive strategy and vegetative growth among invasive populations of a clonal aquatic plant, *Butomus umbellatus* L. (Butomaceae). *Biological Invasions,* 7, 427-444.

LYM, R. G. 2005. Integration of biological control agents with other weed management technologies: successes from the leafy spurge (*Euphorbia esula*) IPM program. *Biological Control*, 35, 366-375.

MACDONALD, I. A. 2004. Recent research on alien plant invasions and their management in South Africa: a review of the inaugural research symposium of the Working for Water programme: working for water. *South African Journal of Science,* 100, 21-26.

MARAIS, C. & WANNENBURGH, A. 2008. Restoration of water resources (natural capital) through the clearing of invasive alien plants from riparian areas in South Africa—costs and water benefits. *South African Journal of Botany*, 74, 526-537.

MARTIN, G. D. & COETZEE, J. A. 2011. Pet stores, aquarists and the internet trade as modes of introduction and spread of invasive macrophytes in South Africa. *Water SA*, 37, 371-380.

MAUVE, A. 1968. The yellow water lily of the Vaal River. *Fauna and Flora*, 19, 24.

MITCHELL, D. 1974. *Aquatic Vegetation and its Use and Control*. United Nations Educational, Scientific, and Cultural Organization. UNESCO), Paris.

MOODY, M. L. & LES, D. H. 2002. Evidence of hybridity in invasive watermilfoil (*Myriophyllum*) populations. *Proceedings of the National Academy of Sciences*, 99, 14867-14871.

MOORHOUSE, T. P. & MACDONALD, D. W. 2015. Are invasives worse in freshwater than terrestrial ecosystems? *Wiley Interdisciplinary Reviews: Water*, 2, 1-8.

MORAN, V., HOFFMANN, J. & HILL, M. 2011. A context for the 2011 compilation of reviews on the biological control of invasive alien plants in South Africa. *African Entomology*, 19, 177-185.

MORAN, V. C., HOFFMANN, J. H. & ZIMMERMANN, H. G. 2005. Biological control of invasive alien plants in South Africa: necessity, circumspection, and success. *Frontiers in Ecology and the Environment*, 3, 71-77.

MORAN, V. C., HOFFMANN, J. H. & ZIMMERMANN, H. G. 2013. 100 years of biological control of invasive alien plants in South Africa: History, practice and achievements. *South African Journal of Science*, 109, 01-06.

MORIN, L., REID, A. M., SIMS-CHILTON, N., BUCKLEY, Y., DHILEEPAN, K., HASTWELL, G. T., NORDBLOM, T. & RAGHU, S. 2009. Review of approaches to evaluate the effectiveness of weed biological control agents. *Biological Control*, 51, 1-15.

MUELLER, U. G. & WOLFENBARGER, L. L. 1999. AFLP genotyping and fingerprinting. *Trends in Ecology & Evolution*, 14, 389-394.

MULCAHY, D. L. 1975. The reproductive biology of *Eichhornia crassipes* (Pontederiaceae). *Bulletin of the Torrey Botanical Club*, 102, 18-21.

MUNTENDAM, J., POVEL, G. & VAN DER VELDE, G. 1996. Morphometric patterns in the *Nymphaea alba-candida* complex. *Acta Botanica Neerlandica*, 45, 279-302.

NETHERLAND, M. 2014. Chapter 11: Chemical control of aquatic weeds. In: Gettys LA, WT Haller & M. Bellaud, (eds.). *Biology and Control of Aquatic Plants: A Best Management Practices Handbook.* Aquatic Ecosystem Restoration Foundation, Marietta GA, 210. Pp. 71-85.

NETHERLAND, M. & JONES, D. 2015. Fluridone-resistant hydrilla (*Hydrilla verticillata*) is still dominant in the kissimmee chain of lakes, FL. *Invasive Plant Science and Management*, 8, 212-218.

NIERBAUER, K. U., KANZ, B. & ZIZKA, G. 2014. The widespread naturalisation of *Nymphaea* hybrids is masking the decline of wild-type *Nymphaea alba* in Hesse, Germany. Flora-Morphology, Distribution, *Functional Ecology of Plants*, 209, 122-130.

NISSEN, S. J., MASTERS, R. A., LEE, D. J. & ROWE, M. L. 1995. DNA-based marker systems to determine genetic diversity of weedy species and their application to biocontrol. *Weed Science*, 43, 504-513.

OBERHOLSTER, P. J. & ASHTON, P. J. 2008. *State of the Nation Report: An Overview of the Current Status of Water Quality and Eutrophication in South African Rivers and Reservoirs*. Parliamentary Grant Deliverable. Pretoria: Council for Scientific and Industrial Research (CSIR).

OLCKERS, T. 2004. Targeting emerging weeds for biological control in South Africa: the benefits of halting the spread of alien plants at an early stage of their invasion: working for water. *South African Journal of Science*, 100, 64-68.

OLIVEIRA, L. O. D., VENTURINI, B. A., ROSSI, A. A. B. & HASTENREITER, S. S. 2010. Clonal diversity and conservation genetics of the medicinal plant *Carapichea ipecacuanha* (Rubiaceae). *Genetics and Molecular Biology*, 33, 86-93.

PANDIT, M. K., POCOCK, M. J. & KUNIN, W. E. 2011. Ploidy influences rarity and invasiveness in plants. *Journal of Ecology*, 99, 1108-1115.

PARISOD, C., HOLDEREGGER, R. & BROCHMANN, C. 2010. Evolutionary consequences of autopolyploidy. *New Phytologist*, 186, 5-17.

PATERSON, I. D. & ZACHARIADES, C. 2013. ISSRs indicate that *Chromolaena odorata* invading southern Africa originates in Jamaica or Cuba. *Biological Control*, 66, 132-139.

PATERSON, I. D., DOWNIE, D. A. & HILL, M. P. 2009. Using molecular methods to determine the origin of weed populations of *Pereskia aculeata* in South Africa and its relevance to biological control. *Biological Control*, 48, 84-91.

PEAKALL, R.O.D. & SMOUSE, P.E., 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, 6, 288-295.

PAYNTER, Q., OVERTON, J. M., HILL, R. L., BELLGARD, S. E. & DAWSON, M. I. 2012. Plant traits predict the success of weed biocontrol. *Journal of Applied Ecology*, 49, 1140-1148.

PERRY, F. 1989. *Waterlilies and Other Aquatic Plants*. Holt, New York.

PHELOUNG, P., WILLIAMS, P. & HALLOY, S. 1999. A weed risk assessment model for use as a biosecurity tool evaluating plant introductions. *Journal of Environmental Management*, 57, 239-251.

PIMENTEL, D., MCNAIR, S., JANECKA, J., WIGHTMAN, J., SIMMONDS, C., O'CONNELL, C., WONG, E., RUSSEL, L., ZERN, J. & AQUINO, T. 2001. Economic and environmental threats of alien plant, animal, and microbe invasions. *Agriculture, Ecosystems & Environment*, 84, 1-20.

PIMENTEL, D., LACH, L., ZUNIGA, R. and MORRISON, D., 2002. Environmental and economic costs associated with non-indigenous species in the United States. In: Pimentel, D., (ed.). *Biological Invasions*. CRC Press, Boca Raton, Pp. 285-303.

PLANCHON, J. E. 1852. *N. ortgiesiano- rubra*. *Flores Des Serres*, 8, 67-73.

POCZAI, P., MÁTYÁS, K. K., SZABÓ, I., VARGA, I., HYVÖNEN, J., CERNÁK, I., GORJI, A. M., DECSI, K. & TALLER, J. 2011. Genetic variability of thermal *Nymphaea* (Nymphaeaceae) populations based on ISSR markers: implications on relationships, hybridization, and conservation. *Plant Molecular Biology Reporter*, 29, 906-918.

POSADA, D. & CRANDALL, K. A. 2001. Intraspecific gene genealogies: trees grafting into networks. *Trends in Ecology & Evolution*, 16, 37-45.

PRING, G. 1934. Hybrid Nymphaeas. Their creation, propagation, and cultivation. *Missouri Botanical Garden Bulletin*, 22, 93-108.

PRITCHARD, J. K., STEPHENS, M. & DONNELLY, P. 2000. Inference of population structure using multilocus genotype data. *Genetics*, 155, 945-959.

PRITCHARD, J. K., WEN, W. & FALUSH, D. 2007. Documentation for STRUCTURE software: Version 2.2. [Online]. Available: http://pritch.bsd.uchicago.edu/software [Accessed month 21/02 2018].

RADICS, F. 1967. A revision of the *Nymphaea* material in Hungarian Natural History Museum. *Pars Bot*, 59, 135-145.

RAMBUDA, T. D. & JOHNSON, S. D. 2004. Breeding systems of invasive alien plants in South Africa: does Baker's rule apply? *Diversity and Distributions*, 10, 409-416.

RAMSEY, J. & SCHEMSKE, D. W. 1998. Pathways, mechanisms, and rates of polyploid formation in flowering plants. *Annual Review of Ecology and Systematics*, 29, 467-501.

RAY, P. & HILL, M. P. 2013. Microbial agents for control of aquatic weeds and their role in integrated management. *CAB Reviews Perspectives in Agriculture Veterinary Science Nutrition and Natural Resources*, 8, 1-9.

REJMÁNEK, M. & RICHARDSON, D. M. 1996. What attributes make some plant species more invasive? *Ecology*, 77, 1655-1661.

RICHARDSON, D. M. & VAN WILGEN, B. W. 2004. Invasive alien plants in South Africa: how well do we understand the ecological impacts? *South African Journal of Science*, 100, 45-52.

RICHARDSON, D. M., PYŠEK, P., REJMÁNEK, M., BARBOUR, M. G., PANETTA, F. D. & WEST, C. J. 2000. Naturalization and invasion of alien plants: concepts and definitions. *Diversity and Distributions*, 6, 93-107.

RICKETSON, J. 1995. Nymphaeaceae Water lily Family. *Journal of the Arizona-Nevada Academy of Science*, 29, 26-28.

RIESEBERG, L. H. 1997. Hybrid origins of plant species. *Annual Review of Ecology and Systematics*, 28, 359-389.

ROBERTSON, M., VILLET, M., FAIRBANKS, D., HENDERSON, L., HIGGINS, S., HOFFMANN, J., LE MAITRE, D. C., PALMER, A., RIGGS, I. & SHACKLETON, C. 2003. A proposed prioritization system for the management of invasive alien plants in South Africa: research in action. *South African Journal of Science*, 99, 37-43.

ROBSON, T. 1974. The control of aquatic weeds: mechanical control. In: Mitchell, D.S. (ed.), *Aquatic Vegetation and its Use and Control*. Unesco, Paris. Pp. 72-84.

RODERICK, G. K. & NAVAJAS, M. 2003. Genes in new environments: genetics and evolution in biological control. *Nature Reviews Genetics*, 4, 889-899.

ROLEY, S. S. & NEWMAN, R. M. 2006. Developmental performance of the milfoil weevil, *Euhrychiopsis lecontei* (Coleoptera: Curculionidae), on northern watermilfoil, Eurasian watermilfoil, and hybrid (northern× Eurasian) watermilfoil. *Environmental Entomology*, 35, 121-126.

ROY, J. 1990. In search of the characteristics of plant invaders. In: di Castri, F., Hansen, A.J., Debussche, M. (eds), *Biological Invasions in Europe and the Mediterranean Basin*. Springer, Netherlands. Pp. 335-352.

RSA 2004. National Environmental Management: Biodiversity Act (Act No. 10 of 2004). Government Gazette, South Africa, 467.

SANKARAN, T. 1990. Strategies for controlling noxious growth of aquatic vegetation. In: Gopal, B. (ed.), *Ecology and Management of Aquatic Vegetation in the Indian Subcontinent*. Springer, Netherlands. Pp. 219-236.

SANS, F., GARCIA-SERRANO, H. & AFÁN, I. 2004. Life-history traits of alien and native *Senecio* species in the Mediterranean region. *Acta Oecologica*, 26, 167-178.

SAPIA 2018. Invasive water lilies in South Africa. *Newsletter of the Southern African Plant Invaders Atlas, an initiative of the Weeds Research Division of Plant Protection Research, an institute within the Agricultural Research Council (ARC)*, 49, 1-3.

SCHEFFER, M., SZABO, S., GRAGNANI, A., VAN NES, E. H., RINALDI, S., KAUTSKY, N., NORBERG, J., ROIJACKERS, R. M. & FRANKEN, R. J. 2003. Floating

plant dominance as a stable state. *Proceedings of the National Academy of Sciences*, 100, 4040-4045.

SCHNEIDER, E. L. & CHANEY, T. 1981. The floral biology of *Nymphaea odorata* (Nymphaeaceae). *The Southwestern Naturalist*, 26, 159-165.

SCHNEIDER, E. & WILLIAMSON, P. 1993. Nymphaeaceae. In: Kubitzki, K., Rohwer, J.G., Bittrich, V. (eds.), *Flowering Plants· Dicotyledons*. Springer, Netherlands. Pp. 486-493.

SESHAVATHARAM, V. 1990. Traditional uses and problem of noxious growth. In: Gopal, B. (ed.), *Ecology and Management of Aquatic Vegetation in the Indian Subcontinent*. Springer, Netherlands. Pp. 201-218.

SHORTHOUSE, D. P. 2010. SimpleMappr, an online tool to produce publication-quality point maps.

SIMBERLOFF, D. 2003a. Eradication—preventing invasions at the outset. *Weed Science*, 51, 247-253.

SIMBERLOFF, D. 2003b. Why not eradication? In: Rapport, D.J., Lasley, W.L., Rolston, D.E., Nielsen, N.O., Qualset, C.O., Damania, A.B. (eds.), *Managing for Healthy Ecosystems*. Routledge Taylor and Francis Group, Milton Park, Abingdon, UK. Pp. 541-548.

SLOCUM, P. D. 2005. *Waterlilies and Lotuses. Species, Cultivars, and New Hybrids*. Timber press, Portland, OR.

SLOCUM, P. D., ROBINSON, P., PERRY, F. & ROBINSON, P. 1996. *Water Gardening: Water Lilies and Lotuses*. Timber Press Portland, OR.

SOKAL, R. R. 1963. The principles and practice of numerical taxonomy. *Taxon*, 12, 190-199.

SONGPANICH, P. & HONGTRAKUL, V. 2010. Intersubgeneric cross in *Nymphaea spp*. L. to develop a blue hardy water lily. *Scientia Horticulturae*, 124, 475-481.

SPIES, J. 1984. A cytotaxonomic study of *Lantana camara* (Verbenaceae) from South Africa. *South African Journal of Botany*, 3, 231-250.

SPIES, J. & PLESSIS, H. D. 1987. Sterile *Lantana camara*: fact or theory. *South African Journal of Plant and Soil*, 4, 171-174.

STATSOFT, I. 2015. STATISTICA (data analysis software system), version 13.2. Tulsa, USA, 150.

STEBBINS, G. L. 1971. *Chromosomal Evolution in Higher Plants*. Edward Arnold Ltd., London.

STRAWN, K. 2016. Hybridization of Hardy Waterlilies. *International Water lily and Water Gardening Society Water Garden Journal*, 31, 3-7.

STRAWN, K. 2017. The Results of Hybridization of Hardy Waterlilies. *International Water lily and Water Gardening Society Water Garden Journal*, 32, 12-13.

SUTTON, G., PATERSON, I. & PAYNTER, Q. 2017. Genetic matching of invasive populations of the African tulip tree, *Spathodea campanulata* Beauv. (Bignoniaceae), to their native distribution: Maximising the likelihood of selecting host-compatible biological control agents. *Biological Control*, 114, 167-175.

TE BEEST, M., LE ROUX, J. J., RICHARDSON, D. M., BRYSTING, A. K., SUDA, J., KUBEŠOVÁ, M. & PYŠEK, P. 2011. The more the better? The role of polyploidy in facilitating plant invasions. *Annals of Botany*, 109, 19-45.

TEAM, R. 2015. RStudio: integrated development for R. RStudio, Inc., Boston, MA. URL: http://www.rstudio.com [Accessed month 22-04-2017].

THILL, D. C., LISH, J. M., CALLIHAN, R. H. & BECHINSKI, E. J. 1991. Integrated weed management–a component of integrated pest management: a critical review. *Weed Technology*, 5, 648-656.

THOMSON, J. D. & BARRETT, S. C. 1981. Selection for outcrossing, sexual selection, and the evolution of dioecy in plants. *The American Naturalist*, 118, 443-449.

TIMM, A.E., GEERTSEMA, H. and WARNICH, L., 2010. Population genetic structure of economically important *Tortricidae* (Lepidoptera) in South Africa: a comparative analysis. *Bulletin of Entomological Research*, 100, 421-431.

TRIVERS, R. 1972. *Parental Investment and Sexual Selection*. Biological Laboratories, Harvard University Cambridge.

VAN DER VELDE, G. 1986. Developmental stages in the floral biology of Dutch Nymphaeaceae (*Nymphaea alba* L., *Nymphaea candida* Presl, *Nuphar lutea* (L.) Sm.). *Acta Botanica Neerlandica*, 35, 111-113.

VAN, T., WHEELER, G. & CENTER, T. 1998. Competitive interactions between Hydrilla (*Hydrilla verticillata*) and Vallisneria (*Vallisneria americana*) as influenced by insect herbivory. *Biological Control*, 11, 185-192.

VAN KLEUNEN, M. & JOHNSON, S. D. 2007. Effects of self-compatibility on the distribution range of invasive European plants in North America. *Conservation Biology*, 21, 1537-1544.

VAN KLINKEN, R. D. & RAGHU, S. 2006. A scientific approach to agent selection. *Australian Journal of Entomology*, 45, 253-258.

VAN VALKENBURG, J. 2011. *Cabomba caroliniana* and *Myriophyllum heterophyllum* a nightmare combination. Robson Meeting February 2011.

VAN WILGEN, B. & DE LANGE, W. J. 2011. The costs and benefits of biological control of invasive alien plants in South Africa. *African Entomology*, 19, 504-514.

VAN WILGEN, B., MORAN, V. & HOFFMANN, J. 2013. Some perspectives on the risks and benefits of biological control of invasive alien plants in the management of natural ecosystems. *Environmental Management*, 52, 531-540.

VAN WYK, E. & VAN WILGEN, B. 2002. The cost of water hyacinth control in South Africa: a case study of three options. *African Journal of Aquatic Science*, 27, 141-149.

VARDIEN, W., RICHARDSON, D., FOXCROFT, L., THOMPSON, G., WILSON, J. & LE ROUX, J. 2012. Invasion dynamics of *Lantana camara* L. (sensu lato) in South Africa. *South African Journal of Botany*, 81, 81-94.

VILA, M., MARON, J. L. & MARCO, L. 2005. Evidence for the enemy release hypothesis in *Hypericum perforatum. Oecologia*, 142, 474-479.

VOLKOVA, P. A. & SHIPUNOV, A. B. 2007. Morphological variation of *Nymphaea* (Nymphaeaceae) in European Russia. *Nordic Journal of Botany*, 25, 329-338.

VOLKOVA, P. A., TRÁVNÍČEK, P. & BROCHMANN, C. 2010. Evolutionary dynamics across discontinuous freshwater systems: rapid expansions and repeated allopolyploid origins in the Palearctic white water-lilies (*Nymphaea*). *Taxon*, 59, 483-494.

VOS, P., HOGERS, R., BLEEKER, M., REIJANS, M., LEE, T. V. D., HORNES, M., FRITERS, A., POT, J., PALEMAN, J. & KUIPER, M. 1995. AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research*, 23, 4407-4414.

WANI, A. A. & BHAT, T. A. 2017. Asynapsis and Desynapsis in Plants. In: Bhat, T.A. and Wani, A.A. (eds.), *Chromosome Structure and Aberrations*. Springer, Netherlands. Pp. 127-140.

WARD, D. 1977. Keys to the flora of Florida: 4. *Nymphaea* (Nymphaeaceae). *Phytologia*, 37, 443-448.

WARD, M., JOHNSON, S. D. & ZALUCKI, M. P. 2012. Modes of reproduction in three invasive milkweeds are consistent with Baker's Rule. *Biological Invasions*, 14, 1237-1250.

WARDILL, T. J., GRAHAM, G. C., ZALUCKI, M., PALMER, W. A., PLAYFORD, J. & SCOTT, K. D. 2005. The importance of species identity in the biocontrol process: identifying the subspecies of *Acacia nilotica* (Leguminosae: Mimosoideae) by genetic distance and the implications for biological control. *Journal of Biogeography*, 32, 2145-2159.

WHITHAM, T. G. 1989. Plant hybrid zones as sinks for pests. *Science*, 244, 1490-1493.

WHITLOCK, M. C. 2008. Evolutionary inference from QST. *Molecular Ecology*, 17, 1885-1896.

WIERSEMA, J. H. 1988. Reproductive biology of *Nymphaea* (Nymphaeaceae). *Annals of the Missouri Botanical Garden*, 75, 795-804.

WIERSEMA, J. & HELLQUIST, C. 1997. Nymphaeaceae. *Flora of North America North Mexico*, 3, 66-77.

WILLIAMS, D. A., OVERHOLT, W. A., CUDA, J. P. & HUGHES, C. R. 2005. Chloroplast and microsatellite DNA diversities reveal the introduction history of Brazilian peppertree (*Schinus terebinthifolius*) in Florida. *Molecular Ecology*, 14, 3643-3656.

WILLIAMS, J. R. 1954. The biological control of weeds. *Report of the Sixth Commonwealth Entomological Congress*, London, United Kingdom, Pp. 95–98.

WILLIAMSON, M., 2002. Chapter six: Alien plants in the British Isles. In: Pimentel, D., (ed.). *Biological Invasions*. CRC Press, Boca Raton, Pp. 91-112.

WILLSON, M. F. 1979. Sexual selection in plants. *The American Naturalist*, 113, 777-790.

WILLSON, M. F. 1994. Sexual selection in plants: perspective and overview. *The American Naturalist*, 144, 13-39.

WILSON, J. R., IVEY, P., MANYAMA, P. & NÄNNI, I. 2013. A new national unit for invasive species detection, assessment and eradication planning. *South African Journal of Science*, 109, 01-13.

WINSTON, R., SCHWARZLÄNDER, M., HINZ, H. L., DAY, M. D., COCK, M. J. & JULIEN, M. 2014. *Biological Control of Weeds: A World Catalogue of Agents and their Target Weeds.* USDA Forest Service, Forest Health Technology Enterprise Team, Morgantown, USA.

WOLFE, L. M. 2002. Why alien invaders succeed: support for the escape-from-enemy hypothesis. *The American Naturalist*, 160, 705-711.

WOODS, K. Y. 2003. *Nymphaea odorata* (Water-lily, Nymphaeaceae): Analyses of molecular and morphological studies. Dissertation Master of Science in Biology, Virginia Polytechnic Institute and State University.

WOODS, K., HILU, K. W., WIERSEMA, J. H. & BORSCH, T. 2005. Pattern of variation and systematics of *Nymphaea odorata*: I. Evidence from morphology and inter-simple sequence repeats (ISSRs). *Systematic Botany*, 30, 471-480.

XU, C. Y., JULIEN, M. H., FATEMI, M., GIROD, C., VAN KLINKEN, R. D., GROSS, C. L. & NOVAK, S. J. 2010. Phenotypic divergence during the invasion of *Phyla canescens* in Australia and France: evidence for selection-driven evolution. *Ecology letters*, 13, 32-44.

YAKANDAWALA, D., GURUGE, S. & YAKANDAWALA, K. 2017. The identity of the violet flowered water lily (Nymphaeaceae) and its hybrid origin in the wetland ecosystems of Sri Lanka. *Journal of the National Science Foundation of Sri Lanka*, 45, 381-392.

YE, W., LI, J., CAO, H. & GE, X. 2003. Genetic uniformity of *Alternanthera philoxeroides* in South China. *Weed Research*, 43, 297-302.

ZACHARIADES, C., PATERSON, I. D., STRATHIE, L. W., HILL, M. P. & VAN WILGEN, B. W. 2017. Assessing the status of biological control as a management tool for suppression of invasive alien plants in South Africa. *Bothalia-African Biodiversity & Conservation*, 47, 1-19.

ZIMMERMANN, H., HOFFMANN, J. & MORAN, V. 2004. Biological control in the management of invasive alien plants in South Africa, and the role of the Working for Water Programme: working for water. *South African Journal of Science*, 100, 34-40.