

Research Article

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Corresponding author:

Julie Angela Coetzee; Email: julie.coetzee@ru.ac.za

Population genetics and invasion history of the invasive pickerelweed (*Pontederia cordata*) in South Africa

Sage Nora Wansell¹ , Sjirk Geerts² , Iain Douglas Paterson³  and Julie Angela Coetzee^{3,4} 

¹Department of Botany, Rhodes University, Makhanda, South Africa; ²Department of Conservation and Marine Sciences, Cape Peninsula University of Technology, Cape Town, South Africa; ³Zoology and Entomology, Rhodes University, Makhanda, South Africa and ⁴National Research Foundation - South African Institute for Aquatic Biodiversity, Makhanda, South Africa

Abstract

Clonality plays an important role in the proliferation of invasive species and can facilitate invasive success by reducing reliance on sexual reproduction and promoting spread of genetically identical individuals. Clonal invasions are advantageous for biological control strategies, as biological control agents may have a uniform, damaging response to invasive populations with low genetic diversity. Agents sourced in the native range, that are locally adapted to the same plant genotype that is invasive, may cause this response. Understanding the population genetics and invasion history of a species in the novel range is paramount to ensure effective biological control agents are introduced. Here we investigated the population genetics of pickerelweed (*Pontederia cordata* L.), a tristylous invasive macrophyte native to North and South America that is causing detrimental impacts in South Africa (SA). SA populations all have only one of three tristylous forms and produce no seeds. Inter simple sequence repeats (ISSR) of *P. cordata* leaf samples from SA and from parts of the native range in the United States were used to determine population structure and potential source populations. *Pontederia cordata* has low genetic diversity within and among invasive populations in SA compared with native populations. This suggests no sexual reproduction and limited gene flow in SA and only one introductory event. Invasive populations shared the closest genetic similarity with native samples from Virginia, USA, and although large parts of the indigenous distribution were not sampled, this close match suggests that the site may be a suitable area to source potential biological control agents. However, genetic sampling in other native areas should also be considered to confidently determine the origin of SA populations. This study corroborates other global findings of highly successful invasive species utilizing clonality and, consequently, resulting in invasive genotypes with low diversity.

Introduction

The study of aquatic plant genetics has become increasingly popular, especially within invasive aquatic plant research (Maréchal 2019; Ward et al. 2008). Genetic studies in aquatic plants, particularly invasive species, can differ slightly from those of terrestrial species, because many aquatic invaders rely on distinct reproductive modes, show variable gene flow, and exhibit local adaptation (Hu et al. 2017). Their gene flow is often shaped by the fragmented nature of aquatic habitats, including isolated still waters and sporadic running waters (Abbasi et al. 2016; Barrett et al. 1993). Dispersal pathways also influence genetic diversity, with vegetative fragments frequently enabling more effective spread and establishment than seeds (Barrett et al. 1993). Consequently, clonal reproduction is considered a major factor underlying the broad distributions of many aquatic species (Barrett et al. 1993).

For example, the invasive macrophyte yellow flag iris (*Iris pseudacorus* L.; Iridaceae) has a high genetic diversity and genetically distinct populations in the invaded distribution, presumably due to numerous introductions from different genetic sources, as well as its ability to sexually reproduce and disperse through seeds (Gaskin et al. 2016). On the contrary, a global-scale population genetic survey conducted on the notorious waterweed water hyacinth [*Eichhornia crassipes* (Mart.) Solms; Pontederiaceae], which generally invades through clonal propagules, indicated low levels of genetic diversity (Zhang et al. 2010). These results have improved understanding of the ecology and invasion genetics of *E. crassipes*, indicating that its success in new regions is likely driven by particular life-history traits that facilitate naturalization, rather than by high genetic diversity or strong local adaptation (Zhang et al. 2010).

Clonal growth is common among invasive aquatic plants and can drive rapid population expansion in invaded ecosystems (You et al. 2016). Yet this mode of spread can often produce

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low genetic diversity among propagules, which may be disadvantageous when invading (Dong et al. 2014). Low genetic diversity can restrict the ability of clonal plants to evolve or adapt to local pressures, including pathogens and herbivores. This limitation can enhance the effectiveness of biological control, as host-specific natural enemies sourced from the plant's native range may have greater impact on genetically uniform invasive populations (Paterson et al. 2009; Ward et al. 2008).

Pickeralweed (*Pontederia cordata* L.; Pontederiaceae) is an ideal study species to investigate the introductory events and genetic diversity of invasive populations in South Africa (SA), because it spreads clonally and does not produce seed (Wansell et al. 2022). We hypothesize that invasive *P. cordata* populations may have low genetic diversity and minimal introductory events, which would be ideal for biological control. Alternative control options such as herbicide and manual control are not sustainable for these types of macrophytes, as the species' dense stands and deep-rooted rhizome structures likely impede control efforts (Paterson et al. 2021). *Pontederia cordata* is an invasive macrophyte in SA that belongs to the same family as *P. crassipes*—one of the world's worst invasive aquatic weeds (Holm et al. 1977; Lowe et al. 2000). Recent studies have shown high biological control success rates for *P. crassipes* in SA (Moffat et al. 2024). This may be a strong predictor that *P. cordata* will be susceptible to biological control like its congener (Paterson et al. 2021).

Pontederia cordata has been categorized as a Category 1b invasive weed in SA and has invaded waterbodies throughout the country (NEM:BA 2004). *Pontederia cordata* derives its common name, "pickerelweed," from the pickerel fish that inhabits part of the plant (Eckenwalder and Barrett 1986). It is native across America and has been recorded in Central, North, and South America; however, the pathway of introduction into SA and dispersal mechanisms within the country remain unknown. We speculate that there was only one introduction event into SA with subsequent asexual spread via rhizomes (Wansell et al. 2022).

Pontederia cordata populations in SA are locally distributed around points of introduction, largely in urban areas (Figure 1). It is likely that dispersal pathways originated from the horticultural trade, as this species is used as a pond ornamental plant (Price and Barrett 1982). Although *P. cordata* is a tristylous macrophyte, populations in SA only possess the short-styled flower (Ornduff 1966; Wansell et al. 2022). The absence of medium-styled and long-styled morphologies may likely be caused by clonal propagation from a single introductory event. For biological control, it is important to determine whether each of these populations came from the same origin, in order to collect biological control agents in the native range that are adapted to the same *P. cordata* genotype. A possible biological control agent, the leaf and petiole mining moth *Xubida infusellus* (Lepidoptera: Pyralidae) has been released in Australia for the biological control of invasive *P. crassipes*, and host-specificity studies have shown that the moth causes considerable damage to both *E. crassipes* and *P. cordata* under high- and low-nutrient regimes (Stanley et al. 2007). *Xubida infusella* and other native herbivores may therefore be of interest during biological control surveys on native *P. cordata* plants in the United States (Harms and Grodowitz 2009; Stanley et al. 2007) and should be adapted to the South African *P. cordata* genotype.

To date, no genetic analyses have been performed on invasive *P. cordata* populations. Understanding the genetic diversity of populations from native regions in North America and invasive regions throughout SA would provide insight into the introduction and dispersal of *P. cordata*. Genetically depauperate populations in

the novel range may be the result of asexual spread. Sexual propagation may not be viable due to "illegitimate" cross-pollination and self-incompatibility among same-morphed flowers in SA (Ornduff 1966; Wansell et al. 2022). Asexual spread could also result in high genetic diversity among invasive populations due to multiple introductions or a wide range of source populations.

This study investigated the genetic diversity of *P. cordata* among populations in the invasive range in SA and compared these with the native range in the United States. This allowed inferences of whether biocontrol is likely to be a successful option for control and guided the collection of potential agents.

Materials and Methods

Sampling and Leaf Preservation

Pontederia cordata comprises three main varieties: *Pontederia cordata* var. *cordata* L. [syn.: *Pontederia cordata* var. *ovalis* (Martius) Solms], and *Pontederia cordata* var. *lanceolata* (Nutt.) Griseb [syn.: *Pontederia cordata* var. *lancifolia* (Muhl. ex Elliott) Torr.]. The characteristics of *P. cordata* plants in SA include tristylous, lilac to blue flowers on a tall spiked inflorescence, and lanceolate to heart-shaped leaves that are similar to the features of *P. cordata* var. *lanceolata* and *P. cordata* var. *cordata* (Lowden 1973; Melton and Sutton 1991).

We collected samples from 13 introduced *P. cordata* populations from natural waterbodies and impoundments in SA in 2018, covering the widest geographic distribution possible in order to obtain an extensive range of genetic variability (Table 1; Figure 1). Native populations were also sampled in 2018 from 13 localities in the United States (Table 1). Within each *P. cordata* population, approximately 5 g of healthy, young leaf material was harvested from 1 plant per meter and placed into ziplock plastic bags. Sample sizes differed between sites and depended on the size of the site. The samples were individually wiped with distilled water to remove any epiphytes and dirt and then wrapped in sterile tissue paper. Samples were then dried using silica gel blue according to Chase and Hills (1991) in sealed plastic bags.

DNA Extraction

DNA from the *P. cordata* samples was extracted using the Qiagen DNeasy® Plant Mini Kit following the manufacturer's protocol to obtain total genomic DNA (Qiagen 2006). Individual dried plant samples (leaf tissue) were ground up in a mortar and pestle under liquid nitrogen before following the Qiagen protocol. In total, we extracted DNA from 63 plant samples across all invasive ($n = 31$) and native sites ($n = 32$), with 1 to 2 plants sampled per site, except for St Francis Marine and Tongaat in the invasive range, where 9 and 10 plant samples were extracted, respectively (Table 1). These two large populations covered a large area and could have contained variable genetic diversity. Belle Haven, VA, and Coconut Creek, FL, had 10 samples collected per site as they were large populations. The resultant DNA samples were stored at -20°C until further use.

Inter Simple Sequence Repeat PCR Protocol and Analyses

Eight primers were screened (McCauley and Ballard 2002; Wolfe et al. 1998; Yockteng et al. 2003). The primers HB13 (GAGGAGGAGGC) and HB15 (GTGGTGGTGGC) produced the highest number of replicable bands and were used in subsequent analyses. Primers were labeled with the fluorescent dye

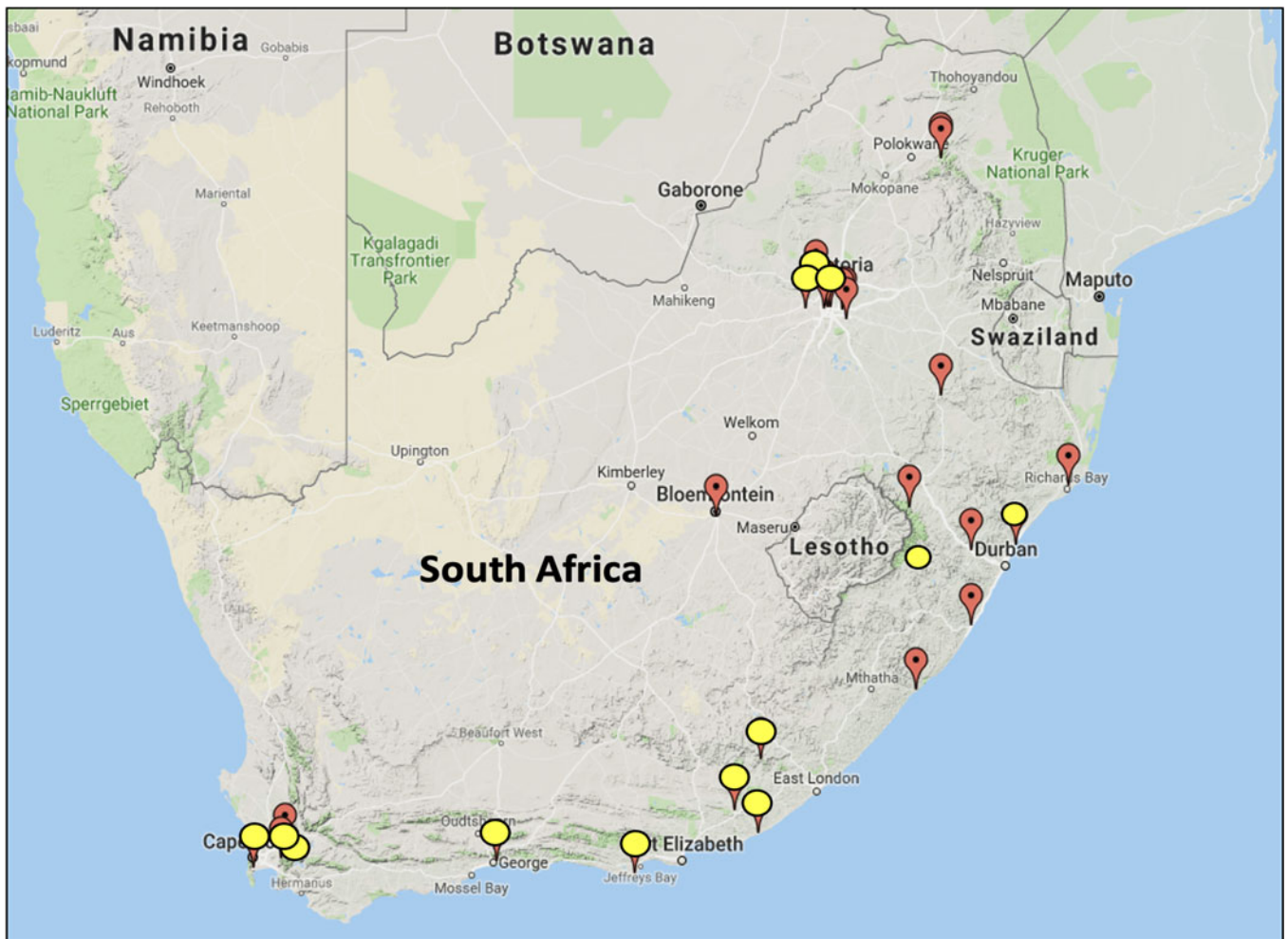


Figure 1. Distribution of invasive *Pontederia cordata* populations in South Africa. Red markers indicate recorded populations (SANBI 2016; SAPIA 2020). Yellow markers indicate genetic sample locations.

6-FAM by the primer manufacturers, so that capillary electrophoresis could be utilized to measure fragment lengths.

PCR reactions of 20 µl were prepared for each sample using 10 µl of iTaQ Universal green SYBR SuperMix™ (Bio-Rad, Hercules, California), 0.8 µl of primer diluted from stock solution (1:9 with ddH₂O), 0.8 µl of MgCl (included in the iTaQ), 5.4 µl of ddH₂O, and 3 µl of template DNA (ranging from 5 to 15 ng µl⁻¹). The PCR protocol for inter simple sequence repeats (ISSR) using DNA from *P. cordata* leaf samples followed the same protocol used by Paterson et al. (2009), with an annealing temperature of 44 °C.

Reactions were replicated from the PCR step in different thermocyclers to verify reproducibility, and the resultant PCR products were sent to the Central Analytical Facility in Stellenbosch, SA, for processing into electropherograms by capillary electrophoresis (3130 genetic analyzer, GS500LIZ size standard, Applied Biosystems).

Data Processing and Analysis

The electropherograms were analyzed in GeneMarker® v. 2.7.4 (SoftGenetics). Only electropherogram peaks or “bands” that were present in both replicates were verified and scored. The absence or presence of bands was used as a scoring process. These scores (as a binary matrix) first originated as electropherograms that we

processed in GeneMarker® using the amplified fragment length polymorphism (AFLP) analysis type on the default settings.

The first 120 bp were removed as unreliable peaks, and all peaks fell with the 1,000-bp range. A peak detection threshold of 50 relative fluorescence units (RFU) was set. These settings were selected because they are considered conservative and would therefore minimize genotyping errors (Whitlock et al. 2008). All default settings mentioned showed negligible effects on scoring during preliminary testing and thus were left as is. The resultant allele report was saved as a.txt file and exported to RawGeno v. 2.0.1 (Arrigo et al. 2012) for band scoring.

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

Thereafter, the following binning parameters were set: maximum bin width: 1.5 bp; minimum bin width: 1.0 bp; scoring range: 100 to 500 bp; low fluorescence bins (std RFU): 100. Electropherogram peaks lower than 100 bp were discarded, as they were likely to be homoplasious and unreproducible (Arrigo et al.

Table 1. Localities and coordinates of *Pontederia cordata* sample sites in South Africa and the United States.

Province/state	Location	Geographic coordinates		
	Invasive range—South Africa	°S	°E	No. of samples
KwaZulu-Natal	Tongaat	29.5881	31.0850	10
KwaZulu-Natal	Underberg Golf Course	29.7861	29.4894	1
North West Province	Pecanwood Estate, Hartbeespoort, Johannesburg	25.7684	27.8575	1
Gauteng	Emmarentia Botanical Gardens, Johannesburg	26.1618	27.9998	2
Gauteng	Glenhazel, Johannesburg	26.1389	28.1020	1
Western Cape	Jonkershoek	33.9568	18.9152	1
Western Cape	Westlake	34.0756	18.4512	1
Western Cape	Heldervue	34.0607	18.8282	1
Western Cape	Eden/Wilderness	33.9657	22.6098	1
Eastern Cape	Royal Port Alfred Golf Course	33.6004	26.8917	1
Eastern Cape	St Francis Marine	34.1484	24.8757	9
Eastern Cape	Grahamstown/Makhanda old golf course	33.2969	26.4993	1
Eastern Cape	Hogsback Arboretum	32.5951	26.9468	1
Native range—United States		°N	°W	
Virginia	Belle Haven Marina boat launch	38.7770	77.0480	10
Louisiana	Blind River	30.0939	90.7624	1
Florida	Coconut Creek	26.3096	80.2035	10
Florida	Apopka Lake	28.6839	81.6735	1
North Carolina	Apex Town Lake	35.7488	78.8178	1
Texas	Lennox Foundation Preserve	25.8493	97.3891	1
Virginia	Cone Marsh Wildlife Management Area, Louisa County	41.3803	91.3957	1
Florida	Okeechobee Lake	26.9329	81.0503	1
North Carolina	Wolf Village NCSU campus	35.7886	78.6863	1
Florida	Everglades	26.3206	80.3300	1
Florida	Kissimmee Lake	27.9899	81.2818	1
Texas	Lewisville Aquatic Ecosystem Research facility	33.0690	96.9580	2
Georgia	Seminole Lake	27.8415	82.7738	1

2012). The binary matrix produced in RawGeno was subsequently exported and consolidated in Microsoft Excel® (Redmond, WA, USA).

A pairwise similarity index using the consolidated binary matrix was generated in FreeTree (Hampl et al. 2001) using Jaccard's index and was subsequently used to output levels of genetic variation. The output datasets were processed in Microsoft Excel® using all within-pairwise values to plot the average genetic variability between the native range and the invasive range with a Mann-Whitney test on the genetic distances of the samples.

The binary matrix was converted to a pairwise similarity matrix in PAST: Paleontological Statistics Package v. 3.26 (Hammer et al. 2001) using Jaccard's coefficient for binary genetic data to produce a principal components analysis (PCA) with a covariance matrix and scatter plot comparing individuals and groups of native and invasive samples.

STRUCTURE v. 2.3.4 (Pritchard et al. 2000) was used to process the binary dataset in a Bayesian model-based cluster analysis to infer genetic structure and clustering. STRUCTURE was used to assign SA and U.S. samples into the appropriate clusters (K) or "populations" according to their genetic similarities (Pritchard et al. 2000). The optimal K -value was determined using StructureSelector (Li and Liu 2018), which presented the number of natural clusters into which the samples were partitioned. The Puechmaile (2016) and Evanno et al. (2005) methods were used in StructureSelector for K -estimation. When population data were not set as priors, the Puechmaile (2016) method produced $K = 2$ at a threshold of 0.5 to 0.8. The Evanno et al. (2005) method reported $K = 5$ for ΔK and $K = 8$ for mean $\text{LnP}(K)$. When the population data were set as priors (i.e., native and invasive samples were labeled/categorized into their countries), the Puechmaile (2016) method still produced $K = 2$. The Evanno et al. (2005) method reported $K = 4$ for ΔK and $K = 5$ for the mean $\text{LnP}(K)$. Because the sample size was uneven and the Puechmaile (2016) method is said

to account for and outperform other sampling methods for unevenness, $K = 2$ was selected for the STRUCTURE output. The Puechmaile method uses four statistical tests to estimate the optimal number of clusters, namely MedMeaK, MedMedK, MaxMeaK, and MaxMedK. The threshold set in StructureSelector was set to a range of 0.5 to 0.8 as recommended (Puechmaile 2016). The correlated allele frequencies and admixed model were applied with settings of 10,000 burn-in iterations and 250,000 Monte Carlo Markov chain iterations with 10 independent runs of the analysis. The admixed model with uncorrelated allele frequencies was used, as this has been demonstrated as the most efficient model to study intraspecific variation (Falush et al. 2003) and is appropriate for dominant markers like ISSRs (Stift et al. 2019). CLUMPAK software (Kopelman et al. 2015) was used to summarize and graphically visualize the resultant STRUCTURE output.

A neighbor-joining tree with bootstrap support (10,000 replications) was constructed from the consolidated matrix in SplitsTree4 v. 4.16.1 (Huson and Bryant 2006). Bootstraps were run for 10,000 runs to record any significant branching of major branches.

Results and Discussion

Overview of ISSR Profiles

A total of 258 loci were amplified, 127 of which were informative polymorphisms. The average number of loci produced per sample was 28.35 (SE $\pm$ 2.05) and only four samples, all from the Tongaat site in SA, had identical ISSR genotypes.

PCA and STRUCTURE analysis

The genetic distance of the native U.S. populations (median = 0.2139 $\pm$ 0.25 (quartile range (Q.R)), $N = 496$) was significantly

Component 2

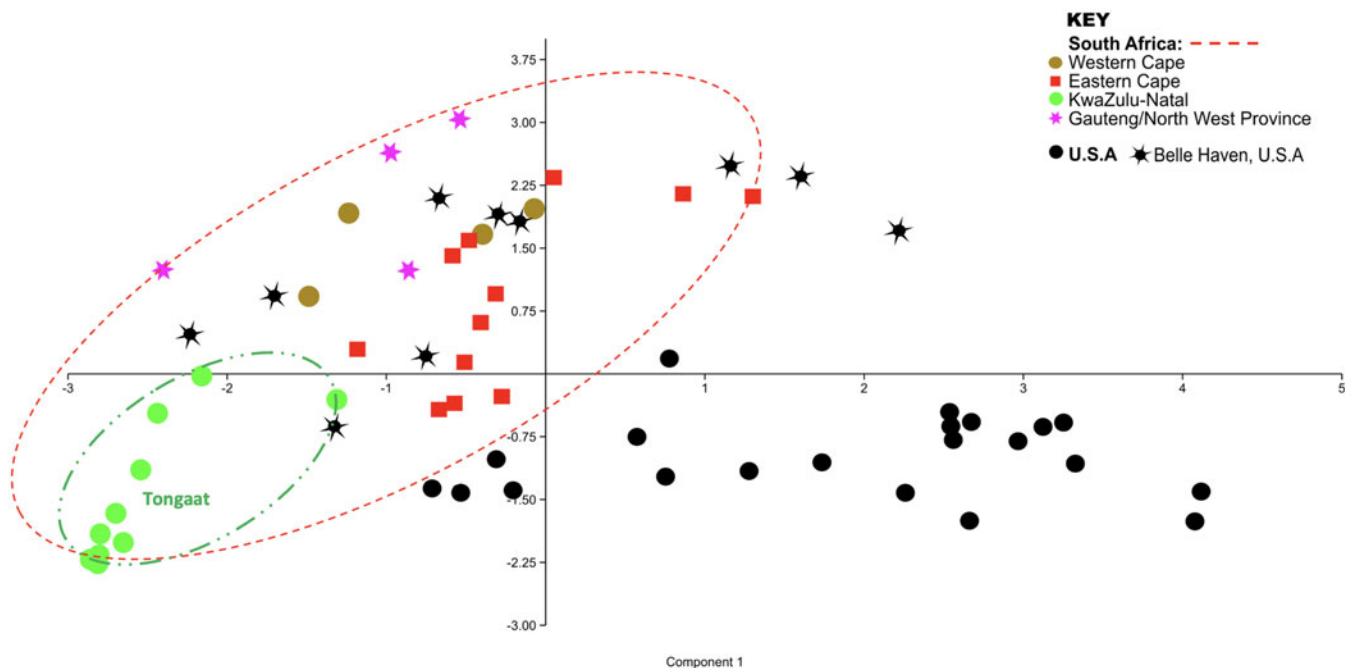


Figure 2. Principal component analysis (PCA) from inter simple sequence repeats (ISSR) binary data demonstrating predominantly one genetic cluster of *Pontederia cordata* in the invasive range (colored symbols) and scattered genetic points in the native range (black symbols). KwaZulu-Natal individuals from Tongaat and Underberg Golf Course, are circled in green. Black stars infer genetic similarity of native to invasive (color) individuals. Black circles infer high genetic diversity among native individuals. Note that 21.9% of the variation is explained by Component 1 and 12.8% by Component 2.

greater than that of the invasive SA populations (median = 0.17 ± 0.31 , $N = 465$) ($U = 963$, $P < 0.0001$). The PCA of the native and invasive populations showed a general cluster of native populations (USA, black symbols), with exception of Belle Haven (USA, black star symbol, Figure 2).

The STRUCTURE analysis found two distinct clusters. SA populations formed one cluster and some of the U.S. populations formed the second cluster, with some populations assigned to the same cluster as SA (Figure 3A). A high proportion of the individuals from Belle Haven were assigned to the same group as the SA populations, and a few individuals from Coconut Creek, Wolf Village, and Cone Marsh in the United States had high or medium ancestry assigned to the same group as the SA populations (Figure 3A).

The $K = 2$ STRUCTURE output for SA populations is presented in Figure 3B. Distinct grouping of Tongaat, Kwa-Zulu Natal (Figure 3B, $n = 10$) is depicted in green, similar to the Tongaat individuals clumped together in the PCA plot (Figure 2).

SplitsTree Analysis

Native U.S. populations grouped together, except for Belle Haven, VA, which was scattered in between invasive range populations (Figure 4). Individuals from Tongaat, KwaZulu-Natal, in the invasive range showed major branching off from the other populations (Figure 4). Within the invasive range, individuals from the St Francis Marine were separated into two branches and KwaZulu Natal, Gauteng, and the Western Cape individuals showed some grouping with these individuals. None of the major branches showed significant bootstrap support, which is not unusual for ISSR datasets (Alfaro et al. 2003; Douady et al. 2003).

These findings confirm that *P. cordata* populations in the native regions of the United States displayed a higher genetic diversity compared with the invasive populations sampled in SA. Belle Haven had a particularly high within-site diversity, but this was expected, as it was a much larger site than any of the other sites in either country, and even if Belle Haven was removed from the analysis, the genetic diversity in the United States is still higher than in SA.

Although not always the case, low genetic diversity is usually present when plants spread asexually via clonal propagules and vegetative fragments (Hollingsworth and Bailey 2000; Meloni et al. 2013). The higher genetic diversity in the native range suggests there is greater gene flow compared with the invasive samples, which appear to be spreading clonally (Gaskin et al. 2011).

Overall, there were two main clusters, a relatively uniform group of SA populations and a more variable group of native U.S. individuals, some of which overlapped with the SA individuals. The Tongaat population in KwaZulu-Natal showed particularly limited ISSR variation, consistent with the monotypic stands and dense rhizome structures commonly observed in the invasive range. While these patterns suggest clonal spread, confirmation will require more detailed analysis.

We speculate that only one or a few propagules were introduced during a single introductory event, and further genetic assessments should be undertaken to confirm this. The low genetic diversity that is recorded suggests limited introduction events, clonal propagation, or both. The genetics therefore corroborates previous work suggesting that clonal growth is prevalent in SA (Wansell et al. 2022).

Low genetic diversity was often considered a barrier to invasion; however, recent findings suggest otherwise (Eckert 2000; Geng

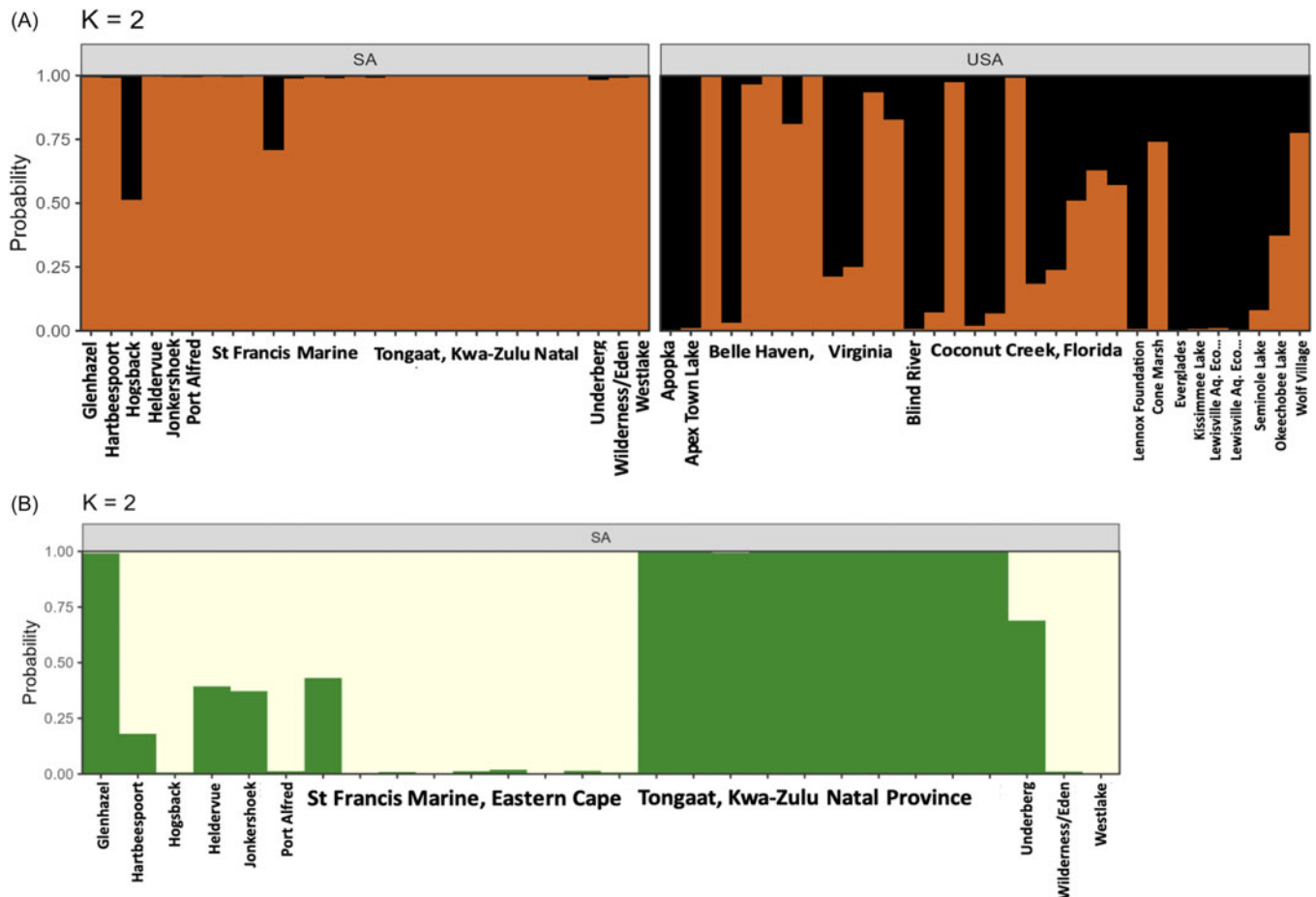


Figure 3. Probability of assignment of *Pontederia cordata* individuals to genetic clusters (K) based on inter simple sequence repeats (ISSR) data for all samples (A) and just invasive populations in South Africa (B). Columns represent the probability of assignment of populations to two genetic clusters using the Bayesian clustering algorithm in STRUCTURE. Individuals are grouped by geographic locality from which they were collected. $n = 10$ for Belle Haven, Coconut Creek, and Tongaat; $n = 9$ for St Francis Marine; $n = 2$ for Lewisville; and $n = 1$ for all other labeled samples.

et al. 2016; Song et al. 2012). Although introduced populations may experience genetic bottlenecks and, in outcrossing species, inbreeding depression that reduces survival and offspring fertility (Charlesworth and Willis 2009; Ellestrand and Elam 1993; Heschel and Paige 1995; Lynch et al. 1995; Mullarkey et al. 2013), several mechanisms can mitigate these effects. Clonal integration, phenotypic plasticity, preadaptation, random genetic mutations, and asexual reproduction (leading to fixed heterozygosity) may all facilitate invasion under low genotypic diversity (Geng et al. 2016; Hodgins et al. 2025; Novak and Mack 2005; Song et al. 2012). Clonal integration enables translocation of resources between interconnected ramets, improving persistence under environmental heterogeneity, disturbances, and local resource shortages (Song et al. 2012; Wang et al. 2017). In this regard, we speculate that clonal integration of *P. cordata* rhizomes could be contributing to the successful spread and rapid proliferation of invasive populations, giving them a competitive edge over native macrophytes in SA.

Additional adaptive processes, such as rapid post-introduction evolution, phenotypic plasticity, and preadaptation may further support invasion (Geng et al. 2016). Phenotypic plasticity may allow invasive plants to colonize diverse habitats (Pichancourt and van Klinken 2012; Sultan 2001). Some degree of preadaptation may also facilitate persistence in a new habitat where a species has no

previous history of adaptation (Jenkins and Keller 2010; MacDougall et al. 2018), which is plausible for *P. cordata*, given climatic similarities between parts of its native range and SA. Epigenetic processes may further influence the traits relevant to invasion, especially in primarily clonal species that are likely to be genetically depauperate (Mounger et al. 2021).

Limited variation does not necessarily restrict spread. For example, 80% of invasive populations of *E. crassipes* consist of a single clone (Zhang et al. 2010), and ornamental jewelweed (*Impatiens glandulifera* Royle; Balsaminaceae) has become widespread in Europe despite very low diversity in its introduced range (Hagenblad et al. 2015). These cases demonstrate that reduced genetic diversity such as that observed in invasive *P. cordata*, is not unusual among successful invaders.

Overall, the findings of our study suggest reduced genetic diversity in SA compared with the indigenous distribution. It is therefore imperative that any additional introductions of genetic material into SA are stopped. Further uncontrolled introductions of *P. cordata* from different regions of the native range into SA could lead to new genotypic invaders with greater invasive potential and disastrous impacts on native ecosystems.

These findings are an important first step to developing a biological control program for *P. cordata* in SA, and while more sampling is needed before one can accurately conclude where the

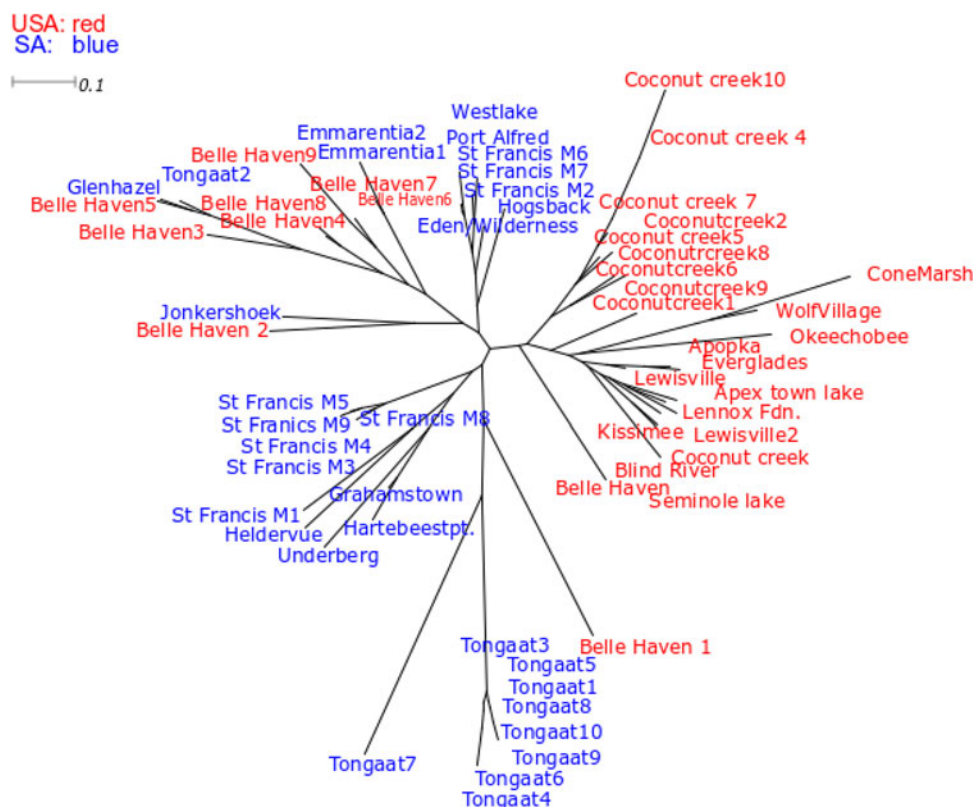


Figure 4. SplitsTree v. 4 graphical output (NeighbourNet method using Jaccard distance) for *Pontederia cordata* individuals from South Africa (SA, invasive range) and United States (USA, native range).

invasive *P. cordata* source populations come from, this study may direct future sampling efforts. The low genetic diversity found in the invasive populations might be advantageous for biological control, given that there may be biological control agents suitably adapted to this invasive genotype. Our results suggest a limited number of introductions, possibly followed by primarily clonal reproduction in SA.

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Competing interests. The authors declare no conflicts of interest.

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