

Exploring the Origin and Genetic Diversity of the Giant Reed, *Arundo donax* in South Africa

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The giant reed, *Arundo donax* is one of the worst invasive alien species globally, including South Africa, where it invades riparian areas across the country. Biological control is being considered to address the invasive potential and negative impacts of the weed. This study investigated the phylogeography of *A. donax* to guide the biological control program. To determine plant haplotype and genetic diversity, three regions of the chloroplast were sequenced and three microsatellite markers were analyzed in 40 samples from across the plant's distribution in South Africa. It was determined that all populations of *A. donax* in South Africa were haplotype M1, which is the most widely distributed haplotype worldwide, believed to originate from the Indus Valley, Asia. In addition, no genetic diversity was found, indicating that all the *A. donax* populations in South Africa are essentially one clone. The results indicate that suitable biological control agents are likely to be found in the ancient native range of haplotype M1. This research has contributed to the global understanding of the phylogeography of *A. donax* and will guide the biological control program in South Africa.

Nomenclature: Giant reed; *Arundo donax* L. ABKDO.

Key words: Biological control, chloroplast, haplotype, microsatellites, tall-statured grasses.

Alien species are often categorized as being either archaeophytes (species introduced at the beginning of the Neolithic period, 1500 B.C.E.) or neophytes (taxa spread after 1500 C.E.) (Pyšek et al. 2004). However, labeling a species as such is complex and necessitates looking at the phylogeography of the species. For example, archaeophytes can become neophytes with the introduction of new genetic material that can lead to novel genotypes (Saltonstall 2002). One such example has been found in the tall-statured grass, giant reed (*Arundo donax* L.), which has long been accepted as an archaeophyte in areas such as the Mediterranean region; however, with improved molecular work, it has become apparent that the reed can become a neophyte when recent and multiple introduction events occur (Tarin et al. 2013). This has important implications concerning the genetic diversity of the species and the subsequent invasive potential in the introduced range that can be used to inform management of the species.

Arundo donax is a tall, perennial, reedlike grass that is one of the most biologically productive plants worldwide, reaching heights of 6 to 8 m (Angelini et al. 2005; Cosentino et al. 2006). It has intentionally been distributed worldwide as a result of its wide range of uses, most notably for erosion control, musical instruments, crafts, and as a building material (Bell 1997; Cosentino et al. 2006; Dudley 2000). In areas where *A. donax* has been introduced, particularly in tropical and warm-temperate regions, it has naturalized, and in many areas, including the subtropical United States through Mexico, the Caribbean islands, South America, the Pacific Islands, Australia, and South Africa, it has become highly invasive (Dudley 2000; Else 1996; Haddadchi et al. 2013; Quinn and Holt 2008). Within these introduced ranges, *A. donax* can only reproduce asexually, but despite this, the reed is highly adaptable to a wide range of conditions such as varying soil types, salinity, and drought (Calheiros et al. 2012; Lewandowski et al. 2003; Tracy and DeLoach 1998).

Arundo donax is widespread and abundant throughout the African continent (Milton 2004). It was deliberately introduced into South Africa in the late 1700s, primarily for erosion control (Guthrie 2007). The reed spread throughout the country as vegetative growth was facilitated by anthropogenic activities, including building of dams and

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Management Implications

The use of molecular-based techniques has proven to be an important tool in managing invasive alien species. In particular, the application of genetics in discerning the native range of invasive weeds has aided in efforts to understand the adaptive potential and population structure of invasive species. Furthermore, this information can play a critical role in guiding biological control programs that rely on determining the native range of an invasive plant to find suitable biological control agents. The invasive giant reed (*Arundo donax* L.) is being considered for a biological control program in South Africa, and this study aimed to use molecular-based techniques to inform this management approach. It was determined that *A. donax* populations have no genetic diversity and belong to haplotype M1, which is known to have an ancient origin in Afghanistan and Pakistan in the Indus Valley and is now found worldwide, including North America. From this, it is now possible to make use of the extensive studies that have already been conducted on haplotype M1 to find suitable biological control agents from the native range to introduce to North America. Much of this research will be applicable to South African populations due to a shared haplotype, and thus a number of potential biological control agents can now be considered for release in the country.

soil stabilization; it has since become one of the worst invasive alien species in the country and is now present in all nine provinces (Guthrie 2007; Van der Merwe et al. 1990; van Wilgen et al. 2007). The reed has been listed as a Category 1 invasive alien species according to the National Environmental Management: Biodiversity Act (NEMBA, Act No. 10 of 2004) (Henderson 2001; van Wilgen et al. 2007). Category 1 plants are prohibited from being sold or planted, and additional efforts are needed to keep the plant under control (Henderson 2001). Using climate envelope models, a study by Rouget et al. (2004) estimated that 79% of South Africa, Lesotho, and Swaziland is potentially suitable for *A. donax* to invade. To address the invasive threat and impacts of *A. donax*, control options are being considered that include investigating the potential of biological control. However, before such management can be considered, it is important to first address the plant's phylogeography in the region to help guide this process.

Arundinoideae is one of the most unresolved grass subfamilies, historically known as the dustbin group by taxonomists (Barker et al. 1995; Hardion et al. 2012; Linder et al. 1997). For *A. donax* this is further complicated by the fact that the reeds are a "cryptogenic species," and thus its true origin is highly debated, as the biogeographic and evolutionary origin of the species is obscured through ancient cultivation (Mariani et al. 2010). Two lineages of *A. donax* have been identified based on genetic analyses: European and Asian/Middle Eastern populations (Mariani et al. 2010). In Asian populations, *A. donax* was found to have viable seeds, and thus there is a relatively high degree of genetic variation (Hardion et al. 2012). In Europe,

however, *A. donax* stands are sterile, and thus there is lower genetic diversity compared with Asian populations (Lewandowski et al. 2003). It is believed that these populations reflect a genetic subset of populations from Asia and thus represent a genetic bottleneck (Ahmad et al. 2008; Hardion et al. 2012). This most likely occurred when particular genotypes were selected and spread such that, over time, a single clone was being cultivated worldwide (Ahmad et al. 2008).

The genetic lineages found in *A. donax* and their species immigration history may have important consequences for the evolutionary processes that regulate the species' geographic range and consequent invasive potential. The evolutionary mechanisms that drive range expansion in invasive alien species are highly complex and for the most part not fully understood; however, it is generally agreed that a plant's ability to adapt is driven by genetic diversity (Holt 2003; Kirkpatrick and Barton 1997; Vendramin et al. 2008). Multiple introductions of a species into an area can overcome bottleneck effects by providing novel alleles and new genetic combinations (Chapman et al. 2000; Pérez de la Vega et al. 1991). With increased genetic diversity, plants are expected to have increased adaptability; for example, the invasive reed canarygrass (*Phalaris arundinacea* L.) in North America has been found to have increased genetic variation due to multiple introductions from the native range, resulting in rapid selection of novel genotypes that allow for adaptation (Lavergne and Molofsky 2007). Similarly, *A. donax* has been found to have increased genetic diversity where there have been multiple introduction events; for example, Tarin et al. (2013) determined that, although there is likely only one clonal lineage in North America, there is microsatellite marker evidence for different populations within this lineage as a result of introductions from different sources. Such immigration history in *A. donax* may be an important factor in the plant's adaptability in the adventive range.

Finally, determining the phylogeography of *A. donax* will serve as an important tool in managing the species, particularly in guiding biological control. The use of molecular techniques to discern the native range of a species has shown to have great potential in biological control, as there is growing evidence that herbivores are sensitive to plant genotype (Bhattarai 2015; Cronin et al. 2016; Lambert and Casagrande 2007). For example, a study by Goolsby et al. (2006) used genetics to investigate the role of coevolution on the invasive Old World climbing fern [*Lygodium microphyllum* (Cav.) R. Br.] and the phytophagous mite (*Floracarus perrepae* Knihinicki & Boczek) in the United States. Herbivore transfer experiments determined that *F. perrepae* were most effective at inducing galls in the climbing fern plant haplotypes from the same native range (Goolsby et al. 2006).

The determination of a strong geographical pattern in mite–fern associations supported the theory of local adaptations (Ehrlich and Raven 1964) and further helped guide biological control to optimize host exploitation in the correct region of origin. Genetic techniques are thus important to help ensure that biological control agents are selected from plants in the correct area of introduction so they will be locally adapted (Goolsby et al. 2006; Roderick 2004).

To date, no work has been carried out on determining the native origin of *A. donax* populations in South Africa and how these populations are genetically structured compared with populations elsewhere in the world. With a biological control program being proposed, this study assessed the phylogeography of *A. donax* in South Africa with the aim of contributing to the knowledge of the founder history of this invasive species and to obtain a better understanding of its reproduction and dispersal mechanisms.

Materials and Methods

Sampling and DNA Extraction. Leaf tissue was collected from the young apical leaves of *A. donax* during the growing season. Samples were collected from across the distribution of *A. donax* in South Africa (Figure 1). Fresh leaves were dried in silica gel according to the protocol of Chase and Hills (1991). DNA was extracted using the Qiagen DNeasy® Plant Mini Kit (Valencia, CA). The Qiagen protocol was modified in that leaf tissue was ground dry in liquid nitrogen before the addition of the extraction buffer.

cpDNA. Plastid DNA diversity was assessed by amplifying and sequencing three intergenic spacers: *trnT* (UGU) to *trnL* (UAA) (Taberlet et al. 1991), *rbcL* to *psaI*, and *trnS* (GCU) to *psbD* (Saltonstall 2001) using the methods of Hardion et al. (2014). Ten picomoles of forward and reverse primers were added to 12.5 µl of Promega MasterMix (Madison, WI) (reaction concentration of 1 U of *Taq*, 1.5 mM

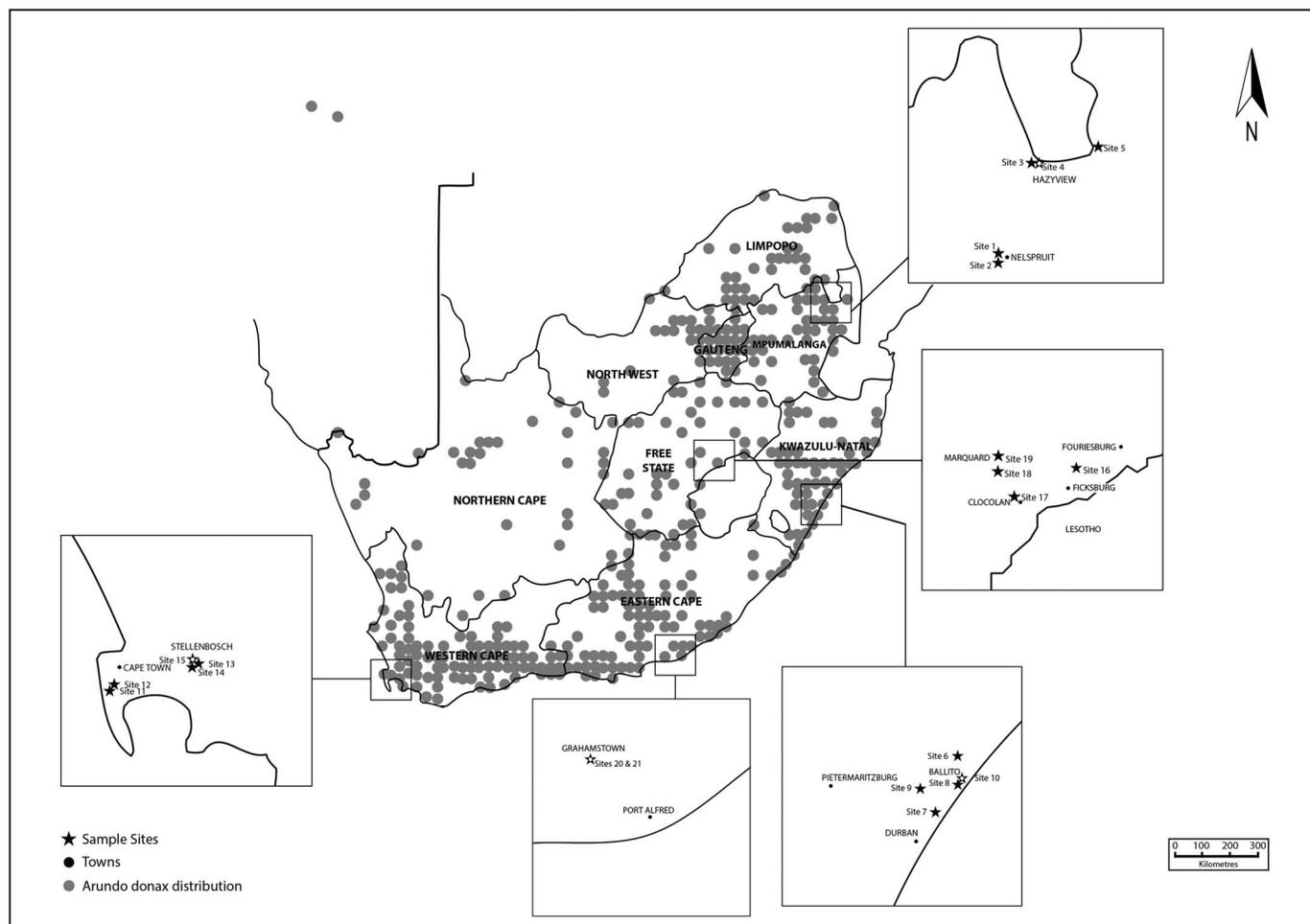


Figure 1. Map of South Africa showing site locations of samples (Appendix S1) and *Arundo donax* distribution according to Fish et al. (2015).

MgCl₂, and 0.2 μM dNTPS), 2 μl of Promega MgCl₂, and 7 μl of template DNA per reaction. Promega nuclease-free water was added to reach a final volume of 25 μl. Amplifications were performed in one of the following machines: Labnet Multigene II (Labnet International, Edison, NJ) or Applied Biosystems 2720 thermal cycler (Applied Biosystems™, Foster City, CA). For the *trnLb* region (*trnT* to *trnL*), the PCR cycling protocol was 94 C for 2 min, 35 cycles of 94 C for 1 min, 56 C for 1 min, 72 C for 2 min, followed by a final extension at 72 C for 5 min. PCR products were cleaned at Inqaba Biotec™, Johannesburg, South Africa. Cycle sequencing reactions were done using BigDye Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems™) with the same primers as used in the PCR reactions. Cycle-sequencing products were purified using ethanol-sodium acetate precipitation. Capillary electrophoresis was done using an ABI 3500® (Applied Biosystems™) genetic analyzer at Inqaba Biotec™.

Microsatellites. Three microsatellite primers developed by Tarin et al. (2013) for *A. donax* in the native (Old World) and introduced (North America) ranges were used (Appendix S2); these markers were selected because they were found to have the highest number of alleles in the Old World and North America. PCR reactions contained 10 pmoles of forward and reverse primers, 12.5 μl of Promega MasterMix (Madison, WI) (reaction concentration of 1 U of *Taq*, 1.5mM MgCl₂, and 0.2 μM dNTPS), 2 μl of Promega MgCl₂, and 7 μl of template DNA per reaction. Promega nuclease-free water was added to reach a final volume of 25 μl. Amplifications were performed in one of the following machines: Labnet Multi Gene II (Labnet International) or Applied Biosystems 2720 thermal cycler (Applied Biosystems™). The PCR cycling protocol was 98 C for 30 s, 30 cycles of 98 C for 10 s, 55–62 C for 30 s, 72 C for 15 s, followed by a final extension at 72 C for 5 min. Primers were fluorescently labeled by Applied Biosystems™, South Africa. PCR products were diluted 20 times with Promega nuclease-free water and sent to Inqaba Biotec™, Johannesburg, South Africa for analysis. Capillary electrophoresis of DNA fragments was done using an ABI 3500® (Applied Biosystems™) genetic analyzer at Inqaba Biotec™.

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

Data Analysis. Chloroplast DNA chromatograms were examined, and contiguous sequences were assembled and manually edited in GeneStudio™ v. 2.2.0.0. (GeneStudio, Suwanee, GA). Alignment of sequences was done in MEGA v. 5.2.2, using ClustalW set to default parameters (Kumar et al.

2012), and included all worldwide haplotypes downloaded from GenBank.

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

Results

The analysis of chloroplast sequences for *A. donax* revealed that all samples from across South Africa were haplotype M1 from Hardion et al. (2014). *TrnT-trnL* samples aligning with TL5 (accession number: KF169820) and *rbcL-psaI* samples aligning with L16 (accession number: KF169810) and *trnS-psbD* samples aligning with accession number: KF169824. No variation in the chloroplast sequences was found across all samples.

Of the four biogeographical clusters determined by Hardion et al. (2014), the South African *A. donax* groups with the Middle East biogeographic cluster. Haplotype M1 is the most common haplotype worldwide and was found in 28 samples in the Mediterranean and Irano-Touranian regions; the haplotype was also found in New Caledonia, Peru, and North America (Hardion et al. 2014). The most closely related haplotypes are M2, M3, and M4, which are found in Afghanistan and Pakistan in the Indus Valley (Hardion et al. 2014).

All plants sampled across South Africa shared a single multilocus genotype. All populations were found to share the same genotype, and in addition, all replicated samples were found to have no variation in the peaks amplified. The study found an error rate of zero. South African samples had a low number of alleles, particularly when considering the allelic diversity from studies of populations in the Old World (native range) and introduced range (New World)

Table 1. The number of alleles found for each marker for *A. donax* in the Old World (native range) compared with the introduced ranges in North America and South Africa.^a

Marker	Number of alleles		
	Old World (<i>n</i> = 203)	North America (<i>n</i> = 159)	South Africa (<i>n</i> = 40)
B7	29	16	1
B1	24	13	1
A3	21	18	2

^a Data for populations outside South Africa were sourced from Tarin et al. (2013).

Table 2. Comparison of the genetic diversity of *A. donax* in New World populations (introduced) and Old World populations (native range) and a comparison of clonality within populations (separate genets in separate locations) and how these compare with populations in South Africa.^a

	Number of samples	Number of genotypes	Nei's genetic diversity	Evenness	Shannon's corrected index
New World	159	6	0.243	0.220	0.266
Old World	203	129	0.929	0.120	2.218
Within-population genetic diversity					
Israel	6	1	0	1	0
California, U.S.A.	5	1	0	1	0
Texas, U.S.A.	14	1	0	1	0
South Africa	40	1	0	1	0

^a Data for populations outside South Africa were sourced from Tarin et al. (2013).

(Table 1). There was no genetic diversity found across all populations of *A. donax* in South Africa. Tarin et al. (2013) also found no genetic diversity within populations in North America and Israel (Table 2). From this, it was determined that using only three of Tarin et al.'s (2013) microsatellite markers was sufficient, as they highlighted the genetic uniformity in populations, and including more markers would most likely not show any significant differences across populations.

Discussion

Arundo donax is believed to represent one of the world's oldest plant invasions (Hardion et al. 2014). For thousands of years, *A. donax* has been a favored plant for a variety of uses and selected genotypes were chosen and distributed worldwide (Goolsby et al. 2009). Within these adventive ranges, *A. donax* does not reproduce sexually and thus relies on vegetative growth (Khudamrongsawat et al. 2009). All of these factors have contributed to a lack of genetic diversity in *A. donax* outside the Asian/Middle Eastern populations (Flores and Wood 2009; Khudamrongsawat et al. 2009; Saltonstall et al. 2010). The results from this study are similar to those found in other parts of the plant's introduced distribution, with only one haplotype (haplotype M1) being found across South Africa and populations being found to be genetically uniform.

The Mediterranean region is the source of about 60% of all naturalized alien grasses in southern Africa (Milton 2004). The success of these Mediterranean plants can be attributed to bioclimatic suitability (Groves and Di Castri 1991) and also to the fact that, with European settlers, there was a high volume of re-introductions of plants (Milton 2004). *Arundo donax* is one such plant that was recorded to have been taken from populations in the Mediterranean in the late 1700s (Milton 2004; Perdue 1958). Support for this was found in this study, wherein *A. donax* in South Africa was found to share the same haplotype (haplotype M1) as

Mediterranean populations. South African populations thus likely belong to the European *A. donax* lineage, which is a genetic subset of populations from Asia (Ahmad et al. 2008; Hardion et al. 2012).

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

Arundo donax genetic diversity reflects a genetic bottleneck and is thus a good model for studying genetically depauperate species (Ahmad et al. 2008). Genetically depauperate plant species can be defined based on the overall species nuclear diversity H being lower than 0.05; this corresponds to the heterozygosity of a locus whose most frequent allele exceeds 0.97 (Vendramin et al. 2008). Genetically depauperate species present challenges to long-established views of genetics and the importance of genetic diversity (Vendramin et al. 2008). The genetic uniformity of *A. donax* is surprising considering

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

Genetically depauperate invasive alien species raise important questions on the role of genetic diversity in invasive potential. Founding plant populations generally have little genetic diversity and generate low intrapopulation genetic variation after range expansion (Burdon and Marshall 1981; Le Roux et al. 2007). It is now believed that their success and ability to adapt can largely be attributed to phenotypic plasticity rather than genetic differentiation (Thompson et al. 1991), which is termed a "general-purpose genotype" (Van Doninck et al. 2002). Successful clones are believed to possess more broadly adapted (general purpose) genotypes compared with sexual taxa (Baker 1967).

The "general-purpose theory" is likely a good model to explain the expansion of *A. donax* in South Africa given its lack of genetic diversity. *Arundo donax* can tolerate a wide range of environmental conditions, including variations in moisture, temperature, and salinity (Saltonstall et al. 2010; Tracy and DeLoach 1998), and is also one of the fastest-growing plants worldwide, having growth rates of up to 10 cm d⁻¹ (Lewandowski et al. 2003; Seawright et al. 2009). This high phenotypic plasticity is believed to have allowed the plant to persist and invade adventive ranges worldwide (Quinn and Holt 2008). There are a number of examples of genetically depauperate invasive alien grasses, including common cordgrass [*Spartina anglica* (C. E. Hubbard)], which has low genetic diversity in the United Kingdom and France (Thompson et al. 1991), and crimson fountain grass [*Pennisetum setaceum* (Forssk.) Chiov.], which has a single invasive haplotype (Le Roux et al. 2007; Thompson et al. 1991). For these species, it is likely that plasticity is the mechanism allowing them to become invasive in the introduced range (Le Roux et al. 2007; Thompson et al. 1991).

Determining the genetic diversity and haplotypes present in *A. donax* populations in South Africa has important implications for the future of the biological control program. First, determining the haplotype present in South Africa allowed insight into the ancestral lineage of these populations. The nearest relative of the invasive haplotype M1 is in Afghanistan and Pakistan along the Indus Valley (haplotypes M2, M3, and M4) (Hardion et al. 2014). A search for biological control agents suitable for South Africa should thus focus on monophagous herbivores in this region. Second, information on genetic diversity should give an indication of how the biological control agents are more likely to adapt and

potentially resist herbivory. Plants with higher genetic diversity are able to adapt and resist herbivory (Fritz and Simms 1992). Therefore, plants that have low genetic diversity are more likely to suffer from herbivory, as they have limited evolutionary potential for adaptability and defense against agents (Muller-Scharer et al. 2004; Tarin et al. 2013).

Biological control agents for *A. donax* in South Africa should be those adapted to the worldwide haplotype M1. These could be sourced from other countries with biological control programs against this haplotype or from the center of origin of the haplotype in the Indus Valley. Agents adapted to haplotype M1 will be suitable for all the *A. donax* in South Africa due to the clonal nature of the plant in the country. Two biological control agents were released on haplotype M1 *A. donax* in the United States, the eurytomid wasp (*Tetramesa romana* Walker) and the armored scale (*Rhizaspidiotus donacis* Leonardi) in 2010 (Goolsby et al. 2011). It is too soon to evaluate their impact on *A. donax* populations in the region; however, laboratory-based impact studies have shown that both agents have the potential to reduce plant growth and spread (Cortes et al. 2011; Moore et al. 2010; Moran et al. 2013). As such, these agents should be considered for biological control in South Africa.

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Supplementary material

To view supplementary material for this article, please visit <https://doi.org/10.1017/inp.2016.5>

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