

## Research Article

# Expansive reed populations—alien invasion or disturbed wetlands?

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**Abstract.** The tall-statured grasses in the genus *Phragmites* are dominant vegetation in wetlands worldwide and thus play a vital role in ecosystem functioning. As a result, *Phragmites* spp. are some of the most widely studied plants; particularly in areas where changes to their abundances have occurred, most notably in Europe and North America. In southern Africa a pattern of reed expansion has occurred in recent decades that has shown a similar trend to cryptic invasions reported in North America. This study used molecular techniques to explore the phylogeography of *P. australis* and *P. mauritanus* in the region to investigate whether the expansion is due to an alien invasion or local factors such as wetland disturbance. Three haplotypes were found and all haplotypes are presently considered African haplotypes (haplotype K for *P. australis* and haplotype V and AP for *P. mauritanus*). Both *Phragmites* spp. were found to have high genetic diversity. Microsatellite and *grass-waxy* analysis also found evidence of hybridization between the two species. No evidence was found for a recent cryptic invasion of non-native haplotypes in southern Africa. The expansion of *P. australis* and *P. mauritanus* is therefore most likely a result of anthropogenic activity. Identifying and mitigating the human-mediated factors that may be contributing to reed growth, such as eutrophication and sedimentation, should be the focus of future management protocols.

**Keywords:** Hybridization; *Phragmites australis*; *Phragmites mauritanus*; phylogeography; reed expansion; tall-statured grasses.

## Introduction

Wetlands are considered one of the most valuable ecosystems as they are areas of naturally high species diversity, a diverse array of biological and physical processes, provide critical habitat structure and improve water quality (Cronk and Fennessy 2016). It is the dominant plants within these ecosystems that are most influential and as such, the greatest functional changes occur if the abundances of these species change (Richardson *et al.* 2007). Species in the genus *Phragmites* are dominant

wetland plants worldwide. There are four species in the genus including *P. australis*, *P. mauritanus*, *P. japonicus* and *P. karka* (Clevering and Lissner 1999). The species are all morphologically similar and presumed to be closely related (Lambertini *et al.* 2006). The abundance and distribution of these *Phragmites* spp. has been found to have important consequence for wetland ecosystem functioning.

The most widely distributed and abundant *Phragmites* species is *P. australis*, which is also one of the most studied

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plants in the world (Pyšek et al. 2008). A large amount of research on the plant's ecophysiology and population dynamics has focused on North American and European populations, primarily due to these regions having had recent changes to *P. australis* abundance and distribution (Meyerson et al. 2016). In Europe, *P. australis* has been the focus of intense research since the 1970s in response to declining reed beds (Tschardt 1992; Brix 1999). In North America, on the other hand, there have been cryptic invasions of *P. australis* that have resulted in rapid expansions of reed populations (Saltonstall 2002; Lambertini et al. 2012a). Such cryptic invasions occurred when non-native haplotypes from Europe were introduced to North America and were able to outcompete native haplotypes (Saltonstall 2002). Since then, the expansion of certain reed populations in other regions has also been attributed to cryptic invasion including parts of Canada (Lelong et al. 2007) and South America (Guo et al. 2013). Such phylogeographic work is however under-represented in Africa (Lambertini et al. 2012b).

The three species of *Phragmites* which occur in Africa are *P. australis*, *P. mauritanus* and *P. karka* (syn. *P. val-latoria*) (Gordon-Gray and Ward 1971; Veldkamp 1994). Despite their importance to wetland ecosystem health, very little is known about any of the species in the region. Yet, in southern Africa where the two species *P. australis* and *P. mauritanus* overlap, there is growing evidence that there is a changing pattern of *Phragmites* spp. abundance and distribution. Although *P. australis* is considered a native species in southern Africa, having been present in the region since the Late Quaternary period according to pollen fossil records (Scott 1982), in recent decades there has been a considerable expansion of the range and abundance of the species in many wetland ecosystems (Weisser and Parsons 1981; Russell 2003; Russell and Kraaij 2008). This expansion is similar to the cryptic invasions in North America whereby reed stands grow to form dense monospecific stands that can negatively impact ecosystem functioning (Chambers et al. 1999; Lambert et al. 2010; Hazelton et al. 2014). South African wetland areas have particularly high diversity, as the region is ecologically diverse with nine terrestrial biomes that have a rich flora and high levels of endemism (Cowling and Hilton-Taylor 1997; Rutherford et al. 2006). However, due to anthropogenic activities these areas are increasingly becoming disturbed and are now currently considered the most threatened ecosystem type (Driver et al. 2011). As such it is important to address the potential for cryptic invasions of non-native haplotypes in the country (Canavan et al. 2014).

In considering the potential for cryptic invasions by non-native *P. australis* genotypes, *Phragmites mauritanus* can serve for comparisons of genetic diversity levels because

it is endemic to Africa and therefore native (Gordon-Gray and Ward 1971). Genetic variation is generally agreed to be structured in space and time (Loveless and Hamrick 1984); with higher levels of genetic diversity being found in plants in their native range (Dlugosch and Parker 2008). To date, *P. mauritanus* remains a poorly studied species where at present there has been no in-depth investigation of its genetic diversity and dispersal mechanisms.

*Phragmites australis* and *P. mauritanus* are closely related and share the same reproductive strategy and plant architecture. Differentiating the two species is difficult; Gordon-Gray and Ward (1971) characterizes *P. mauritanus* from *P. australis* by having lax mature inflorescence with drooping branches, bare internodes with exposed axillary buds after maturation, well-branched stems and stiffer pointed leaves. Both *P. australis* and *P. mauritanus* can reproduce both sexually and asexually (Fanshawe 1972; Ailstock and Center 2000; Saltonstall et al. 2010). Sexual reproduction involves the production of anemochorous seeds from flower panicles; for both reeds seed viability is highly variable (Fanshawe 1972; McKee and Richards 1996; Ailstock and Center 2000). For *P. australis*, seeds do not remain viable for long; however, it has been found that where germination occurs the density of germinated seeds is almost as high as the number of viable seeds produced (~700 seeds per m<sup>2</sup>) (Baldwin et al. 2010; Hazelton et al. 2014). Vegetative reproduction is the primary mode of reproduction with spread occurring from a root system known as a rhizosphere (Fanshawe 1972; Hellings and Gallagher 1992; Clevering and Lissner 1999). Hybridization between *P. australis* and *P. mauritanus* was thought to be impossible due to differences in chromosome number (Lambertini et al. 2006): *Phragmites mauritanus* is tetraploid while *P. australis* is octoploid in southern Africa (Gordon-Gray and Ward 1971). Yet with improved molecular markers, evidence of hybridization among *Phragmites* taxa with different ploidy levels has been uncovered (Chu et al. 2011; Lambertini et al. 2012a; Meyerson et al. 2012; Lambertini 2016).

In this study, we investigated the phylogeography of *P. australis* and *P. mauritanus* in southern Africa. Our aim was to trace, or rule out, a potentially cryptic invasion of *P. australis* in southern Africa, and explore the management of reed beds in wetland areas in light of population dynamics.

## Methods

### Study sites and DNA extraction

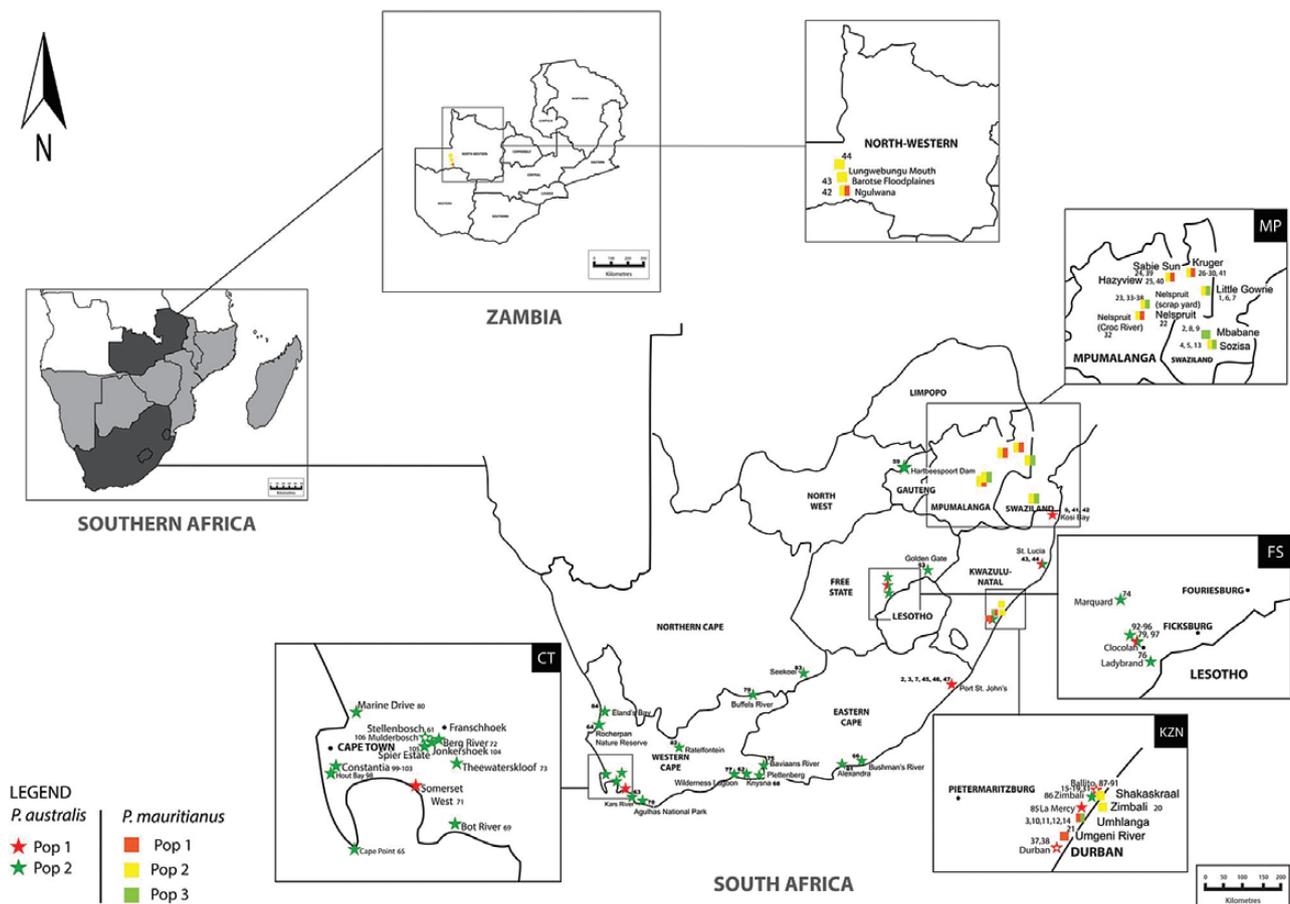
Fresh leaf samples of both *P. australis* and *P. mauritanus* (Fig. 1) were collected from sites in southern

Africa [see [Supporting Information—Table S1](#) and [S2](#)]. For *P. australis*, 39 sites were sampled from wetlands across South African's nine biomes, namely the Albany thicket, grassland, savanna, Nama-Karoo, forest, fynbos, desert, Indian Ocean Coastal Belt and thicket biomes (Rutherford et al. 2006). For *P. mauritianus*, 16 sites were sampled from wetlands within the reed's distribution which is restricted to tropical Africa (Gordon-Gray and Ward 1971) including the Indian Ocean Coastal Belt biome in northeastern South Africa, the subtropical moist forest biome in Swaziland (Rutherford et al. 2006) and the Zambezian cryptosepalum dry forest ecoregion in Zambia (Fig. 1) (Schulze and McGee 1978). The distribution of *P. australis* and *P. mauritianus* was only found to overlap in the KwaZulu-Natal province in South Africa. For both species, a number of populations were chosen at random to take more samples from, to give an indication of intra-site variability. DNA was isolated from samples with the Qiagen DNeasy Plant Mini Kit (Qiagen Inc., Valencia, CA, USA) as described in Lambertini et al.

(2006) from young leaves stored in silica gel, after leaves were crushed using liquid nitrogen.

## Sequences

Two non-coding regions in the chloroplast genome, *trnT-trnL* and *rbcl-psaI*, were amplified (Saltonstall 2002). Ten picomoles of forward and reverse primers were added to 12.5  $\mu$ L of Promega MasterMix (Madison, WI, USA) (reaction concentration of 1 U of *Taq*, 1.5 mM  $MgCl_2$  and 0.2  $\mu$ M dNTPS), 2  $\mu$ L of Promega magnesium chloride and 7  $\mu$ L of template DNA per reaction. Promega nuclease-free water was added to reach a final volume of 25  $\mu$ L. Amplification was run in one of the following machines: Labnet Multigene II (Labnet, Edison, NJ, USA) or Applied Biosystems 2720 thermal cycler (Applied Biosystems, Foster City, CA, USA). For the *trnLb* region, the PCR cycling protocol was 94  $^{\circ}C$  for 1 min, 35 cycles of 94  $^{\circ}C$  for 1 min, 56  $^{\circ}C$  for 1 min, 72  $^{\circ}C$  for 2 min, followed by a final extension at 72 $^{\circ}C$  for 5 min. For the



**Figure 1.** Map of southern Africa showing *P. australis* and *P. mauritianus* sampling sites. Sampling sites for *P. australis* are indicated by a star symbol along with the sample number [see [Supporting Information—Table S1](#)]. Sampling sites for *P. mauritianus* are indicated by a square symbol along with the sample number [see [Supporting Information—Table S2](#)]. Populations found for both reed species are shown (*P. australis*: pop 1—red, pop 2—green; *P. mauritianus*: pop 1—orange, pop 2—yellow, pop 3—green), refer to populations inferred by the Evanno method and based on Bayesian clustering analysis of four microsatellite loci with STRUCTURE (Pritchard et al. 2000).

*rbcl* region, the same cycling protocol was used; however, the annealing temperature was lowered to 45 °C.

PCR products were sent to Stellenbosch University, Stellenbosch, South Africa, or to Inqaba Biotec, Johannesburg, South Africa, for sequencing, using a BigDye Terminator ver. 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). Capillary electrophoresis was done using an ABI 3100 genetic analyser (Applied Biosystems, Foster City, CA, USA) at Stellenbosch University or using an ABI 3500 genetic analyser (Applied Biosystems, Foster City, CA, USA) at Inqaba Biotec.

### Microsatellites

From Saltonstall (2003), four primers (PaGT4, PaGT8, PaGT9 and PaGT22) were selected due to their high variability in the sample set. Primers were fluorescently labelled by Applied Biosystems Inc., UK; PaGT4, PaGT8 and PaGT22 with 6-FAM dye and PaGT9 with NED dye using standard FA parameters (DS-33 Matrix Standard G5 dye set). Eighteen microlitres of Promega Master Mix (Madison, WI, USA), 10 pmol forward and reverse primers, 3 µL of template DNA and Promega nuclease-free water were added to reach a volume of 20 µL. The PCR cycling protocol was 94 °C for 12 min, followed by 35 cycles of 94 °C for 30 s, 50–56 °C for 30 s, 72 °C for 40 s and an extension of 72 °C for 5 min. PCR products were diluted 20× with sterile water prior to capillary electrophoresis (sent to Stellenbosch University, Stellenbosch, South Africa or to the Inqaba Biotec lab, Johannesburg, South Africa) using either an ABI 3100 genetic analyser (Applied Biosystems, Foster City, CA, USA) or an ABI 3500 genetic analyser (Applied Biosystems, Foster City, CA, USA).

An error rate was determined by replicating 10 % of the samples. To further test the accuracy of the results, the same samples were run in different labs. To avoid subjectivity in scoring of peaks, any peaks that were ambiguous and in particular with stutter peaks were scored as missing data. The resulting error rate was 15.22 % and was found due to both false allele amplification and allelic dropout with both factors equally contributing to the error rate. The relatively high error rate is most likely a result of the fact that the duplicated samples were run on different ABI machines. All potential hybrids were amplified twice to ensure reproducibility of the results. The replication of hybrids had an error rate of 0 %.

### Grass-waxy analysis

Two DNA fragments amplified by the *grass-waxy* primers designed by Mason-Gamer et al. (1998) were used as diagnostic DNA fragments for *P. mauritanus*

and Mediterranean *P. australis* by Lambertini et al. (2012b). We designed specific primers [see Supporting Information—Table S3] for the two fragments and amplified all our samples. One microlitre of template DNA was added to 10 µL 2× Mastermix (VWR Amplicon), 10 pmol of forward and reverse primers, and sterile water to reach a total volume of 20 µL. The cycling protocol was 94 °C for 3 min, 40 cycles of 94 °C for 30 s, 62 °C for 40 s, 72 °C for 40 s, followed by 72 °C for 7 min. Products were run in a 1.5 % agarose gel for 1 h 30 min at 125 V, 84 mA and stained with ethidium bromide.

The presence or absence of the resulting bands of 100 bp for *P. mauritanus* and 200 bp for Mediterranean *P. australis* was recorded and the PCR product was then sequenced at the Inqaba Biotec lab, Johannesburg, South Africa using an ABI 3500 genetic analyser (Applied Biosystems, Foster City, CA, USA).

### Sequence data analysis

Sequences were assembled and manually edited in GeneStudio ver. 2.2.0.0 (GeneStudio, Inc.). Alignment of sequences was done in MEGA ver. 5.2.2 including all worldwide haplotypes downloaded from GenBank using ClustalW set to default parameters (Kumar et al. 2012).

### Microsatellite data analysis

Chromatogram alignment and allele sizing was done using Geneious ver. 8.1.7 (Kearse et al. 2012). Given the polysomic nature of the sample set (more than two alleles per locus), the data set was entered into a binary matrix (1 = presence, 0 = absence of homologous alleles) and analysed using GenAIEx ver. 6.5 (Peakall and Smouse 2012). Pairwise genetic distances were calculated based on the number of shared alleles per locus (Euclidean distances). The output matrix of genetic distances was then used to run a principal coordinates analysis (PCoA). Genetic diversity was compared between *P. mauritanus* and *P. australis* by calculating Nei's unbiased genetic identities (Nei 1973) and Shannon information index (Lewontin 1995) and number of effective alleles ( $N_e$ ) with the program PopGene ver. 1.32 population genetic analysis (Yeh and Boyle 1997) as done in previous studies (Lambertini et al. 2012b). To compare the allelic diversity to worldwide lineages in Saltonstall (2003), the average total number of alleles ( $A_o$ ) were recorded across the four loci and observed heterozygosity ( $H_o$ ) was measured for the two species as the percentage of heterozygotes (i.e. genotypes with more than one allele at a locus) across the four loci [see Supporting Information—Table S4].

The samples were initially classified as either *P. australis* or *P. mauritanus* based on their cpDNA matrilineages (*trnT-trnL* and *rbcl-psaI*). The nuclear ancestry of the two populations was subsequently tested based

on the four microsatellite loci using a Bayesian genetic clustering algorithm implemented in STRUCTURE version 2.3.4 (Pritchard et al. 2000). An admixture model was used that assumed independent allele frequencies with 10 iterations for each run. Each run consisted of 1 000 000 MCMC steps and a burn-in period of 100 000. The number of populations ( $K$ ) was tested from 1 to 10 and  $K$  was inferred with STRUCTURE HARVESTER (Earl and vonHoldt 2012) following Evanno et al. (2005). Models were also run including only *P. australis* or *P. mauritanus* samples according to the same parameters.

## Results

### cpDNA

All *P. australis* samples ( $n = 61$ ) were cpDNA haplotype K (Saltonstall 2002), which has also been found elsewhere in Africa (Saltonstall 2002; Lambertini et al. 2012a) and in isolated occurrences in Europe (Spain) (Lambertini et al. 2012b). *Phragmites mauritanus* had two haplotypes; all South African samples ( $n = 41$ ) were found to be haplotype V, while the samples from Zambia were haplotype V ( $n = 1$ ) and haplotype AP ( $n = 2$ ) (Lambertini et al. 2012b). Haplotype V was previously found in the *P. mauritanus* population in South Africa and in one sample from Equatorial Guinea labelled as *P. vallatoria* (syn. *P. karka*), whereas haplotype AP was found only in *P. mauritanus* samples from Zambia (Lambertini et al. 2012b).

### Microsatellites

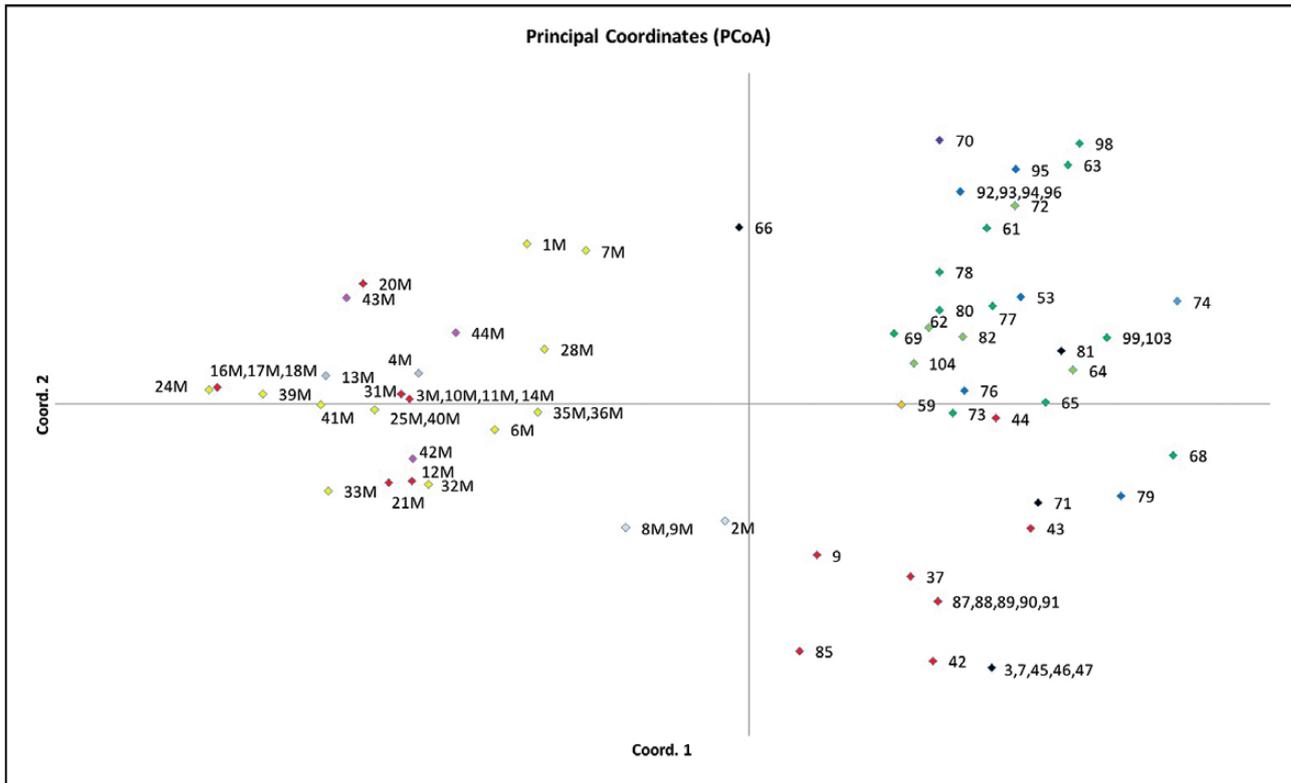
A total of 36 alleles were found in *P. australis* samples and 27 alleles for *P. mauritanus* samples across all four loci [see Supporting Information—Table S4]. The number of alleles amplified in each sample varied for *P. australis* and *P. mauritanus*; *P. australis* samples had an average of  $2.16 \pm 0.94$  alleles amplified compared to only  $1.56 \pm 0.71$  amplified for *P. mauritanus*. For *P. australis*, a maximum of four alleles were found; however, *P. mauritanus* had a maximum of only two alleles. *Phragmites australis* was found to have a higher average of total number of alleles ( $A_0 = 9 \pm 2.83$ ) found at each loci compared to the same loci in worldwide samples from North America and Europe from Saltonstall (2003). However, *P. mauritanus* had a similar average number of total alleles found in the four loci ( $A_0 = 6.75 \pm 1.26$ ) compared to European introduced populations in North America ( $A_0 = 7 \pm 3.56$ ) and native populations in Europe ( $A_0 = 8.5 \pm 4.2$ ) [see Supporting Information—Table S5].

The PCoA separated the two species into two distinct groups but four samples (66, 2M, 8M, 9M) were not found to group with either of the two species and instead clustered halfway between the *P. australis* and *P. mauritanus*

groups (Fig. 2). According to STRUCTURE HARVESTER analysis, *P. australis* and *P. mauritanus* samples have two ancestral populations ( $k = 2$ ) and STRUCTURE was then used to cluster the samples into the two groups (Fig. 3) and also see Supporting Information—Fig. S1. Four samples both within the *P. australis* and *P. mauritanus* clusters had a proportion of their genotype which shares ancestry with the other species. For *P. australis*, sample 66 had a signature of shared ancestry, with 51.8 % membership in the *P. mauritanus* population and 48.2 % membership in the *P. australis* population. The sample shared three alleles with *P. mauritanus* samples (loci: PaGT4—allele 270; PaGT9—alleles 196 and 202). For *P. mauritanus*, sample 2M had 67.2 % membership in the *P. australis* population and 32.8 % membership in the *P. mauritanus* population, whereas samples 8M and 9M had a higher membership in the *P. mauritanus* population than in that of *P. australis*. These samples represent two allelic genotypes (samples 8M and 9M were found to be clones of the same genotype [see Supporting Information—Table S2]) and together had seven shared alleles with *P. australis* samples (loci: PaGT4—allele 276; PaGT8—allele 175; PaGT9—alleles 198, 200; and PaGT22—alleles 173, 180 (samples 8M and 9M) and allele 183 (sample 2M)). The samples were also found to be intermediate between the two species in the PCoA (Fig. 2), suggesting that hybridization may have occurred.

STRUCTURE HARVESTER determined that *P. australis* samples clustered into two populations and *P. mauritanus* samples clustered into three populations [see Supporting Information—Table S1 and S2]. For *P. australis*, the two populations were found to correspond with groupings found in the PCoA. Almost all samples that grouped with Population 1 were found along the coast of South Africa except for one sample (sample 79) (Fig. 1). The remaining samples for Population 2 were found across the range of *P. australis*' distribution in South Africa. A number of samples from this second group, as well as sample 79 from the Free State province, were found to have an admixed ancestry from the two populations showing the occurrence of gene flow between the two populations in the coast and inland. For *P. mauritanus*, STRUCTURE HARVESTER analysis determined that there are three populations in South Africa and when plotted in STRUCTURE it was found that there is no geographic pattern among these populations (Fig. 1 and see Supporting Information—Table S2).

For *P. australis*, two stands sampled in Kosi Bay (samples 9, 41, 42) and St. Lucia (samples 43, 44) were found to have different genotypes [see Supporting Information—Table S1]. For *P. mauritanus*, only two stands did not have variation and most had different



**Figure 2.** Principal coordinates analysis (PCoA) built on Euclidean genetic distances between *P. australis* and *P. mauritianus* samples in Southern Africa using PaGT4, PaGT8, PaGT9 and PaGT22 microsatellite primers. Samples with missing data were excluded. Coordinate 1 accounts for 22.55 % and Coordinate 2 accounts for 12.25 % of the variation. Colours represent geographic area defined by sampling region in Southern Africa; red—KwaZulu-Natal, blue—Free State, yellow—Mpumalanga, black—Eastern Cape, green—Western Cape, orange—North West, purple—Northern Cape, pink—Zambia and grey—Swaziland. *Phragmites mauritianus* samples all clustered to the left axis and *P. australis* samples clustered to the right axis. Samples followed by the letter M denote *P. mauritianus* samples and samples with no letters denote *P. australis* samples. Four samples (66, 2M, 8M, 9M) were found to cluster halfway between the two groups.

genotypes [see Supporting Information—Table S2]. *Phragmites mauritianus* was also found to have a higher Shannon information index ( $I$ ) ( $0.425 \pm 0.204$ ), number of effective alleles ( $N_e$ ) ( $1.446 \pm 0.333$ ) and higher Nei's diversity ( $h$ ) ( $0.273 \pm 0.161$ ) compared to *P. australis* samples ( $I = 0.333 \pm 0.231$ ,  $N_e = 1.339 \pm 0.358$ ,  $h = 0.208 \pm 0.177$ ) (Table 1). However, these differences were not statistically significant.

### Grass-waxy bands

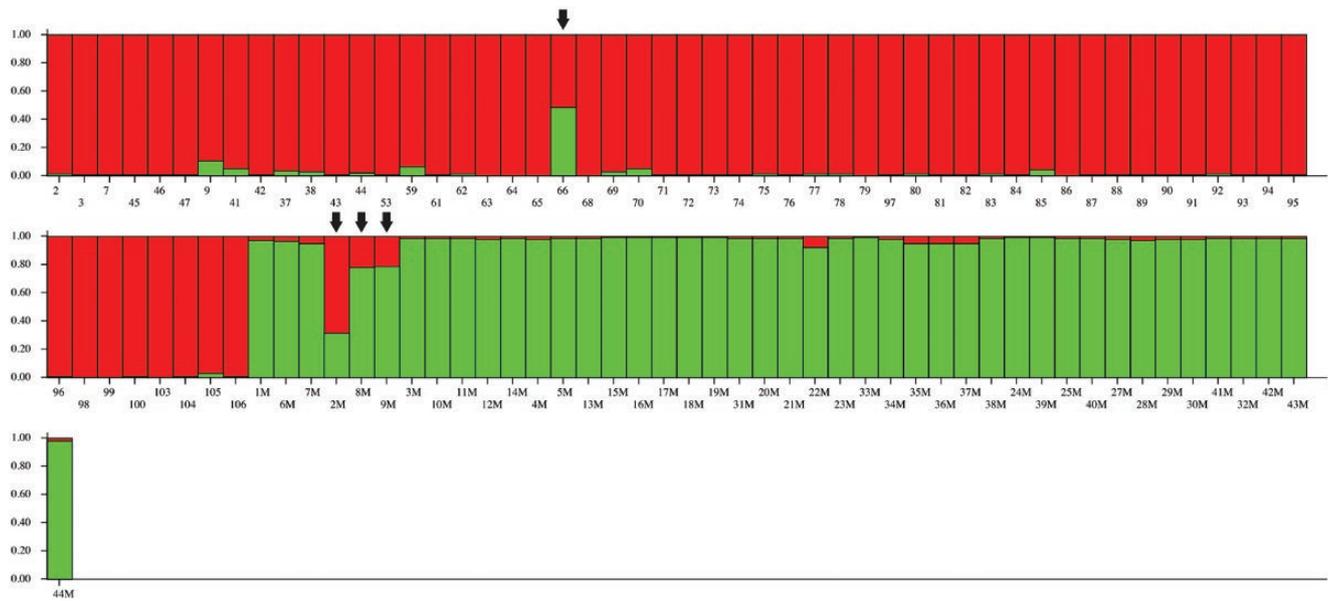
A nuclear DNA band of ~200 bp was amplified in 77 % of the *P. australis* samples [see Supporting Information—Table S1]. This band has previously been found to be exclusive to plants from the Mediterranean region and was used as a marker to trace genotypes of this region (Lambertini et al. 2012b). However, 10 *P. australis* samples did not have this band present. The shorter band of ~100 bp, previously recorded to be exclusive to *P. mauritianus* and associated hybrids with *P. australis* (Lambertini et al. 2012b) was also found in 27 of the *P. australis* samples and for 10 of these samples was

the only band present. For the *P. mauritianus* samples, all reeds were found to have the 100-bp band and none had the 200-bp band [see Supporting Information—Table S2].

The sequence of the 200-bp fragment matched the NCBI accession no. JF317300 (Lambertini et al. 2012a), previously amplified in samples from the Mediterranean, Senegal sample and the Delta-type in the Gulf Coast of North America. The sequence of the 100-bp fragment aligned with *P. mauritianus* NCBI accession no. JF317301 (Lambertini et al. 2012a), and its samples from Uganda, Burkino Faso, the hybrid populations with *P. australis* in Senegal, South America and the Land-type in the Gulf Coast of North America.

### Discussion

Both chloroplast and nuclear markers suggest that *P. australis* and *P. mauritianus* populations should remain being considered native to southern Africa. For both species, the haplotypes found (K, V and AP) are



**Figure 3.** Genetic population structure of *Phragmites australis* and *Phragmites mauritianus* from samples in southern Africa, based on Bayesian clustering analysis of four microsatellite loci with STRUCTURE (Pritchard et al. 2000). According to the Evanno method, two populations were inferred [see Supporting Information—Fig. S1]. Based on cpDNA matrilineage, the red cluster corresponds to *P. australis* individuals, whereas the green cluster represents *P. mauritianus*. The values in the y-axis display the shared ancestry according to percentage membership into *P. australis* or *P. mauritianus* populations [see Supporting Information—Tables S1 and S2]. The downward arrows point to samples with shared alleles with the other species (in order from top left, samples 66, 2M, 8M, 9M).

all recognized as native to the region and microsatellite analysis determined relatively high allelic diversity compared to each worldwide lineages from Saltonstall (2003). The population diversity of *P. australis* was found to be similar to the endemic *P. mauritianus* seen through similar Shannon ( $I$ ) and Nei's diversity ( $h$ ) indices; *P. australis* had a slightly lower diversity although this was not statistically significant. These results suggest that haplotype K populations have a long history in southern Africa and therefore, a cryptic invasion, similar to that found in North America can reasonably be ruled out. The recent expansion of reed beds in southern Africa can most likely be attributed to anthropogenic activities and in such cases *P. australis* can be labelled expansive and not invasive (Pyšek et al. 2004).

Previous phylogeographic studies have determined that haplotype K is not restricted to the African continent and has been found in Mediterranean Europe (Lambertini et al. 2012b). Many of the southern African samples

analysed in this study also carry the diagnostic *waxy* 200-bp band of Mediterranean *Phragmites*, as previously found by Guo et al. (2013), who for this reason, included South Africa in the native range of the Mediterranean population. Although our study does not contradict Guo et al. (2013), further research including samples from the African continent and the Mediterranean region is needed to assess the centre of origin and distribution range of Mediterranean *Phragmites* across Africa and conclusively shed light on haplotype K and the *waxy* 200 band became present in both European and southern African populations.

Two distinct ancestral *P. australis* populations were identified in southern Africa and such populations appeared independent from either the presence/absence of the *waxy* 200-bp band or having shared alleles with *P. mauritianus*. Population 1 had seven out of eight sites found along the coastline of South Africa while Population 2 had sites from across the range of

**Table 1.** Comparison of genetic diversity for *P. australis* (Pa) and *P. mauritianus* (Pm) in southern Africa from this study based on four microsatellite loci (over populations for each loci). Sample size ( $N$ ), observed total number of alleles ( $N_a$ ), number of populations ( $N_{pop}$ ), number of effective alleles ( $N_e$ ), Shannon information index ( $I$ ), Nei's gene diversity ( $h$ ) and SD. No significant differences were found between *P. australis* and *P. mauritianus* in southern Africa ( $P$  values obtained with Mann–Whitney  $U$  test,  $P < 0.050$ ).

	$N$	$N_a$	$N_{pop}$	$N_e$ (SD)	$I$ (SD)	$h$ (SD)
Pa—SA	61	36	39	1.339 ± 0.358	0.333 ± 0.231	0.208 ± 0.177
Pm—SA	44	27	16	1.446 ± 0.333	0.425 ± 0.204	0.273 ± 0.161

areas sampled. The distinction of Population 1 could be a result of local differentiation to a coastal niche and could reflect adaptation to ecological heterogeneity or a different ecotype as *P. australis* is known to have halophytic and glycophytic populations (Turesson 1922; Waisel 1972; Takahashi et al. 2007; Wu et al. 2008; Gao et al. 2012). For example, in the Yellow River Delta, China, *P. australis* was found to have population structuring in microsatellite alleles that is believed to be a result of varying soil salinity (Gao et al. 2012). In such cases, *P. australis* adapts to different saline conditions by adjusting its plant physiology which results in genetic differentiation over time (Takahashi et al. 2007).

For *P. mauritanus*, two haplotypes known as haplotype V and AP were found across all sites. Both haplotypes have been found to be unique to *P. mauritanus* and restricted to the African continent (Lambertini et al. 2012a).

Evidence of gene flow between *P. australis* and *P. mauritanus* populations in southern Africa was found in both microsatellite and *grass-waxy* results. Microsatellite markers determined three genotypes (66, 2M, 8M and 9M) in *P. australis* and *P. mauritanus* monospecific populations that had shared alleles. This may be indicative of recent hybridization events between geographically distant populations. Furthermore, *grass-waxy* analysis highlighted more ancient exchanges between the *Phragmites* species. A 100-bp band that has previously been found to be unique to *P. mauritanus* and its hybrid populations (Lambertini et al. 2012a, b) was also carried by a number of *P. australis* samples in South Africa. Interestingly, the occurrence of the 100-bp band did not affect the microsatellite structure of the *P. australis* populations. It is therefore likely that the high frequency of this band in the *P. australis* population is due to ancient exchanges of genetic material between the two species that have been conserved. This indicates that as previously hypothesized (Chu et al. 2011; Lambertini et al. 2012a, b; Meyerson et al. 2012) hybridization has taken place in the evolution of the genus *Phragmites*. Within *P. mauritanus* populations there was no occurrence of the 200-bp band unique to *P. australis*; this may indicate that *P. australis* populations are more susceptible to the assimilation of *P. mauritanus* genetic material.

*Phragmites australis* and *P. mauritanus* have remained distinct species in southern Africa despite having an overlapping distribution and showing evidence of hybridization; one explanation is that they have different habitat requirements. Gordon-Gray and Ward (1971) observed in KwaZulu-Natal, South Africa, that *P. mauritanus* prefers areas with permanent, moving water, especially in areas with recent disturbance, whereas *P. australis* is mostly found in areas with restricted drainage that

are usually isolated from the main stream. These differences may be either related to different physiological paths in the two species, and/or may be a result of different ploidy levels between the two species in southern Africa. *Phragmites australis* was found to have a higher number of amplified alleles compared to *P. mauritanus* which indicates a higher ploidy level; this agrees with the finding of Gordon-Gray and Ward (1971) who found that South African *P. australis* is octoploid ( $2n = 8x = 96$ ) and *P. mauritanus* is a (disomic) tetraploid ( $2n = 4x = 48$ ). Octoploids generally have increased plant biomass and tetraploids are generally found to be more opportunistic with high plasticity (te Beest et al. 2011).

In addressing the recent expansion of *Phragmites* spp. it is therefore likely that reed encroachment has been facilitated by anthropogenic activities which have disturbed wetlands. Most Southern African wetlands have been heavily degraded, most notably from drainage, eutrophication and input of industrial effluents (Driver et al. 2011; Fernandes and Adams 2016; Lemley et al. 2017; Sieben 2017). For example, Russell and Kraaij (2008) proposed that *P. australis* encroachment in the Wilderness Lakes, Western Cape, South Africa, was a result of a multitude of factors including water stabilization, reduced disturbance by large herbivores and reduced frequency of fire. *Phragmites australis* has the ability to thrive under such disturbance as a result of its ecophysiological strategies, broad ecological amplitude, high evolutionary potential and high phenotypic plasticity (Eller and Brix 2012; Kettenring and Mock 2012; Kettenring et al. 2015). *Phragmites australis* populations in southern Africa belong to the MED lineage, and compared to other lineages respond most positively to eutrophication due to high phenotypic plasticity (Eller and Brix 2012; Eller et al. 2017). Therefore, increasing nutrient loads can result in reeds having increased biomass production, shoot density (Romero et al. 1999; Tho et al. 2016), inflorescence and floret production (Kettenring et al. 2011) as well as seedling success (Kettenring et al. 2015). Reed expansion can also occur in *P. mauritanus* populations in southern Africa and reed encroachment is most often associated with changes to water flow and sedimentation (van Coller et al. 1997; Kotschy et al. 2000).

## Conclusions

A critical part of understanding the status of a plant as an introduced or native species is to have a clear understanding of its evolutionary history (Pyšek et al. 2004). However, understanding such history has often been based on poor data, inappropriate criteria or intuition (Pyšek 1995). The genetic results from this study suggest

that both *P. australis* and *P. mauritanus* have had a long history in southern Africa and thus a cryptic invasion by non-native haplotypes has not occurred. Therefore, unlike in cryptic invasions it is the native populations that are expanding in their range and abundance. In light of this, management of reed encroachment should focus on addressing factors that may be contributing to expansion such as eutrophication and water stabilization while still ensuring the conservation of the species.

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## Contributions by the Authors

All authors conceived the ideas; K.C. collected the data, performed the laboratory work and analysed the results; C.L. led the waxy band laboratory work; all authors led the writing.

## Conflict of Interest

None declared.

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## Supporting Information

The following additional information is available in the online version of this article—

**Table S1.** Sampling sites for collection of *Phragmites australis* genetic material including site location, province in South Africa and GPS coordinates. Membership probability ( $Q$ ) assigns the percentage of shared ancestry for each *P. australis* sample, based on Bayesian clustering analysis of four microsatellite loci (PaGT4, PaGT8, PaGT9, PaGT22) with STRUCTURE (Pritchard et al. 2000). According to the Evanno method, two populations were inferred (pop 1—red; pop 2—green) [see Supporting Information—Fig. S1]. The presence or absence of waxy bands, *Waxy100* and *Waxy200*, is shown, symbols +/- indicate presence/absence, where absent indicates no clear amplification of a waxy band. Genotypes were assigned based on shared alleles from the four microsatellite markers.

**Table S2.** Sampling sites for collection of *Phragmites mauritanus* genetic material including site location, province in South Africa and GPS coordinates. Membership probability ( $Q$ ) assigns the percentage of shared ancestry for each *P. mauritanus* sample, based on Bayesian clustering analysis of four microsatellite loci (PaGT4, PaGT8, PaGT9, PaGT22) with STRUCTURE (Pritchard et al. 2000). According to the Evanno method, three populations were inferred (pop 1—orange, pop 2—yellow, pop 3—green) [see Supporting Information—Fig. S1]. The presence or absence of waxy bands, *Waxy100* and *Waxy200*, is shown, symbols +/- indicate presence/absence, where absent indicates no clear amplification of a waxy band. Genotypes were assigned based on shared alleles from the four microsatellite markers.

**Table S3.** Primer sequences for grass-waxy analysis.

**Table S4.** Summary of allele amplification for *P. australis* and *P. mauritanus*.

**Table S5.** Comparison between the southern African lineages of *P. australis* and *P. mauritanus* and four North American and European lineages of *P. australis* from Saltonstall (2003) in microsatellites genetic traits. The comparison is based on four microsatellite loci (PaGT4, PaGT8, PaGT9, PaGT22) and includes  $n$ : number of samples genotyped;  $A_o$ : observed number of alleles at loci; dominant phenotypes: dominant alleles, values in parentheses are the frequency of each phenotype;  $H_o$ : observed heterozygosity.

**Figure S1.** Graphs of Delta  $K$  values showing the ideal number of populations ( $k$ ) using four microsatellite primer pairs and the Evanno method implemented in STRUCTURE HARVESTER program according to Earl and vonHoldt (2012). (a)  $k = 2$  based on 55 populations of *P. australis* and *P. mauritanus* in southern Africa, (b)  $k = 2$  based on 39 populations of *P. australis* in South Africa, (c)  $k = 3$  based on 16 populations of *P. mauritanus* in South Africa.

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