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PHRAGMITES INVASION

Biogeography of *Phragmites australis* lineages in the southwestern United States

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Abstract The environmental and social impacts of *Phragmites australis* invasion have been extensively studied in the eastern United States. In the West where the invasion is relatively recent, a lack of information on distributions and spread has limited our ability to manage invasive populations or assess whether native populations will experience a decline similar to that in the East. Between 2006 and 2015, we evaluated the genetic status, distribution, and soil properties (pH, electrical conductivity, and soil texture) of *Phragmites* stands in wetlands and riparian systems throughout the Southwest. Native (subspecies *americanus*), Introduced (haplotype M), and Gulf Coast (subspecies *berlandieri*) *Phragmites* lineages were identified in the survey region, as well as watershed-scale hybridization between the Native and Introduced lineages in southern Nevada. Two Asian haplotypes (P and Q) that were previously not known to occur in North America were found in California. The Native lineage was the most frequent and widespread across the region, with four cpDNA haplotypes (A, B, H, and AR) occurring at low densities in all wetland types. Most Introduced *Phragmites* stands were in or near major urban centers and associated with anthropogenic disturbance in wetlands and rivers, and we document their spread in the region, which is likely facilitated by transportation and urban development. Soil pH of Native and hybrid stands was higher (averaging 8.3 and 8.6, respectively) than Introduced stands (pH of 7.5) and was the only soil property that differed among lineages. Continued monitoring of all *Phragmites* lineages in the Southwest will aid in assessing the conservation status of Native populations and developing management priorities for non-native stands.

Keywords Hybridization · Invasive species · Anthropogenic disturbance · Rare species · Riparian · Water resources · Wetlands · Poaceae · Desert spring

Introduction

Human-linked environmental stressors are causing rapid declines in indigenous species worldwide. These byproducts of human society, such as habitat loss, degradation (Blair 2001), or introduction of exotic species (Vitousek et al. 1997; Walck et al. 1999) are often cited as primary causes of native species declines, but frequently, multiple factors (Trentanovi et al. 2013) can impact biological communities

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simultaneously (Czech and Krausman 1997). Compared with natural ecosystems, urban centers tend to have disproportionately higher numbers of non-native species (Kowarik 1995; Trentanovi et al. 2013), and when coupled with other associated anthropogenic disturbances, overwhelm the ability of native species to persist (McKinney 2002). Successful conservation strategies for native plant populations depend on accurate identification and assessment of population trends in the face of environmental change. A critical challenge for plant conservation is obtaining adequate biological and environmental information necessary to evaluate the status of vulnerable species or determine if protection is needed (Schemske et al. 1994). Moreover, misidentification of members of cryptic species complexes during rare species assessment or pest management efforts can have serious unintended effects on native plant populations (Bickford et al. 2007). Collection and dissemination of accurate biogeographical data are essential for timely responses to threats to imperiled species and effective conservation actions.

Wetlands in the Southwest United States are hotspots of biodiversity, and at the same time, are among the most rare and imperiled systems in North America (Hendrickson and Minckley 1985; Unmack and Minckley 2008; Ball-Damerow et al. 2014). These habitats include ephemeral riparian zones, isolated springs and seeps, and less frequently, low gradient rivers with perennial flows. Many of these wetland types (except for perennial rivers) are supported by artesian spring features created where aquifers meet the ground surface or along geologic faults and fractures (Sivinski and Tonne 2011). The most abundant plant species common to these systems include reed grasses (*Phragmites australis* and *Arundo donax*), sedges (*Schoenoplectus americanus* and *S. acutus*), willows (*Salix* spp.), Fremont cottonwood (*Populus fremontii*), seep willow (*Baccharis salicifolia*), mesquite (*Prosopis* spp.), arroweed (*Pluchea sericia*), and Chenopods (*Atriplex* spp.) (Hendrickson and Minckley 1985).

Because of the harsh environmental conditions and isolation of desert wetlands, associated species tend to have narrow geographic ranges and high degrees of endemism (Tiner 2003). These species also tend to have low abundances, which increases their risk of extinction from environmental change (Gaston 1998). This risk is exacerbated by rapid urbanization and groundwater depletion, as well as climate change and encroachment by non-native species occurring in this region (Deacon et al. 2007; Unmack and Minckley 2008). Wetland systems are particularly vulnerable to plant invaders because they are often the ultimate repository of plant propagules from upstream sources (Stohlgren et al. 1998; Zedler and Kercher 2004). Several of the worst invasive plant species are non-native macrophytes that form monocultural stands and reduce native floral and faunal diversity of the wetlands they invade (Daehler and Strong 1996; Dudley 2000; Shafroth et al. 2005). One of the most extensive invasion processes in North America involves various genetic forms of *Phragmites australis* Cav, (Trin.) ex. Steud. (common reed; Saltonstall 2002).

Phragmites is one of the most prevalent species in North American wetlands and riparian systems, with a complex of native and introduced lineages occurring across the continent. The native lineage (*P. australis* subsp. *americanus*; hereafter Native *Phragmites*) is genetically diverse and the most widespread lineage throughout the West (Saltonstall 2002; Meyerson et al. 2010; Kettenring et al. 2012; Kettenring and Mock 2012) where it typically occurs at low densities in mixed wetland plant communities. An invasive European lineage (chloroplast DNA haplotype M; hereafter Introduced *Phragmites*) was introduced into eastern North America at least 150 years ago, and by the 1960s, was widespread in East Coast salt marshes and wetlands (Saltonstall 2002). This lineage has since spread south to the Gulf Coast and west to the Pacific Coast (Saltonstall 2002; Meyerson et al. 2010), but has likely been present in western urban centers for only about 25 years (but see Smith and Kadlec 1983). Hybridization between Native and Introduced *Phragmites* haplotypes has been detected infrequently along the East Coast (Saltonstall et al. 2014; Wu et al. 2015), but remains rare on the continental scale (Saltonstall et al. 2016). Other non-native haplotypes such as M1, which originates from the Mediterranean, continue to be identified as more populations across the continent are analyzed (Hauber et al. 2011; Lambertini et al. 2012). A third lineage, Gulf Coast *P. australis* subsp. *berlandieri* (hereafter Gulf Coast *Phragmites*), is found in the southern United States from Florida to California and also extends into Central and South America. It is unknown whether Gulf Coast *Phragmites* is native or introduced to the region, and is therefore considered cryptogenic (Saltonstall 2002, 2003a, b). Lambertini et al. 2012 suggests that many of the *Phragmites* populations present along the Gulf Coast states appear to be hybrids of Gulf Coast *Phragmites* and *P. mauritianus,* and Gulf Coast *Phragmites* and non-native haplotypes M and M1 (but see Hauber et al. 2011). Saltonstall (2003a) provides a continental-scale description of the genetic structuring of *Phragmites* haplotypes in North America.

Phragmites australis invasion and impacts have been extensively studied in eastern North America, but have received little attention in the West because this species has been a relatively minor problem compared to other invasive riparian and wetland plants, such as *Tamarix* spp. and *Arundo donax*, that have long histories in this region (Shafroth et al. 2005; Lambert et al. 2010a). However, several studies have focused on the genetic diversity and reproductive strategies of Native and Introduced *Phragmites* populations in the Great Salt Lake region, the site of one of the most extensive invasions in the West, and other areas of Utah and southern Idaho where only native haplotypes occur (Kettenring et al. 2012; Kettenring and Mock 2012). It is unclear if the recent *Phragmites* invasion in the West will reach the same magnitude as in the East given the differences in climate and edaphic properties between regions. Recent studies have begun to link biogeographic patterns and latitudinal gradients in Native and Introduced *Phragmites* distribution to community interactions (Chow 2008; Cronin et al. 2015), climate, and anthropogenic disturbance (Hughes et al. 2016).

It is not known if western systems will be as vulnerable to invasion or what role abiotic differences could play in establishment and spread. The temperate climate of eastern North America is similar to that of Europe where Introduced *Phragmites* is native, but a strong contrast to the semi-arid to arid climate of southwestern North America. Soil properties are also dissimilar among regions with acidic, high organic content soils in the East and alkaline, sandy soils with relatively low organic content in the West. The purpose of this study was to (1) evaluate the genetic status (including hybridization events) of *Phragmites* stands in wetlands and riparian systems throughout the arid Southwest, (2) document the distribution of Native *Phragmites* populations across the region, especially in isolated wetlands, (3) assess whether Introduced *Phragmites* is spreading from sites of apparent introduction into ecologically sensitive natural habitats where their impacts could warrant enhanced management efforts, and (4) begin to assess the environmental factors that may influence the distribution patterns of the different *Phragmites* lineages in the southwestern United States.

Materials and methods

Surveys of *Phragmites* populations

Locations of *Phragmites* populations were identified through online herbarium database searches, analysis of aerial imagery in Google Earth (© Google Inc. 2015), and information from scientists and land managers with botanical knowledge of wetland plants in the region. *Phragmites* can be distinguished from other vegetation in aerial imagery by its lighter yellow–green color and linear stem architecture. The authors and collaborators work extensively in riparian areas and wetlands in the region, and surveyed *Phragmites* populations over a 9 year period (2006–2015). Field observations were commonly made along roadways and public right of ways, but extensive segments of riparian corridors were also visited to survey for presence of *Phragmites* plants. Isolated wetlands were surveyed where herbarium records documented *Phragmites* presence or if potential habitat was identified through aerial imagery analysis. If populations were abundant in a given area, as much of the accessible area as possible was surveyed and samples were collected from any stands that appeared to differ morphologically. Over 400 locations with appropriate riparian or wetland habitat were surveyed, with *Phragmites* stands detected and sampled in 97 of these locations (some locations had multiple stands).

Stem density, percent cover, and presence of other species growing alongside *Phragmites* were also recorded for a subset of stands to assist with describing growth habits and relative dominance among the *Phragmites* lineages. Stem density and percent cover were measured in 0.25 m^2 quadrats placed along a transect run as close to the center of the stand as possible. At least seven evenly spaced quadrats were measured in each stand with all quadrats placed at least 5 m apart (average distance of transects depended on stand size and physical barriers).

Genetic analysis/haplotype determination

Green leaf tissue samples were collected from stands throughout Arizona, California, Colorado, Nevada, and Utah during the growing season by the authors and collaborators. Tissue samples were either air dried or dried using silica gel and stored at –70 °C in the laboratory until genetic analysis. Intensive collection occurred across Clark County, Nevada area where hybridization had been earlier detected (Saltonstall et al. 2016). A total of 177 samples were collected and provenance was determined using DNA sequencing and/or microsatellites (151 samples), Restriction Fragment Length Polymorphism (RFLP) analysis (14 samples), or morphological characteristics (12 samples).

DNA for genetic analyses was extracted using a modified 2 % CTAB extraction protocol (Saltonstall 2002). The lineage of the majority of samples was determined by sequencing two non-coding chloroplast DNA (cpDNA) regions on an ABI 3130XL sequencer (Applied Biosystems) as in Saltonstall (2002). Sequences were aligned using Sequencher 4.1 (Gene-Codes Corp.) and compared with known *Phragmites* haplotypes (Saltonstall 2002). Eight microsatellite regions (GT4, GT8, GT9, GT11, GT13, GT14, GT16, GT22) were amplified using the protocols of Saltonstall (2003b), with multiplexing of primer sets to reduce the number of PCR reactions required. As primer set GT22 does not amplify well in Native samples, this locus was only used when comparing Introduced with Gulf Coast samples. Samples were genotyped on an ABI 3130XL sequencer using LIZ 500 as a size standard and allele sizes were estimated using GeneMapper version 3.7 (Applied Biosystems). Previous work (Saltonstall 2003b; Saltonstall, unpublished data) has identified expected microsatellite allele frequencies in the *Phragmites* lineages defined by cpDNA haplotypes and these data were used as a reference. Samples were assigned to a lineage using two methods: Bayesian clustering, as implemented in Structure 2.3.3 using the admixture model (Pritchard et al. 2000; Falush et al. 2007) and Principle Coordinates Analysis based on the band-sharing Lynch distance metric (Lynch 1990), as implemented in the R package Polysat (Clark and Jasieniuk 2011). As *Phragmites* is an allo-polyploid, microsatellite profiles are hereafter referred to as allele phenotypes (Saltonstall 2003b).

Some samples were analyzed using RFLP following the methods of Saltonstall (2003c) to confirm the provenance of plants with distinct morphological characters when genetic sequencing was not available. This test can differentiate between the Native, Introduced, and Gulf Coast lineages and confirm the origin of the maternal parent (seed), but cannot detect hybrids. Any samples that had ambiguous or hybrid characteristics were included in the sequencing analysis above if the DNA was not degraded.

A suite of morphological characters has been developed to distinguish among the three lineages and are reasonably reliable for determining status when molecular methods are not available (Blossey 2015; Saltonstall et al. 2004; Swearingen and Saltonstall 2010). However, stressful environmental conditions can cause variation in several of the stem characters (A. Lambert, personal observation), so caution was used in evaluating character states. Thirteen stands were identified using only the morphological characters described by Blossey (2015) as high quality DNA could not be extracted from them. We excluded any stands where morphological characters were variable or not definitive.

Soil properties

Soil samples were collected from a subset of sites and analyzed to determine if differences in edaphic properties exist among the habitats where the Native, Introduced, and Hybrid plants occurred. Soil samples were not collected from Gulf Coast *Phragmites* populations. Because soil samples were taken across a broad geographical range potentially of different parent materials and over multiple years, resulting data provide only a coarse assessment of potential differences in soil characteristics among sites that may influence *Phragmites* lineage distribution. However, soils are generally stable in this arid region when soil moisture is low (Hultine et al. 2015). We also collected soil samples from several of the same sites over multiple years to evaluate temporal changes in measurements and found that within-site variation in pH and electrical conductivity was very low between years. Soil cores (5 cm diameter × 20 cm depth) were collected from 33 populations during the dry season, although the soils in several of the sites were moist or saturated. Three cores, spaced approximately 1 m apart, were taken in each stand as close as possible to the midpoint of the stand and samples were placed in paper bags for transport. Samples were dried at 60 °C for 2 days, then sieved through mesh to remove particles greater than 2 mm. Particle size (texture) was measured using the hydrometer method in Gee and Bauder (1979). Total soluble salt concentration (salinity) was determined by measuring electrical conductivity (Rhoades 1996). To determine electrical conductivity, 60 ml of 0.1 M calcium chloride was added to 20 g of soil (3:1mixture) and mixed on an orbit shaker for 30 min. Conductivity was measured at 21.0 ± 0.5 °C using an EC Testr 11 + meter (Eutech Instruments Pte LTD.). To determine soil pH, 30 g of soil were mixed with 30 ml deionized water (1:1 mixture) and mixed on an orbit shaker for 30 min. pH was measured with an YSI pH 10 m.

Statistical analysis

To assess variation at the regional level and between wetland types, we focused on cpDNA haplotype diversity as microsatellite profiles showed clear distinction between the Native, Introduced, and Gulf Coast lineages. When determining the percentage of stands from each lineage, locations with multiple samples were only counted once. Soil data were analyzed using a one-way ANOVA to determine if there were detectable differences in soil properties (texture, pH, and electrical conductivity) among the three lineages and hybrid populations. Differences in stem density and percent cover among lineages were also analyzed using a one-way ANOVA. Tukey's test was used for post hoc comparisons of significant main effects.

Results

In general, *Phragmites* was encountered infrequently in Southwest wetland systems and its dominance (stem density or cover) varied considerably among sites (Table 1; Fig. 1). Genetic diversity was high with a total of eight cpDNA haplotypes of native and non-native origin detected in the region. Four Native haplotypes were identified (haplotypes A, B, H, and AR). Introduced haplotype M was the most abundant non-native haplotype, but two previously unidentified haplotypes (P and Q) were also found in California. Gulf Coast *Phragmites* (haplotype I) was the most common haplotype in the southern portion of the sampling area. Hybrids of the Native and Introduced lineages were found in Las Vegas, NV (Saltonstall et al. 2016) but no evidence for hybridization was found between the Introduced (Hap. M) and Gulf Coast (Hap. I) lineages (Fig. 2). Small and ephemeral wetlands tended to have mixed vegetation communities with low Native *Phragmites* densities, while urban wetlands with high levels of disturbance and nutrient rich wastewater inputs had larger and dense monoculture stands of non-native haplotypes and hybrid populations.

Native *Phragmites australis* subspecies *americanus* lineage

Across the region, Native *Phragmites* was the most common and geographically widespread lineage, accounting for 63 % of the populations sampled (n = 101 samples). It was found in all wetland types and was the only lineage outside of urban areas or in remote locations where human disturbance was low. It was often associated with surface hydrologic features fed by groundwater, including alkaline marshes, seeps and springs, with 33 % of Native populations occurring in these habitats. Native stands occurred in mixed plant communities with relatively low cover compared to Introduced or hybrid stands (Fig. 3). However, the Native stands that were associated with anthropogenic disturbance, especially sewage treatment, were more robust (Fig. 3).

Native haplotype H was the most abundant haplotype across the region and occurred in all wetland types typically at elevations below 800 m. It was also found in strongly alkaline (pH > 9.0) soils in Death Valley National Park and the Mojave Desert sink. Haplotype B occurred infrequently and was primarily associated with isolated systems and higher elevation mountain springs and creeks. Interestingly, we found two variants of Haplotype B that can have either 10 or 11 A's in the third microsatellite region of the trnT-trnL locus (Saltonstall et al. 2016). A novel variant of haplotype B was found in samples from California and southern Utah, although this haplotype has been found in samples from six other states (Saltonstall 2003a, this study). Haplotypes A and AR were found in the eastern portion of the sampling area along the upper Colorado River and in Phoenix, Arizona. Haplotype AR is a previously unidentified haplotype (T10/R2;

Table 1 Locations of *Phragmites australis* populations identified during surveys of wetlands and riparian areas in the southwest United States

Latitude	Longitude	Site/location	Lineage	Wetland type	State	County
34.5401	−117.2923	Mojave River, Victorville, population 1	Asian	Riparian	CA	San Bernardino
34.5419	−117.2934	Mojave River, Victorville, population 2	Asian	Riparian	CA	San Bernardino
34.5397	−117.2926	Mojave River, Victorville, population 3	Asian	Riparian	CA	San Bernardino
34.5413	−117.2925	Mojave River, Victorville, population 4	Asian	Riparian	CA	San Bernardino
32.7243	−114.6039	Yuma East Wetlands	Gulf Coast	Riparian	AZ	Yuma
32.9756	−114.4741	Lake Martinez	Gulf Coast	Riparian	AZ	Yuma
33.5731	−114.5333	Ehrenberg	Gulf Coast	Riparian	AZ	La Paz
33.7995	−111.4845	Sycamore Creek, Rte 87	Gulf Coast	Riparian	AZ	Maricopa
32.6867	−115.4998	All American Canal	Gulf Coast	Irrigation canal	CA	Imperial
32.6941	−115.4394	Calexico	Gulf Coast	Irrigation canal	CA	Imperial
33.3453	−114.7001	Cibola National Wildlife Refuge	Gulf Coast	Marsh	CA	Imperial
32.6796	−115.6134	Greeson Wash, Mount Signal	Gulf Coast	Riparian	CA	Imperial
32.7289	−114.6157	Winterhaven	Gulf Coast	Riparian	CA	Imperial
33.4466	−115.8438	Salt Creek	Gulf Coast	Riparian	CA	Imperial
36.0912	−115.0070	Along Duck Creek at bridge opposite side of creek from US886	Hybrid	Alkaline marsh/wastewater	NV	Clark
36.0912	−115.0070	Along Duck Creek at bridge, across road from US885	Hybrid	Alkaline marsh/wastewater	NV	Clark
36.0913	−115.0069	Along Duck Creek at bridge	Hybrid	Alkaline marsh/wastewater	NV	Clark
36.0921	−115.0144	Las Vegas Wash 24 Duck Creek #10	Hybrid	Alkaline marsh/wastewater	NV	Clark
36.0932	−115.0152	Las Vegas Wash 19 Below pond #7	Hybrid	Alkaline marsh/wastewater	NV	Clark
36.0937	−115.0153	Below Pond #7, Sample 12	Hybrid	Alkaline marsh/wastewater	NV	Clark
36.0382	−115.0531	Cornerstone Park, Railroad Lake, Las Vegas	Hybrid	Artificial pond	NV	Clark
36.0912	−115.0070	Las Vegas, Duck Creek @ wash South Side	Hybrid	Alkaline marsh/wastewater	NV	Clark
36.0912	−115.0070	Las Vegas, Duck Creek @ wash North Side	Hybrid	Alkaline marsh/wastewater	NV	Clark
36.0659	−115.0619	Las Vegas 7	Hybrid	Drainage ditch	NV	Clark
36.1023	−114.9343	Lake Las Vegas	Hybrid	Reservoir	NV	Clark
36.1039	−114.9333	LLV2	Hybrid	Reservoir	NV	Clark
36.1039	−114.9328	Lake Las Vegas west population	Hybrid	Reservoir	NV	Clark
36.1043	−114.9320	LLV1	Hybrid	Reservoir	NV	Clark
36.1202	−114.8602	Las Vegas Bay	Hybrid	Reservoir	NV	Clark
36.0455	−115.0581	Pittman Wash 1, Las Vegas	Hybrid	Riparian	NV	Clark
36.1219	−114.8634	Burned stand on top of cliff near LV Bay	Hybrid	Riparian	NV	Clark
36.0868	−114.9844	Las Vegas 1	Hybrid	Riparian/wastewater	NV	Clark

Table 1 continued

Latitude	Longitude	Site/location	Lineage	Wetland type	State	County
36.1214	−114.9058	Las Vegas 4	Hybrid	Seep	NV	Clark
36.0660	−115.0618	Las Vegas 14, Whitney Mesa	Hybrid	Seep runoff	NV	Clark
36.0647	−115.0630	Sunset Blvd. Site 1	Hybrid	Seep/golf course runoff	NV	Clark
36.0649	−115.0640	Las Vegas 12	Hybrid	Seep/golf course runoff	NV	Clark
36.0650	−115.0640	Whitney Mesa, Hybrid along Sunset Blvd	Hybrid	Seep/golf course runoff	NV	Clark
36.0682	−115.0566	Whitney Mesa, Neighborhood Park, Sample 3	Hybrid	Seep/golf course runoff	NV	Clark
36.0690	−115.0559	Whitney Mesa, Neighborhood Park, Sample 2	Hybrid	Seep/golf course runoff	NV	Clark
36.0693	−115.0558	Whitney Mesa, Neighborhood Park Sample 1	Hybrid	Seep/golf course runoff	NV	Clark
36.0960	−114.9471	Las Vegas Wash 22 Demonstration Pond #4	Hybrid	Water treatment pond	NV	Clark
36.0659	−115.0619	Las Vegas Wash 10	Hybrid	Water treatment runoff	NV	Clark
36.0912	−114.9692	Bostick, Las Vegas	Hybrid	Water treatment runoff	NV	Clark
36.0922	−114.9646	Calico Ridge Weir, LV Wash	Hybrid	Water treatment runoff	NV	Clark
36.0923	−114.9641	Calico Ridge Weir, LV Wash	Hybrid	Water treatment runoff	NV	Clark
36.0958	−114.9474	Las Vegas 15	Hybrid	Water treatment runoff	NV	Clark
36.0961	−114.9470	Las Vegas Wash, Upstream of North Shore Bridge	Hybrid	Water treatment runoff	NV	Clark
36.1017	−114.9407	Las Vegas Wash 23 PWL #3	Hybrid	Water treatment runoff	NV	Clark
36.1020	−114.9397	Las Vegas, Powerline	Hybrid	Water treatment runoff	NV	Clark
36.1220	−114.9045	North Shore Bridge Sample 2, nearer to water	Hybrid	Water treatment runoff	NV	Clark
37.4361	−122.1045	Palo Alto salt marsh, population 2	Introduced	Brackish marsh	CA	San Mateo
37.4362	−122.0997	Palo Alto	Introduced	Brackish marsh	CA	San Mateo
37.4367	−122.1049	Palo Alto marsh, population 1	Introduced	Brackish marsh	CA	San Mateo
38.0231	−122.1004	McNabney Marsh, Martinez	Introduced	Brackish marsh	CA	Contra Costa
38.0246	−122.1350	Martinez Wildlife Refuge, Suisun Bay	Introduced	Brackish marsh	CA	Contra Costa
38.0262	−122.1359	Martinez marina west	Introduced	Brackish marsh	CA	Contra Costa
38.0477	−122.0775	Point Edith, Concord	Introduced	Brackish marsh	CA	Contra Costa
38.0826	−122.1066	Suisun Marsh, Pop 2	Introduced	Brackish marsh	CA	Solano
38.0826	−122.1067	Suisun Marsh, Pop 1	Introduced	Brackish marsh	CA	Solano
38.0827	−122.1069	Suisun Marsh, Pop 4	Introduced	Brackish marsh	CA	Solano
38.0849	−122.1064	Suisun Marsh, Pop 3	Introduced	Brackish marsh	CA	Solano
35.3071	120.6616	San Luis Obispo, Cal Poly Brae Lab Reservoir	Introduced	Reservoir	CA	San Luis Obispo
35.3159	120.6839	San Luis Obispo, Cal Poly Vineyard pond	Introduced	Reservoir	CA	San Luis Obispo
32.7613	−117.2058	San Diego River	Introduced	Riparian	CA	San Diego

Table 1 continued

Latitude	Longitude	Site/location	Lineage	Wetland type	State	County
34.7158	−114.4886	Interstate 40, Needles	Introduced	Riparian	CA	Riverside
35.4993	−120.6520	Salinas River, Atascadaro	Introduced	Riparian/wastewater	CA	San Luis Obispo
35.5402	−120.7093	Atascadero	Introduced	Riparian/wastewater	CA	San Luis Obispo
36.0647	−115.0630	Whitney Mesa, Henderson	Introduced	Seep/golf course runoff	NV	Clark
36.0647	−115.0632	Las Vegas 5	Introduced	Seep/golf course runoff	NV	Clark
36.0656	−115.0620	Whitney Mesa, Sample 4	Introduced	Seep/golf course runoff	NV	Clark
41.1980	−112.1969	Ogden	Introduced	Marsh	UT	Box Elder
41.4690	−112.0822	Brigham City	Introduced	Marsh	UT	Box Elder
37.0855	−113.5537	St George, River Bridge near gas station	Introduced	Riparian	UT	Washington
37.0865	−113.5561	St. George, River Bridge (2011)	Introduced	Riparian	UT	Washington
37.0866	−113.5561	St George, W of River Bridge, Virgin River (2014)	Introduced	Riparian	UT	Washington
37.2162	−112.9659	Pine Creek 1, Zion National Park	Introduced	Riparian	UT	Washington
34.7427	−114.4877	Catfish Paradise, Topock Marsh	Native	Marsh	AZ	Mohave
33.4174	−112.0220	Salt River, Phoenix	Native	Riparian	AZ	Maricopa
33.5677	−114.5316	Ox Bow Road	Native	Riparian	AZ	La Paz
33.6041	−114.5298	I-10 Bridge AZ	Native	Riparian	AZ	La Paz
34.9681	−110.6473	McHood State Park, Winslow	Native	Riparian	AZ	Navajo
36.0800	−112.1261	Indian Gardens, population 1	Native	Riparian	AZ	Grand Canyon
36.0800	−112.1261	Indian Gardens, population 2	Native	Riparian	AZ	Grand Canyon
36.1089	−111.2371	Tuba City	Native	Riparian	AZ	Coconino
33.1025	−116.4545	Senetac Marsh, Anza Borrego	Native	Alkaline marsh	CA	San Diego
33.8374	−116.3101	Coachella Valley Preserve	Native	Alkaline marsh	CA	Riverside
34.3553	−119.0064	Santa Clara River, Santa Paula	Native	Alkaline marsh	CA	Ventura
36.4178	−117.8239	Route 190	Native	Alkaline marsh	CA	Inyo
37.3896	−118.4999	Route 395, Bishop, CA	Native	Alkaline marsh	CA	Inyo
33.5401	−116.0992	Torres Martinez Indian Reservation, Salton Sea	Native	Drainage ditch	CA	Riverside
33.6291	−116.1293	Rte 111, Thermal	Native	Drainage ditch	CA	Imperial
32.9489	−116.3049	Agua Caliente, Anza Borrego 2	Native	Hot spring runoff	CA	San Diego
32.9536	−116.3107	Agua Caliente, Anza Borrego 1	Native	Hot spring runoff	CA	San Diego
34.5403	−119.6200	Little Caliente Hot Spring, Los Padres NF	Native	Hot spring runoff	CA	Santa Barbara
35.8560	−116.2293	Tecopa Hot Springs	Native	Hot spring runoff/sink	CA	Inyo
38.0414	−121.6943	Jersey Island, Antioch	Native	Levee	CA	Contra Costa

Table 1 continued

Latitude	Longitude	Site/location	Lineage	Wetland type	State	County
33.5731	−114.5401	Goose Flats, site 2	Native	Riparian	CA	Riverside
33.5761	−114.5413	Goose Flats, site 1	Native	Riparian	CA	Riverside
34.5339	−119.5639	Agua (Big) Caliente Hot Spring, Los Padres NF	Native	Riparian	CA	Santa Barbara
36.4480	−117.8369	Owen's Lake	Native	Riparian	CA	Inyo
36.6403	−118.0793	Owen's River, Lone Pine	Native	Riparian	CA	Inyo
37.1827	−120.6550	San Joaquin River	Native	Riparian	CA	Merced
37.3893	−118.4988	Inyo Co., N. Bishop, Rte 395 near Pleasant Valley Farm Rd	Native	Riparian	CA	Inyo
37.3896	−118.4999	Owens Valley, Mill Creek	Native	Riparian	CA	Inyo
37.4412	−118.5731	Lower Rock Creek, Bishop	Native	Riparian	CA	Inyo
37.4884	−117.9222	Oasis	Native	Riparian	CA	Mono
34.9022	−114.7682	Sacramento Spring, Route 95	Native	Seep	CA	San Bernardino
35.1724	−116.1095	Zzyzx Road	Native	Seep	CA	San Bernardino
35.3357	−120.8281	Morro Bay	Native	Seep/salt marsh	CA	San Luis Obispo
33.4962	−115.8515	Dos Palmas Preserve 2	Native	Spring	CA	Riverside
33.4970	−115.8516	Dos Palmas Preserve	Native	Spring	CA	Riverside
33.4984	−115.8492	Dos Palmas Preserve 5	Native	Spring	CA	Riverside
33.8297	−116.3126	Thousand Palms Canyon Road	Native	Spring	CA	Riverside
39.1040	−108.5486	Grand Junction, CO	Native	Drainage ditch	CO	Mesa
40.8130	−108.9567	Browns Park NWR, Butch Cassidy Marsh, Maybell	Native	Marsh	CO	Moffat
37.0385	−108.7351	Mancos River, Ute Mountain Tribal Park area	Native	Riparian	CO	Montezuma
37.4376	−107.8692	Durango, CO	Native	Riparian	CO	La Plata
38.4455	−107.8650	Montrose, CO	Native	Riparian	CO	Montrose
38.6813	−108.9804	Dolores River, Gateway	Native	Riparian	CO	Mesa
39.1682	−108.8680	P1, Colorado River west of Grand Junction	Native	Riparian	CO	Mesa
39.1768	−108.2791	Plateau Creek, near confluence with Colorado River	Native	Riparian	CO	Mesa
39.2046	−108.9314	P4, Colorado River west of Grand Junction	Native	Riparian	CO	Mesa
36.7786	−108.6924	Shiprock, near San Juan River	Native	Riparian	NM	San Juan
37.2734	−115.1187	Parhanagat NWR; Headquarters Road	Native	Alkaline marsh	NV	Lincoln
37.2769	−115.1164	Pahranagat Nat. Wildlife Refuge, Black Canyon	Native	Alkaline marsh	NV	Lincoln
37.2770	−115.1164	Parhanagat NWR, Black Canyon	Native	Alkaline marsh	NV	Lincoln
36.0921	−115.0144	Las Vegas Wash 24—deformed flowers	Native	Alkaline marsh/wastewater	NV	Clark
36.0947	−115.0220	Wetland Park, Las Vegas	Native	Alkaline marsh/wastewater	NV	Clark

Table 1 continued

Latitude	Longitude	Site/location	Lineage	Wetland type	State	County
36.0980	−115.0217	Near Pond #3, Stand 7, along road	Native	Alkaline marsh/wastewater	NV	Clark
36.0982	−115.0212	Near Pond #3, Stand 6,along road	Native	Alkaline marsh/wastewater	NV	Clark
36.0982	−115.0215	Near pond #3, Sample 18	Native	Alkaline marsh/wastewater	NV	Clark
36.0983	−115.0214	Near Pond #3, Stand 1, bordering pond & road	Native	Alkaline marsh/wastewater	NV	Clark
36.0983	−115.0214	Near Pond #3, Stand 2, bordering pond & road	Native	Alkaline marsh/wastewater	NV	Clark
36.0984	−115.0208	Near Pond #3, Stand 5,along road	Native	Alkaline marsh/wastewater	NV	Clark
36.0984	−115.0208	Near Pond #3, Stand 4, along road	Native	Alkaline marsh/wastewater	NV	Clark
36.0987	−115.0209	Near Pond #3, Stand 3, along road	Native	Alkaline marsh/wastewater	NV	Clark
36.0992	−115.0156	DR Wetlands #2, Sample 11	Native	Alkaline marsh/wastewater	NV	Clark
36.1006	−115.0246	Tropicana #9	Native	Alkaline marsh/wastewater	NV	Clark
36.1089	−115.0241	CCWRD	Native	Alkaline marsh/wastewater	NV	Clark
36.0839	−115.0083	Burn St. #8	Native	Drainage ditch	NV	Clark
36.0460	−115.0568	Pittman Wash 2, Las Vegas	Native	Riparian	NV	Clark
36.6031	−114.4842	Muddy River, Overton	Native	Riparian	NV	Clark
36.7938	−114.0832	Hughes School, Mesquite, 28	Native	Riparian	NV	Clark
36.7938	−114.0828	Hughes School, Virgin River, Mesquite	Native	Riparian	NV	Clark
36.9236	−116.7451	Amaragosa River, Beatty	Native	Riparian	NV	Clark
36.0771	−115.0637	Cattail Marsh Park	Native	Seep	NV	Clark
36.1214	−114.9059	Las Vegas 11	Native	Seep	NV	Clark
36.1216	−114.9047	Las Vegas 6	Native	Seep	NV	Clark
36.1218	−114.9046	Las Vegas 3, North Shore Bridge	Native	Seep	NV	Clark
40.0832	−118.5783	Humboldt Sink	Native	Seep	NV	Churchill
36.0648	−115.0632	Las Vegas 2	Native	Seep/golf course runoff	NV	Clark
36.0649	−115.0639	Sunset Blvd. Site 2	Native	Seep/golf course runoff	NV	Clark
36.0653	−115.0633	Whitney Mesa, Sample 2	Native	Seep/golf course runoff	NV	Clark
36.0656	−115.0631	Las Vegas 9	Native	Seep/golf course runoff	NV	Clark
36.3769	−114.4436	Rogers Hot Spring, Lake Mead	Native	Spring	NV	Clark
36.0964	−115.0209	Wetland Park, end of duck creek trail	Native	Water treatment pond	NV	Clark
36.0773	−114.9980	Demonstration Pond 9	Native	Water treatment runoff	NV	Clark
36.0877	−114.9747	Las Vegas Wash 25 C-1 Channel 6	Native	Water treatment runoff	NV	Clark
36.0886	−114.9924	Site 108—Desert Wetlands Park	Native	Water treatment runoff	NV	Clark
36.0941	−115.0124	Las Vegas 8	Native	Water treatment runoff	NV	Clark

Table 1 continued

Latitude	Longitude	Site/location	Lineage	Wetland type	State	County
36.1220	−114.9045	Las Vegas Wash, Downstream of North Shore Bridge along bank	Native	Water treatment runoff	NV	Clark
39.8440	−113.3523	Fish Springs NWR, Avocet Unit, Dugway	Native	Marsh	UT	Juab
39.8816	−113.3929	Fish Springs NWR, Pintail Unit, Dugway	Native	Marsh	UT	Juab
37.0854	−113.5539	St George, N of River Bridge, Virgin River	Native	Riparian	UT	Washington
37.0862	−113.5573	St George, S of River Bridge	Native	Riparian	UT	Washington
37.2163	−112.9664	Pine Creek 2, Zion NP	Native	Riparian	UT	Washington
37.2169	−112.9676	Virgin River, Route 9	Native	Riparian	UT	Washington
37.2728	−112.9418	Virgin River, Zion NP	Native	Riparian	UT	Washington
37.2757	−112.9372	Zion, 7 Sandbar in Virgin River near Weeping Rock	Native	Riparian	UT	Washington
38.0267	−109.5405	Canyonlands National Park	Native	Riparian	UT	San Juan
38.2446	−109.8154	P14, Colorado River south of Moab	Native	Riparian	UT	San Juan
38.7992	−109.2041	Rio Mesa Station, Delores	Native	Riparian	UT	Grand
38.9230	−110.4012	Green River	Native	Riparian	UT	Emery
38.9938	−110.1441	San Rafael River	Native	Riparian	UT	Emery
37.2719	−112.9366	Zion NP, Weeping Rock	Native	Seep	UT	Washington
38.7345	−109.5192	Arches National Park	Native	Seep	UT	Grand
36.6389	−114.2477	Gold Butte	Native	Seep/spring	UT	Washington

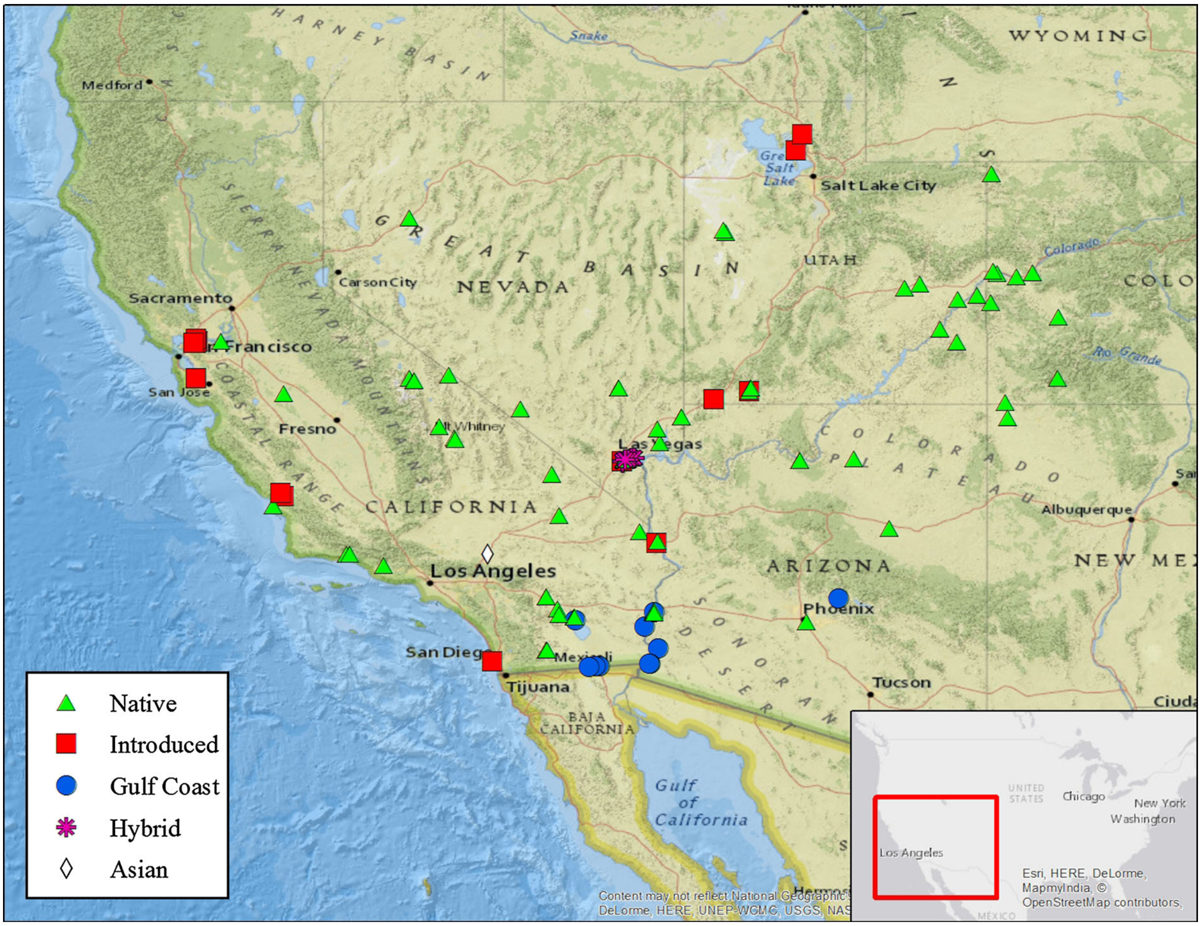

Fig. 1 Distribution of *Phragmites australis* lineages and hybrids in the southwestern United States

GenBank Accession No. AF457397/AY016333), but is closely related to Haplotypes A, B, and H.

Non-native *Phragmites australis* haplotypes

Introduced *Phragmites* stands (n = 26) were associated with wetland modification and disturbance in or near urban centers, and often with systems where wastewater effluent provided permanent flows in historically ephemeral rivers. While it was rare throughout much of the study area, extensive monocultures were found throughout the San Francisco Bay Delta and northern San Joaquin Valley in California. The San Francisco area populations were most extensive (covering many hectares) in the highly disturbed brackish marshes of the Delta. Since the beginning of this study, Introduced *Phragmites* has been spreading south in the San Joaquin Valley, especially in areas where riparian restoration is occurring (J. Rentner, personal communication). In 2006, a relatively small population (less than 0.25 ha) was found to the south of this region near a sewage treatment facility in the Salinas River, Atascadero, CA. In 2014, new populations were identified in previously surveyed areas 20 km away growing along the banks of man-made reservoirs in San Luis Obispo, CA. In 2007, the San Diego, CA, population was localized to a small island near the mouth of the San Diego River, but new populations have recently established along coastal rivers and marshes to the north (J. Rebman, personal communication). Several Introduced populations were found along the Virgin River, a tributary to the lower Colorado River. A small stand was present in Saint George, Utah in 2010 and additional stands were found in 2014. In 2014, a large stand was identified in Pine Creek (tributary to the Virgin River) in Zion

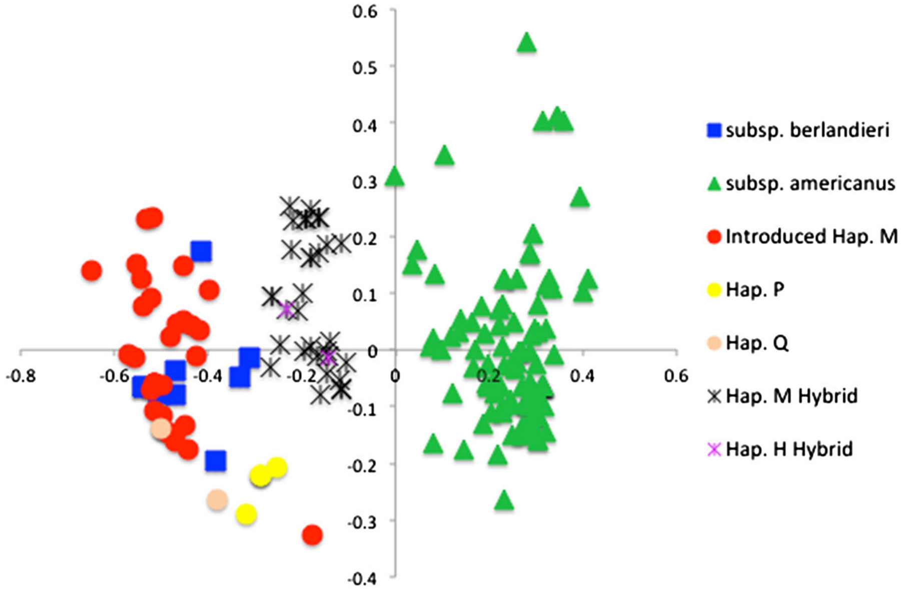

Fig. 2 Principle Coordinates Analysis plot of 201 *Phragmites australis* individuals from western North America based on microsatellite profiles at seven loci. cpDNA lineage of origin is indicated in the *legend*

National Park that was not present at the same location during a survey of the area in 2007. Two Introduced *Phragmites* populations were found in Las Vegas, Nevada, very close to each other on debris and fill at a new housing development. An extensive Introduced population was identified south of Las Vegas along the Colorado River in Needles, California. These populations are linked by the Colorado River, but no additional Introduced populations were detected between Las Vegas and Needles, or south of the Needles population, although there are inaccessible parts of the river in these locations.

Two haplotypes that are native to Asia were identified for the first time in North America. Haplotype P was found in the Mojave Narrows along the Mojave River in Victorville, CA. Several robust populations occurred along this wet river reach, but no other populations were found in the dry reaches to the north or south. All four of the unique stands with this cpDNA haplotype that we tested had the same microsatellite phenotype suggesting that the lineage is spreading clonally along the river. It also appears that this haplotype is octoploid, based on its microsatellite phenotype, which showed four alleles at locus GT4. In addition, two samples identified as haplotype Q were collected from large stands in Bayland Park, Palo Alto, California. These stands were considered invasive by park staff and treated with herbicide in 2007, however recent aerial imagery shows that the stands continue to expand. The two samples that we tested had unique, but closely related, microsatellite phenotypes also suggestive of the plants being octoploid.

Hybrids in the Las Vegas Wash watershed

Hybrid *Phragmites* populations were widespread in the Las Vegas, Nevada area, and are likely first-generation hybrids based on their microsatellite allele phenotypes which displayed alleles common to both the Native and Introduced lineages at nearly all loci (Saltonstall et al. 2016; Fig. 2). Most of these hybrids had cpDNA Haplotype M (n = 34 samples), indicating that their maternal parent was an Introduced plant. These plants were extremely robust and found growing along the lower reaches of the Las Vegas Wash, as well as in surrounding remnant creeks and drainage channels. Two hybrid samples had cpDNA Haplotype H (Native maternal parent) and microsatellite profiles suggesting that they might be first-generation hybrids as well. These haplotype H hybrids were localized in the upper Wash and were smaller in stature than other hybrids,

Fig. 3 (*Top*) Native *Phragmites australis* stand at Little Caliente Hot Springs in the Los Padres National Forest, Santa Barbara County, California. This population typifies the size and density of native stands observed in the southwest. (*Bottom*) Native *Phragmites* stand along the Las Vegas Wash, Clark County, Nevada. The density and robust size of this stand is atypical of southwest populations and is most likely facilitated by the nutrient-rich effluent in which it grows

but still grew in large patches. All hybrid populations were found predominately in areas with heavy soil disturbance, including residential developments, within the Las Vegas Wash and its tributaries, and upper Lake Mead where extensive flood control and riparian restoration projects are occurring. An in-depth analysis of the hybridization we documented in Las Vegas is provided by Saltonstall et al. (2016).

Table 2 Soil pH and electrical conductivity measurements taken from a subset of *Phragmites australis* stands throughout the survey area

Location	Lineage	pH	Electrical conductivity (mS)	% Sand	% Silt	% Clay
Agua Caliente Hot Spring, Santa Barbara, CA	Native	8.3	0.09	72	19	9
Coachella Valley Preserve, Thousand Palms, CA	Native	7.98	0.59	58	22	20
Santa Clara River, Santa Paula, CA	Native	8.04	0.51	51	21	28
Tecopa Hot Spring, Death Valley, CA	Native	9.56	1.21	58	31	11
Zzyzx Road, Baker, CA	Native	9.1	1.86	84	6	10
Morro Bay, CA	Native	6.12	5.84	–	–	–
Rogers Hot Spring, Lake Mead Nat Rec Area, NV	Native	8.64	5.6	88	6	6
Muddy River, Overton, NV	Native	7.72	3.85	68	17	15
Hughes School, Mesquite, NV	Native	7.78	0.27	72	18	10
Whitney Mesa Native, Henderson, NV	Native	8.6	4.03	70	12	18
Native below pond 7, sample 1, Henderson, NV	Native	8.43	5.71	48	36	16
Native below pond 7, sample 2, Henderson, NV	Native	7.98	4.59	46	35	19
North Shore Bridge Seep, Lake Mead Nat Rec Area, NV	Native	7.38	2.33	90	0	10
Dos Palmas Preserve, Mecca, CA	Native	8.29	3.61	79	17	4
Wetland Park Native, Henderson, NV	Native	8.5	1.01	62	22	16
Saratoga Spring, Route 95, Needles, CA	Native	8.2	0.41	–	–	–
Northshore Bridge, Lake Mead Nat Rec Area, NV	Native	7.59	2.08	74	16	10
Cattail Park, Henderson, NV	Native	8.78	4.4	66	18	16
River Bridge Native, St. George, UT	Native	7.93	0.41	88	3	9
Whitney Mesa Introduced, Henderson, NV	Introduced	8.09	4.96	80	10	10
Las Vegas Bay, Lake Mead Nat Rec Area, NV	Introduced	7.86	0.43	78	12	10
Salinas River, Atascadero, CA	Introduced	7.53	–	76	12	12
Suisun Marsh, CA	Introduced	6.7	5.38	–	–	–
River Bridge Introduced, St. George, UT	Introduced	7.89	0.16	70	16	14
Whitney Mesa Hybrid, sample 1, Henderson, NV	Hybrid	7.95	2.95	55	35	10
Lake Las Vegas Hybrid, NV	Hybrid	7.61	2.54	78	12	10
Whitney Mesa Hybrid, sample 2, Henderson, NV	Hybrid	7.98	2.45	78	11	11
Hybrid below pond 7, sample 1, Henderson, NV	Hybrid	8.12	4.86	46	34	20
Hybrid below pond 7, sample 2, Henderson, NV	Hybrid	8.4	4.26	46	35	19
Wetland Park Hybrid, sample 1, Henderson, NV	Hybrid	8.05	0.9	66	25	9
Wetland Park Hybrid, sample 2, Henderson, NV	Hybrid	8.1	0.6	77	11	12
Salt Creek, Salton Sea, CA	Gulf Coast	8.01	2.42	–	–	–

Gulf Coast *Phragmites australis* subspecies *berlandieri* lineage

The Gulf Coast lineage (n = 10) was restricted to latitudes below 33.8°N and was generally associated with agricultural canals and modified wetlands linked to the lower Colorado River. A very small stand was found in Cottonwood Creek, a dry river wash north of Phoenix, AZ. All samples from this lineage shared Haplotype I and have unique allele phenotypes across the majority of microsatellite loci, suggesting that they are hexaploid. No evidence for hybridization with the Introduced lineage was detected in either the Structure (results not shown) or PCoA analyses (Fig. 2).

Soil properties

Soil properties were variable across the survey area and among habitat types, with only soil pH showing consistent differences among lineages. Soil pH of

Table 3 Stem density and percent cover of *Phragmites australis* lineages in the southwestern United States

	Number of stems per m^2 (±SD)	Post-hoc[a]	Percent cover (±SD)	Post-hoc[a]
Native	69.3 ± 23.9	a	36.5 ± 23.2	a
Introduced	118.3 ± 31.7	b	76.4 ± 12.2	b
Hybrid	113.5 ± 43.6	b	73.1 ± 23.0	b
Haplotype H hybrid	104.6 ± 24.4	b	31.4 ± 5.6	a

[a] Post hoc comparisons analyzed using Tukey's test. Different letters represent significant differences among lineages

Native (mean 8.3 ± 0.7 [SD]) and Hybrid (mean 8.6 ± 0.6) stands was generally higher than that of soils collected in Introduced stands (mean 7.5 ± 1.5), although this result was not significant ($F_{2,52} = 1.47$, $p = 0.24$; Table 2). Electrical conductivity varied substantially among sites, but no significant difference among lineages was detected. Soil texture (% sand/silt/clay) was highly variable and did not differ significantly among lineages, although sand constituted the majority fraction (at least 48 %) of the soil volume for all samples.

Stem density and percent cover

Stem density was significantly different among lineages ($F_{(3,87)} = 15.09$, $p < 0.001$; Table 3). Introduced, hybrid, and haplotype H hybrid stands contained 71, 63, 51 % (respectively) more stems per meter than Native stands.

Percent cover was also significantly different among lineages ($F_{(3,87)} = 28.02$, $p < 0.001$; Table 3). Introduced and hybrid stands had 109 and 100 % greater cover, respectively, than Native stands. However, the haplotype H hybrid had a similar cover to that of Native stands.

Discussion

Phragmites has been a component of southwestern US wetland plant communities for thousands of years (Goman and Wells 2000; Hansen 1978; Kiviat and Hamilton 2001). Today, wetlands in the Southwest face multiple threats from urbanization and associated reductions in water availability, especially through groundwater overdraft, that have caused regional declines in wetland extent and dependent vegetation (Patten et al. 2008). The future of plant populations, including the Native *Phragmites* lineage, in these systems is of conservation concern, particularly when considering the fragmented nature of wetland habitats in the xeric habitats of the Southwest. There is a need for ecological and distributional data for these communities at the regional level, yet to date, little information is available. Here, we show broad patterns of regional overlap among Native, Introduced, and Gulf Coast *Phragmites* lineages in the Southwest, which is the only region of the United States where the three lineages co-occur (Saltonstall 2002, 2003a; Saltonstall et al. 2004; Meyerson et al. 2010). Native *Phragmites* has high genetic diversity, as we found four cpDNA haplotypes including one new one, which may also reflect the high diversity of habitats in the region. We also document two novel introductions and hybridization between the Native and Introduced lineages. These findings suggest that (1) Native *Phragmites* remains widely distributed across wetland habitats and is maintaining its genetic diversity; (2) Introduced *Phragmites* is uncommon but spreading, and where found, is associated with disturbed and urbanized wetlands or those adjacent to transportation corridors; (3) Native and Introduced *Phragmites* coexist at many sites, but appear genetically isolated everywhere except in southern Nevada where hybrids are common at the watershed scale; (4) Gulf Coast *Phragmites* is restricted to wetlands associated with human-modification along the lower Colorado River and shows no evidence for hybridization with Introduced *Phragmites;* and (5) Two haplotypes likely originating from Asia have been introduced to California, but thus far appear to be restricted to two river drainages.

Native *Phragmites australias* subspecies *americanus* lineage

Native *Phragmites* was the most common lineage detected, but generally at low densities. This may reflect the rarity of appropriate wetland habitat types and severity of the edaphic conditions in the region. However, genetic diversity of Native *Phragmites* in the Southwest region is higher than in the Midwest and eastern parts of North America (Saltonstall 2003a, b) and displays many unique haplotypes and allele phenotypes as well. This high diversity is perhaps due to its long history in the region, as well as adaptation to the relictual nature of the wetlands it inhabits (Minckley et al. 2013). In another study of *Phragmites* populations in Utah and southern Idaho, Kettenring and Mock (2012) found that Native clones had lower genetic diversity than Introduced clones, possibly due to a greater dependency of Introduced populations on establishment by seed rather than clonal expansion.

Native stands were associated with all wetland habitats and over the range of human disturbance, and was the only lineage present in locations away from urban centers or transportation corridors. It appears that Native *Phragmites* is the only lineage currently associated with the isolated seeps, springs, and oases in the Southwest, which provide critical habitat for wildlife (Fleishman and Murphy 2005; Fensham et al. 2011). These remote stands had low stem densities and were always mixed with other native wetland plant species.

Non-native *Phragmites australis* haplotypes

It is unknown how long the Introduced lineage has been present in the western United States or whether multiple introductions have occurred, but it is generally accepted that populations were established in this region in the late twentyth century whereas the eastern invasion began in the 1800's (Saltonstall 2002). The oldest sample in our dataset was collected in August, 1995 in San Diego, CA below an Interstate highway 5 overpass (D. Hauber pers. comm). Introduced *Phragmites* is already widespread and expanding in some western systems, including the San Francisco Bay Delta (Grossinger et al. 1998) and around the Great Salt Lake (Kulmatiski et al. 2010; Kettenring et al. 2012; Kettenring and Mock 2012). Kulmatiski et al. (2010) dated the first Introduced *Phragmites* herbarium samples in the Salt Lake City, Utah area to 1993, and found that current populations expanded to cover 56 % of the extensive wetlands within 27 years. We found the Introduced lineage primarily associated with urban wastewater and highly impacted wetlands in the San Francisco Bay Delta. However, the two populations in Zion National Park and along the Colorado River in Needles, California are in locations with relatively low human disturbance (but near major roads) suggesting that invasion is possible away from urban centers, although it is unclear if alterations occurred in these areas that may have led to establishment. We identified Introduced populations in the Virgin River and tributaries in Southwest corner of Utah and suspect that these represent relatively new establishment events likely facilitated by transport (see Brisson et al. 2010) in the Interstate 15 highway corridor, a major route between Salt Lake City and Las Vegas, Nevada, as well as channel modification for flood control. Kettenring et al. (2012) provide a similar explanation for the widespread *Phragmites* invasion around the Great Salt Lake in northern Utah. In 2014, we found a new population along the main corridor through nearby Zion National Park, which was not present when we surveyed the area in 2007. Similar range expansions are occurring in coastal California south of San Francisco and in San Diego which suggests that this is an ongoing invasion and expansion into new habitats will continue. There is also concern that Introduced *Phragmites* will continue to expand its range as water resources are modified along with the growing human population, as well as replace other invasive riparian plants that are primary targets for eradication (Lambert et al. 2010b; Meyerson et al. 2010). The presence and continued spread of the Introduced lineage is a previously unrecognized threat to isolated wetlands in the region, but it is unclear if this lineage can successfully invade these systems, which have substantially different abiotic (especially soil) properties than the temperate regions of Europe where it is native or the Northeastern United States where it has reached its greatest extent.

Hybrids in the Las Vegas Wash watershed

Previously, hybridization between Native and Introduced lineages had only been detected in eastern North America and appeared to occur as infrequent and localized events (Saltonstall et al. 2014; Wu et al. 2015). Saltonstall (2003a, b, c) found no evidence for hybridization across North America and Kettenring and Mock (2012) did not find evidence of hybridization in their analysis of Native and Introduced

Phragmites populations in Utah and southern Idaho. The hybrid stands we found in the Las Vegas area of southern Nevada are the first documented evidence that hybridization is occurring at the landscape or watershed level. Further, their abundance and propensity to spread with human disturbance is concerning. Hybrid stands were observed throughout the Las Vegas Wash, an effluent discharge system for regional wastewater that was once an ephemeral wash. Extensive hybrid populations grow immediately adjacent to the river banks, while Native *Phragmites* is limited to higher terrace locations, and dispersal of clonal fragments appears to be a major source of spread along these rivers. Hybrid stands have also been detected in newly constructed artificial wetlands in the area, but it is unclear if establishment occurred by the wind-borne seeds or movement of rhizomes during construction. It is very possible that hybrids will continue to spread throughout the lower Colorado River Basin. Saltonstall et al. (2016) more fully describe the *Phragmites* distribution patterns and hybridization observed in southern Nevada.

Gulf Coast *Phragmites australis* subspecies *berlandieri* lineage

The geographical origin and taxonomic designation of the Gulf Coast lineage (subspecies *berlandieri*) has been the subject of much debate (Saltonstall 2002; Jones et al. 1997; Saltonstall and Hauber 2007; Ward 2010; Lambertini et al. 2012), and although it is considered potentially native in the very southern portion of our sampling area (Saltonstall 2002; Saltonstall and Hauber 2007), it may have been introduced to the habitats in which we document it. We found this lineage restricted to the lower Colorado River and canal systems that convey water for agricultural use in southern California and Arizona. Continued population expansion associated with water management is considered a significant concern for resource managers and agricultural interests (C. Bell, personal communication) as these stands appear large and grow as dense monocultures (Lambert and Saltonstall, Personal observation). For example, *Phragmites* from the Gulf Coast lineage was planted at Yuma Crossing, Arizona over 20 years ago for erosion control, but is now the target of control efforts in that area and much of the lower Colorado River because of its rapid spread and facilitation of fire in riparian corridors (Fred Phillips Consulting 2011).

Soil properties

We expect that differences in soil properties between the eastern and western United States will influence the relative scope of the invasion in the Southwest. The Introduced lineage has evolved under a temperate, high precipitation climate in Europe, and appears capable of invading the majority of wetlands in eastern and central North America with a similar climatic regime. In the West, it appears to be most abundant where excess fresh water (and nutrients) is added to wetland systems and/or where human activities have created a disturbance. The pH of soils collected from Introduced populations was less than 7.6, below the average pH levels of the sites with Native and hybrid stands, although more data are necessary to confirm this trend (we sampled all possible Introduced stands in our study). The highly basic pH of desert wetlands, which at some of our sites exceeded 9.0, may limit or even prevent the spread of the Introduced lineage, but not necessarily hybrid populations, which may have inherited genetic material from their Native parent making them pre-adapted to the desert climate. Kettenring et al. 2012, suggest that other climatic factors, such as the elevated carbon dioxide and temperature conditions expected in the Southwest under a climate change scenario could also facilitate colonization of saline habitats by invasive genotypes.

Conclusions

Although desert wetland ecosystems have been recognized as critical habitats for protecting biodiversity, they are underrepresented as conservation targets (Minckley et al. 2013). Further, the paucity of ecological and environmental information for these habitats contributes to a lack of awareness of the threats of invasive species and human disturbance to associated biota. Native *Phragmites* is still the most common lineage in the Southwest, but it is unclear how invasion of non-native *Phragmites* haplotypes in this region will ultimately affect wetland habitats, or whether the scale of invasion and spread occurring in the Great Salt Lake (Kulmatiski et al. 2010; Kettenring et al. 2012; Kettenring and Mock 2012) and the San

Francisco Estuary (Grossinger et al. 1998), and most recently the Las Vegas area, will continue across this arid region. It is also disturbing that we found two novel introductions in California that appear to be spreading vegetatively. We suggest that these stands should be a priority for control efforts as they currently are isolated to certain watersheds and it may be possible to eradicate them at this time before they spread. Continued monitoring of Native population trends and spread of Introduced and hybrid populations is critical for determining population trajectories, as well as assessing whether the Native lineage requires management or protected status in the Southwest.

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