MORPHOLOGICAL AND GENETIC ANALYSES OF HERBARIUM SPECIMENS CLARIFY THE ARRIVAL OF NON-NATIVE COMMON REED (*PHRAGMITES AUSTRALIS* SUBSP. *AUSTRALIS*) IN KANSAS (U.S.A.)

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ABSTRACT

The common reed, *Phragmites australis,* is a globally distributed grass species with numerous named subspecific taxa. In North America, a non-native Eurasian subspecies has established and is rapidly expanding its range at the expense of a native subspecies. Our understanding of the common reed invasion in North America is possible because diagnostic data can be obtained from freshly collected and herbarium material, allowing the geography of subspecies to be understood through time. In this study, these morphological and genetic tools were used to diagnose subspecies in a set of specimens from Kansas collected between 1936–2024. Morphological and genetic diagnoses of subspecies agreed in 92% of cases. The non-native subspecies is present in Kansas and arrived in the 1970s or 1980s based on morphological or genetic data, respectively. The last native subspecies specimen was observed in the late 1990s, suggesting that statewide invasion could have been rapid. However, relatively recent specimens are not available for large portions of the state, particularly western Kansas. Additional sampling of both herbarium and freshly collected material is needed to fully understand the historic and current distribution of common reed subspecies in Kansas.

RESUMEN

El carrizo común, *Phragmites australis*, es una especie herbácea de distribución global con numerosos taxones subespecíficos. En Norteamérica, una subespecie euroasiática no nativa se ha establecido y está expandiendo rápidamente su área de distribución a expensas de una subespecie nativa. Nuestra comprensión de la invasión del carrizo común en Norteamérica es posible gracias a la obtención de datos de diagnóstico a partir de material recién recolectado y de herbario, lo que permite comprender la geografía de las subespecies a lo largo del tiempo. En este estudio, se utilizaron herramientas morfológicas y genéticas para diagnosticar subspecies en un conjunto de especímenes de Kansas recolectados entre 1936 y 2024. El diagnóstico morfológico y genético de las subespecies coincidió en el 92% de los casos. La subespecie no nativa está presente en Kansas y llegó en las décadas de 1970 o 1980, según el tipo de datos. El último ejemplar de subespecie nativa fue observado a finales de la década de 1990, lo que sugiere que la invasión estatal podría haber sido rápida. Sin embargo, no se dispone de especímenes relativamente recientes de grandes áreas del estado, particularmente del oeste de Kansas. Se necesitan más muestras de herbario y material recién recolectado para comprender plenamente la distribución histórica y actual de las subespecies comunes de carrizo en Kansas.

Key Words: Great Plains, herbarium DNA, invasive species, Poaceae

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INTRODUCTION

The common reed, *Phragmites australis* (Cav.) Trin. ex Steud., is one of North America's most widespread and easily recognizable grasses (Allred 2003). Although fossil and herbarium data establish that it was present in North America during the pre- and early settlement periods (reviewed in Saltonstall 2002), it rapidly increased in frequency in the 20th century (Chambers et al. 1999). The common reed's ability to reproduce vegetatively through rhizomes allows it to form extensive, dense monocultures which result in a variety of detrimental effects (Ketterning et al. 2012). This feature of its life history, combined with its expanding frequency, has caused many state and local agencies to consider it a problematic weed and/or ban its sale (Tilley & St. John 2012). The common reed has a worldwide distribution, and a number of subspecific taxa have been proposed (reviewed in Lambertini et al. 2012). A variety of datasets have established that three of these taxa are present in North America (Saltonstall et al. 2004; Saltonstall & Hauber 2007). These include a native taxon widespread across the U.S. and Canada [*Phragmites australis* (Cav.) Trin. ex Steud. subsp. *americanus* Saltonst., P.M. Peterson & Soreng], a second native taxon found in Central America, Mexico, and the U.S. Gulf Coast, [*Phragmites australis* (Cav.) Trin. ex Steud. subsp. *berlandieri* (E. Fourn.) Saltonst. & Hauber], and a third taxon native to Eurasia [*Phragmites australis* (Cav.) Trin. ex Steud. subsp. *australis*]. Existing studies have also indicated that the non-native *P. australis* subsp. *australis* has dramatically expanded its range in North America since 1900 and has now largely replaced native *P. australis* in some areas (Saltonstall 2002; Hauber et al. 2011; Zuiderveen et al. 2015; Melchior & Weaver 2016; Zuzak et al. 2018).

As with all invasive species, a full understanding of the invasion requires data across both space (geography) and time. The common reed provides a unique opportunity to obtain both. Morphological and genetic tools allow for diagnosis of native vs. non-native *P. australis* subspecies in both freshly collected and herbarium tissue (Saltonstall 2003; Saltonstall & Hauber 2007). In this study we use these tools to diagnose subspecies, and thus native vs. non-native status, in a set of 53 herbarium specimens from Kansas spanning the years 1936–2024. We seek to answer the following questions: 1) Is the non-native *P. australis* subsp. *australis* present in Kansas? 2) If present, when was it first collected? 3) If present, has this non-native subspecies replaced the native *P. australis* subsp. *americanus*?

MATERIALS AND METHODS

A small amount (ca. 15 mg) of leaf tissue was sampled from 53 Kansas *P. australis* herbarium specimens, 52 from the University of Kansas Ronald L. McGregor Herbarium (KANU), and one from the Wichita State University Arthur Youngman Herbarium (WICH). All specimens had been previously identified to subspecies using the morphological characters outlined in Saltonstall and Hauber (2007). In addition, specimens were collected in 2024 from three populations in the city of Wichita, Kansas. A small amount of tissue from each of these freshly collected specimens was preserved in silica gel desiccant. Vouchers for these three specimens were deposited at WICH, and each specimen was identified to subspecies as above. Collection information for all 56 specimens is presented in Appendix 1. DNA extractions were performed with a standard CTAB protocol modified for 96 well plates (Beck et al. 2012), and a Qubit fluorometer (Life Technologies, Carlsbad, CA) was used to establish DNA concentration for seven extracts. A ca. 290 bp portion of the *rbcL-psaI* intergenic spacer was PCR-amplified using the primers "rbcL" and "rbcL3R" (Saltonstall 2001). Each 25 µL reaction contained 2 µL undiluted DNA, 1.5 µL each primer (each at 10 µM), 12.5 µL Apex Taq RED master mix (Genesee Scientific, San Diego, CA, USA), and 7.5 µL water. Cycling conditions included an initial denaturation step (94°C for 2 minutes) followed by 35 denaturation/annealing/elongation cycles (94°C for 45 seconds, 52°C for 45 seconds, 72°C for 90 seconds) and a final elongation step (72°C for 2 minutes). Each amplicon was digested with the restriction enzyme *HhaI* (New England Biolabs, Ipswich, MA) according to the manufacturer's protocol. Digested amplicons were visualized on a 1% agarose gel stained with ethidium bromide under UV light. The presence/absence of the *HhaI* restriction site was scored according to Saltonstall (2003). In order to confirm the presence/absence of the restriction site, amplicons from seven samples (two cut by

HhaI, five uncut- see Appendix 1) were sequenced with the forward primer "rbcL" at the University of Chicago Sanger DNA Sequencing Core. The package ggplot2 (Wickham 2016) on the R statistical platform (R Core Team 2024) was used to construct Figures 1 and 2.

RESULTS

Amplification of the *rbcL-psaI* intergenic spacer was successful for 50 *P. australis* herbarium specimens and the three silica-dried samples (Appendix 1). Of these 53 samples, 26 were morphologically identified as *P. australis* subsp. *americanus*, ranging in collection year from 1936–1987 (Fig. 1A). Twenty-seven were morphologically identified as *P. australis* subsp. *australis*, ranging in collection year from 1973–2024 (Fig. 1A). Thirty samples were not cut by *HhaI*, indicating that they are one of the two native taxa- *P. australis* subsp. *americanus* or *P. australis* subsp. *berlandieri* (Saltonstall 2003). These samples ranged in collection year from 1936–1998 (Fig. 1B). Twenty-three samples were cut by *HhaI*, indicating that they are *P. australis* subsp. *australis* (Saltonstall 2003). These samples ranged in collection year from 1989–2024 (Fig. 1B). Sanger sequencing confirmed the presence of the *HhaI* restriction site in two samples cut by *HhaI* and its absence in five samples uncut by *HhaI* (Appendix 1).

DISCUSSION

Both morphological and genetic data establish that non-native *P. australis* subsp. *australis* is present in Kansas, the earliest collections of which are from either the 1970s (morphological data) or 1980s (genetic data). Although the presence of the *HhaI* restriction site in the *rbcL-psaI* intergenic spacer cannot discriminate between the two native subspecies (Saltonstall 2003), no specimens were morphologically determined to be *P. australis* subsp. *berlandieri*. In the U.S., this taxon is widely viewed as limited to the Gulf Coast, Texas, and the southwest (Saltonstall & Hauber 2007; Lambert et al. 2016), although recent genetic data suggest it may be present in Michigan (Zuiderveen et al. 2015). Both datasets indicate that *P. australis* subsp. *australis* spread rapidly following introduction to the state, as the last collection of *P. australis* subsp. *americanus* in our sample set occurred in 1998. This invasion timeline is broadly consistent with that seen in a study of 45 herbarium specimens from the Ottawa district of Canada (Catling & Carbyn 2006). Using similar morphological criteria, these authors documented the first appearance of *P. australis* subsp. *australis* in 1976, with this non-native taxon found in 74% of populations surveyed in 2003 (Catling & Carbyn 2006). In the four cases of the current study where morphological and genetic determinations did not agree (Appendix 1), morphological determinations were based on limited observable characters and often equivocal. Two of these specimens (*Stephens 87393; Brooks 12467*) were vegetative, each with a distal stem portion, with the remaining two specimens featuring an inflorescence and a medial stem portion. Each of the four specimens had only one measurable ligule, and measurements of floret characters often fell in the range of overlap of the two subspecies. It should be noted that a broader suite of morphological characteristics has recently been identified that reliably discriminate the native and non-native subspecies in live populations from the Great Lakes region (McTavish et al. 2023). Additional study will be required to demonstrate that this strategy can be routinely applied to the limited material routinely seen on herbarium specimens. New, and potentially more rapid, genetic diagnostic approaches have also been recently developed (Lindsay et al. 2023), although their applicability to the degraded DNAs extracted from herbarium specimens remains unexplored.

A plot of subspecies by collection location suggests that *P. australis* subsp. *australis* is not present in large portions of western Kansas (Fig. 2A). However, specimens from these areas are older (Fig. 2B), and the geographic pattern seen in Fig. 2A may be in part an artifact of the dataset. The distribution of man-made lakes may also contribute to this pattern. Although natural lakes in Kansas are quite rare, a number of sizeable artificial lakes have been built by the U.S. Army Corps of Engineers (18 lakes), the U.S. Bureau of Reclamation (7), and the State of Kansas (>40 "fishing lakes"). Most of these man-made lakes were constructed after 1930 (Stene 1946; Schoewe 1953), and few of them are in the portions of western Kansas in which *P. australis* subsp.

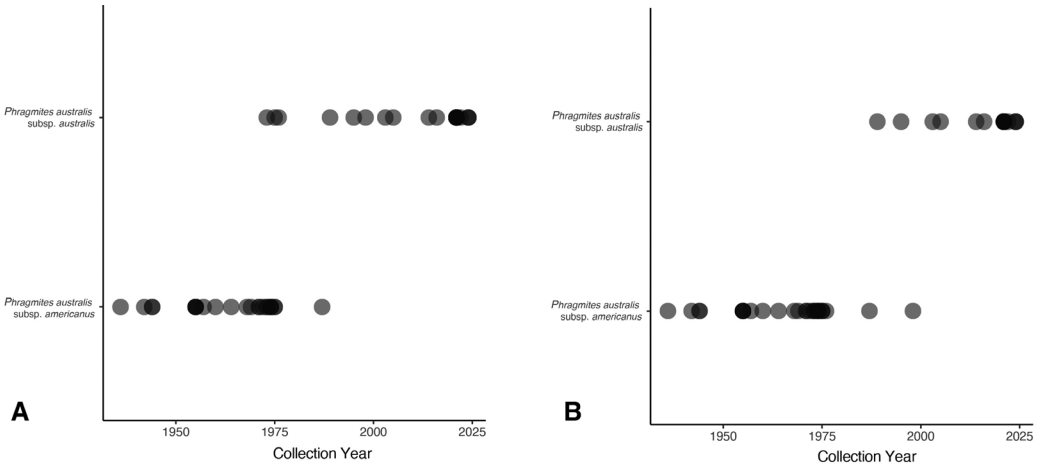

Fig. 1. Age distribution of Kansas *Phragmites australis* subsp. *americanus* and *Phragmites australis* subsp. *australis* specimens. Darker circles indicate overlap among multiple samples. A. Age distribution based on morphological data. B. Age distribution based on genetic data.

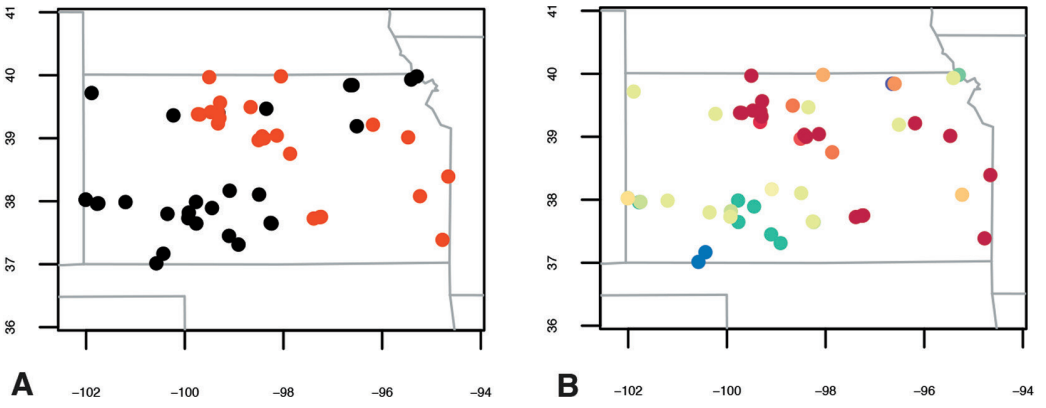

Fig. 2. Kansas *Phragmites australis* specimens. A. Distribution of *P. australis* subsp. *americanus* (black) and *P. australis* subsp. *australis* (red) specimens as diagnosed by genetic data. B. Collection dates, with warmer colors indicating more recent collectionss—see Appendix 1 for collection years.

australis specimens were not observed (Fig. 2A). The relative lack of these large, man-made palustrine habitats could perhaps have delayed the establishment of *P. australis* subsp. *australis* in western Kansas. A final area of interest is the potential for hybrid genotypes resulting from gene flow between the native and non-native subspecies (Meyerson et al. 2010; Saltonstall et al. 2016). Our results suggest that the two subspecies co-existed in Kansas for at least some time (Fig. 1), and the presence of hybrids should be explored. Fully establishing the historic and current distribution of common reed subspecies in Kansas will require morphological and genetic examination of additional material, both from herbarium specimens and newly collected samples.

APPENDIX 1

Sample information. "Taxon" refers to subspecies defined by morphology. An asterisk denotes samples for which identification based on genetic analysis conflicted with that based on morphology.

Taxon	Herbarium	Collector	Collection #	County	Date	Digest	GenBank #
P. australis subsp. *americanus*	WICH	Aquinas-Stiefferman	s.n.	Sedgwick	1939	no amplification	
P. australis subsp. *americanus*	KANU	Snow	3522	Douglas		no amplification	
P. australis subsp. *americanus*	KANU	Booth	s.n.	Marshall	1936	uncut	
P. australis subsp. *americanus*	KANU	Horr	E468	Miami	1942	uncut	XXXXXX
P. australis subsp. *americanus*	KANU	Horr	3348	Meade	1944	uncut	
P. australis subsp. *americanus*	KANU	Horr	3349	Meade	1944	uncut	XXXXXX
P. australis subsp. *americanus*	KANU	McGregor	10898	Ford	1955	uncut	
P. australis subsp. *americanus*	KANU	McGregor	10870	Edwards	1955	uncut	
P. australis subsp. *americanus*	KANU	McGregor	10936	Hodgeman	1955	uncut	
P. australis subsp. *americanus*	KANU	McGregor	10983	Barber	1955	uncut	
P. australis subsp. *americanus*	KANU	McGregor	10994	Kiowa	1955	uncut	
P. australis subsp. *americanus*	KANU	McGregor	13457	Hamilton	1957	uncut	
P. australis subsp. *americanus*	KANU	Horr	4963	Meade	1957	no amplification	
P. australis subsp. *americanus*	KANU	McGregor	15986	Doniphan	1960	uncut	
P. australis subsp. *americanus*	KANU	Kolstad	1863	Rooks	1964	uncut	
P. australis subsp. *americanus*	KANU	Stephens	29653	Ford	1968	uncut	XXXXXX
P. australis subsp. *americanus*	KANU	McGregor	20743	Kingman	1969	uncut	
P. australis subsp. *americanus*	KANU	Stephens	50266	Hamilton	1971	uncut	
P. australis subsp. *americanus*	KANU	Stephens	50373	Hamilton	1971	uncut	
P. australis subsp. *americanus*	KANU	Stephens	63063	Gray	1972	uncut	
P. australis subsp. *americanus*	KANU	Seiler	5970	Kingman	1973	uncut	
P. australis subsp. *americanus*	KANU	Seiler	6013	Stafford	1973	uncut	XXXXXX
P. australis subsp. *australis**	KANU	Stephens	73999	Ford	1973	uncut	
P. australis subsp. *americanus*	KANU	Brooks	8976	Brown	1974	uncut	
P. australis subsp. *americanus*	KANU	McGregor	26076	Mitchell	1974	uncut	
P. australis subsp. *americanus*	KANU	McGregor	26117	Sheridan	1974	uncut	
P. australis subsp. *americanus*	KANU	McGregor	26158	Cheyenne	1974	uncut	
P. australis subsp. *americanus*	KANU	Brooks	10813	Pottawatomie	1975	uncut	
P. australis subsp. *americanus*	KANU	Stephens	86940	Rooks	1975	uncut	
P. australis subsp. *australis**	KANU	Stephens	87393	Kearny	1975	uncut	
P. australis subsp. *australis**	KANU	Brooks	12467	Pawnee	1976	uncut	
P. australis subsp. *americanus*	KANU	McGregor	38451	Hamilton	1987	uncut	
P. australis subsp. *australis*	KANU	Moody	s.n.	Anderson	1989	cut	XXXXXX
P. australis subsp. *australis*	KANU	Elliott	1048	Jewell	1995	cut	
P. australis subsp. *australis**	KANU	Freeman	11804	Marshall	1998	uncut	XXXXXX
P. australis subsp. *australis*	KANU	Elliott	1822	Saline	2003	cut	
P. australis subsp. *australis*	KANU	Scott	s.n.	Barton	2005	cut	
P. australis subsp. *australis*	KANU	Morse	24329	Russell	2014	cut	
P. australis subsp. *australis*	KANU	Freeman	26372	Rooks	2016	cut	
P. australis subsp. *australis*	KANU	Morse	28054	Crawford	2021	cut	
P. australis subsp. *australis*	KANU	Freeman	28195	Graham	2021	cut	
P. australis subsp. *australis*	KANU	Freeman	28460	Rooks	2021	cut	
P. australis subsp. *australis*	KANU	Freeman	28473	Rooks	2021	cut	
P. australis subsp. *australis*	KANU	Freeman	28474	Russell	2021	cut	
P. australis subsp. *australis*	KANU	Freeman	28475	Lincoln	2021	cut	
P. australis subsp. *australis*	KANU	Freeman	28479	Lincoln	2021	cut	
P. australis subsp. *australis*	KANU	Freeman	28485	Pottawatomie	2021	cut	
P. australis subsp. *australis*	KANU	Freeman	28488	Douglas	2021	cut	
P. australis subsp. *australis*	KANU	Freeman	28207	Rooks	2021	cut	
P. australis subsp. *australis*	KANU	Freeman	28208	Rooks	2021	cut	
P. australis subsp. *australis*	KANU	Freeman	28213	Osborne	2021	cut	
P. australis subsp. *australis*	KANU	Freeman	28214	Lincoln	2021	cut	
P. australis subsp. *australis*	KANU	Freeman	28715	Miami	2022	cut	
P. australis subsp. *australis*	WICH	Beck	1822	Sedgwick	2024	cut	
P. australis subsp. *australis*	WICH	Beck	1823	Sedgwick	2024	cut	XXXXXX
P. australis subsp. *australis*	WICH	Beck	1824	Sedgwick	2024	cut	

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DATA AVAILABILITY

GenBank numbers had not been assigned at the time of publication, even though sequences were submitted to NCBI on 2 Oct 2025. The delay is presumably due to the government shutdown. Once GenBank accession numbers are assigned they will be published as an Addendum in a future issue of *J. Bot. Res. Inst. Texas.*

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