

Research Article

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Morphological traits for rapid and simple separation of native and introduced common reed (*Phragmites australis*)

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Abstract

Effective management of the introduced invasive grass common reed [*Phragmites australis* (Cav.) Trin. ex Steud.] requires the ability to differentiate between the introduced and native subspecies found in North America. While genetic tools are useful for discriminating between the subspecies, morphological identification is a useful complementary approach that is low to zero cost and does not require specialized equipment or technical expertise. The objective of our study was to identify the best morphological traits for rapid and simple identification of native and introduced *P. australis*. A suite of 22 morphological traits were measured in 21 introduced and 27 native *P. australis* populations identified by genetic barcoding across southern Ontario, Canada. Traits were compared between the subspecies to identify measurements that offered reliable, diagnostic separation. Overall, 21 of the 22 traits differed between the subspecies, with four offering complete separation: the retention of leaf sheaths on dead stems; a categorical assessment of stem color; the base height of the ligule, excluding the hairy fringe; and a combined measurement of leaf length and lower glume length. Additionally, round fungal spots on the stem occurred only on the native subspecies and never on the sampled introduced populations. The high degree of variation observed in traits within and between the subspecies cautions against a “common wisdom” approach to identification or automatic interpretation of intermediate traits as indicative of aberrant populations or hybridization. As an alternative, we have compiled the five best traits into a checklist of simple and reliable measurements to identify native and introduced *P. australis*. This guide will be most applicable for samples collected in the late summer and fall in the Great Lakes region but can also inform best practices for morphological identification in other regions as well.

Introduction

Introduced common reed [*Phragmites australis* (Cav.) Trin. ex Steud.] is a tall perennial grass that forms dense, near-monospecific stands in a variety of wetland, riverine, and roadside habitats (Mozdzer et al. 2013; Packer et al. 2017). Three subspecies of *P. australis* occur in North America (Saltonstall 2016): *Phragmites australis* ssp. *australis* Trin. ex Steud. (hereafter “introduced *P. australis*”) is a widespread nonnative, invasive lineage; *Phragmites australis* ssp. *americanus* Saltonst., P.M. Peterson & Soreng (“native *P. australis*”) is a desirable native lineage endemic to North America (Saltonstall 2002; Saltonstall et al. 2004); and *Phragmites australis* ssp. *berlandieri* (E. Fourn.) Saltonst. & Hauber (“Gulf Coast *P. australis*”) is an endemic lineage distributed along the Gulf Coast (Colin and Eguiarte 2016; Saltonstall and Hauber 2007). In the Great Lakes Region of northern North America, only the native and introduced *P. australis* subspecies occur (Lindsay et al. 2023). Pollination studies indicate that the introduced and native *P. australis* subspecies can hybridize (Meyerson et al. 2010), but hybrids have rarely been found in the field (Paul et al. 2010; Saltonstall et al. 2014, 2016; Wu et al. 2015).

The introduced subspecies of *P. australis* is considered one of the most invasive plants in Canada and is managed using physical, chemical, cultural, and biological control methods (Blossey and Casagrande 2016; Hazelton et al. 2014; Nichols 2020). Unfortunately, a lack of awareness of the subspecies or of practical tools to reliably identify them can result in accidental management of native *P. australis* (Hunt et al. 2017), misallocating limited weed control resources and inadvertently harming desirable native flora.

Genetic tools are useful for discriminating between different *P. australis* lineages and are reviewed by Lindsay et al. (2023), including a method to screen for native-introduced *P. australis*



Management Implications

To effectively manage the invasive introduced subspecies of *Phragmites australis* (common reed) and avoid misallocating resources, land managers require practical, reliable tools to differentiate it from the desirable native subspecies. While genetic tools are extremely useful for identification, morphological identification is a valuable complementary tool that is easier, cheaper, available in the field, and thus more accessible for many land managers and researchers. From a survey of 22 morphological measurements of native and introduced *P. australis* populations across southern Ontario, we identified five easy to measure traits that offer reliable discrimination between native and introduced *P. australis*. These traits include the presence or absence of round stem fungal spots, the presence of dark red pigmentation on lower internodes, leaf sheath retention on dead stems, ligule height, and a combined measurement of lower glume length and leaf length. We provide a checklist of these traits and recommend evaluating all measurements to help identify a specimen. Any samples for which traits are not consistent with the checklist should be evaluated further with genetic identification tools when possible. “Common wisdom” about the differences between native and introduced *P. australis* was often true (e.g., introduced subspecies tend to have taller, wider stems with higher stem densities); however, many populations can exhibit variation beyond these expectations, and these generalizations are not sufficient for reliable identification. Given the geographic variation in *P. australis* populations, this checklist of traits is most applicable for samples collected in the late summer and early fall in the Great Lakes region.

hybrids (Wendell et al. 2021). However, for land managers, availability of genetic tools can be limited by a lack of access to laboratory resources and technical expertise, financial costs, and the challenges of proper collection and preservation of tissue samples to extract high-quality genetic material (Lambert et al. 2016). These limitations may be particularly pronounced for community science programs (Hunt et al. 2017).

Morphological identification can also be a useful approach to differentiate between *P. australis* lineages, supplementing genetic tools with a low- to zero-cost option that does not require specialized equipment or technical expertise (Allen et al. 2017). *Phragmites australis* subspecies differ in vegetative, floral, and growth properties that can be measured from *P. australis* specimens in the field, lab, and herbaria (Mozdzer et al. 2013; Swearingen et al. 2022). These differences inform various identification guides and keys based on several individual morphological measurements (e.g., Blossey 2003; Catling and Mitrow 2011; Catling et al. 2007; Nichols 2020; Saltonstall and Hauber 2007; Saltonstall et al. 2004; Swearingen et al. 2022) or composite indices of multiple traits determined from principal component analysis (e.g., Allen et al. 2017).

Despite the existence of these guides, reports have emerged from land managers around the Great Lakes region of unknown *P. australis* populations with “unusual” traits that they worry may be hybrids (Lindsay et al. 2023; MJM, personal observation). However, genetic surveys have not detected hybrids within any of these “unusual” populations (Tippery et al. 2020; Warren 2020), suggesting that land managers are encountering normal native and introduced *P. australis* populations beyond their expected range of variation for the subspecies and signaling a need to improve current morphological identification tools.

At the subspecies level, routine morphological identification of native and introduced *P. australis* can be difficult for several reasons. First, *P. australis* exhibits high phenotypic variability within and across genotypes resulting from multiple introductions and haplotypes (Meyerson and Cronin 2013); seasonal changes (Allen et al. 2017; Blossey 2003; Catling et al. 2007); and environmental stress and disturbance, including management (Blossey 2003; Lambert et al. 2016). Given the high degree of variation and overlap within and between subspecies, many of these traits (e.g., stem density, stem height) can be unreliable predictors of subspecies identity (Blossey 2003; Hunt et al. 2017). Second, some traits are subjective and prone to user bias (e.g., categorical assessment of stem color or texture) (Allen et al. 2017; Saltonstall et al. 2004) or measurement error (e.g., ligule height) (Catling et al. 2007) and may be especially difficult if working from herbarium specimens that are incomplete, discolored, or damaged (Allen et al. 2017; Catling and Mitrow 2011). Third, observers may be ignoring existing *P. australis* guides and quantitative measurements altogether—especially more complex ones—in favor of a more “common sense” or “general wisdom” approach (e.g., “introduced *P. australis* is taller and has higher stem density and larger patches”).

Here we report on research to identify the best morphological traits for rapid and simple identification of native and introduced *P. australis*. The goal was to assess traits that ideally provide good separation of introduced and native *P. australis*, can be quantified objectively and accurately, and are quick and easy to use. This study includes a large-scale field survey of native and introduced *P. australis* populations across southern Ontario, Canada, and individually compares a large group of 22 morphological traits to identify those that differ between lineages.

Materials and Methods

Phragmites australis Field Surveys

We surveyed a total of 63 sites across southern Ontario, Canada in early fall (September 2019). Sampling was conducted within a single year and season to limit other differences between populations beyond subspecies identity. Sites were chosen from a 2016 to 2017 survey of introduced and native *Phragmites* populations (deJonge et al. 2022) supplemented by additional sites identified by partner organizations (Ducks Unlimited Canada), historical records (Catling and Mitrow 2011), community science resources (e.g., <https://www.iNaturalist.org>), and opportunistic observations while traveling between sites. Of the 63 initial sites, 15 were unsuitable for sampling (private property, population no longer present, etc.) and were not included in the final study. The 48 remaining suitable sites were confirmed by subsequent genetic identification (see next section) to include 21 introduced and 27 native *P. australis* populations. Given the sampling approach, most sites were roadside ditches (73%), while others occurred off-road in larger marshes (21%) or in open fields (6%). The sampled sites encompassed 3.6° of latitude (42.0°N to 45.6°N) and 7.7° of longitude (74.8°W to 82.5°W). All sites were located roughly within a triangular area reaching from the Ottawa area in the northeast, down to Point Pelee in the southwest, and up to the Bruce Peninsula in the northwest.

At each replicate sampling site, five subsamples were collected to account for within-population variation. Five circular plots (0.6-m diameter, 0.28 m²) were spaced equidistantly along the

navigable perimeter of each patch and ~5 m inside each patch to minimize edge effects. Within each plot, the total numbers of living and dead stems were counted. A single random living and a single dead stem from the center of each plot was cut at ground level and removed. The total number of nodes and retained leaf sheaths were counted on the dead stems, which were then discarded. A single leaf was removed from near the top of each green stem, torn into small (~5-cm) pieces and placed in sealed plastic bags with silica gel to preserve the samples for DNA extraction (Chase and Hills 1991; Saltonstall 2002). The rest of the stems were folded, labeled, and placed in large paper yard waste bags for transport back to the lab ($n = 5$ stems per site).

Genetic Identification

A total of 272 leaf tissue samples were collected. DNA was isolated using the NucleoSpin Plant II DNA isolation kit (Macherey and Nagel GmbH & Co. KG, Allentown, PA, USA). We amplified the noncoding chloroplast region *rbcL-psaI* using primers 5'-TGTA CAAGCTCGTAACGAAGG-3' and 5'-TAAGCCTACTAAAG GYACG-3' (Saltonstall 2001), and a thermal cycle program of 95 C for 4 min, 34 cycles of 94 C for 30 sec, 55 C for 1 min, 72 C for 1 min, followed by 72 C for 10 min. Sequences were aligned by eye using Geneious (v. 11.1.5; Biomatters, Auckland, New Zealand) and matched to reference locus haplotype sequences as described by Saltonstall (2016).

Morphological Analysis

Data collected in the field were combined with measurements taken in the lab for a total of 22 characteristics described below, presented roughly in the order of measurement (Table 1). Most characteristics were selected from existing guides and literature (noted in Table 1). This resulted in the most comprehensive single list of traits for comparison to date. Unless otherwise noted, each measurement was averaged across 5 stems (one from each of five field plots) to create a mean value for each of 48 sites.

Most of the measurements were conducted as outlined in the referenced guides and keys (see Table 1). Old and living stem densities, stem height, stem diameter (at the base, middle, and top of the stem), and inflorescence fullness are less common measurements but were included because they have been descriptively compared between subspecies (Nichols 2020; Swearingen et al. 2022) and inform some of the “general wisdom” about differences between native and introduced *P. australis* (MJM, personal observation). All traits have been operationalized as quantitative (continuous or ordinal) data to facilitate analytical comparison, including categorical assessments of stem texture, color, and inflorescence fullness (sensu Allen et al. 2017), and we generated quantitative versions of traits that have previously been descriptive only, including the percent of leaf sheaths retained on an old stem and stem spot fungus occurrence across multiple stems.

Other measurements that have been studied or proposed but were not assessed in this study include leaf color, stem toughness, timing of senescence, rhizome biomass (Blossey 2003), herbivore attack rate (Allen et al. 2015; deJonge et al. 2022; Lambert and Casagrande 2007; Lambert et al. 2007; Park and Blossey 2008), vegetation diversity (Swearingen et al. 2022), inflorescence branch length (Allen et al. 2017), aboveground–belowground biomass ratio (Mozdzer et al. 2013), rhizome internode length, and internode leaf sheath coverage (League et al. 2006). Traits were omitted if they required too much effort to easily measure (e.g., biomass, vegetation and insect diversity) and to prioritize more commonly used traits that already appear in existing guides.

While stem color is one of the most common diagnostic traits for comparing introduced and native *P. australis* descriptively (e.g., Blossey 2003; Catling and Mitrow 2011; Catling and Robichaud 2003; Catling et al. 2007; Nichols 2020; Swearingen et al. 2022) or numerically/categorically (e.g., Allen et al. 2017), it is a highly subjective trait that can vary throughout the season (Saltonstall et al. 2004). Therefore, we used photographs of *P. australis* stems and image analysis to produce an objective, quantitative assessment of stem color and to validate the subjective categorical stem color observations. In the lab, the second-lowest complete internode was photographed against a plain white backdrop under standard indoor lighting conditions using a Canon EOS Rebel XTi/400D DSLR camera (Canon Canada Inc., Brampton, ON, Canada). Images were imported into ImageJ software (National Institutes of Health, Bethesda, MD, USA), which was then used to mask and isolate the internode tissue from the background and extract average RGB values. RGB values were then converted into the three parameters of the HSL (hue, saturation, lightness) color system using a free online tool (<https://www.w3schools.com/colors>). Spearman's rank correlations for data lacking bivariate normality were used to assess the associations between the standard categorical measure of stem color and each of the three HSL parameters.

Data Analysis

For each measurement, the mean (\pm SD) is given along with the range for each subspecies. To assess differences in traits between the subspecies, the effects of subspecies genetic ID on each of the 22 morphological characteristics were tested using one-way ANOVA or Welch's test for data that violated the assumption of equal variance (Welch 1951). Both tests were run using the *oneway* function from R package USERFRIENDLYSCIENCE (Peters 2018). Multiple univariate analyses were prioritized over multivariate statistics or other data reduction techniques (e.g., Allen et al. 2017; Mozdzer et al. 2013) to better address our research objective of identifying individual diagnostic traits that can be used for simple and rapid identification, ideally in the field. Omega-squared (ω^2) was calculated (using the function *omega_squared* from R package EFFECTSIZE; Ben-Shachar et al. 2020) as an effect size and used to rank characteristics in order of the proportion of trait variation that was explained by subspecies. We also calculated the amount of overlap in the distribution of observations for a given trait between the subspecies, that is, what percent of total observations fell within the range of data shared between subspecies.

Our univariate analyses identified a set of “primary” variables that individually provided clear discrimination between the subspecies. We then conducted a linear discriminant analysis (LDA) of the remaining variables. The objective of this supplemental analysis was to identify combinations of variables that were useful in distinguishing between the subspecies in order to confirm identifications when the primary variables were not available or were difficult to assess. Starting from the full set of variables, we removed any for which there was no overlap between the two subspecies (i.e., the primary variables). We also removed all color variables, as these are difficult to assess in the field and may vary over the course of the growing season. We assessed correlations among the remaining variables and selected a set for which no two variables had a pairwise correlation >0.8 .

At the end of this process, eight variables remained: stem height, leaf length, leaf width, dead stem density, lower glume length, ligule length, inflorescence height, and stem base diameter. Excluding specimens that were missing one or more of these measurements, we

Table 1. Overview of 22 measurements taken from native and introduced *Phragmites australis* samples collected in southern Ontario, Canada, including measurement name, units of measurement, a general description, and literature that has used the same or similar measurements.

Measurement ^a	Description and reference(s)
1. Old stem density (m ⁻²)	Density of old, standing, dead stems (m ⁻²) (Nichols 2020; Swearingen et al. 2022)
2. Living stem density (m ⁻²)	Density of living, green stems (m ⁻²) (Nichols 2020; Swearingen et al. 2022)
3. Old stem leaf retention (%)	Percent (%) of internodes on a dead stem with leaf sheaths attached (Blossey 2003; Nichols 2020; Saltonstall et al. 2004; Swearingen et al. 2022)
4. Stem texture (1–4)	Categorical classification of the roughness of the second fully complete internode from the base of the stem (1 = very smooth; 2 = smooth with gentle ridges; 3 = lightly coarse/ridged; 4 = very coarse/ridged) (Allen et al. 2017; Blossey 2003; Nichols 2020; Saltonstall et al. 2004; Swearingen et al. 2022)
5. Stem spot fungus (%)	Percent (%) of five collected stems with any fungal spots on the internodes (Blossey 2003; Swearingen et al. 2022)
6. Stem color (1–4)	Categorical classification of the redness of the second fully complete internode from the base of the stem (1 = no redness; 2 = tinges of light redness; 3 = patches of darker red over <1/2 of internode; 4 = dark red over ≥1/2 of internode) (Allen et al. 2017; Blossey 2003; Catling and Mitrow 2011; Catling and Robichaud 2003; Catling et al. 2007; Nichols 2020; Swearingen et al. 2022)
7. Stem color hue	Hue (0–360 position on a color wheel) of the second fully complete internode from the base of the stem assessed by image analysis (see “Materials and Methods” for additional details)
8. Stem color saturation (%)	Saturation (% pigment intensity) of the second fully complete internode from the base of the stem assessed by image analysis (see “Materials and Methods” for additional details)
9. Stem color lightness (%)	Lightness (% whiteness of the color) of the second fully complete internode from the base of the stem assessed by image analysis (see “Materials and Methods” for additional details)
10. Stem height (m)	Height (m) from the base of the stem to the base of the inflorescence, measured using a meter stick (Nichols 2020)
11. Basal stem diameter (mm)	Diameter (mm) at the bottom of the stem, measured with calipers (Nichols 2020)
12. Mid-stem diameter (mm)	Diameter (mm) halfway up the stem, measured with calipers (Nichols 2020)
13. Top stem diameter (mm)	Diameter (mm) at the top of the stem at the base of the inflorescence, measured with calipers (Nichols 2020)
14. Inflorescence fullness (1–4)	Categorical classification of the fullness of the inflorescence, omitted if no inflorescence present (1 = small and spindly; 2 = small but filled out; 3 = large but sparse; 4 = bushy and full) (Nichols 2020; Swearingen et al. 2022)
15. Inflorescence height (cm)	Height (cm) from the base of the inflorescence to its highest point, measured using a meter stick (Allen et al. 2017; Nichols 2020)
16. Leaf length (cm)	Length (cm) of a leaf blade collected from the middle of the stem, measured from the center top of the ligule to the leaf tip (i.e., excluding the sheath), measured using a ruler (Allen et al. 2017)
17. Leaf width (cm)	Width (cm) of the same leaf measured for length at the widest point, measured using a ruler (Allen et al. 2017)
18. Ligule base height (mm)	Height (mm) of the dark tissue of the ligule, excluding the hairy fringe, measured with calipers under a microscope (Allen et al. 2017; Catling and Mitrow 2011; Catling et al. 2007; Nichols 2020)
19. Ligule full height (mm)	Height (mm) of the center of the ligule, including the dark tissue and hairy fringe, measured with calipers under a microscope (Catling et al. 2007; Saltonstall et al. 2004; Swearingen et al. 2022)
20. Lower glume length (mm)	Mean length of the lower glume (mm) from two random florets per sample, measured using calipers under a microscope (Allen et al. 2017; Catling and Mitrow 2011; Catling and Robichaud 2003; Catling et al. 2007; Nichols 2020; Saltonstall et al. 2004; Swearingen et al. 2022)
21. Upper glume length (mm)	Mean length of the upper glume (mm) from two random florets per sample, measured using calipers under a microscope (Allen et al. 2017; Nichols 2020; Saltonstall et al. 2004; Swearingen et al. 2022)
22. Lemma length (mm)	Mean length of the lemma (mm) from two random florets per sample, measured using a scale bar under a microscope (Allen et al. 2017; Nichols 2020; Saltonstall et al. 2004)

^aStems were sampled from 0.6-m-diameter circular plots (0.28 m²). Old and living stem density measurements (measurements 1 and 2) were counted across the whole plot, while the remaining measurements were taken from a single dead stem (measurement 3) or a single living stem (measurements 4 to 22) from the center of the plot.

retained 41 samples, including 18 introduced *P. australis* and 23 native *P. australis*.

The eight variables were standardized to mean = 0, SD = 1. We tested for a significant difference between subspecies with a multivariate analysis of variance (MANOVA), and then proceeded to an LDA using the *lda* function in the R package MASS (Ripley et al. 2023). We used the absolute scaling values to rank the variables' discriminating power.

All analyses were completed in R (R Core Team 2019), and tables and figures were produced in Microsoft Excel.

Results and Discussion

Genetic Identification

Of the 272 tissue samples analyzed, 222 yielded sequences adequate for haplotyping. Of these, 126 matched *rbcL* locus haplotype r2, which is associated with seven combined haplotypes by Saltonstall (2016), all of which are native. The second most abundant haplotype was r4, which was present in 94 samples. Saltonstall

(2016) documents this haplotype as part of nine combined haplotypes, all nonnative, and including the widespread invasive haplotype M. A third locus haplotype, r7, was present in two samples. This haplotype has been documented in a single native combined haplotype, S (Saltonstall 2016). We conclude that all samples with haplotype r4 are nonnative, and most likely the invasive combined haplotype M, and samples with haplotypes r2 and r7 are native.

The native haplotypes r2 and r7 co-occurred at two locations. The native haplotype r2 co-occurred with the invasive haplotype at two locations. The remaining 44 sites had only a single haplotype present (native r2 or invasive r4), and there were no locations with only the native r7 haplotype present. For the two sites with co-occurring native and introduced *P. australis*, we identified the numerically dominant subspecies in the samples (accounting for four out of five subsamples) and dropped the nonmatching subsamples from further analyses. The two locations with different native haplotypes were treated as ordinary native *P. australis* sites for the purpose of this study, and all subsamples were averaged together.

Table 2. Morphological measurement comparison between introduced ($n = 21$) and native *Phragmites australis* populations ($n = 27$) in southern Ontario, Canada.^a

Measurement	F-statistic and P-value	ω^2 effect size	% Overlap	Introduced	Native
				mean \pm SD (range)	
Old stem leaf retention (%)	$F(1, 45) = 1026.3, P < 0.001$	0.96	0	92 ± 10 (53–100)	8 ± 8 (0–27)
Stem color (1–4)	$F(1, 28) = 419.7^*, P < 0.001$	0.87	0	1.0 ± 0.1 (1.0–1.4)	3.4 ± 0.6 (1.6–4.0)
Ligule base height (mm)	$F(1, 27) = 207.6^*, P < 0.001$	0.76	0	0.11 ± 0.02 (0.08–0.15)	0.63 ± 0.19 (0.37–0.96)
Stem color hue	$F(1, 36) = 183.6^*, P < 0.001$	0.76	4	41.6 ± 7.7 (26.4–51.6)	-12.4 ± 18.8 (–50.8–27.2)
Stem texture (1–4)	$F(1, 31) = 126.8^*, P < 0.001$	0.74	15	3.0 ± 0.6 (1.8–4.0)	1.4 ± 0.3 (1.0–2.2)
Lower glume length (mm)	$F(1, 46) = 111.9, P < 0.001$	0.70	19	3.55 ± 0.45 (2.82–4.47)	4.99 ± 0.49 (3.96–5.95)
Upper glume length (mm)	$F(1, 46) = 83.1, P < 0.001$	0.63	31	5.58 ± 0.60 (4.59–6.90)	7.19 ± 0.61 (5.91–8.56)
Stem spot fungus (%)	$F(1, 25) = 105.4, P < 0.001$	0.63	49	0 ± 0 (0)	64 ± 32 (0–100)
Leaf length (cm)	$F(1, 41) = 7.05, P < 0.001$	0.62	28	46 ± 4 (40–55)	36 ± 4 (28–44)
Stem color lightness (%)	$F(1, 46) = 48.8, P < 0.001$	0.50	42	52 ± 8 (40–61)	38 ± 7 (27–51)
Mid-stem diameter (mm)	$F(1, 46) = 31.4, P < 0.001$	0.39	60	5.7 ± 0.9 (4.1–7.6)	4.5 ± 0.6 (3.2–5.9)
Top stem diameter (mm)	$F(1, 33) = 27.3^*, P < 0.001$	0.37	69	2.9 ± 0.7 (1.5–3.8)	2.0 ± 0.5 (1.3–3.0)
Ligule full height (mm)	$F(1, 46) = 22.7, P < 0.001$	0.31	54	0.82 ± 0.15 (0.59–1.06)	1.05 ± 0.18 (0.73–1.40)
Inflorescence fullness (1–4)	$F(1, 46) = 21.6, P < 0.001$	0.30	92	2.6 ± 0.8 (1.0–3.8)	1.6 ± 0.6 (1.0–3.4)
Lemma length (mm)	$F(1, 46) = 21.4, P < 0.001$	0.30	79	9.5 ± 1.0 (7.3–11.6)	10.7 ± 0.6 (9.1–12.1)
Stem color saturation (%)	$F(1, 28) = 13.1^*, P = 0.003$	0.22	83	14 ± 4 (8–23)	11 ± 2 (7–16)
Leaf width (cm)	$F(1, 25) = 10.9^*, P = 0.003$	0.20	77	2.4 ± 0.5 (1.4–3.2)	1.9 ± 0.3 (1.3–2.6)
Stem height (m)	$F(1, 28) = 5.7^*, P = 0.024$	0.09	88	2.30 ± 0.45 (1.63–3.35)	2.05 ± 0.23 (1.62–2.60)
Inflorescence height (cm)	$F(1, 46) = 5.6, P = 0.022$	0.09	90	26 ± 7 (11–40)	21 ± 5 (15–35)
Basal stem diameter (mm)	$F(1, 46) = 4.9, P = 0.032$	0.08	88	7.0 ± 1.2 (4.6–9.1)	6.3 ± 0.9 (5.0–8.0)
Old stem density (m^{-2})	$F(1, 44) = 4.6, P = 0.037$	0.07	98	36 ± 33 (0–132)	17 ± 27 (0–134)
Living stem density (m^{-2})	$F(1, 44) = 3.2, P = 0.083$	0.04	91	65 ± 28 (24–129)	52 ± 23 (4–101)

^aMeasurements per site are based on an average of 5 plots (living and old stem density) or 5 stems per site (all other measurements). Statistical output is from a one-way ANOVA or Welch's test ($*$) assessing the effects of subspecies on each individual measurement. Results are ordered by decreasing effect size and also include the amount of overlap between subspecies measurements, mean \pm SD, and ranges for each subspecies. P-values in bold denote statistically significant differences at $\alpha = 0.05$.

Measuring Traits of Native and Introduced *Phragmites australis*

Of the 22 measured variables, a statistically significant difference was found between the subspecies for all but one variable (living stem density) (Table 2). However, high effect size (ω^2 : 0.76 to 0.96) and zero overlap were observed for only three measurements: old stem leaf retention, categorical stem color, and ligule base height. A fourth variable, the proportion of stems with fungal spots, was notable, in that fungal spots only appeared on native *P. australis* (although not all native plants had them); these spots never appeared on introduced *P. australis*. All other measurements included some amount of overlap ranging from 4% to 98% (Figure 1).

After primary variables were excluded, the MANOVA revealed there was a statistically significant difference between the subspecies for the eight variables retained for the LDA (Wilks $\Lambda = 0.131$, $df = 8$, $P < 0.001$). Lower glume length and leaf blade length had the highest absolute scaling values, and while variables overlapped for the subspecies when plotted individually, they show clear separation when plotted together (Figure 2).

The Best Morphological Traits for Separating Introduced and Native *Phragmites australis*

We have identified four individual traits (old stem leaf retention, ligule base height, stem color, and stem spot fungus) and one combination of two traits (lower glume length + leaf length) that provide rapid and simple separation of the 48 introduced and native *P. australis* populations that we examined.

The individual trait that was best explained by subspecies ($\omega^2 = 0.96$) and had zero overlap between lineages was the percent of leaf sheaths retained on old dead standing stems (Table 2). Despite being starkly different between subspecies and easy to measure, leaf sheath retention is underrepresented in identification guides compared with other traits such as stem color. While some

researchers have highlighted the value of this trait (e.g., Blossey 2003; Swearingen et al. 2022), it may be especially underused in comparisons of native and introduced *P. australis* traits using herbarium or voucher specimens, which may not always include older stems (Allen et al. 2017).

The second individual trait of interest was ligule height, which is commonly regarded as one of the most useful distinguishing traits, though with disagreement about whether or not to measure the height of the full ligule (e.g., Saltonstall et al. 2004; Swearingen et al. 2022) or to exclude the hairy fringe that can form above the darkened basal tissue (Allen et al. 2017; Catling and Mitrow 2011; Nichols 2020). Our results support excluding the fringe, because it can introduce additional variation to the measurement from damaged or particularly long hairs (Catling et al. 2007); while the base height of the ligule had no overlap between introduced and native *P. australis*, full ligule height had 54% overlap (Table 1) and was therefore less diagnostic in our samples.

The third individual trait that offered complete subspecies separation was a categorical assessment of stem color. While stem color is one of the most commonly cited differences between introduced and native *P. australis*, it is also one of the least reliable traits, because it is subjective and prone to observer bias (Allen et al. 2017; Saltonstall et al. 2004). However, while individual stems of both subspecies had no to light red pigmentation, dark red pigmentation was more diagnostic and less subjective. Dark red coloration (i.e., color categories 3 to 4) was only observed on native stems and can be used as strong evidence for an identification. Additionally, image analysis objectively supported the perceived color differences, with strong correlations between the categorical color values and actual stem hue (Spearman's rank correlation, $r_s(46) = -0.90$, $P < 0.001$), lightness ($r_s(46) = -0.81$, $P < 0.001$), and to a lesser extent, saturation ($r_s(46) = -0.30$, $P = 0.04$). While image analysis was useful to validate the descriptive color differences and to compare samples within our study, this approach is less useful for general identification of individual

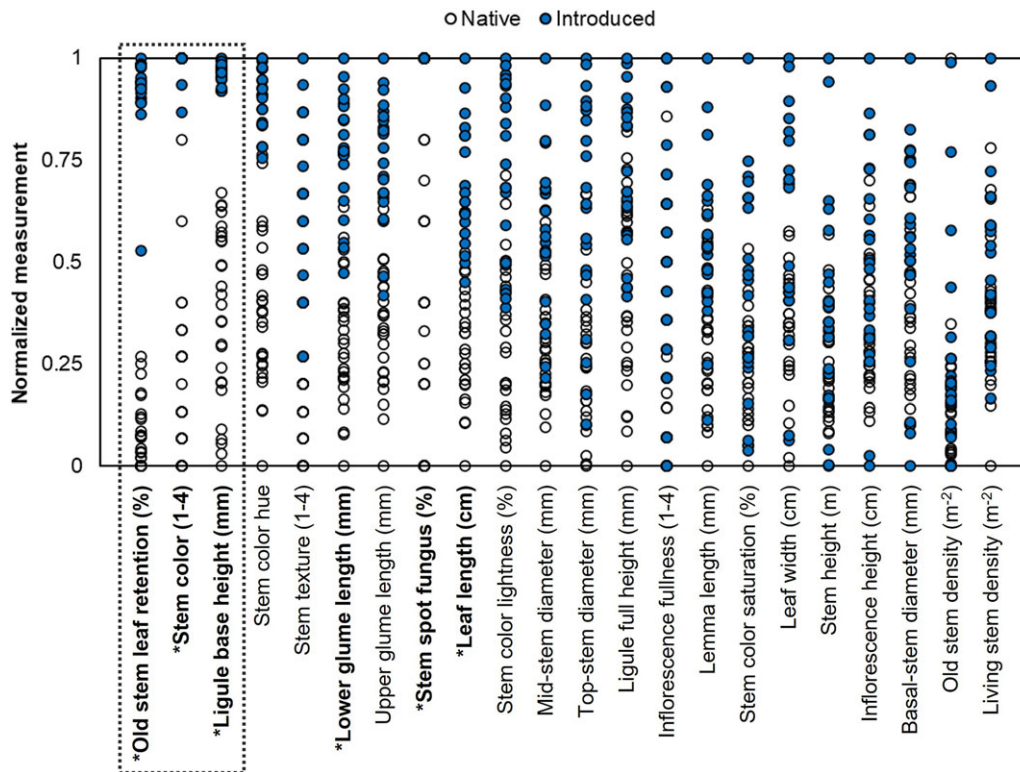


Figure 1. Dot plot of 22 morphological traits measured for introduced ($n = 21$ sites, filled blue circles) and native *Phragmites australis* ($n = 27$ sites, empty white circles), presented as normalized measurements (range 0–1), arranged to consistently present introduced values at the higher end of the range. Traits are presented along the x axis, with bolded and starred traits used for further identification purposes and traits with 0% overlap highlighted by the dotted box.

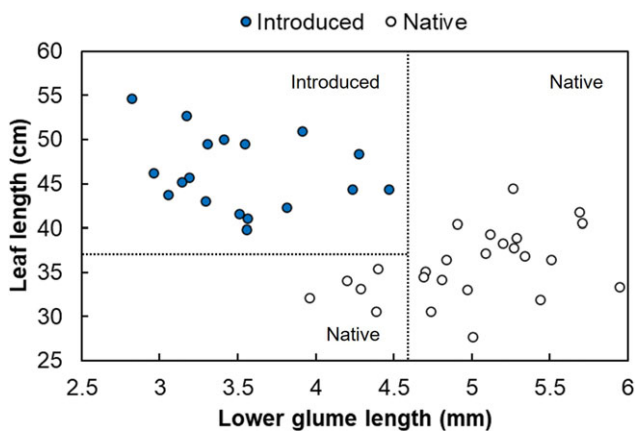


Figure 2. Scatter plot of lower glume length (mm) and leaf length (mm) averaged from 5 stems per site from 18 introduced (filled blue circles) and 25 native *Phragmites australis* populations (empty white circles). The dashed lines indicate the threshold values used to separate subspecies samples using a combination of the two measurements (lower glume length: 4.6 mm; leaf length: 37 cm).

samples, as differences in camera and lighting conditions will limit comparability of HSL values and the method is logistically demanding compared with other measurements.

Although both subspecies had some stems with no round fungal stem spots (Table 2), the presence of these spots is nevertheless a useful fourth individual characteristic for separating them. The spots form on some stems of native *P. australis* (average 64% in this study) but never appear on the introduced lineage (Swearingen et al. 2022). Thus, while the absence of stem spots is not diagnostic, the presence of spots can

provide a quick and easy positive identification of native *P. australis*. While stem spot fungus is only rarely recommended as a diagnostic trait (e.g., Blossey 2003; Swearingen et al. 2022), the spots are clear and easy to distinguish from the other fungi that appear on introduced *P. australis* as a dark smudge or smear on the leaf sheaths (Blossey 2003). To date, the presence or absence of fungal spots on hybrid *P. australis* has not been documented.

Finally, LDA identified lower glume length and leaf blade length as combined traits that were not sufficiently diagnostic on their own but could be used together to separate introduced and native *P. australis*. While differences in leaf length are only rarely considered (e.g., Allen et al. 2017), lower glume length has been suggested as a useful trait (Catling and Robichaud 2003; Catling et al. 2007; Saltonstall et al. 2004). Additional effort is required to measure two traits rather than one, but the combined assessment of lower glume length and leaf length using a two-step binomial key remains accessible, as it does not require statistical analysis or calculation of additional indices (Table 3).

All five traits can be measured with moderate to high confidence and ease. The only tools required will be a meter stick or measuring tape for leaf length and a pair of calipers for the smaller measurements of ligule height and glume length. All can also be conveniently measured in the field or lab, except for lower glume length, which is most accurately measured under a microscope.

Recommendations for Identification

To facilitate rapid and simple identification of introduced and native *P. australis*, we have organized the five diagnostic traits/trait

Table 3. Binomial key for differentiating native and introduced *Phragmites australis* using lower glume length and leaf length measurements.

1A. Lower glume length > 4.6 mm: native <i>Phragmites australis</i> ssp. <i>americanus</i>
1B. Lower glume length < 4.6 mm:
2A. Leaf length > 37 cm: introduced <i>Phragmites australis</i> ssp. <i>australis</i>
2B. Leaf length < 37 cm: native <i>Phragmites australis</i> ssp. <i>americanus</i>

combinations into a checklist (Figure 3). For ease of use and based on the trait ranges observed in this study (Table 2), we describe the difference between subspecies for each trait as either a clear binary or with convenient numerical thresholds. While our results indicate that any one of these five traits/double traits can be sufficient to diagnostically separate introduced and native *P. australis*, we suggest examining all traits for increased confidence. Multiple stems should be examined per suspected population of *P. australis* (e.g., minimum 5 stems) to capture within-site variation. Complete consensus among all measured traits in this guide across samples should provide morphological identification with high confidence. Incomplete consensus—which could emerge due to measurement error, within-subspecies variation beyond what was documented in our populations, multiple subspecies co-occurring at a single site, or hybrids with intermediate traits (Williams et al. 2019)—should be considered inconclusive and followed by genetic testing where possible.

In general, identification will be easiest and most consistent with this checklist in the late summer or fall. Many morphological traits develop over the course of the growing season, after which time they can fade or degrade as stems senesce (Blossey 2003; MJM, personal observation). One useful exception is old stem leaf retention, which is expected to remain an informative trait year-round. Old, greying stems of native *P. australis* will drop most of their leaf sheaths by the end of the growing season and remain bare while old stems of introduced *P. australis* can retain their leaf sheaths for multiple seasons and years (Blossey 2003).

Because *P. australis* traits are known to vary regionally (Lambert et al. 2016), this guide will be most valuable for populations located in the Great Lakes region. Notably, similar research from eastern Ontario (Catling et al. 2007) and western Canada (Allen et al. 2017) observed overlap in traits (e.g., ligule height) that were fully separable in our southern Ontario specimens, suggesting strong regional variation at larger geographic scales. In particular, this guide should not be used in regions with Gulf Coast *P. australis*, which has morphological traits intermediate to the introduced and native lineages and occurs much further south beyond the Great Lakes region (Colin and Eguarte 2016; Saltonstall and Hauber 2007; Swearingen et al. 2022). We hope that this study will guide continuing investigation to determine the best practices for morphological identification of *P. australis* subspecies in additional geographic areas.





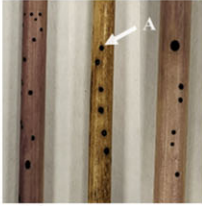


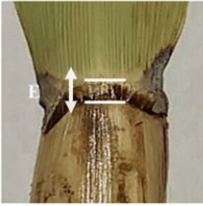

Trait	Stem spot fungus	Stem colour	Leaf retention	Ligule base height	Lower glume length + leaf length
How to measure	Check living stems for dark round fungal spots (arrow A).	Check the base of the stem for dark red colouration.	Inspect greying dead stems (i.e., <u>not</u> living stems) to determine how much is still covered by attached leaf sheaths (arrow C). When leaf sheaths have fallen off, the stem below will be bare (arrow D).	Remove a leaf from the middle of the plant. Use calipers or a ruler to measure the height of the dark membranous band where the leaf meets the stem (i.e., the ligule), excluding any light-coloured, hairy fringe at the top of the band (arrow E).	Press a floret under glass and measure lower glume length (arrow F) using calipers or a ruler under a microscope. Find a leaf near the middle of the stem. Measure its length from ligule to tip (arrow G) using a ruler.
Introduced <i>Phragmites australis</i>	Stems <u>without</u> round fungal spots (arrow A) or dark red may be either introduced or native <i>P. australis</i> . Dark smudges (arrow B) are not diagnostic. 	<input type="checkbox"/> > 50% attached (stem mostly covered) 	<input type="checkbox"/> ≤ 0.15 mm 	<input type="checkbox"/> Lower glume < 4.6 mm and leaf length > 37 cm 	
Native <i>Phragmites australis</i>	<input type="checkbox"/> Round spots present 	<input type="checkbox"/> Dark red, up to 100% coverage 	<input type="checkbox"/> < 30% attached (stem mostly bare) 	<input type="checkbox"/> > 0.35 mm 	<input type="checkbox"/> Lower glume > 4.6 mm, OR lower glume < 4.6 mm and leaf length < 37 cm 

Figure 3. Checklist of the best traits for distinguishing between introduced and native *Phragmites australis*. For each trait, follow the instructions for “How to measure” and mark off the corresponding check box. Measurements will be most comparable if taken in the Great Lakes region in late summer or fall and should be compared across a minimum of 5 samples per suspected *P. australis* population. Complete consensus between all traits and samples with introduced or native *P. australis* should provide morphological identification with high confidence. Incomplete consensus should be considered inconclusive and followed by genetic testing where possible.

Relying on Diagnostic Traits Rather Than “Common Wisdom”

This study reinforces the importance of reliable identification features to distinguish between introduced and native *P. australis*. While 21 of our 22 measured traits differed between the two lineages and were generally in accordance with “common wisdoms” (e.g., on average, introduced *P. australis* was indeed taller, had larger panicles, broader leaves), all but three of the measurements had overlap up to 98%. Thus, common observations of plants with “intermediate” traits are not automatically indicative of aberrant populations or hybridization. Instead, they are representative of the normal range of variation within and between the subspecies. We hope that this checklist of five easy to use traits (Figure 3) will provide a practical, affordable, and objective assessment tool to supplement genetic methods (Lindsay et al. 2023) and help land managers and researchers identify *P. australis* subspecies around the Great Lakes region.

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