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Discovery of a dune-building hybrid beachgrass (*Ammophila arenaria* × *A. breviligulata*) in the U.S. Pacific Northwest

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Abstract. The production of novel hybrid zones is an ecologically important consequence of globally increasing rates of species introductions and invasions. Interspecific hybridization can facilitate gene flow between parent species or produce novel taxa that may alter invasion dynamics or ecosystem services. The coastal sand dunes of the U.S. Pacific Northwest coast are densely populated by two non-native, congeneric, dune-building beachgrasses (*Ammophila arenaria* and *A. breviligulata*). Here, we present morphological, cytological, and genetic evidence that the two beachgrass species have hybridized in this globally unique range overlap. The *A. arenaria* × *A. breviligulata* hybrid has been found at 12 coastal sites in Washington and Oregon. It is a first-generation hybrid between the beachgrass species as evidenced by genome size comparisons and single nucleotide polymorphism genotyping. It is intermediate between the parent grasses in many morphological characters but exceeds both parents in shoot height, a trait associated with dune-building potential. Understanding the ecological and population genetic consequences of this novel hybridization event is of the utmost importance in a system where any change in dominant beachgrass species can have large effects on both biodiversity management and coastal protection.

Key words: *Ammophila*; beachgrass; ecosystem engineer; hybrid zone; hybridization; invasive grass; U.S. Pacific Northwest.

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INTRODUCTION

The rate of human-mediated species introductions is increasing (Seebens et al. 2017), posing threats to biodiversity, human health, and global economies (Simberloff et al. 2013) and affecting biogeography at a global scale (Capinha et al. 2015). Introductions can produce unique species co-occurrence patterns, and if they generate range overlaps between related species, novel interspecific hybridization events are possible (Chunco 2014). Hybridization is common in both the evolutionary history and modern ecology of plants (Mallet 2005) and can serve as a driver of speciation and adaptation (Anderson and Stebbins 1954, Wissemann 2007). Hybridization may increase the invasiveness of plants by producing evolutionarily novel phenotypes or phenotype combinations, increasing genetic variation (in the parent species through backcrossing, or in the hybrid itself), facilitating genetic load dumping (i.e., masking deleterious alleles accumulated in small or isolated populations), and fixing heterosis (i.e., mechanisms that stabilize hybriditidy, for example, allopolyploidy or clonal spread; Ellstrand and Schierenbeck 2000). Transgressive segregation, or the tendency to express extreme phenotypes compared with the parent species, is common in plant hybrids and may contribute to rapid adaptation and range expansion of hybrid lineages (Rieseberg et al. 2001). For example, hybridization within the genus *Spartina* (cordgrass) has produced a variety of highly invasive taxa including hybrid polyploids (e.g., *S. anglica* in France, Salmon et al. 2005), sterile first-generation (F1) hybrids (e.g., *S. densiflora* × *maritima* on the Iberian Peninsula in Spain, Castillo et al. 2010), and multiple backcrossed hybrids (e.g., *S. alterniflora* × *foliosa* in San Francisco, California, Ayres et al. 2008*b*). These hybrids have stabilized sediment and expanded salt marsh habitats, to the detriment of native mudflat communities (Hacker and Dethier 2006, Strong and Ayres 2009). Thus, when hybridization occurs in plants that are highly invasive and have strong ecosystem engineering properties, the genetic and ecological consequences can be significant and unpredictable.

In this paper, we provide the first documented evidence of a novel hybrid formed between two non-native, dune-building *Ammophila* beachgrass species, both of which dominate the U.S. Pacific Northwest coast. Coastal dunes are dynamic and harsh ecosystems at the land-sea interface characterized by strong winds and waves, salt spray, and sand burial. These conditions reduce the likelihood of plant invasions (Maun 2009) but regular disturbance events (e.g., overwash and blowouts) can produce openings and opportunities for appropriately adapted plants to establish (Hesp 2002, David et al. 2015, Charbonneau et al. 2020). Coastal ecosystems are especially vulnerable to the geomorphic changes caused by ecosystem engineering invaders (Fei et al. 2014). In addition, given the shifting and unstable nature of coastal dunes, non-native dune plants have been used over the last century for stabilization purposes. One of the most often used is *Ammophila* spp., a grass that has been intentionally introduced to sandy coastlines all over the world where it specializes in sand capture and stabilization, resulting in tall and wide foredunes and increased coastal protection (Wiedemann and Pickart 1996, Hertling and Lubke 1999, Hilton et al. 2006, Hacker et al. 2012).

One such intentional introduction of *Ammophila* occurred on the U.S. Pacific coast, where dune habitat is widespread (i.e., 45% of the Oregon and Washington coastline and 25% of the California coastline; Cooper 1958, 1967). In the early 1900s, U.S. Pacific coast dunes were systematically planted with the non-native European beachgrass *Ammophila arenaria* to stabilize a shifting sand environment (Seabloom and Wiedemann 1994). By the 1950s, *A. arenaria* had spread from Mexico to Canada building tall, continuous coastal foredunes (Wiedemann and Pickart 1996). Meanwhile, in the 1930s, another non-native beachgrass, American beachgrass *Ammophila breviligulata* was planted in dunes near the Columbia River and, by the 1980s, had spread and dominated northward along the Washington coast (Seabloom and Wiedemann 1994). The spread of these beachgrasses has had a positive impact on coastal development; they stabilize the naturally shifting sand environment, build tall stable dunes, and increase coastal protection (Hacker et al. 2012, Seabloom et al. 2013, Ruggiero et al. 2018). However, they also negatively impact biodiversity management by outcompeting native plants and reducing habitat value for native shorebirds, particularly the threatened Western snowy plover (Zarnetske et al. 2010, Biel et al. 2017).

The two congeneric beachgrasses differ in their dune building and competitive abilities. *A. breviligulata* is a better competitor (Zarnetske et al. 2013) but *A. arenaria* creates taller dunes, even after accounting for differences in sand supply (Hacker et al. 2012, Zarnetske et al. 2012, Biel et al. 2019). The variation in competitive ability and dune shape is ultimately the result of differences in grass density, morphology, growth form, and their effects on sand capture (Hacker et al. 2012, Zarnetske et al. 2012, 2013). *A. arenaria* exhibits a vertical growth form with high stem density that results in deeper sand deposition for a given area, and thus taller and narrower dunes (Hacker et al. 2012, Biel et al. 2019). In comparison, *A. breviligulata* exhibits more lateral growth with a lower stem density, resulting in better space occupation, but less sand accretion over a larger area, creating shorter and wider dunes. The intentional introduction and subsequent spread of the two beachgrass species over the last century has resulted in overlapping distributions along the U.S. Pacific Northwest coast, a globally unique occurrence pattern.

In 2012, a putative hybrid was discovered in the geographic overlap between the two

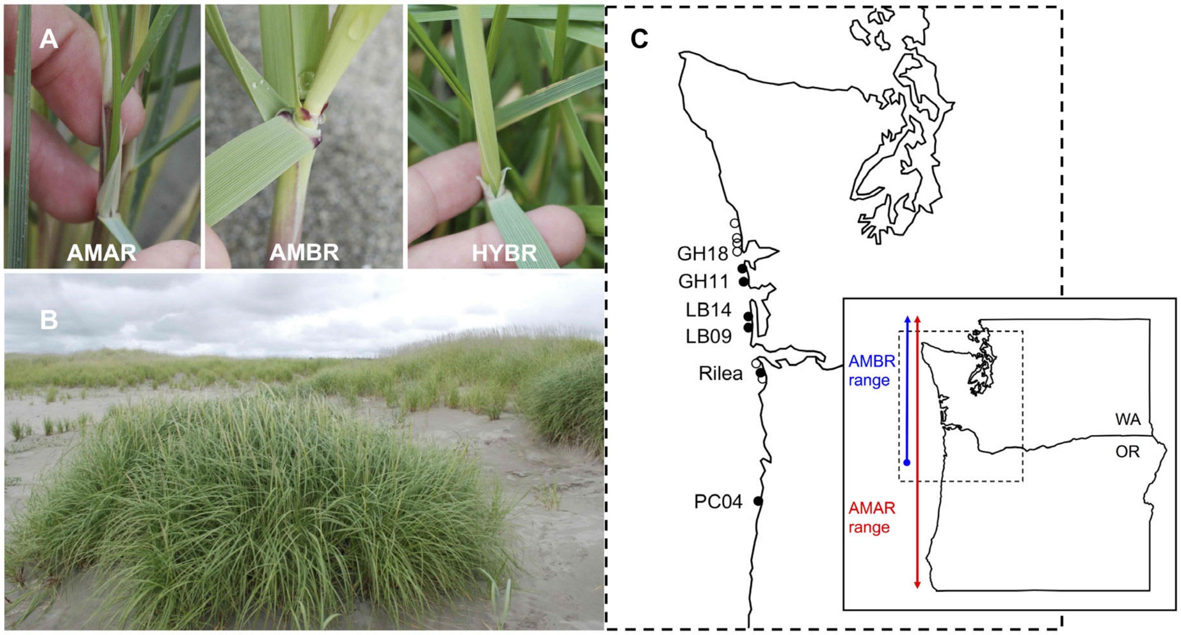

Fig. 1. (A) Photographs of the distinctive ligules of the *Ammophila* taxa. *Ammophila arenaria* (AMAR) has a long, pointed ligule, *Ammophila breviligulata* (AMBR) has a short, flat-topped ligule, and the *Ammophila* hybrid (HYBR) has a ligule of intermediate length with a flat top. (B) A patch of *Ammophila* hybrid (foreground) at the toe of an *A. breviligulata*-dominated dune. (C) Map of all known *Ammophila* hybrid stands. Labeled, closed circles are sites discovered between 2013 and 2018, from which hybrid samples were collected. Open circles are sites discovered between 2018 and 2019, after data analysis for this study had begun. Inset shows the range overlap between AMAR and AMBR in Washington and Oregon. See Appendix S1: Table S1 for site and transect abbreviations and locations.

Ammophila species (Hacker et al. 2012, Zarnetske et al. 2013; Fig. 1). Individual stands of this morphologically unusual grass have since been found at 12 sites between Pacific City, Oregon, and Ocean Grove, Washington (hybrids from six sites were sampled for this study, Fig. 1C). The putative hybrid individuals were initially discovered because they were morphologically similar to *A. breviligulata* but had ligules (the thin tissue at the base of the leaf) intermediate in length between the two *Ammophila* species (Fig. 1A). Ligule length is a distinguishing character for these two beachgrass species (*A. arenaria* ~25 mm and *A. breviligulata* ~2 mm in length; Hitchcock and Chase 1951). At the time of discovery, other morphological differences observed included intermediate leaf width, which suggested that these plants could be hybrids.

Here, we present genetic and cytological evidence that confirms the hybrid origin of the newly discovered beachgrass and a morphological comparison between the hybrid and its parents. We aim to (1) verify the hybrid origin using cytology (genome size estimation with flow cytometry) and single nucleotide polymorphism (SNP) genotyping and (2) compare morphological characters which may aid in hybrid identification and affect sand capture and dune building. If the putative hybrid exceeds its parents in traits associated with dune-building potential (i.e., stem density, plant height, or biomass), which is possible via heterosis, then its spread could affect dune morphology and have substantial ecosystem-scale consequences. Moreover, hybridization between the *Ammophila* beachgrasses could produce a highly invasive taxon or increase the invasive potential of either parent species, leading to a change in invasion dynamics or dominant beachgrass.

METHODS

Between 2013 and 2018, we collected whole plants, inflorescences, and leaf tissue samples of

A. arenaria, *A. breviligulata*, and/or the putative hybrid from as many as eight sites spanning a 190 km stretch of Oregon and Washington coastline (Fig. 1C; see Appendix S1: Tables S1, S2 for collection information). We air-dried the individual plants (shoots attached to a single rhizome; $N = 523$) and inflorescences (a single shoot with flowers; $N = 249$) prior to conducting morphological measurements. Leaf tissue samples were either stored on ice and processed within 24 h for flow cytometry ($N = 24$) or desiccated and stored in silica gel for DNA extraction ($N = 32$).

To measure the morphology and growth form of the plants, we measured individual plant weight including aboveground biomass (all biomass 1 cm above the primary node) and belowground biomass (all biomass, including roots and rhizomes, below 1 cm above the primary node) ligule length, number of vertical and horizontal shoots, shoot length, leaf number, leaf length, leaf width, total rhizome length, number of rhizome nodes, and rhizome internode length (see Hacker et al. 2019 for methods). For each inflorescence, we measured culm length (from the node to tip of the inflorescence), panicle biomass, panicle length (from the base of the inflorescence to the tip), panicle width (measured at the widest point), floret length, callus hair (hairs at the base of the floret) length, and rachilla (small stem baring the spikelet) length. We analyzed the resulting data using R version 3.6.1 (R Core Team 2019). Outliers were assessed for all traits using a threshold of 10 times the mean Cook's distance (Cook 1977). Outliers were detected and subsequently removed from two traits (panicle length and plant height). We used one-factor ANOVAs or Kruskal–Wallis tests (including Tukey's honestly significant difference [HSD] post hoc test, when appropriate), in addition to principal component analysis (PCA), to determine whether the two *Ammophila* species and the putative hybrid differed in their morphology and growth form.

To test for hybridization, we measured the genome size of the plants using flow cytometry. Leaf tissue from three individuals of each of the two *Ammophila* species and the putative hybrid was analyzed (see Appendix S1: Table S1 for collection information). Leaf tissue (~0.3 cm^2) was placed in a plastic petri dish with the same area of leaf tissue from *Pisum sativum* Ctirad, which served as an internal standard with known diploid genome size (2C = 8.75 pg; Greilhuber et al. 2007). Samples were mixed with 400 μL of nuclei extraction buffer (CyStain ultraviolet Precise P Nuclei Extraction Buffer; Sysmex Partec, Görlitz, Germany), chopped with a razor blade, and then filtered through a 50-μm nylon mesh filter. The nuclei were stained by adding 1.6 mL 4′,6-diamidino-2-phenylindole (DAPI) to the tissue mixture. Samples were analyzed on a Partec PA II flow cytometer (Munster, Germany) and run until at least 3000 nuclei were counted. Genome size was calculated as 2C = genome size of standard × (mean fluorescence value of sample ÷ mean fluorescence value of standard). Differences between the genome sizes of each taxon were assessed using one-factor ANOVA and Tukey's HSD tests.

Finally, we conducted SNP genotyping to measure the potential genetic contribution of the two *Ammophila* species to the hybrid. Leaf tissue from 32 total plants at five sites was used (see Appendix S1: Table S2 for sample information). DNA was extracted from 15 mg of leaf tissue using the E.Z.N.A. Plant DNA Kit (Omega Bio-Tek, Norcross, Georgia, USA) following the manufacturer's instructions. DNA quality was assessed with 1% agarose gel electrophoresis, and DNA quantity was measured using an AccuBlue NextGen dsDNA Quantitation Kit (Biotium, Fremont, California, USA) in the SpectraMax M3 plate reader (Molecular Devices, San Jose, California, USA).

Genotyping libraries followed the 2b Restriction Site-Associated DNA (2b-RAD) protocol from Wang et al. (2012) with minor modifications (Appendix S2). Libraries were pooled in equimolar amounts and multiplexed in a single lane as 100-bp single reads on an Illumina HiSeq 3000 at Oregon State University's Center for Genome Research and Biocomputing (Corvallis, Oregon, USA). After quality-filtering the reads, de novo loci were generated by clustering across samples and genotypes called for each individual. These steps were performed using methods described in Wang et al. (2012) with publicly available scripts (https://github.com/Eli-Meyer/2brad_utilities). For the full details of SNP genotyping methods, see Appendix S2.

From the resulting dataset of 1870 SNPs, we identified markers that would be informative for verifying the parentage of the putative hybrids. We used the R package SNPRelate (Zheng et al. 2012) to calculate the minor allele frequency for both *Ammophila* species at each locus and then identified nearly fixed differences (major allele frequency >80%) between the two species. This filtering revealed 47 SNPs that were diagnostic between the two *Ammophila* species. We then used the program STRUCTURE (Pritchard et al. 2000) to test for evidence of hybridization by clustering the putative hybrids based on their genotypes at these 47 loci. The population origin of each sample belonging to the two *Ammophila* species was pre-specified and STRUCTURE was used to predict the population of origin of the hybrid samples. The number of groups was set to 2, lambda was fixed at 1, and the migration prior was set to 0.05. Fifteen iterations were run with 50,000 burn-in steps (sufficient for key parameters to reach equilibrium) and 50,000 run steps. The STRUCTURE output from all runs was collated using CLUMPP (Jakobsson and Rosenberg 2007) and visualized using DISTRUCT (Rosenberg 2003). The same SNP dataset was also examined via PCA on their covariance matrix using SNPrelate (Zheng et al. 2012).

RESULTS

Several notable morphological differences were identified between the two beachgrass species and the putative hybrid (Fig. 2; Appendix S3: Table S1). The putative hybrid was intermediate between the two *Ammophila* species in ligule length (Fig. 2A) and leaf width (Fig. 2B), characters important for field identification. The putative hybrid exceeded both *Ammophila* species in shoot height (Fig. 2C) and was intermediate in shoot number (Fig. 2D). Additionally, there were differences in inflorescence morphology: the panicles of the putative hybrid were heavier than either *Ammophila* species (Fig. 2F) although they were of intermediate length (Fig. 2E). A PCA grouped samples by taxon with the putative hybrid clustering between the two *Ammophila* species (Fig. 3). Differences in morphology within each taxon (PC1) explained the most variation (70.3%) while differences among taxa (PC2) explained 25.5% of the variation. Notably, *A. arenaria* had the greatest within-species morphological variation (Fig. 3).

The genome sizes of *A. arenaria*, *A. breviligulata*, and the putative hybrid all differed from one another (mean ± standard deviation: 6.39 pg ± 0.05, 7.26 pg ± 0.07, and 6.80 pg ± 0.05, respectively). Compared with its parents, the putative hybrid genome is intermediate in size (Appendix S3: Fig. S1). Moreover, there was no overlap in estimated genome size among the three taxa. Finally, the 95% confidence interval of the hybrid genome size (6.76–6.84 pg) encompasses the expected midparent genome size (6.83 pg), the predicted genome size for an F1 diploid hybrid. Using the known chromosome counts and genome sizes of the parent species, we can estimate the 2N chromosome count for the hybrid (Ayres et al. 2008*a*). Both *Ammophila* parent species have 2N = 28 chromosomes (Bowden 1960, Zonneveld et al. 2005). The estimated average mass per chromosome for each species is 0.228 pg for *A. arenaria* (for a total of 6.39 pg across 28 chromosomes per nucleus) and 0.259 pg for *A. breviligulata* (7.26 pg per nucleus). A first-generation hybrid cross with a genome comprised of 1N from *A. arenaria* and 1N from *A. breviligulata* would have an estimated genome size of 6.82 pg ((14 chromosomes × 0.228 pg per chromosome) + (14 chromosomes × 0.259 pg per chromosome)). These calculations imply that the hybrid, found here to have a genome size of 6.80 pg, is a first-generation cross between *A. arenaria* and *A. breviligulata* and is not an allopolyploid.

To verify the origin of the putative hybrid, we calculated the hybrid allele frequency at genome sites diagnostic for both *Ammophila* species. The expected allele frequency for an F1 hybrid at SNPs that are fixed between parent species is 0.5 (Fitzpatrick 2012). For all hybrid samples, the average minor allele frequency at these diagnostic SNPs was between 0.44 and 0.49 (mean = 0.45). The PCA on the parental diagnostic SNP dataset groups hybrid individuals between the two *Ammophila* species, which are clearly differentiated from each other (Fig. 4A). As with the morphological data (Fig. 3), the *A. arenaria* cluster has the widest spread, indicating that this group contains the highest level of SNP diversity. In contrast, the hybrids cluster together closely, indicating that there are very few SNP differences between individuals. Four *A. arenaria* samples and one *A. breviligulata* sample are clustered

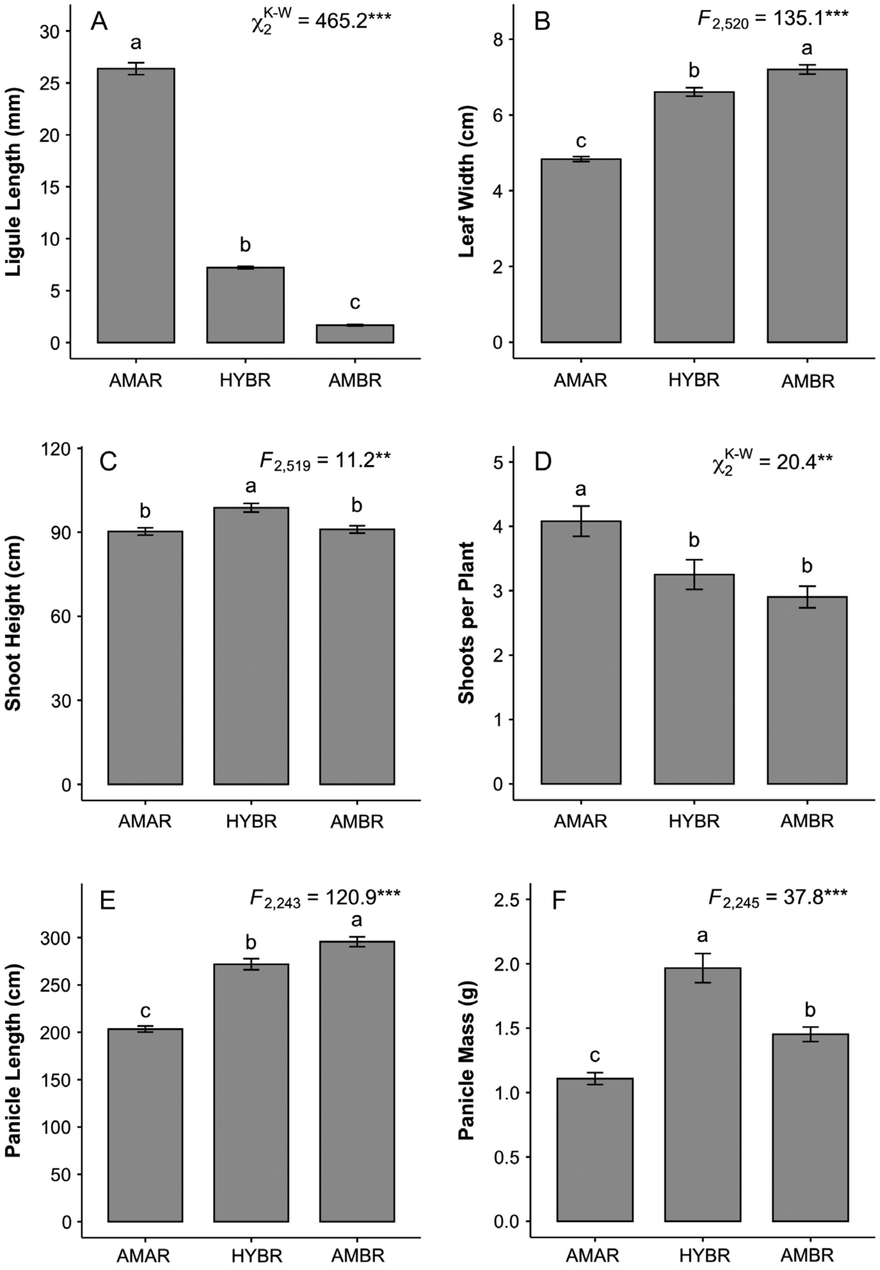

Fig. 2. Comparisons (mean ± standard error) of grass species morphology, including (A) ligule length, (B) leaf width, (C) shoot height, (D) number of shoots per plant, (E) panicle length, and (F) panicle weight of the two beachgrass species and the hybrid (abbreviations in Fig. 1). Results of one-factor ANOVAs and Kruskal–Wallis rank sum tests are given in plots. Bars that do not share letters are statistically different (Tukey's honestly significant difference $P \leq 0.05$). $^{**}P < 0.0001$, $^{***}P < 0.00001$.

with the hybrids. Four of these five samples are missing significant data (i.e., some of the species-differentiating SNPs were not sequenced for this sample), which results in their positioning near zero on both PCA axes. Finally, the STRUCTURE analysis suggested that the hybrids are of

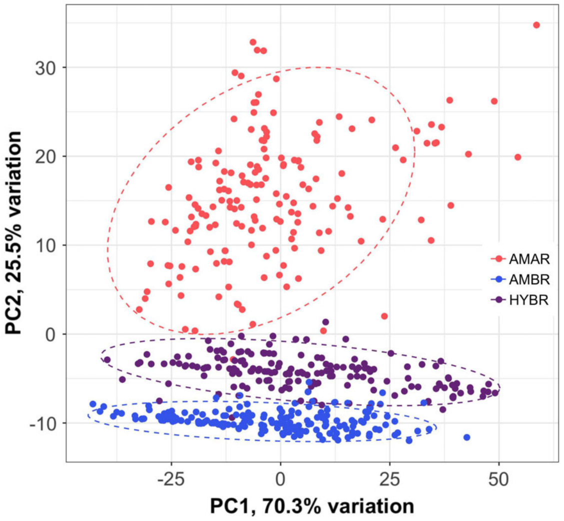

Fig. 3. Dimensional reduction on morphological data using principal component analysis. Dashed lines represent 95% confidence ellipses for each species (species abbreviations in Fig. 1) based on a multivariate *t* distribution. The first principal component is most strongly correlated with shoot height while the second principal component is most strongly correlated with ligule length.

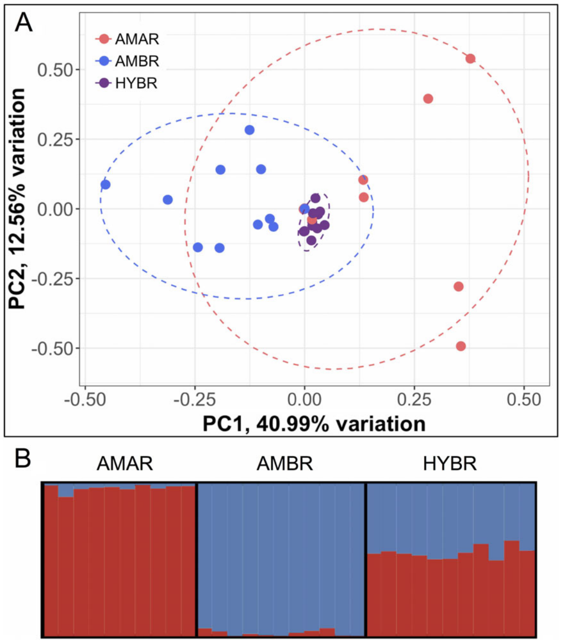

Fig. 4. Genomic ancestry of hybrids. Both (A) principal component analysis and (B) STRUCTURE analysis were performed with 47 species-diagnostic single nucleotide polymorphism loci. Dashed lines in A represent 95% confidence ellipses for each species (abbreviations in Fig. 1) based on a multivariate *t* distribution.

intermediate population ancestry (Fig. 4B), with an average ancestry of 55% *A. arenaria* and 45% *A. breviligulata*.

DISCUSSION

The morphological, cytological, and genomic data presented here all support the hypothesis that the distinct *Ammophila* morphotype recently discovered on the Pacific Northwest coast is an F1 hybrid between *A. arenaria* and *A. breviligulata*. The hybrids we identify are undoubtedly a unique and unintended consequence of the sequential introduction and subsequent invasion of two non-native beachgrass species to the Pacific Northwest over the last century. Although both species have been introduced to a number of coastlines (Hertling and Lubke 1999, Hilton et al. 2006), as far as we know the Pacific Northwest is the only place in the world where the two *Ammophila* species significantly overlap in their range and thus have had the opportunity to hybridize.

We show that the hybrid has some morphological traits that are intermediate between the two *Ammophila* species (i.e., ligule length, leaf width, and callus hair length; Fig. 2A, B, Appendix S3: Table S1) and a few traits for which it exceeds both parents (i.e., tiller height, panicle width and biomass, and spikelet and rachilla length; Fig. 2C, F, Appendix S3: Table S1). F1 hybrids may experience heterosis, or hybrid vigor, which can produce exceptional traits and likely explain some of these novel phenotypic values. The taller shoots and larger inflorescences of the hybrid suggest greater energy input into growth and reproduction.

The cytological and genomic evidence is concordant with our hypothesis that the putative hybrid is an F1 cross between the two *Ammophila* species. The genome sizes of the three taxa are significantly different and non-overlapping, and the hybrid genome size and number of chromosomes are as predicted for a F1 hybrid

(Appendix S3: Fig. S1). Although we do not yet know if the hybrid can sexually reproduce, the lack of allopolyploidy (chromosome duplication following hybridization) and the large differences between average chromosome sizes of the parent species suggest that the hybrid is likely sterile (Rieseberg and Willis 2007). Allopolyploidy can lead to previously sterile hybrids developing sexual reproduction, which can increase spread and speciation as has been seen in invasive *Spartina* (Ayres and Strong 2001) and *Salsola* (Ayres et al. 2009). Further research into the potential for sexual reproduction and monitoring for changes in the ploidy level of the hybrid will be increasingly important as we consider the possibilities of speciation, backcrossing, and spread.

The species-diagnostic SNP data clearly indicate that the putative hybrid is an F1 cross between the two beachgrass species (Fig. 4). At this point, we have found no evidence of later generation hybrids or backcrossing, which would produce genome sizes intermediate to the F1s and their parent species. Genetic variation among hybrids and their current geographic range of 190 km of the coast suggests that there have likely been multiple independent hybridization events. However, both *Ammophila* parent species have been shown to disperse long distances during winter storm events, which erode rhizomes and stems from beaches and deposit them in different locations (Maun 1984, Konlechner et al. 2016). This mechanism could contribute to further spread of the hybrid without additional hybridization events. Ayres et al. (2004) documented the dramatic spread, a 100-fold increase in area in 30 yr, of the hybrid cordgrass, *S. alterniflora* × *S. foliosa*, in San Francisco Bay, California. The authors attributed this spread to both high asexual growth rate and seedling germination, an example that further emphasizes the need to understand the sexual and asexual reproductive potential of the *Ammophila* hybrid.

The low genetic diversity observed in *A. breviligulata* in this study is congruent with previous studies of out-planting using this species. Clonal propagules typically come from limited source populations (Carlson et al. 1991), resulting in field populations with low genetic diversity (Slaymaker et al. 2015). Although propagated using the same technique, *A. arenaria* has a much longer history of planting on the U.S. Pacific coast (Seabloom and Wiedemann 1994, Wiedemann and Pickart 1996), which perhaps explains the higher genetic diversity we observed in that species. Further study is needed to understand the relationship between planting patterns in the Pacific Northwest, the population genetic diversity of both *A. arenaria* and *A. breviligulata* in their invasive ranges, and the impact of these patterns on hybridization success.

The direction of hybridization (i.e., which parent species is the pollen donor and which is the seed parent) is unknown. To infer the direction of parentage, we are planning to sequence sections of the chloroplast genome, which is exclusively maternally inherited in most grasses (Harris and Ingram 1991). The majority of the hybrid stands are found on dunes that are dominated by *A. breviligulata* with very low *A. arenaria* abundance (Hacker et al. 2012, Biel et al. 2019). We hypothesize that in these sites, the high ratio of *A. breviligulata* to *A. arenaria* flowers leads to pollen swamping, likely producing hybrids with an *A. arenaria* seed parent and an *A. breviligulata* pollen parent. The only known hybrid stand that is on an *A. arenaria*-dominated dune is found in Pacific City, Oregon. Comparison of patterns of chloroplast genome inheritance between hybrids from *A. arenaria*- and *A. breviligulata*-dominated sites will help us better determine the direction of hybridization and the ecological and biological controls on hybrid formation.

Besides the population genetic consequences of the hybridization, there are ecological and ecosystem service implications as well. Past research shows that beachgrass shoot density, height, and biomass are important characteristics in determining species-specific differences in sand capture (Zarnetske et al. 2012, Biel et al. 2019, Hacker et al. 2019) and competition (Zarnetske et al. 2013). The number of shoots per plant was found to be highest in *A. arenaria* and lowest in *A. breviligulata* (Fig. 2D), in agreement with earlier studies of beachgrass functional morphology (Hacker et al. 2012). The hybrid had an intermediate number of shoots per plant but was not statistically different from the trait average for *A. breviligulata* (Fig. 2D). Additionally, the hybrid exceeded both parents in shoot height, belowground biomass, and average rhizome internode distance (Appendix S3: Table S1). This combination of taller plants with intermediate density and a greater investment in rhizomes has the potential to produce dunes that are as wide as those produced by *A. breviligulata* but significantly taller. Proliferation of the hybrid could result in dunes intermediate in morphology between those dominated by *A. arenaria* and *A. breviligulata*. However, both *Ammophila* species have been shown to be phenotypically plastic (Gray 1985) and to have growth rates and competition interactions that respond strongly to their environment (Zarnetske et al. 2013). Therefore, studies of the morphological differences between the parent species and their hybrid under controlled environmental conditions, especially sand deposition and climate, will be critical for predicting the effect of this novel taxon on dune morphology and related ecosystem services. The two beachgrass species both negatively impact native coastal shorebird and plant populations but do so to different degrees (Hacker et al. 2012, Biel et al. 2017). Specifically, *A. arenaria*-dominated dunes tend to have lower plant species diversity than those dominated by *A. breviligulata* (Hacker et al. 2012) and removal of both species improves breeding habitat for the threatened Western snowy plover (Zarnetske et al. 2010, Biel et al. 2017). Given the intermediate morphological characteristics of the hybrid, we hypothesize that it will also negatively affect both native plant biodiversity and plover habitat value. The effects of a possible spread of the *Ammophila* hybrid on biodiversity conservation are unknown but potentially detrimental.

It is interesting to consider how the spread of the hybrid beachgrass might affect dune morphology. Expansion of the hybrid beachgrass into areas dominated by *A. arenaria* (i.e., spread to the south of its current range) might decrease dune height and compromise coastal protection. However, expansion of the hybrid on dunes currently dominated by *A. breviligulata* would likely increase dune height and increase coastal protection. A more nuanced understanding of the likelihood of spread of the hybrid beachgrass, and the consequences for dune shape and coastal protection is of critical management importance, especially with relation to the different invasion contexts posed by the two parent species.

There is a history of hybrid grasses being preferentially planted for their value as sediment stabilizers. In Europe, a hybrid between *A. arenaria* and *Calamagrostis epigejos* is more vigorous than *A. arenaria*, growing more laterally with longer and wider leaf blades, and is therefore preferentially planted in dune stabilization projects (Rihan and Gray 1985). *S. anglica*, a polyploid hybrid species of cordgrass, has been intentionally planted in marshes in Puget Sound, Washington, USA (Hacker et al. 2001), and across China (Zhang et al. 2004, Chung 2006) to capture sediment and minimize erosion. *A. arenaria* × *breviligulata* may have properties that make it an exceptional or valuable dune builder and could be of interest to coastal planners and land managers. However, any plans to intentionally plant the hybrid must consider its invasion potential and effects on the genetic diversity of its parent species. Additionally, introduction of the hybrid into the Great Lakes native range of *A. breviligulata* could have irreversible negative consequences for populations already threatened by land use change and genetic swamping from restoration (Fant et al. 2008, Slaymaker et al. 2015). Both *Ammophila* species are still intentionally planted in their native and invasive ranges as dune stabilizers. Considering the ability of these two species to hybridize, introducing them into the same range should be carefully considered. Clearly, more research into the effects of the hybrid on important ecosystem functions and services is necessary to fully understand the management implications of this discovery.

Monitoring the size and expansion of this new hybrid zone is of critical management importance along the Pacific Northwest coast, where any change in dominant beachgrass species as a result of the spread of this new invader could have substantial effects on dune geomorphology, coastal protection, and biodiversity conservation (Seabloom et al. 2013, Biel et al. 2017, 2019). Additionally, further study into the reproductive interactions between the hybrid and its parents, including studies of flowering phenology, reproductive compatibility, and tests for introgression, is essential for determining the potential effects on the population genetics and invasibility in beachgrasses, a critical ecosystem engineer in coastal dune systems.

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