Black needlerush (*Juncus roemerianus* Scheele; Juncaceae) is a clonal, gynodioecious macrophyte found in salt marshes from the mid-Atlantic in Maryland and Delaware to the western coast of the Gulf of Mexico in Texas (Godfrey and Wooten, 1979). The species has a high salt tolerance and dominates areas of low tidal flux, such as the Gulf Coast, forming large monotypic stands through sexual and clonal reproduction (Eleuterius, 1984). *Juncus roemerianus* is an ecosystem engineer and forms the foundation of the salt marsh, creating habitat for other marsh species by accumulating and stabilizing sediment (Pennings and Bertness, 2001). Genetic diversity of foundation species has an elevated importance in maintaining ecosystem health and resilience in monotypic ecosystems such as salt marshes (Reusch and Hughes, 2006; Hughes et al., 2008). Restored macrophyte populations with higher genetic diversity are more resilient and have greater overall restoration success (Reynolds et al., 2012). Across the Gulf Coast, *J. roemerianus* habitat has been fragmented by human development and is vulnerable to future losses and degradation from pollution and sea level rise. Information on the genetic diversity and population structure of *J. roemerianus* is essential for salt marsh conservation.

While transplant studies suggest the existence of distinct populations of *J. roemerianus*, no molecular population genetic studies have been conducted on the species (Eleuterius, 1989). We address this need by developing and characterizing 19 microsatellite markers for *J. roemerianus* suitable for population studies. Microsatellites are highly variable and useful in characterizing the scale of population structure necessary for successful restoration and management.

**METHODS AND RESULTS**

Microsatellite markers were developed using an Illumina NextSeq sequencing system (Illumina, San Diego, California, USA). Genomic DNA was extracted from a leaf sample collected from the same site as the voucher specimen at the Grand Bay National Estuarine Research Reserve (NERR) in Moss Point, Mississippi, USA, using a QIAGEN DNeasy Plant Maxi Kit (QIAGEN, Hilden, Germany) (Appendix 1). Library preparation was completed using a KAPA LTP Library Preparation Kit (KAPA Biosystems, Wilmington, Massachusetts, USA) for Illumina platforms following the manufacturer’s protocol. Reads totaling 872,449 sequences were paired by name using Geneious version 8.1.2 (Kearse et al., 2012) and archived in the GenBank Sequence Read Archive (SRR5076849). Illumina TruSeq adapters and bases with an error probability limit above 0.04 were trimmed, and de novo assembly was performed on sequences greater than 150 bases. Unused reads were extracted to MSATCOMMANDER version 1.0.8beta (Faircloth, 2008) and queried for microsatellite loci. MSATCOMMANDER identified 4237 loci with perfect repeats of 3–6 nucleotides using default minimum lengths and melting temperatures, and combining loci less than 50 bp apart. Five hundred and two loci had unique sequences surrounding the repeats with sufficient length for primer design. We selected 96 primer pairs...
with a pair penalty assigned by Primer3 (Rozen and Skaletsky, 1999) below six
that had a diversity of repeat lengths and nucleotide motifs. One primer for
each locus was tagged with either a CAG (CAGTCGGGCGTCATCA) or M13
(GGAAACAGCTATGACCAT) sequence tagged locus-specific primer, 0.5 μM GTTT
M CAG (CAGTCGGGCGTCATCA)–tagged or M13
tions containing 0.05
KCl, 1.5 mM MgCl2, 0.5 units AmpliTaq Gold DNA polymerase (all from Ap-
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Jr72 F
Jr68 N
Jr66 P
Jr65 P
Jr64 P
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Jr61 P
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Jr7 P
Jr6 P
Jr5 P
Jr4 P
Jr3 P
Jr2 P
Jr1 P
Table 1. Traits and thermal cycling conditions for 19 microsatellite loci for Juncus roemerianus.a

| Locus  | Primer sequences (5′−3′) | Repeat motif | Allele size range (bp) | GenBank accession no. |
|--------|--------------------------|--------------|------------------------|-----------------------|
| Jr01   | F: 4GGTGATGCTGGAAATTTCCAAG  | (AAAG)9       | 226–268                | KX398592              |
|        | R: 5AGCATTCCATCCGGCTTC   |              |                        |                       |
| Jr02   | F: 4CTCTGGAAGGCTGTTTC    | (AAAG)9       | 213–237                | KX398593              |
|        | R: 5TTTGGATTCACTTGGCAAC  |              |                        |                       |
| Jr03   | F: 4CACCTTACAGCGGCAATC  | (AAAT)8       | 112–128                | KX398594              |
|        | R: 5GCAGATGTTAAATGTCGCCAG |              |                        |                       |
| Jr05   | F: 4CTCTTCATGTTAGCCTCTTC | (AAAT)8       | 255–271                | KX398595              |
|        | R: 5AGTGGCATGTGTTTTGGACG |              |                        |                       |
| Jr12   | F: 4CCCTCTGGTCGTTCTCCTTC | (ACT)9        | 200–215                | KX398596              |
|        | R: 5AGGCTTACATCTCCTTCCC  |              |                        |                       |
| Jr13   | F: 4AGAAAGTTAGCTCGGAGG   | (AAC)8        | 175–193                | KX398597              |
|        | R: 5ATCCTGCTCCACGGTACAC  |              |                        |                       |
| Jr16   | F: 4CGCTGACGTGTTGATTCAG | (AAG)11       | 192–207                | KX398598              |
|        | R: 5GGATCGTAGATGTTGGGCC  |              |                        |                       |
| Jr19   | F: 4ATACGGAGGAGAGGATTTCT | (AGG)12       | 156–183                | KX398599              |
|        | R: 5ATCCACCTCCTCCGCCAG   |              |                        |                       |
| Jr29   | F: 4AACCTGAGCAACCGAAGGCC | (AAAT)9       | 139–154                | KX398600              |
|        | R: 5TTTGGATGCAACACCACCC |              |                        |                       |
| Jr33   | F: 4CTTGGCCCTAAACCTCCTCC | (AAT)12       | 179–218                | KX398601              |
|        | R: 5CCCTGCAAGCCTCTCAGC   |              |                        |                       |
| Jr41   | F: 4ACCTGCCCTCTCACAACC  | (AGG)12       | 168–204                | KX398602              |
|        | R: 5TCTGGAGGCTCCGGTGCTC  |              |                        |                       |
| Jr42   | F: 4ACCTGTCTTACCTGGAGG   | (ACTGG)9      | 168–208                | KX398603              |
|        | R: 5TGTTGATGAGCCGGGTGTT |              |                        |                       |
| Jr46   | F: 4TCAAAGCTCTCCTACCCTCC | (AAAAT)9      | 157–197                | KX398604              |
|        | R: 5CCGACGTTTTACAGTGAGCC |              |                        |                       |
| Jr58   | F: 4TTCGCTGGTCAGGTGTTAGG | (AAAT)9       | 149–175                | KX398605              |
|        | R: 5CCGGAGCCTGGAATCAGAC  |              |                        |                       |
| Jr72   | F: 4GCTGGAGCCATTTCTACCGC  | (AAAT)9       | 333–341                | KX398606              |
|        | R: 5GCCCTGTTGAGTTTGGTT  |              |                        |                       |
| Jr73   | F: 4TCCTAGCAGGCTACCTTCC  | (AGG)11       | 159–180                | KX398607              |
|        | R: 5CTAACGGGACTGGGGGGGCGG  |              |                        |                       |
| Jr80   | F: 4CCAGAATGAAGCCTGGAGG  | (AAAAG)5      | 133–148                | KX398608              |
|        | R: 5CTAGCTGGAAGGAGACACCC  |              |                        |                       |
| Jr86   | F: 4ACCTGGAGTAGGCTCCCTTG | (AGCAGG)9     | 160–187                | KX398609              |
|        | R: 5ATCCCTGAGGCTCTGATCC |              |                        |                       |
| Jr87   | F: 4ATAATGATGCCCCACAGC  | (ACCTG)9      | 304–314                | KX398610              |
|        | R: 5CCGAGTGAAGGAGACAGCATC |              |                        |                       |

Values are based on 66 samples from the northeastern Gulf of Mexico in North America located in eastern Mississippi and Florida (N = 20–24).

Fluorophore used to label M13- and CAG-tagged primers: F = FAM, V = VIC, N = NED, P = PET.

Thermal cycling conditions for all loci were set at two annealing temperatures, 60°C for 25 cycles and 52°C for 25 cycles.

GTTT tag addition to 5′ terminus.

CAG tag (CAGTCGGGCGTCATCA) addition to 5′ terminus.

M13 tag (GGAAACAGCTATGACCAT) addition to 5′ terminus.

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http://www.bioone.org/loi/apps

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Applications in Plant Sciences 2017 5(3): 1600141 Tumas et al.—Juncus roemerianus microsatellites
do:10.3732/apps.1600141

Table 2. Genetic diversity metrics for three populations of Juncus roemerianus located in the northern Gulf of Mexico.²

| Locus | Grand Bay NERR (N = 24) | Apalachicola NERR (N = 20) | Choctawhatchee Bay (N = 22) |
|-------|------------------------|---------------------------|-----------------------------|
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**APPENDIX 1**. Voucher specimen information for the three populations of *Juncus roemerianus* and one population of *J. effusus*.

| Species | Collector no. | Location            | Population   | Geographic coordinates |
|---------|---------------|---------------------|--------------|------------------------|
| *J. roemerianus* Scheele | Mark Woodrey 1 | Moss Point, MS, USA | Grand Bay NERR | 30°21.761′N, 88°27.023′W |
| *J. roemerianus* | Mark Woodrey 2 | Moss Point, MS, USA | Grand Bay NERR | 30°22.229′N, 88°24.429′W |
| *J. roemerianus* | Mark Woodrey 3 | Moss Point, MS, USA | Grand Bay NERR | 30°23.712′N, 88°23.981′W |
| *J. roemerianus* | Hayley Tumas 3 | East Point, FL, USA | Apalachicola NERR | 29°40.303′N, 84°51.101′W |
| *J. roemerianus* | Hayley Tumas 4 | East Point, FL, USA | Apalachicola NERR | 29°40.300′N, 84°51.106′W |
| *J. roemerianus* | Hayley Tumas 2 | Santa Rosa Beach, FL, USA | Choctawhatchee Bay | 30°23.918′N, 86°13.771′W |
| *J. effusus* L. | Hayley Tumas 1 | Lillian, AL, USA | Perdido Bay | 30°25.947′N, 87°24.843′W |

*Note*: NERR = National Estuarine Research Reserve.

Herbarium vouchers are deposited at the University of Georgia Herbarium (GA), Athens, Georgia, USA.