PRIMER NOTE

DEVELOPMENT AND CHARACTERIZATION OF 11 MICROSATELLITE PRIMERS FOR THE SEDGE TRICHOPHORUM PLANIFOLIUM (CYPERACEAE)1

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• **Premise of the study:** Microsatellite loci were developed for *Trichophorum planifolium* (Cyperaceae), an endangered woodland sedge protected under federal and provincial legislation in Canada, to explore patterns of population genetic diversity and differentiation in the species.
• **Methods and Results:** Sixty-three primer pairs were evaluated for amplification consistency and screened for polymorphisms with 96 samples collected from 12 populations of *T. planifolium* distributed through the range of the species. Of these, 11 loci were shown to be polymorphic, displaying two to six alleles. Mean observed heterozygosity across loci ranged from 0.00 to 0.06 among populations tested.
• **Conclusions:** The results suggest that the 11 primer pairs developed in this study will be useful for future studies of broad-scale genetic variation in *T. planifolium* and in guiding management protocols for the species in Canada.

**Key words:** conservation genetics; Cyperaceae; microsatellites; *Trichophorum planifolium.*

*Trichophorum planifolium* (Spreng.) Palla (Cyperaceae) is a woodland sedge that occurs on dry, rocky slopes in northeastern North America. In Canada, there is only one known extant occurrence, located in a remnant natural area within one of the most highly urbanized regions in the country. Due to its limited Canadian range and an apparent decline in population size (COSEWIC, 2000), *T. planifolium* is listed as endangered under the Canadian Species at Risk Act (SARA). Like many species-at-risk in Canada (Yakimowski and Eckert, 2007; Gibson et al., 2009), *T. planifolium* reaches the northern limit of its range in Canada, but is relatively common farther south. Although there is some debate over whether, or when, peripheral populations merit national concern (Gibson et al., 2009), empirical evidence suggests that they may be important reserves of genetic diversity (Eckert et al., 2008), providing adaptive and evolutionary potential for the species (Lesica and Allendorf, 1995) and facilitating species’ responses to climate change (Etterson and Shaw, 2001; Parmesan, 2006; Gibson et al., 2009).

An understanding of the population genetic structure of *T. planifolium* is needed to guide management strategies for this species. At present, no molecular markers appropriate for studies of intraspecific genetic variation have been developed for *T. planifolium.* To this end, we isolated and characterized 11 polymorphic microsatellite loci.

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METHODS AND RESULTS

Genomic DNA (~6 μg) was extracted from the silica gel–dried leaf tissue of one individual of *T. planifolium* collected from Daniel Boone National Forest (Kentucky, USA) in 2011 using a NucleoSpin Plant II Kit (Machery-Nagel, Bethlehem, Pennsylvania, USA) following the manufacturer’s protocol. The sample was submitted to the Georgia Genomics Facility at the University of Georgia (Athens, Georgia, USA) for isolation of microsatellite loci and primer development. DNA was fragmented using the Bioruptor UCD-300 sonication device (Diagenode, Denville, New Jersey, USA). Libraries compatible with Illumina TruSeq HT were prepared using the KAPA Library Preparation Kit (KR0453–v2.13; Kapa Biosystems, Wilmington, Massachusetts, USA) with custom indexes from Faircloth and Glenn (2012). Libraries were quantified with Qubit (Life Technologies, Burlington, Ontario, Canada) and sequenced using an Illumina MiSeq v3 600-cycle kit (Illumina, San Diego, California, USA). A total of 6,391,132 reads were imported and paired in Geneious 7.0.6 (Biomatters, Auckland, New Zealand). Illumina TruSeq adapters and bases with an error probability limit above 0.05 were trimmed. A de novo assembly was performed on the first 1,000,000 sequences where both reads of any pair were ≥200 bases. Consensus sequences between 200 and 420 bp were exported from Geneious as FASTA files and imported into MSATCOMMANDER 1.0.8 beta (Faircloth, 2008). A total of 721 loci with perfect di-, tri-, or tetranucleotide repeats were designed at default minimum lengths (i.e., eight repeats for di- and trinucleotide motifs, six repeats for tetranucleotide motifs) and combining loci ≤50 bp apart. Sixty-three CAG-tagged primer pairs for di- (9), tri- (47), and tetranculeotide (7) microsatellite loci with the greatest number of motif repeats were selected for further testing.

Loci were subsequently evaluated for amplification consistency and screened for polymorphisms with 96 samples of *T. planifolium* collected from 12 populations distributed through the range of the species in May 2014: (1) Tarrywile Park, Connecticut, USA; (2) Bare Mountain, Massachusetts, USA; (3) Dan’s Mountain, Maryland, USA; (4) Big Spring State Park, Missouri, USA; (5) Sutton Hollow, Missouri, USA; (6) Elmer G. Raymond Park, New Hampshire, USA; (7) Mendon Ponds Park, New York, USA; (8) Strait Creek Prairie Bluffs Preserve, Ohio, USA; (9) Royal Botanical Gardens, Ontario, Canada; (10) Gifford Pinchot State Park, Pennsylvania, USA; (11) Huckleberry Trail, Virginia, USA; and (12) Fisher Mountain, West Virginia, USA (Appendix 1). These collections yielded substantially higher DNA quality than the samples obtained...
from the Kentucky population used in microsatellite isolation. Consequently, we chose to use the more recent collections of *Trichophorum planifolium* for further testing of the microsatellite loci. Voucher specimens were obtained for each population, except when population size was estimated to be below 100 individuals, or if permits did not allow destructive sampling. For sites where a voucher was not collected, a representative voucher has been assigned if available. The deposition of vouchers is provided in Appendix 1.

Reactions were carried out in 12.5-μL reaction volumes containing 2.5 μL 5× Phusion High-Fidelity Buffer (New England Biolabs, Whitby, Ontario, Canada), 0.25 μL dNTPs (10 mM), 0.625 μL untagged primer (10 mM), 0.0625 μL tagged primer (10 mM), 0.5625 μL dye-labeled CAG Tag (10 mM), 0.375 μL DMSO, 0.125 μL Phusion High-Fidelity Polymerase (2 U/μL, New England Biolabs), 5.5 μL ddH₂O, and 2.5 μL DNA (10 ng/μL) using a T-100 Thermal Cycler (Bio-Rad, Hercules, California, USA). To obtain high-quality amplification product, we used the thermocycling profile of touchdown PCR (TD-PCR) (Korbie and Mattick, 2008) with some modifications. Thermal cycling began with a 5-min denaturation at 95°C; followed by the touchdown phase with 15 cycles of 30 s denaturation at 95°C, 30 s annealing from 57°C to 72°C (−1°C per cycle), and 30 s elongation at 72°C; followed by a generic amplification stage of 20 cycles of 30 s denaturation at 95°C, 30 s annealing at 57°C, and 30 s elongation at 72°C; followed by a 5-min final elongation at 72°C. Amplification products with incorporated fluorescent labels (6-FAM and VIC; Life Technologies) were pooled into groups of four and sequenced by capillary electrophoresis using a 3130xl Genetic Analyzer (Life Technologies) and verified with manual scoring. Standard measures of intra- and inter-population genetic diversity including average number of alleles (*Hо*) and the observed heterozygosity (*Hо*) and expected heterozygosity (*Hₑ*) were calculated with the R package ‘adegenet’ version 1.4-2 (Jombart, 2008) and ‘PopGenReport’ version 2.11 (Adamack and Gruber, 2014) (Table 2). Of the 96 samples initially screened, 16 failed at one or more loci and were excluded from the population genetic analysis. In total, 31 alleles were observed for 11 microsatellite loci in 80 individuals from 12 populations of *Trichophorum planifolium*. The number of alleles per locus ranged from two to six (overall mean = 2.82 alleles), with the highest number detected in the population at Fisher Mountain, West Virginia. The mean *Hо* per site ranged from 0.00 and 0.06, whereas the mean *Hₑ* varied between 0.00 and 0.19. Although these values are low, they are comparable to those documented in other woodland sedges (e.g., *Carex breviculmis* R. Br. and *C. hebes* Nelmes; M’Baya et al., 2013), and may be explained by limited pollen and seed dispersal among populations (Crins, 1989).

CONCLUSIONS

The primer pairs developed in this study successfully amplified 11 polymorphic microsatellite loci in populations distributed across the species’ range and, as such, will be a useful tool with which to examine patterns of genetic diversity and differentiation in *Trichophorum planifolium*. An understanding of genetic variability and structure within the Canadian population, and between the Canadian and core populations in the United States, is necessary to guide the development of effective management and monitoring protocols for the species.

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http://www.bioone.org/loi/apps
### TABLE 2. Results of initial primer screening and genotyping in 80 individuals from 12 populations of *Trichophorum planifolium*.

| Locus     | Tarrywile Park, CT (n = 8) | Bare Mountain, MA (n = 6) | Dan’s Mountain, MD (n = 7) | Big Spring State Park, MO (n = 7) | Sutton Hollow, MO (n = 6) | Elmer G. Raymond Park, NH (n = 6) | Gifford Pinchot State Park, WA (n = 5) | Hackberry Trail, VA (n = 5) |
|-----------|-----------------------------|---------------------------|-----------------------------|-----------------------------------|--------------------|-----------------------------------|----------------------------------------|---------------------------|
| A         | A                           | A                         | A                           | A                                 | A                  | A                                 | A                                      | A                         |
| B         | B                           | B                         | B                           | B                                 | B                  | B                                 | B                                      | B                         |
| He        | 0.00                        | 0.00                      | 0.00                        | 0.00                              | 0.00              | 0.00                              | 0.00                                   | 0.00                      |
| Ho        | 0.00                        | 0.00                      | 0.00                        | 0.00                              | 0.00              | 0.00                              | 0.00                                   | 0.00                      |

**Notes:**
- **A** = number of alleles;
- **He** = expected heterozygosity;
- **Ho** = observed heterozygosity;
- **n** = number of individuals sampled.

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APPENDIX 1. Voucher information for *Trichophorum planifolium* populations used in this study.

| Locality                          | Geographic coordinates | Collection date | Collector(s)                     | Deposition |
|-----------------------------------|------------------------|-----------------|----------------------------------|------------|
| Tarrywile Park, CT, USA           | 41.37140, −73.45273    | May 23, 2014    | V. J. Nowell & T. W. Smith       | DAO        |
| Daniel Boone National Forest, KY, USA | 37.59063, −83.78146   | May 19, 2011    | Tyler W. Smith                   | EKY        |
| Bare Mountain, MA, USA            | 42.30170, −72.52946    | May 26, 2014    | V. J. Nowell & T. W. Smith       | DAO        |
| Dan’s Mountain, MD, USA           | 39.56760, −78.91190    | May 10, 2011    | P. M. Peterson, J. M. Saarela & C. Frye | US         |
| Big Spring State Park, MO, USA    | 36.94750, −90.99314    | May 9, 2014     | V. J. Nowell & T. W. Smith       | DAO        |
| Sutton Hollow, MO, USA            | 37.19962, −91.32581    | May 6, 2014     | V. J. Nowell & T. W. Smith       | DAO        |
| Elmer G. Raymond Park, NH, USA    | —                     | June 12, 2011   | J. Sachs, C. Kane, P. Hines & D. Allen | NHA        |
| Mendon Ponds Park, NY, USA        | 43.03012, −77.57369    | May 28, 2014    | V. J. Nowell & T. W. Smith       | DAO        |
| Strait Creek Prairie Bluffs Reserve, OH, USA | 39.05830, −83.38857 | May 11, 2014    | V. J. Nowell & T. W. Smith       | Edge of Appalachia |
| Royal Botanical Gardens, ON, Canada | —                     | June 7, 2000    | P. G. O’Hara                     | HAM        |
| Gifford Pinchot State Park, PA, USA | 40.07460, −76.90913   | May 17, 2014    | V. J. Nowell & T. W. Smith       | DAO        |
| Huckleberry Trail, VA, USA        | 37.18850, −80.41925    | May 14, 2014    | V. J. Nowell & T. W. Smith       | DAO        |
| Fisher Mountain, WV, USA          | 38.66417, −79.24028    | —               | —                                | —          |

*Coordinates for populations sampled in Ontario and New Hampshire have been omitted as *Trichophorum planifolium* is of conservation concern in these jurisdictions.

*Herbaria abbreviations are per Index Herbariorum (http://sweetgum.nybg.org/ih/), except for Edge of Appalachia, 4274 Waggoner Riffle Road, West Union, OH 45693, USA.*