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Development of Microsatellite Markers in the Hexaploid Aquatic Macrophyte, Myriophyllum spicatum (Haloragaceae)\(^1\)

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- Premise of the study: We developed microsatellite primers to investigate genetic diversity and population genetic structure of the cosmopolitan submerged plant Myriophyllum spicatum.
- Methods and Results: Twenty microsatellite loci were identified in *M. spicatum* using the microsatellite-enriched library method. The numbers of alleles per locus ranged from one to 13, and the expected heterozygosity varied from 0 to 0.873 with a mean of 0.504 in two Chinese populations of *M. spicatum*. All of the loci were also found to be amplifiable in the related species *M. verticillatum* and *M. sibiricum*.
- Conclusions: The results indicate that these markers will be significant for studies of population genetic structure and evolutionary history of *M. spicatum* as well as some of its related species.

Key words: Haloragaceae; microsatellite markers; Myriophyllum spicatum; polyploid; population genetic structure.

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Eurasian watermilfoil (*Myriophyllum spicatum* L.) is a perennial submerged macrophyte native to Europe, Asia, and northern Africa (Couch and Nelson, 1985). In North America, *M. spicatum* has been recognized as a noxious invasive plant mainly due to the rapid spread of this species (Reed, 1977; Jacono and Richerson, 2003). *Myriophyllum spicatum* is hexaploid, and the chromosome number (2*n* = 6x = 42) was reported for plants from Europe and North America (Löve, 1961; Aiken et al., 1979), whereas there was no report about the polyploid types of *M. spicatum* because its chromosomes were found to be too small to disclose morphological characteristics for karyotype analysis (Aiken, 1981). There have been numerous studies concerned with the ecology and management of *M. spicatum*, and only a few studies have revealed DNA sequence variation among different individuals (e.g., Moody and Les, 2007). No investigation has been carried out to examine genetic variation in *M. spicatum* at the population level; the evolutionary processes of this species are more likely distinctive due to its occurrence in exclusively aquatic habitats (Barrett et al., 1993). Therefore, we isolated 20 microsatellite markers from *M. spicatum* for use in investigations of genetic variation, population genetic structure, and evolutionary history of this cosmopolitan submerged species.

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METHODS AND RESULTS

Total genomic DNA was extracted from the dried leaves of one individual of *M. spicatum* sampled from the Tai Lake population (Appendix 1) using the DNAsecure Plant Kit (Tiangen Biotech, Beijing, China). A microsatellite-enriched library was developed following the protocol of Glenn and Schable (2005). The genomic DNA was digested into ~500-bp fragments with *RsaI* and *XmnI* (New England Biolabs, Ipswich, Massachusetts, USA) and ligated to the SuperSNX24 adapters (F: 5’-GGTAAAGGCCTAGCTAGCAGAATC-3’, R: 5’-pGATTTCCTAGCTAGGCCTTTAACA-3’). The digestion-ligation mixture was hybridized with 3’ biotinylated oligo probes (AC)\(_5\)/(AG)\(_5\) (ATG)\(_5\), and captured by Dynabeads M-280 streptavidin (Invitrogen, Dynal AS, Oslo, Norway) for enrichment of simple sequence repeat (SSR) sequences. The products were recovered by PCR amplification with the SuperSNX24 forward primer, ligated into the pEASY-T1 Simple Cloning Vector (Transgen, Beijing, China), and then transformed into competent cells of *E. coli*. Eighty-three positive clones were selected and sequenced with the ABI 3730XL DNA analyzer (Applied Biosystems, Foster City, California, USA). Forty-three clones, or approximately 50% of the positive clones, contained SSRs.

PCR primers were designed for all 43 sequences using the program Primer Premier 5.0 (PREMIER BioSoft International, Palo Alto, California, USA) and evaluated in 20 individuals from different populations of *M. spicatum* (Appendix 1). Twenty pairs of primers (Table 1) that showed single and clear bands were chosen and labeled with the fluorescent dyes 6-FAM, HEX, or ROX. Characterization of the SSR loci was estimated in two distant populations in China (Bosten Lake population and Liangzi Lake population; Appendix 1), each with 20 individuals. PCR amplifications were performed in 15 μL total volume containing ~50 ng genomic DNA, 0.33 μM of each primer, and 1× PCR Mix (Tiangen Biotech). Microsatellites were amplified under the following conditions: 5 min initial denaturation at 94°C; 35 cycles of 30 s at 94°C, 30 s at 52–60°C (Table 1), and 1 min at 72°C; and a final extension at 72°C for 10 min. PCR products were analyzed on the ABI 3730XL and genotyping was performed using GeneMapper version 4.0 software (Applied Biosystems).

Because *M. spicatum* is hexaploid, up to six alleles per locus should be expressed in one single plant. Of all 20 loci, however, most showed no more than four alleles per individual (Table 2); no reliable explanation could be provided for this considering that the inheritance pattern of *M. spicatum* was ambiguous. The allele dosage of partial heterozygotes is difficult to identify, thus the presence/absence of the peaks was used to calculate the frequencies for Nei’s expected heterozygosity. The locus Mrynsp12 showed the highest polymorphism...
with 13 alleles in the Liangzi Lake population, whereas Myrsp17 and Myrsp20 were monomorphic in both populations. The expected heterozygosity ranged from 0 to 0.873 with a mean of 0.407 and 0.601 in the two populations, respectively (Table 2).

Cross-species amplification was conducted in M. verticillatum L. (10 individuals, Appendix 1) and M. spicatum Kom. (20 individuals, Appendix 1), both of which are in the same section of Myriophyllum as M. spicatum (Moody and Les, 2010). All of the loci were amplified successfully in these two related species.

**CONCLUSIONS**

The polymorphism observed for the microsatellite loci we isolated is high enough to support genetic studies in M. spicatum. Cross-species amplification also reveals that these markers are suitable to use in two related species. We conclude that these primers will facilitate the investigation of genetic diversity, population structure, and evolutionary history of M. spicatum as well as some of its related species.

**LITERATURE CITED**

Aiken, S. G. 1981. A conspectus of Myriophyllum (Haloragaceae) in North America. Brittonia 33: 57–69.

Aiken, S. G., P. R. Newrath, and I. Wolfe. 1979. The biology of Canadian weeds. 34. Myriophyllum spicatum L. Canadian Journal of Plant Science 59: 201–215.

Barrett, S. C. H., C. G. Eckert, and B. C. Husband. 1993. Evolutionary processes in aquatic plant populations. Aquatic Botany 44: 105–145.

Couch, R., and E. Nelson. 1985. Myriophyllum spicatum in North America. In L. W. J. Anderson [ed.], Proceedings of the First International Symposium on watermilfoil (Myriophyllum spicatum) and related Haloragaceae species, 8–18. Aquatic Plant Management Society, Vicksburg, Mississippi, USA.

Glenn, T. C., and N. A. Scharle. 2005. Isolating microsatellite DNA loci. Methods in Enzymology 395: 202–222.

Jacono, C. C., and M. M. Richerson. 2003. Myriophyllum spicatum L. nonindigenous aquatic species. Website: http://nas.er.usgs.gov/taxgroup/plants/docs/my_spica.html [accessed 7 May 2012].

Love, A. 1961. Some notes on Myriophyllum spicatum. Rhodora 63: 139–145.
TABLE 2. Results of initial primer screening in two populations of *Myriophyllum spicatum*.

| Locus   | \( A_m \) | \( A \) | \( H_e \) | \( A \) | \( H_e \) |
|---------|-----------|--------|--------|--------|--------|
| Myrsp1  | 3         | 4      | 0.661  | 3      | 0.591  |
| Myrsp2  | 3         | 3      | 0.594  | 9      | 0.847  |
| Myrsp3  | 2         | 1      | 0      | 5      | 0.417  |
| Myrsp4  | 4         | 2      | 0.496  | 10     | 0.782  |
| Myrsp5  | 5         | 4      | 0.703  | 8      | 0.817  |
| Myrsp6  | 3         | 5      | 0.568  | 4      | 0.681  |
| Myrsp7  | 2         | 1      | 0      | 5      | 0.687  |
| Myrsp8  | 2         | 2      | 0.496  | 2      | 0.496  |
| Myrsp9  | 3         | 1      | 0      | 8      | 0.716  |
| Myrsp10 | 2         | 4      | 0.543  | 4      | 0.543  |
| Myrsp11 | 2         | 1      | 0      | 2      | 0.466  |
| Myrsp12 | 4         | 3      | 0.667  | 13     | 0.873  |
| Myrsp13 | 3         | 2      | 0.496  | 3      | 0.631  |
| Myrsp14 | 4         | 4      | 0.543  | 10     | 0.773  |
| Myrsp15 | 4         | 4      | 0.543  | 10     | 0.784  |
| Myrsp16 | 4         | 3      | 0.665  | 4      | 0.689  |
| Myrsp17 | 1         | 1      | 0      | 1      | 0      |
| Myrsp18 | 2         | 3      | 0.665  | 4      | 0.727  |
| Myrsp19 | 2         | 2      | 0.496  | 2      | 0.496  |
| Myrsp20 | 1         | 1      | 0      | 1      | 0      |
| Mean    | 3         | 2.55   | 0.407  | 5.4    | 0.601  |

**Note:** \( A = \) number of alleles; \( A_m = \) maximum allele number per individual; \( H_e = \) expected heterozygosity.

**APPENDIX 1.** Geographic location and voucher information of *Myriophyllum* populations in this study. All voucher specimens are deposited at the Wuhan University Herbarium (WH).

| Species     | Population | Location       | Geographic coordinates       | Voucher no. |
|-------------|------------|----------------|-----------------------------|-------------|
| *M. spicatum* | FY         | Fuyang, Zhejiang | 29°59'40"N, 119°41'40"E | Xu et al., 1051 |
| *M. spicatum* | TJ         | Tongjiang, Heilongjiang | 47°30'06"N, 133°05'10"E | Xu et al., 201 |
| *M. spicatum* | BM         | Bomu, Tibet      | 29°54'58"N, 95°38'05"E     | Xu et al., 2464 |
| *M. spicatum* | Tai Lake   | Suzhou, Jiangsu  | 31°13'22"N, 120°26'46"E    | Xu et al., 1017 |
| *M. spicatum* | Liangzi Lake | Ezhou, Hubei    | 30°15'30"N, 114°33'30"E    | Xu et al., 2616 |
| *M. spicatum* | Bosten Lake | Bohu, Xinjiang  | 41°54'24"N, 86°43'53"E     | Xu et al., 2570 |
| *M. verticillatum* | Xinkai Lake | Mishan, Heilongjiang | 45°20'43"N, 132°22'16"E  | Xu et al., 137 |
| *M. sibiricum* | DQ         | Deqin, Yunnan   | 28°30'22"N, 98°54'41"E     | Xu et al., 2450 |