Isolation of Polymorphic Microsatellite Loci in the New Zealand Endemic Sand-Binder, Ficinia spiralis (Cyperaceae)

Authors: Heugten, Rachel A. van, Hale, Marie L., Bryan, Stacey, Griensven, Bart van, Satter, Sophia, et. al.

Source: Applications in Plant Sciences, 5(9)

Published By: Botanical Society of America

URL: https://doi.org/10.3732/apps.1700039
Isolation of polymorphic microsatellite loci in the New Zealand endemic sand-binder, Ficinia spiralis (Cyperaceae)  

Rachel A. van Heugten, Marie L. Hale, Stacey Bryan, Bart van Griensven, Sophia Satter, Lily Brailsford, and Hannah L. Buckley

Abstract: Ficinia spiralis (A. Rich.) Muasya & de Lange (Cyperaceae), otherwise known as pīngao, pikao, or golden sand sedge, is a sand-binding sedge that occurs on the foredunes of New Zealand sandy beaches (Bergin and Herbert, 1998). Small remnants and numerous human-planted restoration populations are all that remain of this previously widespread plant (Courtney, 1983; Bergin, 2011). Despite significant effort being put into conservation and restoration activities, this species is currently ranked as “Declining” in the New Zealand Threat Classification System (de Lange et al., 2013). Pīngao is of great cultural and ecological value and significance for New Zealand because it provides habitat for other native sand dune specialists and is an important weaving fiber, being highly valued for its beautiful golden color when dry. It exhibits pronounced morphological, physiological, and ecological variation observed in this species. Ficinia spiralis Microsatellite primers were developed in F. spiralis to investigate how population genetic structure is related to the pronounced morphological, physiological, and ecological variation observed in this species.

Key words: Cyperaceae; Ficinia nodosa; Ficinia spiralis; pīngao; polyploidy; microsatellites.

Methods and Results: A 454 shotgun-sequencing approach was used to generate 157,274 raw sequence reads, 536 of which contained microsatellites. After initial primer testing for 40 loci, 14 polymorphic loci were isolated, containing five to 27 alleles per locus. Ten of these loci also amplified in a congener, F. nodosa.

Conclusions: These loci will enable the assessment of the population genetic structure of F. spiralis, improving our understanding of the population processes underlying the observed morphological, physiological, and ecological variation in this endemic species. As the first microsatellites developed in Ficinia, these loci are a valuable resource for population genetic studies within this genus.

Key words: Cyperaceae; Ficinia nodosa; Ficinia spiralis; pīngao; polyploidy; microsatellites.

Applications in Plant Sciences 2017 5(9): 1700039; http://www.bioone.org/loi/apps © 2017 van Heugten et al. Published by the Botanical Society of America. This is an open access article distributed under the terms of the Creative Commons Attribution License (CC-BY-NC-SA 4.0), which permits unrestricted noncommercial use and redistribution provided that the original author and source are credited and the new work is distributed under the same license as the original.
Ficinia spiralis (Cyperaceae) is a declining, endemic species of great cultural and ecological value to New Zealand. Here, we describe one monomorphic and 14 polymorphic microsatellite loci that will enable us to assess genetic variation across the geographic range of this species, and to determine whether this variation correlates with morphological and/or physiological variation. Ten of these loci also amplified in F. nodosa, suggesting these loci may be useful for assessing genetic variation and the chance of polymorphism while minimizing the likelihood of excessive stutter. Fourteen di-, 15 tri-, six tetra-, two penta-, and three hexanucleotide repeat regions were chosen for the forward primer. The forward primer for each locus was appended with an M13 sequence (5′-TGTAAAACGACGGCCAGT-3′) at the 5′ end to allow the use of universal labeled primers (Schuelke, 2000), and PG-tails (5′-GGTACCC-3′) attached to the forward primer. 

The primers (Invitrogen, Carlsbad, California, USA) were initially tested for amplification against four F. spiralis individuals from two sample sites. PCRs used the following protocol: 15 μL reactions containing 1 μL of template DNA, 1× PCR buffer (Bioline, London, United Kingdom), 2 mM MgCl₂, 0.08 mM dNTPs, 0.33 μM reverse primer, 0.33 μM fluorescent M13 tag (Applied Biosystems, Carlsbad, California, USA) using an additive M13 sequence (5′-TGTAAAACGACGGCCAGT-3′) at the 5′ end to reduce incomplete A-addition during PCR (Brownstein et al., 1996).

The primers (Invitrogen, Carlsbad, California, USA) were initially tested for amplification against four F. spiralis individuals from two sample sites. PCRs used the following protocol: 15 μL reactions containing 1 μL of template DNA, 1× PCR buffer (Bioline, London, United Kingdom), 2 mM MgCl₂, 0.08 mM dNTPs, 0.33 μM reverse primer, 0.33 μM fluorescent M13 tag (Applied Biosystems, Carlsbad, California, USA) using an additive M13 sequence (5′-TGTAAAACGACGGCCAGT-3′) at the 5′ end to reduce incomplete A-addition during PCR (Brownstein et al., 1996).

The primers (Invitrogen, Carlsbad, California, USA) were initially tested for amplification against four F. spiralis individuals from two sample sites. PCRs used the following protocol: 15 μL reactions containing 1 μL of template DNA, 1× PCR buffer (Bioline, London, United Kingdom), 2 mM MgCl₂, 0.08 mM dNTPs, 0.33 μM reverse primer, 0.33 μM fluorescent M13 tag (Applied Biosystems, Carlsbad, California, USA) using an additive M13 sequence (5′-TGTAAAACGACGGCCAGT-3′) at the 5′ end to reduce incomplete A-addition during PCR (Brownstein et al., 1996).

The primers (Invitrogen, Carlsbad, California, USA) were initially tested for amplification against four F. spiralis individuals from two sample sites. PCRs used the following protocol: 15 μL reactions containing 1 μL of template DNA, 1× PCR buffer (Bioline, London, United Kingdom), 2 mM MgCl₂, 0.08 mM dNTPs, 0.33 μM reverse primer, 0.33 μM fluorescent M13 tag (Applied Biosystems, Carlsbad, California, USA) using an additive M13 sequence (5′-TGTAAAACGACGGCCAGT-3′) at the 5′ end to reduce incomplete A-addition during PCR (Brownstein et al., 1996).

The primers (Invitrogen, Carlsbad, California, USA) were initially tested for amplification against four F. spiralis individuals from two sample sites. PCRs used the following protocol: 15 μL reactions containing 1 μL of template DNA, 1× PCR buffer (Bioline, London, United Kingdom), 2 mM MgCl₂, 0.08 mM dNTPs, 0.33 μM reverse primer, 0.33 μM fluorescent M13 tag (Applied Biosystems, Carlsbad, California, USA) using an additive M13 sequence (5′-TGTAAAACGACGGCCAGT-3′) at the 5′ end to reduce incomplete A-addition during PCR (Brownstein et al., 1996).
population structure in other species within this genus. Although the polyploidy of *F. spiralis* limits the range of population genetic analyses available to those that can accommodate dominant data, numerous other studies have used similar data to investigate population structure and how it relates to other factors such as the environment (i.e., Brito et al., 2016; Wu et al., 2016). The high number of alleles at many of these loci should provide sufficient information for the assessment of fine-scale genetic structure throughout the species’ range.

### LITERATURE CITED

BERGIN, D. O. 2011. Pingao golden sand sedge: Ecology distribution and habitat. Dune Restoration Trust of New Zealand Technical Article No. 73. Coastal Restoration Trust of New Zealand, Wellington, New Zealand.

BERGIN, D. O., AND J. W. HERBERT. 1998. Pingao on coastal sand dunes. Coastal Dune Vegetation Network Technical Bulletin No. 1. New Zealand Forest Research Institute, Rotorua, New Zealand.

| Locus | Kaitorete (n = 20) A | Matakana (n = 30) A | New Brighton (n = 29) A | Tumbledown Bay (n = 21) A | Total (n = 100) A |
|-------|------------------|------------------|------------------|------------------|------------------|
| FSP1  | 7                | 1.000            | 13               | 1.000            | 16               |
| FSP2  | 5                | 0.875            | 9                | 1.000            | 11               |
| FSP4  | 10               | 0.950            | 8                | 1.000            | 11               |
| FSP6  | 11               | 0.950            | 21               | 0.818            | 14               |
| FSP7  | 4                | 0.308            | 4                | 0.964            | 1                |
| FSP11 | 8                | 0.950            | 12               | 1.000            | 8                |
| FSP16 | 6                | 1.000            | 10               | 1.000            | 3                |
| FSP21 | 2                | 0.235            | 4                | 0.966            | 1                |
| FSP29 | 7                | 1.000            | 4                | 0.138            | 8                |
| FSP30 | 4                | 0.600            | 4                | 0.793            | 5                |
| FSP44 | 4                | 0.950            | 4                | 0.778            | 3                |
| FSP45 | 7                | 0.842            | 9                | 0.750            | 7                |
| FSP50 | 4                | 0.850            | 9                | 0.967            | 5                |
| FSP51 | 5                | 0.833            | 5                | 0.929            | 6                |

Note: A = number of alleles; $H_o$ = observed heterozygosity; n = number of individuals sampled.

*Geographic coordinate locations of the populations are: Kaitorete Spit (−43.827°S, 172.655°E), Matakana Island (−37.563°S, 176.081°E), New Brighton (−43.523°S, 172.739°E), Tumbledown Bay (−43.852°S, 176.683°E). New Zealand Threat Classification Series 3. New Zealand Department of Conservation, Wellington, New Zealand."

### Table 3. Cross-amplification of 15 nucleic microsatellite loci developed for *Ficinia spiralis* in *F. nodosa* (n = 6).a

| Locus | A | Allele size range (bp)b |
|-------|---|-------------------------|
| FSP1  | 1 | 186                     |
| FSP2  | 1 | 243                     |
| FSP4  | 1 | 211                     |
| FSP6  | — | —                       |
| FSP7  | 1 | 238                     |
| FSP11 | 1 | 236                     |
| FSP16 | — | —                       |
| FSP19 | 2 | 219–229                 |
| FSP21 | 1 | 226                     |
| FSP29 | 1 | 256                     |
| FSP30 | — | —                       |
| FSP44 | 1 | 273                     |
| FSP50 | — | —                       |
| FSP51 | 2 | 203–259                 |

Note: A = number of alleles.

*aGeographic coordinates for the sample sites are: −43.486°S, 172.693°E and −36.956°S, 174.468°E.

*bFragment sizes include the M13 tag (5’TGTAAACGACGCGCCAGT-3’) attached to the forward primer.

BRITO, V. L. G., G. M. MORI, B. B. Z. VIGNA, M. AZEVEDO-SILVA, A. P. SOUZA, AND M. SIZIMA. 2016. Genetic structure and diversity of populations of polyploid *Tibouchina pulchra* Cogn. (Melastomataceae) under different environmental conditions in extremes of an elevation gradient. *Tree Genetics & Genomes* 12: 101.

BROWNSTEIN, M. J., J. D. CARPENT, AND J. R. SMITH. 1996. Modulation of nontemplated nucleotide addition by Taq DNA polymerase: Primer modifications that facilitate genotyping. *Biotechniques* 20: 1004–1010.

CLARK, L. V., AND M. JASHEK. 2011. POLYSAT: An R package for polyploid microsatellite analysis. *Molecular Ecology Resources* 11: 562–566.

CLARK, L. V., AND A. D. SCHEEERER. 2017. Resolving microsatellite genotype ambiguity in populations of allopolyploid and diploidized autopolyploid organisms using negative correlations between allelic variables. *Molecular Ecology Resources* doi:10.1111/1755-0998.12639.

COURTNEY, S. P. 1983. Aspects of the ecology of Desmoschoenus spiralis (A. Rich.) Hook. f. M.Sc. thesis, University of Canterbury, Canterbury, New Zealand.

DE LANGE, P., J. ROFFE, P. CHAMPION, S. COURTNEY, P. HEENAN, J. BARKLA, E. CARROLL, ET AL. 2013. Conservation status of New Zealand indigenous vascular plants. 2012. New Zealand Threat Classification Series 3. New Zealand Department of Conservation, Wellington, New Zealand.

FAIRCLOTH, B. C. 2008. MSATCOMMANDER: Detection of microsatellite repeat arrays and automated, locus-specific primer design. *Molecular Ecology Resources* 8: 92–94.

KORESSAAR, T., AND M. REMM. 2007. Enhancements and modifications of primer design program Primer3. *Bioinformatics (Oxford, England)* 23: 1289–1291.

R C ORE TEAM. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Website http://www.R-project.org/ [accessed 18 August 2017].

SCHULKIE, M. 2000. An economic method for the fluorescent labelling of PCR fragments. *Nature Biotechnology* 18: 233–234.

UNTERGAESSER, A., I. CUTICUTACHE, T. KORESSAAR, J. YE, B. C. F A IRCLOTH, M. REMM, AND S. G. ROZEN. 2012. Primer3—New capabilities and interfaces. *Nucleic Acids Research* 40: e115.

WEBING, K., H. NVRHOM, K. WOLFE, AND W. MEYER. 1995. DNA fingerprinting in plants and fungi. CRC Press, Boca Raton, Florida, USA.

WU, Z., D. YU, X. LI, AND X. XU. 2016. Influence of geography and environment on patterns of genetic differentiation in a widespread submerged macrophyte, Eurasian watermilfoil (Myriophyllum spicatum L., Haloragaceae). *Ecology and Evolution* 6: 460–468.

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