MICROSATELLITE MARKERS FOR STUDIES WITH THE CARNIVOROUS PLANT *PHILCOXIA MINENSIS* (PLANTAGINACEAE)

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- *Premise of the study:* Microsatellite markers were developed for the critically endangered carnivorous species *Philcoxia minensis* (Plantaginaceae) for further population genetic studies aiming at its conservation.
- *Methods and Results:* We identified 29 clones containing 40 microsatellites from a genomic enriched library. A total of 27 primer pairs were developed and evaluated in 30 individuals of a natural *P. minensis* population. Seventeen markers successfully presented amplification products within the expected size range, of which 12 were polymorphic. The expected and observed heterozygosities ranged from 0.03 to 0.65 and from 0.00 to 0.77, respectively. Positive transferability with the related species *P. bahiensis* was observed for the same 17 markers.
- *Conclusions:* The 12 polymorphic microsatellite markers are suitable for studies in genetic diversity and structure, mating system, and gene flow in *P. minensis* and also may be useful for similar issues regarding the related species *P. bahiensis*.

**Key words:** conservation genetics; Espinhaço Range; Gratioleae; *Philcoxia minensis*; Plantaginaceae; simple sequence repeat (SSR).

*Philcoxia* T. P. & V. C. Souza is a carnivorous plant genus, endemic to the Brazilian cerrado and caatinga biomes (Pereira et al., 2012; Carvalho and Queiroz, 2014). The genus is characterized by terrestrial herbs with underground stems and leaves under or on the soil surface. It comprises five species: *P. bahiensis* V. C. Souza & Harley and *P. tuberosa* M. L. S. Carvalho & L. P. Queiroz, from Bahia; *P. goiásensis* P. Taylor, from Goiás; *P. minensis* V. C. Souza & Giul., from Minas Gerais (Taylor et al., 2000); and a newly described species (Scatigna et al., unpublished), also from Minas Gerais. Only a small population of *P. minensis* is known from a sand patch at the Serra do Cabral State Park, in Minas Gerais State (Souza and Giulietti, 2009). Due to its rarity and the vulnerability of the formation where it occurs, this species could be treated as critically endangered. In addition, very little is known about the general biology of *P. minensis*. One important step for studies supporting its conservation is the characterization of its genetic diversity and structure, gene flow, breeding ratio of populations, and mating system.

The use of microsatellite markers for population genetics and biodiversity conservation has increased in the past decade (Ouborg et al., 2010) and is now a well-established technique. Therefore, the purpose of this study was to develop microsatellite markers or simple sequence repeats (SSR), aiming to elucidate questions concerning aspects of *P. minensis* biology, such as its genetic structure, variability, and mating system.

**METHODS AND RESULTS**

A silica gel–dried inflorescence of a single individual of *P. minensis* was used in the initial DNA extraction, using the Nucleospin Plant II Kit (Macherey-Nagel, Düren, Germany), following the manufacturer’s protocol. A voucher specimen of that individual (Scatigna, A. V. 42) was deposited at the UEC herbarium at the Universidade Estadual de Campinas, Brazil. The inflorescence of *Philcoxia* individuals is used for DNA extraction to (1) avoid killing the plant, as the leaves are situated on or below the soil surface; and (2) prevent contamination of the template DNA by the abundant nematodes stuck on the leaf surfaces. A microsatellite-enriched genomic library was constructed following the protocol proposed by Billotte et al. (1999), with slight modifications. Genomic DNA was digested with *AfaI* enzyme (Invitrogen, Carlsbad, California, USA) for 3 h incubation at 37°C and ligated to the double-stranded adapters 5′-CTCTTGCTTACGCGTGGACTA-3′ and 5′-TAGTCCACCGCTAAGCA-AGAGCACA-3′ for 2 h incubation at 20°C. Enrichment was performed using a hybridization-based capture with (CT)8 and (GT)8 biotin-linked probes and streptavidin-coated magnetic beads (MagneSphere Magnetic Separation Products, Promega Corporation, Madison, Wisconsin, USA). The enriched fragments were amplified by PCR, and the amplification products were cloned into pGEM-T Easy Vector (Promega Corporation). Competent XL1-Blue Escherichia coli (Stratagene, Agilent Technologies, Santa Clara, California, USA) were transformed with the recombinant plasmids and cultivated on agar medium containing ampicillin and 100 μg/mL of X-galactosidase. Eighty recombinant colonies were selected using blue/white screening and sequenced in an automated ABI 3500xl Genetic Analyzer (Perkin Elmer–Applied Biosystems,

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