Utilizing DNA barcoding to conserve Canada's endangered populations of red mulberry (Morus rubra L., Moraceae)

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Background: Red mulberry is a wind-pollinated tree species that is distributed throughout eastern North America. In Canada, previous studies show that hybridization with the introduced white mulberry (M. alba L.) is leading to genetic assimilation of the remaining red mulberry populations. Due to a lack of diagnostic morphological characters, the confirmation of red, white, and hybrid mulberry remains an elusive tool for the conservation management of this endangered tree species. This is mostly due to costly, molecular-based tools that are required to genetically identify red and white mulberry for preservation and removal, respectively. Results: In August of 2014, we collected 61 putative red, white, and hybrid mulberry from two National Park sites in southern Ontario, Canada: Middle Island and Point Pelee. To circumvent costly and time-consuming sequence analysis to evaluate the frequency of hybrids at each site, we developed speciesspecific amplification (SS-PCR) of the nuclear ITS2 barcode region. Using two primer pairs, we were able to obtain consistent SS-PCR products that generated products of two sizes: 313 bp for M. rubra and 174 bp for M. alba. Hybrids yielded both products. Furthermore, we found 17 samples (27.9%) were M. rubra, 21 (34.4%) were M. alba, and 23 (37.7%) were hybrids. To confirm these results as well as determine the maternal parentage of hybrids, the rbcL barcode gene region was sequenced for all samples. All species identifications were confirmed; among the 23 samples identified as hybrids, 20 (87.0%) of them share the red mulberry rbcL haplotype, while 3 (13%) share the rbcL haplotype with white mulberry. Significance: Collectively, our results not only demonstrate an efficient use of DNA barcoding to identify the red, white, and hybrid mulberries in Canada, but also confirm previous studies that suggest hybridization between red and white mulberry is bidirectional but asymmetric, and likely continues to be a threat to remaining populations.