

Characterization and microsatellite marker development for a common bark and ambrosia beetle associate, *Geosmithia obscura*

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Abstract

Background. Symbioses between *Geosmithia* fungi and wood-boring and bark beetles seldom result in disease induction within the plant host. Yet exceptions exist such as *Geosmithia morbida*, the causal agent of Thousand Cankers Disease (TCD) of walnuts and wingnuts, and *Geosmithia* sp. 41, the causal agent of Foamy Bark Canker disease of oaks. Isolates of *G. obscura* were recovered from black walnut trees in eastern Tennessee and at least one isolate induced cankers following artificial inoculation. Due to the putative pathogenicity and lack of recovery of *G. obscura* from natural lesions, a molecular diagnostic screening tool was developed using microsatellite markers mined from the *G. obscura* genome. **Results.** A total of 3,256 candidate microsatellite markers were identified (2236, 789, 137 di-, tri-, and tetra- motifs were identified, respectively), with 2011, 703, 101 di-, tri-, and tetra- motifs containing markers with primers. From these, 75 microsatellite markers were randomly selected, screened, and optimized, resulting in 28 polymorphic markers that yielded single, consistently recovered bands which were used in downstream analyses. Five of these microsatellite markers were found to be specific to *G. obscura* and did not cross-amplify into other, closely related species. Although the remaining tested markers could be useful, they cross-amplified within different *Geosmithia* species, making them not reliable for *G. obscura* detection. **Conclusion.** Five novel microsatellite markers (GOBS9, GOBS10, GOBS41, GOBS43, GOBS50) were developed based on the *G. obscura* genome. These species-specific microsatellite markers are available as a tool for use in molecular diagnostics and can assist future surveillance studies.

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