

Using Restriction Enzyme Digests as a New Method for Species Identification in *Diorhabda* Species

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Diorhabda species (tamarisk leaf beetles) were introduced into the United States as a biological control agent for the invasive tamarisk species, with some populations successfully controlling the spread of tamarisk and others failing. It has been proposed that this disparity in efficacy of different populations could be tied directly to the species of each population. The identification of *Diorhabda* spp. through traditional methods is taxing on researcher time, budgets, and laboratory materials. This research has developed a new method for performing species identification of *Diorhabda* spp. that can be performed cheaper, faster, entirely in-house, and with less of an environmental impact than the traditional protocol.

Species identification is traditionally performed by Sanger sequencing of the mitochondrial gene, cytochrome c oxidase subunit 1 (COI), which costs \$17.76 per sample and takes 10-14 days to accomplish. Through this research, a method for species identification of the *Diorhabda* genus has been developed that utilizes restriction fragment length polymorphism (RFLP) to target nucleotide sequences within the COI gene that are unique to each species. By performing two restriction enzyme digests that target these non-conserved sites, a significant difference in DNA fragment lengths can be observed through gel electrophoresis that allows for positive species identification. This method allows species identification to be performed in as little as two days, and at a 72% reduction in cost (\$4.96 per sample) when compared to the traditional method.

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