

Novel Applications of iDNA Tool for Measuring Small Mammal Community Biodiversity via Silphid Beetles

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Abstract.—Environmental DNA (eDNA) and invertebrate DNA (iDNA) have become popular techniques in biodiversity surveys. Established iDNA sources to survey mammals include biting invertebrates, but *Silphidae* (carrion feeders) have yet to be reported in literature. These beetles could provide a less invasive tool to survey mammalian communities. To validate use of iDNA methods using silphids, fieldwork was conducted in Michigan Technological University's Ford Forest in the Northern Hardwood-Silviculture Experiment to Enhance Diversity (NH-SEED). Modified pitfall trapping was conducted and we successfully sequenced diet DNA from silphids, showing their potential as an iDNA tool. Additionally, trapping in NH-SEED showed a potential treatment selection by silphids in different harvesting methods and ground treatments, though no statistically significant results were determined in our preliminary data collection. Further studies are necessary to conduct more extensive sampling to determine silviculture treatment preferences and to further explore potential applications for silphids as an iDNA tool.

INTRODUCTION

Environmental DNA (eDNA) is a novel tool where DNA of plants, animals, or bacteria are extracted from environmental samples, such as soil or streams, to assay community-wide biodiversity. A subset of eDNA is invertebrate DNA (iDNA), where typically, vertebrate DNA is extracted from stomach contents of invertebrates to determine the vertebrate communities they feed upon. These sampling methods can provide an accurate representation of the vertebrate diet species composition (Andersen et al. 2012, Calvignac-Spencer et al. 2013). eDNA and iDNA samples provide information on the organisms in that environment or diet and are tools used in conservation biology without having to directly sample the target wildlife (Bohmann et al. 2013). The introduction of cutting edge genetic tools has shifted the focus from traditional trapping to these newer methods. With iDNA techniques, blood- or dung-feeding insects become a helpful tool for estimating wildlife diversity (Calvignac-Spencer et al. 2013). Methods have been developed to use dung beetles (Scarabaeidae: Coleoptera), or mosquitoes and flies (Diptera), or ticks (Ixodidae), and other blood-feeding invertebrate groups to study wildlife. Silphids (Silphidae: Coleoptera) are made up of two major subfamilies, carrion beetles (*Silphinae*) and burying beetles (*Nicrophorinae*), with carrion beetles being indiscriminate in host-carcass preference and burying beetles preferring small mammal carcasses to bury for reproduction and consumption. No research has yet focused on utilizing silphid beetles to extract small-mammal DNA for small mammal population studies. Silphids have been mentioned in reviews as a potential iDNA source, though no literature has explored the actual use of these species (Calvignac-Spencer et al. 2013).

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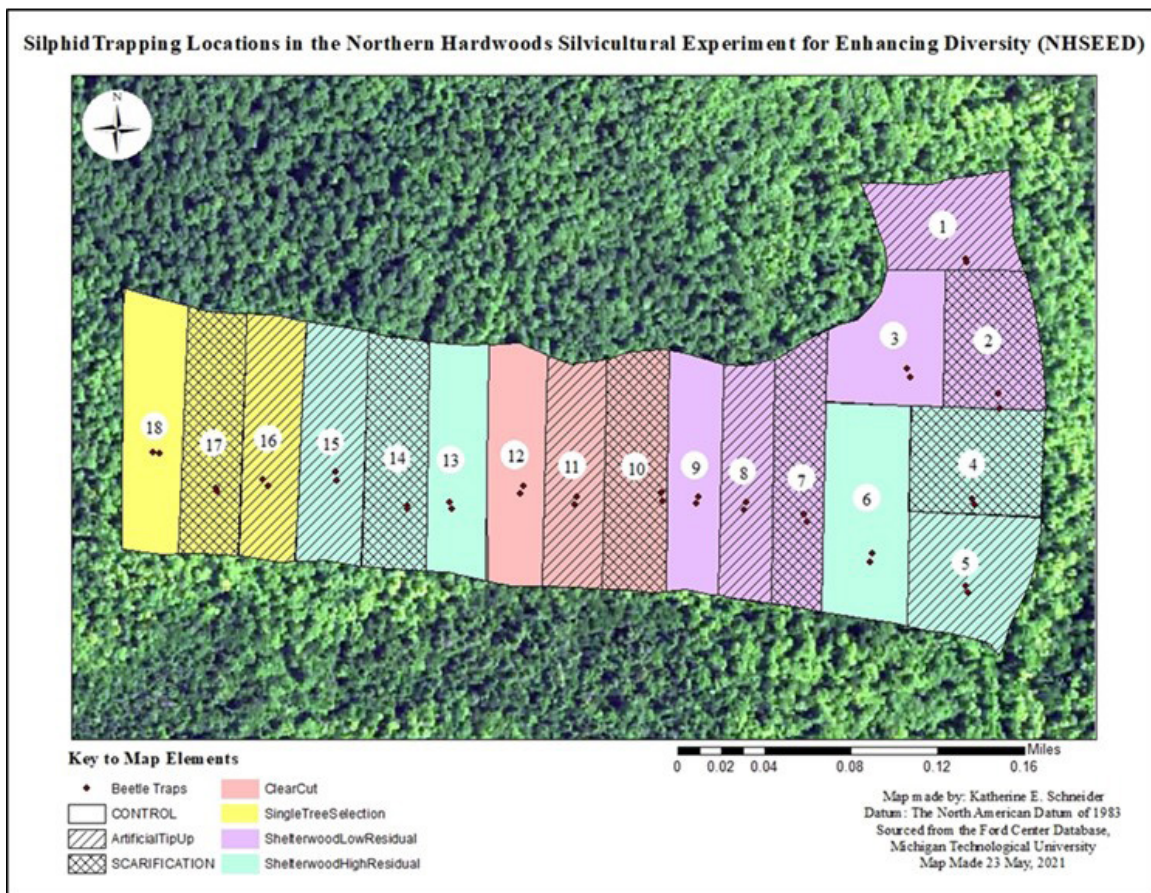


Figure 1.—Silphid trapping locations from 2019 in a portion of the Northern Hardwood Experiment to Enhance Diversity (NH-SEED) cutting trials in the Ford Forest, Alberta, MI.

Our research was focused on evaluating the utility of silphid beetles as an iDNA source, specifically looking at how useful these invertebrates may be for estimating small mammal community composition across varying silviculture practices.

PRELIMINARY FINDINGS

Silphid Beetle Trapping

To test how silphid diets and populations might be influenced by silviculture practices, pitfall traps were set in May through October 2019 in one-third (18 total) of the experimental treatment plots in the Northern Hardwoods-Silviculture Experiment to Enhance Diversity (NH-SEED) on Michigan Technological University's Ford Forest (Fig. 1). Treatment plots were a mix of clearcut, shelterwood, and selection systems with different ground-disturbance intensities (Hupperts et al. 2020). Each treatment plot had two traps installed. The pitfall traps were cups buried so that the opening was level to the soil's surface, with a lid placed loosely over them to prevent rainwater incursion and captured invertebrates from flying out; a classic method for sampling ground dwelling invertebrates (e.g., Niemälä et al. 1990). Bait was catfish bait and raw beef, with pinkie mice used in the last month of trapping. The 36 total traps were baited and monitored in six different sampling sessions over the summer lasting approximately 14 days each, checked weekly. We incorporated a sterilized two-tiered mesh layer in the trap prior to entering the field to separate the beetles from the bait, avoiding contamination as much as possible.

Traps were checked once a week after they were set and each trapped silphid beetle's exterior was swabbed in the field for a body sample and a fecal sample, then frozen for 3 weeks until a dissection was performed to remove stomach samples. For each of the sample types, DNA was extracted with a modified Qiagen (Hilden, Germany) Dneasy® blood and tissue extraction method, then diet DNA was amplified via a polymerase chain reaction (PCR) using the vertebrate primer pair 12S-V5F/R. Samples with a successful PCR, as determined by an agarose gel, were Sanger sequenced to identify the diet item. Of the 20 adult beetles sampled, five DNA sequences of mammalian diet items were successfully identified, including star-nosed mole (*Condylura cristata*), deer mouse (*Peromyscus maniculatus*), canid (*Canis spp.*), and cow (*Bos taurus*), with the cow likely due to bait contamination.

Future applications

For specific trapping locations, the average number of beetles trapped per each silvicultural treatment indicates trends for preference of ground and silviculture treatments. Previous studies have found more significant impacts of overall forest management regimes on ground dwelling beetle abundance and presence than on canopy density or tree species at sites (Werner and Raffe 2000). In our initial study here, more beetles were trapped in the single-tree selection systems that retained higher-basal areas, and in treatments with tip-ups compared to scarification. Though these are not statistically significant findings, we are continuing sampling efforts in 2021 and 2022. The preliminary data presented here paves the way for future data collection to continue with more intensive trapping and iDNA analysis throughout the full NH-SEED treatment and control area. Silphid iDNA sampling methods may be an important tool for monitoring mammals that are at risk or difficult to sample with traditional-trapping methods. Due to the importance of silphids as nutrient cyclers through their aid in the decomposition process and their beneficial role reducing fly species through carcass competition (Steele 1927), understanding their preferences for silviculture treatments can also contribute to management plans that consider a diversity of species. Additionally, use of iDNA to better understand diets can better elucidate how invertebrates, including burying beetles and others, and vertebrates, specifically small mammals, may be using different forest systems with variable silvicultural treatments. Silvicultural practices that affect silphids may be linked to a web of other organisms, leading to the development of management plans and practices that are more comprehensive regarding the biodiversity of species impacted.

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