Genetic Diversity of *Rhyacophila fuscula* (Walker) throughout the Upper Susquehanna River Catchment

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**ABSTRACT**

Caddisfly larvae are aquatic insects that can be found in eastern North America. Certain species of caddisflies can be used as bioindicators, such as *Rhyacophila fuscula* (Walker), due to their sensitivity to environmental changes. Populations with high genetic diversity are more likely to persist in a changing environment, so examining the diversity of populations is important for assessing population sustainability. The genetic diversity of *Rhyacophila fuscula* was examined by sequencing the gene Cytochrome Oxidase I from 240 individuals (20 individuals for all 12 sites). A high number of unique haplotypes were found, many with a frequency at the minimum detection level. This suggests that the actual level of genetic diversity is even higher than sampled.

**METHODS**

- Twenty larval *Rhyacophila* were collected from each of the 12 locations (Fig 1).
- Resampled Oneonta Creek in Jan 2023.
- Genomic DNA was extracted from muscle tissue using a Qiagen DNEasy extraction kit, according to the manufacturer’s instructions.
- Cytochrome Oxidase I was amplified using degenerated Folmer primers (Astrin & Stuben 2008) and the thermal cycler protocol of Astrin et al. (2016).
- PCR products were purified with Omega Bio-Tek NGS purification beads (2015) as per manufacturer’s instruction and were sequenced by Eurofins Genomics.
- Sequences were trimmed by hand and compared to those on GenBank using a nucleotide BLAST search
- Haplotype frequencies were calculated for each population.
- MrBayes was used to predict evolutionary relationships, this was visualized through FigTree.
- Bray-Curtis was calculated for each pair of locations to assess dissimilarity

**RESULTS**

A total of 255 individuals were successfully sequenced. The sizes of the individuals collected varied greatly (Fig 2). There were 67 unique haplotypes, where only one haplotype was shared among all locations (Fig 3). The resampling of Oneonta Creek revealed two more unique haplotypes. Many of the haplotypes had the minimum detection limit of 0.05. MrBayes showed approximately 10 clades of these haplotypes (Fig 4). Bray-Curtis showed that the range of dissimilarity is 0.3-0.95.

**DISCUSSION**

The size difference of these individuals calls into question whether this species of *Rhyacophila* is truly univoltine. This means that the “populations” sampled might be composites of multiple populations. Having such a high number of unique haplotypes at the minimum detection limit suggests that the full genetic diversity was not captured with the sample size. This would suggest that a much larger number of individuals should be sampled, though 20 is more than many current populations genetic studies in the literature. Bray Curtiss showed that there is a high degree of difference between sites, and it doesn’t immediately appear to be explained by geography.

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