

## Comparison of four species-delimitation methods applied to a DNA barcode data set of insect larvae for use in routine bioassessment

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### ABSTRACT

Species delimitation (grouping individuals into distinct taxonomic groups) is an essential part of evolutionary, conservation, and molecular ecology. Deoxyribonucleic acid (DNA) barcodes, short fragments of the cytochrome c oxidase subunit I (COI) gene, are being used in environmental bioassessments to assign specimens to putative species, but no method for delimiting DNA barcodes into species-level entities is universally accepted. We investigated the effect of delimitation methods on outcomes of bioassessments based on DNA barcodes. We used 2 tree-construction methods (neighbor joining [NJ], maximum likelihood [ML]) and 4 classes of species-delimitation criteria (distance-based, bootstrap support, reciprocal monophyly, and coalescentbased) with a DNA barcode data set consisting of 3 genera and 2202 COI sequences. We compared species delimitations for *Baetis* (Ephemeroptera:Baetidae), *Eukiefferiella* (Diptera:Chironomidae), and *Simulium* (Diptera: Simuliidae) from different streams. We assessed congruence among trees and compared species abundances and estimated species richness among methods. NJ followed by use of a standard barcoding distance cutoff (2%) yielded the greatest number of putative species. All other delimitation methods yielded similar, but lower, richness. Differences in species delimitations produced by various methods might have been caused by confounding factors, such as possible parthenogenesis in *Baetis* and rare haplotypes in abundant species of *Baetis* and *Simulium*. *Eukiefferiella* presented the fewest discrepancies among delimitations. Each method can be regarded as producing a separate line of evidence contributing to the delimitation of separately evolving lineages. The increased resolution offered by DNA barcoding can yield important insights into the natural history of organisms, but the power of these observations is limited without the use of multigene and multilocus data sets. Key words: DNA barcoding, bioassessment, biomonitoring, species delimitation, coalescent theory, cytochrome oxidase I, COI, benthic macroinvertebrates, GMYC

Full text:

[Species Delimitation.pdf](#)