

BUILDING DNA BARCODE REFERENCE LIBRARIES FOR BARK AND WOODBORERS

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ABSTRACT

DNA barcoding is a molecular diagnostic tool that utilizes a standardized segment of the mitochondrial gene, cytochrome c oxidase subunit 1 (COI), for species identification. Attributes of the COI gene, including: the absence of introns; rarity of indels; ease of amplification by polymerase chain reaction; availability of universal primers; and ease of alignment after sequencing are of benefit for the development of diagnostic systems for arthropods. In order to identify unknown specimens, one must first create reference libraries of COI sequences from reliably identified voucher specimens.

In 2008 we focused on developing a library of reference sequences for bark- and wood-borers (Buprestidae, Cerambycidae, Scolytinae, and Siricidae). Single legs were removed from recently collected specimens and the associated collection and identification data was extracted from the specimen labels and data-based. All tissue samples were submitted for extraction, amplification and sequencing to the DNA analysis platforms at the Ontario Biodiversity Institute (University of Guelph, Guelph, ON). The resultant COI sequences, associated sequencer trace files and primer sequences were uploaded into the online BOLD and the BOLD-MAS platforms (<http://www.boldsystems.org/views/login.php>) used to analyze the resultant sequences and

generate neighbor-joining trees. Preliminary results for selected taxa are presented.

To date more than 2,300 specimens from the target taxa have been sampled: 1,800 samples have been processed and 1,370 sequences >500 bp in length have been generated. An example of a neighbor-joining tree for Scolytinae generated by the BOLD analysis platform is presented to illustrate previously unrecognized divergences within taxa. Large intraspecific sequence divergence within specimens identified as *Trypodendron lineatum* suggests that recent morphological observations of the existence of more than one species within the current concept of “*T. lineatum*” are correct. Similar large intraspecific sequence divergences in *Xyleborinus saxesenii* suggest that it also may also be a species complex.

DNA barcoding provides a powerful tool for the identification of unknowns and, because COI is present in all life stages, will be of great utility in identifying immature life stages of bark and wood-borers. The ability to reliably identify immature life stages from regulatory interceptions will be of great benefit in the analysis of introduction pathways for nonindigenous species.