

DNA barcoding: an efficient means of identifying agricultural pests and biological control agents



Christa D. Maitland and Robert H. Hanner
Department of Integrative Biology, University of Guelph



INTRODUCTION

- Agricultural pests and biological control agents are of great economic and environmental significance. Ensuring that pest species are not spread and that biological control species are used responsibly are important endeavours.
- Identifying these species with any certainty often requires an expert taxonomist and can be a time consuming process, especially when immature specimens must be reared in the lab prior to identification.
- DNA barcoding provides an efficient, reliable means of identification by non-specialists. As an integral part of this process, we are establishing and testing a reference database of these species.

METHODS

- Insects and mites comprising pest species, control agents, host species and other insects, including unidentified specimens were collected from the field or sampled from previously existing collections.
- DNA was extracted from 1-3mm³ of tissue, using pieces of legs from larger specimens and whole organisms for tiny specimens. Silica-based Pall plates were used (1).
- DNA extracts were re-suspended in 30µL of dH₂O and a 658 base pair (bp) region from the 5' end of COI was amplified using LepF1/LepR1 primers and following standard protocols (2).
- Where amplification was not successful, internal mini-primers (MLepF1/LepR1 and LepF1/MLepR1) were used (3).
- Analyses were performed using the tools provided by the Barcode of Life Database (4). The Kimura 2Parameter distance model was used to calculate divergences. Sequences of <350bp or with >1% ambiguity were excluded from analysis.

RESULTS

- The primers designed for Lepidoptera yielded sequences for 80% of the samples. Several taxa failed to amplify, notably members of the families Psyllidae and Phlaeothripidae and the genera Myzus and Thrips, for which there were multiple representatives.
- Sequences with >350bp and <1% ambiguity were obtained for a total of 333 samples, including 69 known families and 58 known species. Many older samples yielded shorter sequences.
- DNA barcodes successfully delimited all 58 identified species, except within the genus *Villa*, where two species exhibit haplotype sharing (see Figures 1 and 5). The Kimura 2Parameter rankings tended to cluster together as expected (see Figure 5).

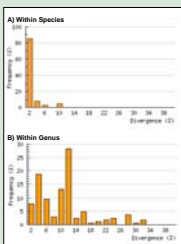


Figure 1: Distance summaries of samples included in analysis. All congeneric species exhibited divergences of at least 2% and are easily distinguished by barcodes, with the exception of two species in the genus *Villa* that exhibit haplotype sharing.

SPECIES IDENTIFICATION

- Unidentified specimens from a wide variety of taxonomic groups have been matched with those that have been expertly identified (See Figures 2 and 5).

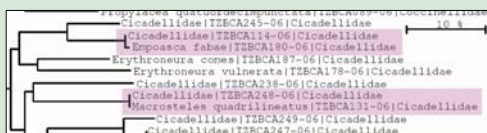


Figure 2: A section of NJ-tree (K2P) showing two cases where unknown Cicadellids share a barcode with expertly identified species (highlighted).

MULTIPLE LIFE STAGES

- Barcodes were successfully retrieved from both adult and immature specimens. The same species had similar barcodes, indicating that positive identifications can be made on eggs and immature specimens in the absence of adults by using barcoding, without the need to rear specimens in the lab.



Figure 3: NJ-tree (K2P) of adult and immature specimens from five different orders, showing that identifications can be made for eggs and immature specimens from a range of taxonomic groups through the use of barcodes.

CORRECTING TENTATIVE IDs

- Confusing larval stages lacking the identifying characteristics of adult specimens may be assigned a "best guess" identification that is later corrected when tested against reference barcodes.

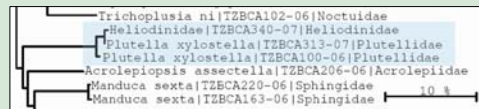


Figure 4: A section of NJ-tree (K2P) showing a caterpillar, originally thought to be a Heliodinid, sharing its barcode with a Plutellid species (highlighted).

CONCLUSIONS

- The ability to identify specimens using DNA barcodes is only as good as the reference database. Taxonomic experts are needed to classify organisms as the reference database is established.
- Although primers originally designed for Lepidoptera successfully amplified the majority of taxa, the failure of several taxonomic groups suggests the need for further primer development.
- However, the beginning of a reference database has been built and shown to be effective in classifying specimens from a wide variety of taxonomic groups.

REFERENCES

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Figure 5 (right): Neighbour-joining analysis (K2P distances) of DNA barcodes (>350bp and <1% ambiguous) of 333 insects and mites comprising pest species, control agents, host species and other insects, including unidentified specimens, with 69 families and 58 species identified.

