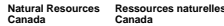


# DNA Barcoding of Pests and Invasive Species: Aphids, Adelgids and Scales

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## INTRODUCTION

- DNA barcoding promises rapid species identification using a standard methodology by non-taxonomic specialists. One of the areas in which such a technology could potentially have the greatest benefit to society is in the monitoring of harmful species, such as agricultural and forestry pests.
- The insect order Hemiptera (true bugs and their allies) contains many species of significant pest status for gardeners, agriculture and forestry, including the aphids (superfamily Aphidoidea), adelgids (superfamily Adelgoidea) and scale insects (superfamily Coccoidea).
- As part of the Canadian Barcode of Life Network, the Pests and Invasive Species Campaign aims to build a database of COI barcode sequences from species with significant impact as pests, currently with a main focus on North America.

## METHODS

- Specimens from the same clonal colony were kept for identification and voucher specimens. Usually, larvae were used for barcoding and the associated adults kept as vouchers.
- Specimens were preserved in ethanol and sent to BIO in matrix boxes.
- Individual animals were placed in proteinase K buffer for lysis, and total genomic DNA was extracted using the PALL Glass Fibre protocol (ref 1).
- The barcode region (658-bp fragment at the 5' end of the COI gene) was PCR-amplified using standard invertebrate primers, and sequenced using an ABI 3730 platform.
- Data analysis was carried out using SeqScape, BOLD (Barcode of Life Data Systems - ref 2) and MEGA software.

## RESULTS - GENERAL

- Full-length sequences have been generated from 1945 specimens, covering 543 species.
- A further 117 sequences were partial or mini-barcode sequences (398 bp).
- In some cases eggs or egg masses were able to yield sequences.



Figure 1 (above): Distribution map of hemipteran COI sequences currently deposited in BOLD within the Pests and Invasive Species Campaign.

## RESULTS – DIGITAL IMAGES



(a) CNCHEM040058 *Cinara* sp. – Aphididae, Lachninae



(b) CNCHEM040078 *Cinara* sp. – Aphididae, Lachninae



(c) CNCHEM056128 *Pemphigus* sp. – Aphididae, Entosomatinae



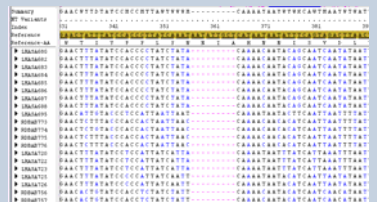
(d) CNCHEM56132 *Chaitophorus* sp. – Aphididae, Chaitophorinae

Figure 2 a-d (above): A selection of digital images of aphids taken through a dissecting microscope. These images are deposited on BOLD and serve as digital vouchers for the specimens after DNA extraction.

Figure 4 (right): A section of a COI sequence alignment showing a 9-nucleotide deletion in all 20 scale insect sequences examined in comparison with the reference sequence (*Aphis* sp.)

## RESULTS – SCALE INSECTS

- The scale insects (Coccoidea) are a superfamily within the Hemiptera comprising around 7,355 species worldwide. They are most important as pests of perennial plants and can cause serious damage to nut and fruit trees, forest vegetation, greenhouse plants, and house plants.
- Other researchers have reported great difficulty in sequencing COI from scales, and there are indications that they are considerably divergent in sequence from their nearest relatives (aphids); it appears that scales lack the standard primer sites used for PCR-amplification. All existing scale COI sequences are from a different region of the gene which does not overlap with the barcode region (ref 3).
- We were able to successfully generate COI sequences from scales using internal mini-barcode primers (3' end only).
- One unusual feature found is a deletion of nine nucleotides compared to the aphid reference sequence, seen in all of the scales examined in the same position around the middle of the sequenced region. Since this corresponds to three amino acids, it does not alter the subsequent translation frame, though it should result in a shorter protein.



## CONCLUSIONS/FUTURE DIRECTIONS

- We consider that these results represent a promising beginning for the use of DNA barcoding in the identification of pest species.
- The fact that DNA sequences may be generated, not only from adults, but also from larvae or even eggs, suggests that barcoding could play a significant role in the early detection of invasions, by allowing the identification of life-stages which cannot be distinguished by morphology.
- One group, the scale insects, proved a more difficult target for COI amplification and may require new primer design to reliably obtain longer sequences. The sequences which were obtained here suggest an unusual pattern of molecular evolution, and further work could yield significant insights into the reasons behind this.
- In addition, projects are under way to barcode Orthoptera (locusts, grasshoppers and crickets) and Coleoptera (bark beetles) as well as increasing our coverage of Hemiptera.

- A neighbour-joining tree was constructed from the 20 scale sequences. While not all specimens had species-level identifications the results showed families and genera grouping together as expected. The host plant from which each specimen was collected was also mapped on the tree.

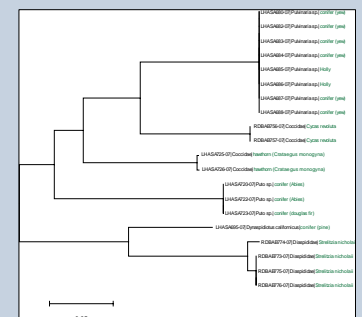


Figure 5 (above): Neighbour-joining tree, based on K2P distance, of 20 scale insect COI sequences. Host plant for each specimen is displayed in green.

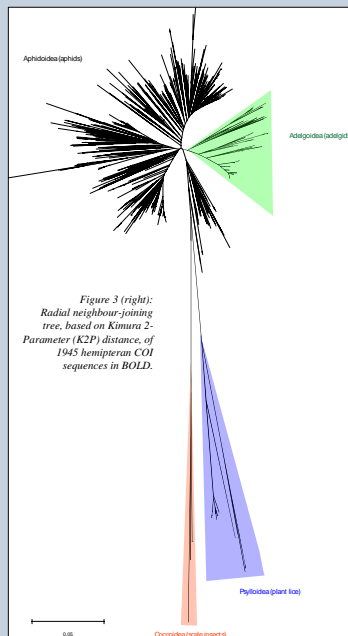


Figure 3 (right): Radial neighbour-joining tree, based on Kimura 2-Parameter (K2P) distance, of 1945 hemipteran COI sequences in BOLD.

- A neighbour joining tree analysing all hemipteran sequences simultaneously indicates the separation of the main groups. The scale insects appear to be the most divergent from the other groups, as indicated by long branches.

## REFERENCES

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