

THE ANTS OF NORTH AMERICA: A Flagship Taxon for CO1 DNA Barcoding

M. Alex Smith¹, Gary J. Umphrey², Brian L. Fisher³, Lloyd R. Davis⁴, and Paul D. N. Hebert¹

¹Biodiversity Institute of Ontario, University of Guelph, ²Department of Mathematics and Statistics, University of Guelph, ³California Academy of Sciences, San Francisco, CA, ⁴Gainesville, Florida



INTRODUCTION

- Ants (Hymenoptera: Formicidae) are important components of global ecosystems. In the tropics they can comprise up to 15% of the total animal biomass of an area. Temperate species richness is lower – but impact in ecosystems remains high (1).
- North American ant taxonomy is well developed and there is a strong contingent of ant taxonomists. Since the publication of the comprehensive “Ants of North America” by Creighton (2), many genera or species groups have been subsequently revised. But many taxonomic problems remain. Sibling species complexes are common, where subtle morphological differences between species are masked by complex patterns of intraspecific variation in morphological, ecological and behavioural characters.
- CO1 barcoding provides a rapid method for testing taxonomic hypotheses proposed in taxonomic revisions. At the same time, North American ant taxonomy is sufficiently mature to offer robust tests of the efficacy of barcoding for (i) identifying ant species and (ii) contributing to resolving taxonomic knots.
- The approximately 1000 recognized ant species in North America (north of Mexico) are classified into 73 genera and 10 subgenera.
- About 200 ant species reside in Canada. Some of the most common Canadian ants belong to taxonomically difficult genera (e.g., *Formica*, *Myrmica*).
- Most taxonomic keys to ants are restricted to the worker caste. Identification of less frequently encountered males and queens is often difficult even for specialists.
- As part of an on-going project to barcode the ants of North America using the cytochrome c oxidase subunit I (CO1), we present data on the identification of ant species from a subset of specimens collected throughout North America.
- We have CO1 DNA barcoded nearly 2000 ants from more than 300 ant species and present preliminary data here, for 180 species, regarding the efficacy of identification, variation within the CO1 barcode region and ease of amplification.

METHODS

- Specimens were collected from across North America. This effort is ongoing.
- Total genomic DNA was extracted from small pieces (≤1 mm long) of leg using the NucleoSpin® 96 Tissue kit (Macherey-Nagel Duren, Germany), following manufacturer’s protocols, or silica-based Pall plates (3).
- Extracts were re-suspended in 30 µl of dH2O, and a 658-base pair (bp) region near the 5’ terminus of the CO1 gene was amplified following standard protocol for capturing CO1 barcodes (for more detail see 4).
- When a species showed deep genetic divergences, more specimens were sequenced to provide a better understanding of the distribution of this variation and its relationship to morphology, geography and natural history.
- In the cases where CO1 barcode divergences were slight but there was evident morphological differentiation, ecological specialization, restricted sample size, we amplify the internal transcribed spacer region (ITS1) of the ribosomal RNA, and/or, the D2 expansion segment of the ribosomal large subunit (28S).



RESULTS

- Standard primers, developed for Lepidoptera, amplify the vast majority of the ant fauna. In cases where we suspect DNA degradation due to specimen preservation or age, internal primers can be used to generate overlapping shorter sequences.
- **BARCODE VARIATION**
- Inter-specific variation in the CO1 DNA barcode range from exceedingly low (e.g. within the *Formica* species complexes) to a maximum of 28% (Average = 16.35% SE = 0.173).
- Intra-specific variation is generally less than 2%. As with any taxon where alpha-taxonomy is difficult, or incomplete, this calculation must be taken as provisional.
- There is evident (and expected) inter-specific hybridisation within some species (see *Lasius* (*Acanthomyops*)).
- Sub-families are not monophyletic with CO1. This is likely due to substitution saturation.

CONCLUSIONS

- Ant species can often be distinguished only on minor morphological details. It now seems that some widespread species may actually represent multiple species.
- Barcoding these difficult to identify specimens will be a great help in both reducing synonymy and identifying cryptic species.
- Ant keys are most frequently based on the worker caste. CO1 barcoding has been helpful in linking the less frequently discovered sexuals with their worker.
- While we do not present data here regarding 28S or ITS1 divergences, these independent nuclear markers have been very useful in evaluating surprising (and shallow) or expected (and missing) intraspecific CO1 barcode divergences.
- However, due to length variation and complexities of alignment we do not consider these nuclear markers as a replacement for the CO1 barcode – but rather as a complementary tool.
- Many of the species complexes most commonly found in Canada (e.g. *Formica*, *Myrmica* and *Leptothorax*) are acknowledged need of taxonomic revision. DNA barcoding should be able to assist substantially in this effort. Our efforts with conventional taxonomic and DNA based species identification continue.
- Donations of specimens or tissue samples representing geographic range extensions are welcome.

Figure 1: Neighbour-joining tree of 180 specimens of North American ants representing nearly 20% of the North American ant fauna. Tree built using a single specimen per species where the CO1 barcode is greater than 600 bp long.



Figure 3: Map indicating collection locations for each of the 180 specimens detailed in Figure 1.



REFERENCES

- 1 Hölldobler B., Wilson E.O. (1990) *The Ants*. Belknap Press of Harvard University Press, Cambridge, MA. 732 pp
- 2 Creighton W.S. (1950) *The Ants of North America*. Bulletin of the Museum of Comparative Zoology 104. 585 p
- 3 Ivanova, N. V., DeWaard, J. R. & Hebert, P. D. N. (2006) *Mol. Ecol. Notes* 6, 998-1002.
- 4 Smith, M. A., Wood, D. M., Janzen, D. H., Hallwachs, W. & Hebert, P. D. N. (2007) *PNAS* 104, 4967-4972

ACKNOWLEDGEMENTS

This research was supported through funding to grants from the Gordon and Betty Moore Foundation, the Canada Research Chairs program, the Canadian Barcode of Life Research Network from Genome Canada through the Ontario Genomics Institute, NSERC and other sponsors listed at www.barcodinglife.org. We thank our many colleagues at Guelph, especially those at the Canadian Centre for DNA Barcoding and the Biodiversity Institute of Ontario. In particular, Taika von Königslöw and Kate Crosby have provided invaluable assistance in the lab. We thank April Noble for photography and ANTWEB (www.antweb.org) for permissions to use images.

For more information see Smith, M. A. et al (2007) *PNAS* 104, 4967-4972, Smith et al *Phil Trans B* (2005) 360, 1825-1834, or contact MAS at salex@uoguelph.ca

Figure 2: ABOVE – Examples of four North American ant species barcoded here. Location on the NJ tree is indicated with a yellow circle. From the top: *Neivamyrmex wilga*, *Cephalotes texanus*, *Formica rufa*, *Lasius niger*. Images courtesy of ANTWEB (www.antweb.org)