

RAPID ASSESSMENT OF ANT DIVERSITY IN A NORTHERN WORLD HERITAGE SITE USING DNA BARCODES

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INTRODUCTION

"Ant species can often be distinguished only on minor morphological details; it now seems that many remaining widespread "species" are in fact clusters of siblings." (1)

- As part of an on-going project to barcode the ants (Hymenoptera - Formicidae) of North America using the cytochrome c oxidase subunit I (CO1), we present data on the successful identification of ant species from a subset of specimens collected from a protected area in northern Canada.
- The ant fauna of the north is largely unknown and a DNA based system of identification would facilitate the identification of species areas of high biological diversity.
- Ants were collected from the first natural UNESCO-World Heritage site, the Nahanni National Park Reserve (NNPR) that lies within Taiga Cordillera and Taiga Shield ecozones at the southwestern NWT-Yukon border in Canada.
- The NNPR likely contains a high biological diversity due to the absence of glacial ice during the Wisconsinian glacial period, and a large number of highly specialized habitats.
- Mining development makes the NNPR one of the most threatened protected areas in Canada.

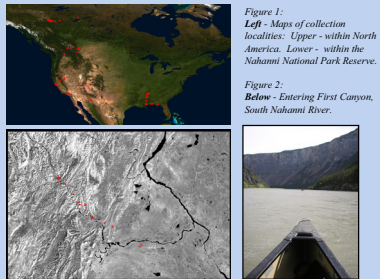


Figure 1: Left - Maps of collection localities. Upper - within North America. Lower - within the Nahanni National Park Reserve.

Figure 2: Entering First Canyon, South Nahanni River.

METHODS

- DNA extracts were prepared from small pieces of leg using the GeneElute DNA miniprep Kit (Sigma, St. Louis, Missouri, United States), following the manufacturer's protocols.
- DNA extracts were resuspended in 20 µl of dH₂O, and a 600-bp region near the 5' terminus of the COI gene was amplified using primers (LF1-LR1) following standard protocols (2).

- Composite sequences were generated using internal primer pairs (LF1-AMLR) and (MLF1-LR) when initial primers were not successful.
- Sequence divergences were calculated using the K2P distance model (3) and a NJ tree of distances (4) was created to provide a graphic representation of the among-species divergences using MEGA2 (5).

RESULTS

- PCR products were generated for 377 of 394 specimens (96%) from 104 species.
- PCR product greater than 500bp was generated for 303 individuals (81%). A 350 bp sequence was recovered for 74 (19%) of the remaining individuals.
- Avg. conspecific pairwise divergence = 1.9% (Fig. 3).
- Avg. congeneric pairwise divergence = 8.5 %.
- Provisional Species** (Shown in green on Fig. 4-A)
- 9% divergence between eastern and western populations of *Tapinoma sessile*.
- 4% divergence between *Dorymyrmex bureni* from northern and southern Florida.
- 6% within the cluster of sibling species *Aphaenogaster rudis-texana* (6).

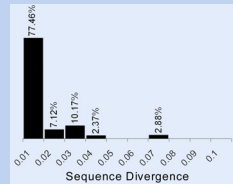


Figure 3: Frequency histogram of within-species pairwise comparisons of sequence divergence (S90). The majority of the divergences are less than 1%.

Unrecognised synonymy

- Acanthomyops* occurs within *Lasius* (7). Early results suggest the possible synonymy of *Camponotus americanus* with *C. castaneus*.

Species Complexes

- Formica fusca* and *F. sanguinea* complexes are characterized by divergences less than 2%. Monophyletic groupings are resilient.

Phylogeny

- Most genera and tribes appeared as monophyletic lineages concordant with current taxonomy (Fig. 4-A).

NNPR

- Preliminary results indicate 15 species of ant (Fig. 4-B). Conventional taxonomic efforts continue.
- No relationship between sequence divergence and geography (Fig. 5).

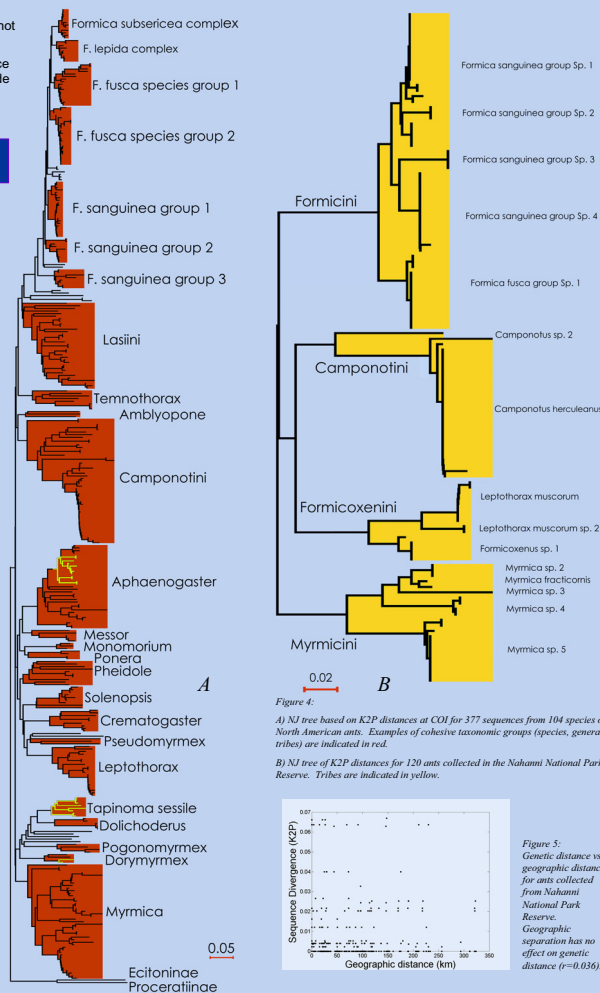


Figure 4: A) NJ tree based on K2P distances at COI for 377 sequences from 104 species of North American ants. Examples of cohesive taxonomic groups (species, genera and tribes) are indicated in red.

B) NJ tree of K2P distances for 120 ants collected in the Nahanni National Park Reserve. Tribes are indicated in yellow.

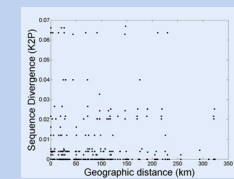


Figure 5: Genetic distance vs. geographic distance for ants collected from Nahanni National Park Reserve. Geographic separation has no effect on genetic distance ($r=0.036$).

CONCLUSIONS

Species Identification

- CO1 sequence diversity can successfully resolve species-level differences between Formicidae across North America.
- The groups most commonly found in the North are in dire need of taxonomic revision. DNA barcoding should be an enormous help in this effort. Our efforts with conventional taxonomic and DNA based species identification continue.

Biological Diversity of the NNPR

- Importantly, our analysis demonstrates the utility of rapid species identification by revealing that the ant species diversity of this understudied UNESCO world heritage site rivals the known ant species diversity of an entire neighboring territory (8).
- This suggests that current estimates of ant diversity in the North are restricted by sampling and expertise.
- DNA barcoding will greatly assist in the rapid enumeration of the diversity of the North.
- Efforts to expand taxonomic and geographic coverage continue.

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