

The astonishing diversity of parasitic wasps (Hymenoptera, Ichneumonidae) at Churchill, Manitoba

Jose L. Fernandez, M. Alex Smith, Dirk Steinke and P. D. N. Hebert
Biodiversity Institute of Ontario, University of Guelph, CANADA



INTRODUCTION

- The Polar Research Observatories for Biodiversity and the Environment (PROBE) is a multidisciplinary initiative that uses DNA-based approaches to identify and characterize the molecular and species diversity in Canada's polar regions (Polar Barcode of Life, 2007). In August 2006 the PROBE-2006 team conducted a comprehensive collecting effort focused in Churchill, Manitoba.
- The taxonomic composition of the Arctic arthropod fauna – 75 % of which are insects – has been documented by Danks (1981). The North American Arctic contains over 2100 species of terrestrial arthropods in nearly 1000 genera and 273 families. The actual number of fauna living in the Arctic is expected to be at least double this estimate (Danks, 1981).
- The family Ichneumonidae (Hymenoptera) is one of the most diverse in the Arctic – represented by 162 species – along with two fly families (Diptera: Chironomidae and Muscidae). As parasitoids the ichneumonids are a key component to the regulation of many food webs involving different arthropod groups (e.g. Diptera, Coleoptera, Lepidoptera, other Hymenoptera, spiders, etc.).
- Ichneumonidae is not only the largest family of living things in Canada (BSC, 2007) but also has many taxonomic impediments related to such high diversity; making identification a daunting task. There is no published list of ichneumonids for Churchill. The most up-to-date information (Yu et al., 2005) records 494 species for the entire province of Manitoba and 834 for North America north of 60° N.
- This research will provide a preliminary list of ichneumonidae from Churchill (based on classical alpha-taxonomical examination of morphology and comparison to the CNC collection). Additionally, we use CO1 DNA barcoding as a complementary tool to test these alpha-taxonomic groupings and accelerate the rate at which the resident diversity of Churchill can be described for a complex group such as parasitic wasps.

METHODS

- Ichneumonids were collected as part of the PROBE-2006 effort (Barcode of Life Initiative, 2007). Collecting efforts were located in an area delimited by 58.3°-58.5° N and 93.3°-94.2° W (Figure 1).
- DNA extracts were prepared from small pieces of leg using a glass fibre protocol (Ivanova et al., 2006). Extracts were resuspended in 30 µl of dH₂O, and a 658-bp region near the 5' terminus of the COI gene was amplified using primers (LepF1–LepR1) following standard protocols. Composite sequences were generated using internal primers when initial amplification was not successful. Sequence divergences were calculated using the K2P distance model and a NJ tree of distances was created to provide a graphic representation of the among-species divergences (Figure 2). See Smith et al. (2007), for full methodology. Each individual was assigned to a provisional species using a sequence threshold of 2 %. When a diverse cluster of barcode species were identified within a morphologically determined species those specimens were also sequenced for 28S – an independent nuclear marker (Figure 3).
- Specimens were also studied using the literature available (a full list of taxonomical references for Nearctic ichneumonids can be found in Yu et al., 2005). Identification was provided to subfamily (100% of cases), genera (75%) and species (~30%) level, whenever possible. Additional information was gathered from extensive review of the Canadian National Collection in Ottawa and other sources. Orthocentrinae, the second best represented subfamily of Ichneumonidae, was chosen for further analysis and comparisons with molecular techniques. Time spent to accomplish all non-molecular tasks was approximated to provide a comparison to the molecular results.

RESULTS

- Hundreds of ichneumonid wasps have been collected thus far. Within the samples studied to date, the family comprises 60 % of all the parasitic wasps (with Proctotrupoidea *sensu lato* and Braconidae the 2nd and 3rd largest groups respectively). Combined they represent about 90 % of Hymenoptera. Chalcid wasps, another diverse group, are not well represented in the collection.
- To date 11 families, 75 genera and more than 123 species of parasitic wasps have been identified and named from the PROBE collection. In addition to the 35 new species records for Manitoba, we have recorded the presence of a new subfamily (Ichneumonidae: Phrudinae) and several species new to Canada.
- We have currently recorded 71 species of Ichneumonidae – almost half of the total recorded from the entire Arctic zone. Due to the proportion of the collection yet to be processed and the new collecting efforts underway, final estimates are expected to be much higher. Within the subfamily Orthocentrinae, 17 species were identified.

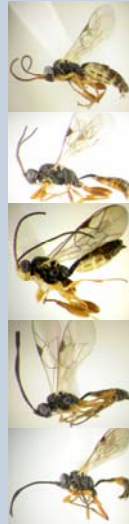
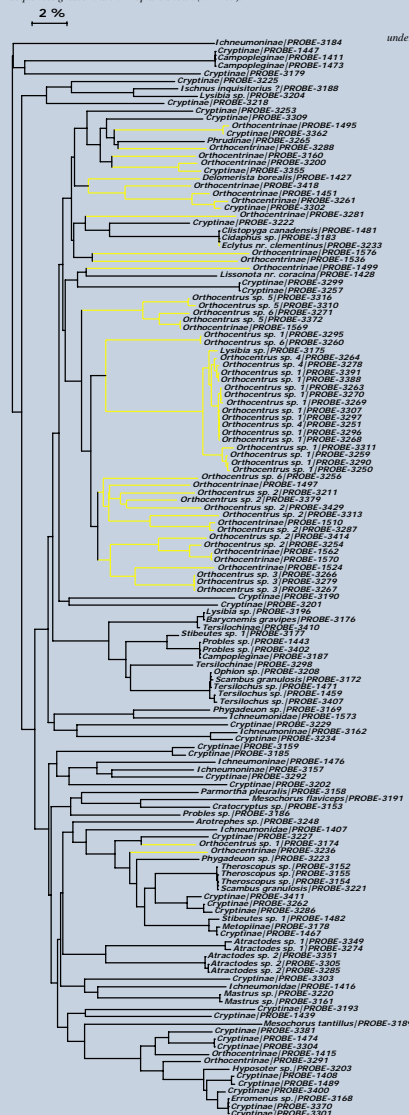


Figure 4: Exemplar Ichneumonidae collected in Churchill. From the top: Orthocentrus sp. 4, Tersilochus sp. 1, Orthocentrus sp. 1, Atractodes sp. 2, undetermined Cryptinae

Figure 2: Neighbour-joining analysis of K2P distance for specimens of Ichneumonidae collected in Churchill, Manitoba in the summer of 2006. Orthocentrinae are represented on yellow branches. Only those sequences greater than 600 bp are shown (n = 165).



RESULTS

- A total of 103 Ichneumonidae species were identified using DNA barcoding (Figure 2). This represents a 1.4x increase from the estimates arising from a traditional taxonomical approach. As for Orthocentrinae, 38 provisional species were recognized after barcoding (Figure 2 yellow), almost double the morphological estimate. For example, see the molecular diversity within *Orthocentrus* sp. 2 in Figure 3. Diversity estimates are under further review to provide a final tally integrating both approaches.
- Many more specimens are currently being processed (several hundreds of ichneumonids). It is expected that this material will significantly increase the current diversity estimates of parasitic wasps in Churchill.
- Time devoted to tasks not related with the molecular techniques – mostly carried out by a taxonomist – were significant, and easily exceeded the time devoted to DNA barcoding production and analysis – calculated according to the standards outlined in Ivanova et al. (2006). This extra time requirement for formalized alpha-taxonomy is especially critical for the ichneumonids where taxonomical keys are either outdated or not available, and where the diversity is extraordinary (e.g. Cryptinae, with many species impossible to assign even to genera; or Ichneumoninae, Banchinae and Mesochorinae, with very difficult to use keys).

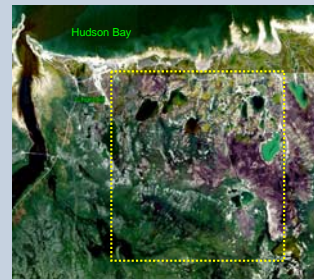


Figure 1: Satellite map of Churchill and surroundings. The yellow dash line indicates the area sampled for terrestrial insects during the PROBE-06 collecting.

CONCLUSIONS

- The diversity of parasitic wasps in Churchill is remarkable. Based on our preliminary results, samples being examined now and the new collecting efforts, it is reasonable to expect an eventual species number 2-3 times higher than present.
- DNA barcodes identified 1.4x more provisional species than those identified using traditional taxonomy (2x more for Orthocentrinae).
- DNA barcodes allow the rapid identification of functional units of diversity. This could be applied to the problem of identifying arthropods in a more rapid fashion – as rapid identifications are often required by conservation groups responding to increases rates of habitat destruction and degradation.
- In the absence of a completed alpha-taxonomy, DNA barcode provisional species can be quickly compared to those groups revealed using an independent nuclear marker (such as 28S) (Figure 3).
- Unfortunately, if the end-product of such an assessment remains a formal taxonomic description, the time and costs associated with the non-molecular part of the work; mounting, labelling, databasing, housing and most importantly: identifying/ assigning names to the species collected will remain formidable. However, beyond the independent estimate of diversity DNA barcoding can reduce this time by creating a *priori* bundles of specimens to be considered by the taxonomist.
- For hyperdiverse and taxonomically difficult groups – such as ichneumonids – those alpha-taxonomic inputs are extremely important and should be taken into account. Our preliminary data show that the high diversity of ichneumonids uncovered in Churchill necessitates much more time spent in the effort of traditional taxonomy. DNA barcoding will always allow a more rapid estimate of diversity and summarily allow the results to be contrasted, refined and validated with traditional taxonomy. Ultimately, each method complements the other.

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Figure 3: Neighbour-joining analysis of genetic distance for *Orthocentrus* sp. 2. Top tree is built using COI DNA barcode data, K2P distance. Lower tree is built using 28S rRNA data using p-dist. Each gene differentiates 10 provisional species. One case of 28S variation without paired COI variation is under further investigation but likely represents heteroplasmy within the 28S sequence. Specimen images are approximately 10x life-size.