

DNA barcodes affirm that 16 species of apparently generalist tropical parasitoid flies (Diptera, Tachinidae) are not all generalists

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INTRODUCTION

- Many species of tachinid flies are viewed as generalist parasitoids because what they apparently a single species of fly has been reared from many species of caterpillars.
- However, an ongoing inventory of the tachinid flies parasitizing thousands of species of caterpillars in Area de Conservación Guanacaste (ACG), northwestern Costa Rica, has encountered more than 400 species of specialist tachinids with only a few generalists.
- We CO1 DNA barcoded 2,134 flies belonging to what appeared to be the 16 most generalist of the reared tachinid morphospecies and encountered 73 mitochondrial lineages separated by an average of 4% sequence divergence.
- These lineages are supported by collateral ecological information and, where tested, by independent nuclear markers (28S and ITS1) and we therefore view these as provisional species.
- Each of the 16 apparently generalist species dissolved in one of four patterns: a single generalist species, a pair of morphologically cryptic generalist species, a complex of specialist species plus a generalist, or a complex of specialists with no remaining generalist.
- In sum, there remained nine generalist species among the 73 mitochondrial lineages that we analyzed, demonstrating that a generalist life style is possible for a tropical caterpillar parasitoid fly.

METHODS

- Wild-caught caterpillars were kept individually in plastic bags at ambient temperatures until the insects produce an adult moth or butterfly, produce a parasitoid, or die.
- Newly eclosed flies are killed by freezing, pinned, oven-dried on site, individually databased, and uniquely coded.
- The dry flies are deposited as permanent vouchers at ambient temperature in the Diptera Unit of the Canadian National Collection, Agriculture and Agri-food Canada, Ottawa, Canada.
- Total genomic DNA was extracted from small pieces (<1 mm long) of fly leg using the NucleoSpin® 96 Tissue kit (Macherey-Nagel Duren, Germany), following manufacturer's protocols, or silica-based Pall plates (1).
- 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 2).
- When a morphospecies showed deep genetic divergences, more specimens were sequenced to provide a better understanding of the distribution of this variation and its relationship to morphology and natural history.
- In the cases where CO1 barcode divergences were slight but there was evident ecological specialization, and in cases where sample size was limited, we amplified the internal transcribed spacer region (ITS1) of the ribosomal RNA, and/or, the D2 expansion segment of the ribosomal large subunit (28S).

RESULTS

- COI sequences were recovered from 89.9% (2,134) of the 2,374 specimens analyzed. Specific primer combinations and electropherogram trace files are available at www.barcodinglife.org.
- When distinct barcode clusters of CO1 sequences within a described generalist morphospecies differed by more than 0.5% and/or were derived from different host(s), they were treated as different entities, hypothesized to have independent evolutionary histories).
- 14 of the 16 generalist morphospecies were readily distinguishable from all others by their DNA barcodes but barcodes of two morphospecies were only very slightly divergent from each other (though they were easily distinguishable from all others – *Blepharipa albicauda* and *B. fimbriata*).
- When the CO1 barcode information was added to the ecological and nuclear sequence information, what appear to be 73 species fell into four patterns.



Figure 1: Right – Provisional species of *Hyphantrophaga* identified in this study. From the top, *Hyphantrophaga blandaDHJ06*, *H. blandaDHJ03* and *H. blandaDHJ02*. *H. blandaDHJ06* is a generalist, while *H. blandaDHJ02* and *H. blandaDHJ03* are specialists.

RESULTS

- **Pattern 1: A barcoded generalist morphospecies remains a generalist**
 - *Hyphantrophaga virilis* and *Lespesia aletiae*. We barcoded flies from 135 *H. virilis* rearings (of at least 153 caterpillar species from 15 families) and 221 rearings of *L. aletiae* spread across at least 55 species of caterpillars and in each case found no barcode variation.
- **Pattern 2: The barcoded generalist becomes two generalists**
 - *Chetogena scutellaris* splits into two generalists after barcoding. These two provisional species are sympatric within the ACG dry forest and each uses a multi-family list of hosts that overlap substantially. ITS1 sequence data suggests there may be a further provisional species not apparent using CO1 barcoding. These putative ITS1 species all tested positive for *Wolbachia* – which may explain the lack of CO1 variation.
- **Pattern 3: The barcoded generalist becomes multiple specialists and one generalist**
 - *Patelloa xanthura*, *Hyphantrophaga blanda*, *Hemisturmia tenuipalpis*, *Lespesia postica*, *Blepharipa fimbriata* and *B. albicauda*. Here, *Patelloa xanthuraDHJ01* was found to be one provisional species reared from at least 145 species of caterpillars in 19 families – unambiguously a generalist – and five specialists; where each parasitizes a very restricted number of species in a different family of caterpillars
- **Pattern 4: The barcoded generalist is a complex of specialists**
 - *Winthemia tricolor*, *Anoxynops auratus*, *Siphosturmia rataeli*, *Lespesia parviteres*, *Eucelatoria armigera*, *Drino rhoeo* and *D. piceiventris*. Each 'generalist' split into host-specific lineages. For instance, *D. rhoeo* barcodes into three specialists on different species of *Manduca*; where each provisional species are ~2% different in their CO1 barcodes

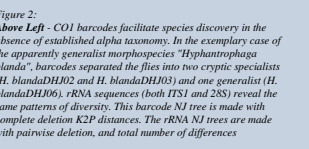


Figure 2: Above – Left – CO1 barcodes facilitate species discovery in the absence of established alpha taxonomy. In the exemplary case of the apparently generalist morphospecies 'Hyphantrophaga blanda', barcodes separated the flies into two cryptic specialists (*H. blandaDHJ02* and *H. blandaDHJ03*) and one generalist (*H. blandaDHJ06*). rRNA sequences (both ITS1 and 28S) reveal the same patterns of diversity. This barcode *NJ* tree is made with complete deletion K2P distances. The rRNA *NJ* trees are made with pairwise deletion, and total number of differences

CONCLUSIONS

- Species Identification and Discovery
- Barcoding is not only an effective identification tool for these small and similar parasitoids, but it has also played a major role in discovering the existence of many provisional species among them. It has helped to bring clarity to the degree of host specificity within the 16 morphospecies of flies and suggests where seemingly small variation in morphology reflects distinguishing traits of cryptic lineages.

Figure 3: Left – Neighbor-joining tree of genetic distance (K2P) for 73 specimens, each representing one of the barcode clusters – provisional species – encountered among 16 generalist morphospecies. Yellow circles flag species that remained generalist after analysis and a star indicates those lineages represented by only one specimen.

CONCLUSIONS

- Subsequent iterative morphological examination of the provisional tachinid species located with our barcoding is finding that some of these provisional species do indeed have distinguishing morphological traits, traits that were previously ascribed to intraspecific rather than interspecific variation.
- Nuclear sequence data is not used here as a substitute for the CO1 barcode. We apply it here to species complexes or pairs with slight CO1 differentiation where ecological data suggested that the slight divergence was meaningful.
- It would be computationally and methodologically complex to conduct taxonomically broad sequencing and subsequent comparisons and identifications with ITS1 sequence data, and there is unlikely to be sufficient resolving power within 28S for many species.
- However, ITS1 and 28S are useful independent (from CO1) genetic covariates to help interpret hybridization and branching patterns of young species when a mitochondrial marker alone is insufficient
- The remaining genuine generalists pose difficult questions. How do they manage to use so many different taxa as hosts? Why are generalist parasitoids no more abundant than are most of the hundreds of host specialists? Work on these questions continues.
- Our results suggest that barcoding a large number of presumed generalist temperate tachinids reared from many species and families of carefully identified caterpillars might modify the conclusion/hypothesis of polyphagy.
- These results reinforce the emerging suspicion that estimates of global species richness are likely underestimates for parasitoids (which may constitute as much as 20% of all animal life), and furthermore that the strategy of being a tropical generalist parasitoid fly may be yet more unusual than has been envisioned for tachinids.

REFERENCES

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For more information see Smith, M. A., Wood, D. M., Janzen, D. H., Hallwachs, W. & Hebert, P. D. N. (2007) *PNAS* 104, 4967-4972, or contact MAS at: salex@uoguelph.ca