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

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Many species of tachinid flies are viewed as generalist parasitoids because what is 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, northwestern Costa Rica, has encountered >400 species of specialist tachinids with only a few generalists. We 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 lineages as provisional species. Each of the 16 apparently generalist species dissolved into one of four patterns: (i) a single generalist species, (ii) a pair of morphologically cryptic generalist species, (iii) a complex of specialist species plus a generalist, or (iv) a complex of specialists with no remaining generalist. In sum, there remained 9 generalist species among the 73 mitochondrial lineages we analyzed, demonstrating that a generalist lifestyle is possible for a tropical caterpillar parasitoid fly. 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 that the strategy of being a tropical generalist parasitic fly may be yet more unusual than has been envisioned for tachinids.

Parasitoid insects are currently believed to comprise as much as one quarter of all insect species (1, 2) and, because insects comprise ~80% of all named animal species, up to 20% of all animal life (3). However, accurate evaluations of parasitoid species richness (2), and subsequent determinations of parasitoid host-specificity, are impeded by the very large number of morphologically similar species and the resultant difficulty in identifying them. This situation further complicates the determination of host-parasitoid relationships. There may be many more species of insect parasitoids than currently believed if host-specificity has been underestimated (4, 5). After the Hymenoptera, Diptera (flies) are the most species-rich group of parasitoids, and the obligate parasitoid family Tachinidae is among the most species-rich of Diptera families, with nearly 10,000 described species (1, 6–8). Within this diversity, many described species of Tachinidae are extremely similar morphologically, and it is a taxonomically challenging family.

It is a widely held view that many species of tachinid parasitoids are relatively generalist (polyphagous) in the species of hosts they parasitize (7–10). However, a 20-year inventory of >400 species of tachinid flies reared from >390,000 wild-caught caterpillars of >3,500 species in Area de Conservación Guanacaste (ACG) in northwestern Costa Rica indicates that at least 90% of the tachinid species from this tropical site are host-specific to one or a few related species (specialists) (ref. 11 and http://janzen.sas.upenn.edu). However, there are conspicuous exceptions. To ascertain whether these exceptions are truly generalists, we cytochrome c oxidase 1 (CO1) DNA-barcoded (e.g., as in ref. 12) the 16 most generalist morphospecies, all being species that have been reared by the inventory from many species of caterpillars in a few to many families and all being species reared tens to hundreds of times [see supporting information (SI) Table 1 and SI Appendices 1 and 2]. When barcoded, the select group of exceptionally generalist morphospecies dissolved into 64 species of specialists and 9 generalists (Fig. 1 and SI Table 1). This outcome mirrors and magnifies the result recorded when we barcoded the 20 relatively specialist morphospecies of Belvosia, another tachinid genus living in the same ACG habitats; its three somewhat generalist species were each found to be complexes of specialists (12).

As of October 2006, the ACG caterpillar and parasitoid inventory had reared tachinid flies from 16,500 of >390,000 wild-caught caterpillars (~4.2% rate of infection). For the past 17 years, D.M.W. (8, 13) has iteratively assigned these flies to morphospecies and identified them with scientific names when available. The first taxonomic assignment was completed without knowledge of the host caterpillar. Taxonomic assignments were flagged for reexamination when the fly’s host caterpillar species did not match what appeared to be the host-use pattern of that fly species. Although all flies were placed to genus (described or undescribed), <10% of the morphospecies appeared to match a known type specimen and thus could be presently “named” with any confidence. Inasmuch as it is believed that only 7–20% of insect species have been scientifically described (3, 14), such a low level of taxonomic allocation is not surprising. However, all of the 16 most generalist morphospecies were sufficiently distinctive among the hundreds of species-, genus-, or family-level specialists that they did receive a tentative scientific name through this process; on average, these names are all >100 years old (SI Table 2). We then added sequence data from CO1 barcoding to the 16 morphologically defined and host-checked units already recognized to determine whether each generalist morphospecies comprised specimens with little intraspecific barcode variability. Such a protocol determines whether these 16 species can


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Abbreviations: ACG, Area de Conservación Guanacaste, Costa Rica; CO1, cytochrome c oxidase 1; N, neighbor-joining.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database (CO1: accession nos. EF180450–EF182583; 28S and ITS1: accession nos. EF183546–EF184019 and EF189688–EF189703 and two representative sequences of Chetogena scutellarisDHJ01 Wolbachia, accession nos. EF192042 and EF192043).

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be easily distinguished by their barcodes, as has been effective for other taxa (12, 15–21), and is also the first step for using barcodes in the discovery of cryptic species (12, 15).

Results

We successfully barcoded one fly from each of 2,134 generalist tachinid rearings (among >4,500 barcoded flies of >100 morphospecies) and found that 14 of the 16 generalist morphospecies were readily distinguishable from all others by their DNA barcodes (Fig. 1, SI Table 1, and SI Appendix 1) but that barcodes of two morphospecies were only very slightly divergent from each other (although they were easily distinguishable from all others, i.e., *Blepharia albicauda* and *B. fimbriata*). Each of the 14 morphospecies is represented by a distinct, nonoverlapping cluster of sequences in the neighbor-joining (NJ) tree (SI Appendix 1). These barcode clusters had >5% sequence divergence among them, but the sequence divergence within each morphospecies was quite high, in some cases even greater than 10%. This finding suggested that the applied morphospecies name actually referenced multiple cryptic species. We then overlaid an array of ecological correlates [host caterpillar, caterpillar food plant, ecosystem (see SI Appendix 2)] and independent nuclear genetic markers (ITS1, 28S) to determine whether these data as a whole supported the hypothesis that each of these within-morphospecies barcode clusters represents a morphologically cryptic provisional species. This was unambiguously the case. Sixteen generalists became 73 cryptic provisional species. Note that the “*Blepharia albicauda*” species complex is here represented by only one specimen due to extremely restricted barcode divergences among its potential provisional species. Alternating red and black text does not represent any taxonomic pattern and is only to facilitate visual discrimination between adjacent morphospecies.

![Fig. 1. NJ tree of genetic distance (K2P) for 73 specimens, each representing one of the barcode clusters (provisional species) encountered among 16 generalist morphospecies. See SI Appendix 1 for an NJ tree containing all >500-bp barcoded individuals. The number of species of Lepidoptera parasitized by each parasitoid is shown in parentheses.](image-url)
In those cases in which a barcode sequence cluster within 1 of the 16 morphospecies did not correlate with ecological information, or alternatively, a barcode cluster was only slightly distinct from its immediate neighbor, we also sequenced two independent nuclear markers. As with barcoding of *Balvesia*, we used the first nuclear rRNA internal transcribed spacer region (ITS1) (12), as well as the D2 region of 28S. A nuclear marker was not sequenced for all specimens for which we have CO1 barcodes because our purpose was species identification and the detection of cryptic provisional species within generalist morphospecies, which was often achieved with the standardized CO1 barcode alone.

When the CO1 barcode information was added to the ecological and nuclear sequence information, what appear to be 73 species fell into four patterns. SI Table 3 enumerates the intramorphospecific CO1 DNA barcode variation.

**Pattern 1: A Barcoded Generalist Morphospecies Remains a Generalist.** Just 2 of the 16 most generalist morphospecies encountered by the ACG tachnoid inventory, *H. virilis* and *L. aletiae*, barcoded as single biological units. Because there is no evidence that they are more than two species, we feel confident in applying a scientific name to these Costa Rican specimens, even though these widespread species were described from the United States. We barcoded flies from 135 *H. virilis* specimens for which we have CO1 barcodes because our purpose was the other cryptic species discussed below.

**Pattern 2: The Barcoded Generalist Becomes Two Generalists.** "*Chetogena scutellaris*" contained two barcode groups: *C. scutellaris-DHJ01* and *C. scutellaris-DHJ02*. These groups differ by eight (12.3%) characteristic nucleotide substitutions within the CO1 barcode (one C-G transversion and seven transitions; six of the transitions are of synonymous third-position substitutions, and one is a nonsynonymous first position) (Fig. 1, SI Table 1, and SI Appendices 1 and 2). These two provisional species are sympatric within the ACG dry forest, and each uses a multifamily list of hosts that overlap substantially. These two provisional species have been encountered with equal frequency in the tachnoid inventory.

Could there be cryptic species that are not distinguishable by their CO1 barcodes hidden within these two generalists? Specimens of *C. scutellaris-DHJ01* and *C. scutellaris-DHJ02* were also sequenced for 28S and for ITS1 (SI Appendix 3). There is just 1 bp difference between *C. scutellaris-DHJ01* and *C. scutellaris-DHJ02* within 28S (SI Appendix 3b), and the sequences are homogeneous within each of the two provisional species. Additionally, there are five heteroplasmic individuals at that locus, which suggests that the two provisional species are capable of producing hybrids. The more-variable rRNA gene region (ITS1, SI Appendix 3a) supports the divergence between *C. scutellaris-DHJ01* and *C. scutellaris-DHJ02* (≈7% divergent) and further suggests that there is a division within *C. scutellaris-DHJ01* that is not apparent to the CO1 barcode.

The absence of mtDNA variation with evident nuclear variation, as displayed here by *C. scutellaris-DHJ01*, can sometimes be caused by the presence of the cytoplasmic bacteria *Wolbachia*. *Wolbachia* are obligate intracellular endosymbiotic bacteria that cause reproductive incompatibility between infected and uninfected lineages, which results in an increased proportion of infected maternal lineages that cannot reproduce (22, 23). If closely related species hybridize and one member of this pair is infected with *Wolbachia*, the bacteria-caused cytoplasmic incompatibility can result in the lack of interspecific mtDNA variability with little effect on nuclear genes (24). Using a standard PCR diagnostic for the presence of *Wolbachia* (25), we determined that at least 73.9% of 23 specimens of *C. scutellaris-DHJ01* contained *Wolbachia*. Until more is known about the relationships within the “*C. scutellaris*” provisional species, we hypothesize that a *Wolbachia* infection may have caused the barcode to be identical between two morphologically cryptic species that can otherwise be differentiated by their nuclear DNA. In short, the two clearly cryptic generalists without "*C. scutellaris*" might even be three cryptic generalists.

**Pattern 3: The Barcoded Generalist Becomes Multiple Specialists and One Generalist.**

- **"Patelloa xanthura":** *P. xanthura-DHJ01* was found to be one provisional species reared from at least 145 species of caterpillars in 19 families, unambiguously a generalist, and five specialists. Each of these specialists parasitizes a very restricted number of species in a different family of caterpillars (Fig. 1, SI Table 1 and SI Appendices 1 and 2).

- **“Hyphantaephora blanda”:** *H. blanda-DHJ06* was found to be one barcode group reared from at least 21 species of caterpillars in seven families-unambiguously a generalist- and two specialists: *H. blanda-DHJ02* and *H. blanda-DHJ03*. Each of the latter use a very narrow and nonoverlapping range of caterpillar species, species that are not attacked by *H. blanda-DHJ06* (see SI Table 1 and SI Appendix 2). These three provisional species are supported by distinct haplotypes within both ITS1 and 28S (Fig. 2 and SI Appendix 4 a and b).

- **“Hemisturia tenuipalpis”:** *H. tenuipalpis-DHJ03* was found to be one generalist reared from at least 10 species and five families. However, *H. tenuipalpis-DHJ01* is a specialist on *Immidia*; *H. tenuipalpis-DHJ02* specializes on one species, *Hylaea umbrata* (Saturniidae); and *H. tenuipalpis-DHJ04* specializes on two members of one genus, *Climiodes* (Crambidae). All of these species of caterpillars occur at high densities, but only on a few individuals of their food plants. *H. tenuipalpis-DHJ03* also contains shallow CO1 divergences that we hypothesized might be two marginally separated species, but this was not supported by ecological information or independent genetic markers (ITS1, 28S (see SI Appendix 5 a and b)). We therefore conclude that *H. tenuipalpis-DHJ03* is one provisional species.

- **“Lespesia postica”:** One morphospecies splits into six when barcoded, and each provisional species parasitizes a different species or a small closely related group of species of caterpillars.

- **“Blepharipa fimbriata” and “Blepharipa albicauda”:** Upon barcoding, “*B. fimbriata*” becomes four distinct groups, with *B. fimbriata-DHJ04* appearing to be a generalist feeding on at least 24 species in two families. However, all but one of the *B. fimbriata-DHJ04* host records is from a large hesperine Heresiidae caterpillar feeding on a monocot (from grasses to palms) (SI Appendices 1 and 2). We therefore conclude that this species is a generalist but only within one subfamily of hosts. The single exceptional host species record is *Manataria maculata* (Nymphalidae), which feeds side-by-side with the usual hosts. The other three species of “*B. fimbriata*” each specialize on very taxonomically restricted groups of caterpillars. *B. fimbriata-DHJ01* contains intraspecific CO1 DNA barcode variation. Intraspecific CO1 DNA barcode variation is more than all of the other provisional specialist barcode species, including some instances of heteroplasmacy, and the CO1 barcode variation of all species are mingled among those of “*B. albicauda*.” *B. albicauda* is a putative generalist species that is morphologically quite distinctive because of a whitish tip (tergite 5) to an otherwise mostly black abdomen, whereas “*B. fimbriata*” has the classical gray and black striped/banded pattern of tachinids (SI Appendix 6d). The CO1 barcodes of some individuals of *B. fimbriata-DHJ11* are too similar to those
of “B. albicauda” to be separated from them (Fig. 1 and SI Appendices 1 and 6d). There is no overlap between the two arrays of caterpillars that are parasitized by these two totally sympatric and barcode-indistinguishable provisional species (SI Appendix 1). The morphospecies “B. albicauda” is a morphologically distinct generalist that has been reared from 18 species in seven families of caterpillars (but see below).

Because “B. albicauda” and B. fimbriataDHJ11 are a special case from both a taxonomic and a molecular perspective, they were investigated in more detail. ITS1 and 28S were sequenced for 137 individuals from “B. albicauda” and B. fimbriataDHJ11. The two morphologically defined species showed <0.05% divergence at ITS (three transitions), reinforcing their close affinities because such variation fell within that seen in “B. albicauda” (including indels, which range from 0 to 11 differences). Furthermore, within the D2 region of 28S, B. fimbriataDHJ11 possesses the same haplotype as approximately half of the “B. albicauda” (there are two haplotypes within B. albicauda differing by a 1-bp insertion) (SI Appendix 6).

Considering only molecular data, it appears that the morphospecies “B. albicauda” is one internal member of the “B. fimbriata” species complex. However, both the absence of overlap in host use (none of the B. fimbriataDHJ11 hosts, i.e., all Lirimiris, Heterocampa, and Farigia in the Notodontidae, are parasitized by B. albicauda) and the distinctive color pattern suggest that “B. albicauda” can be comfortably viewed as its own evolutionary lineage.

We do not here consider “B. albicauda” to be a true generalist because 28S and ITS1 divergences are correlated with the fly’s host species. The D2 region for 28S supports a division between flies that parasitize the Hemiceras pallidula/Encruphion leena group of hosts and those that primarily parasitize Hemiceras nigrescens, in that there is an insertion characteristic of the latter (SI Appendix 6 b and d). Furthermore, there is a different ITS1 haplotype for flies reared from Phoebis sennae (Pieridae), another for Hylesia lineata (Saturniidae), and a third for Pentobesa pinna and Moresa valkeri (Notodontidae) together (SI Appendix 6). We think it likely that there are cryptic species within B. albicauda (SI Appendix 6d) and that these are characterized by extremely shallow CO1 divisions. However, elucidation of this possibility will require larger sample sizes and other data to confirm. The observed heteroplasmy could be caused by the differential amplification of nuclear pseudogenes (26), by paternal leakage (27), or by somatic mutation (28). If these CO1 sequences are pseudogenes, it might explain why the evident diversity within ITS1 is not reflected within the barcode, given that nucleotide substitutions are not expected to occur as rapidly in the nucleus as in the mitochondrial genome (29). However, as pseudogenes are freed from functional constraint, nonsynonymous and synonymous substitutions should occur at the same rate. However, in this case the alternative base at each heteroplasmic site represents a synonymous substitution. This observation does not support the pseudogene hypothesis. Additional information that makes unintentional amplification of pseudogenes unlikely includes uniform electrophoretic bands, lack of indels, stop codons, or a transversion bias. The CO1 trace files of those likely hybrid individuals (see panel B of SI Appendix 6 b and d) display examples in which the evident CO1 heteroplasmy was the nucleotide of the provisional species that is the
other inferred parent. This finding supports the hypothesis that there has been leakage of small amounts of paternal mtDNA. Irrespective of whether this heteroplasmy is a pseudogene or the outcome of paternal leakage, from the perspective of species identification by barcoding, the single mtDNA barcode alone is not sufficient to differentiate these two species. None of the specimens tested positive for Wolbachia.

Pattern 4: The Barcoded Generalist Is a Complex of Specialists. Although the above eight morphospecies that appeared to be generalists retained at least one generalist lineage (except for the suspiciously complex “B. albicauda”), the other eight morphospecies that were barcoded appear to be constituted entirely of complexes of very similar species distinguishable by barcode and host caterpillar correlates (Fig. 1, SI Table 1, and SI Appendices 1 and 2). The scientific names of the remainder of the morphospecies are given in quotation marks because we have no idea which, if any, of the barcode sequence clusters truly match the holotype.

• “Winthemia tricolor”: W. tricolorDHJ01 and W. tricolorDHJ02 are each specialists on a different species of Arctiidae, whereas W. tricolorDHJ03 and W. tricolorDHJ04 are specialists on each of two species of Geometridae. Specific ITS1 and 28S haplotypes are associated with each of the CO1-distinctive provisional species identified here, including the two provisional species represented by a single collection (W. tricolorDHJ03) (see SI Appendix).

• “Anoxylops auratus”: Four of the specialists (A. auratus-DHJ01, A. auratusDHJ03, A. auratusDHJ04, A. auratus-DHJ05) parasitize four quite different sets of Nymphalidae. A. auratusDHJ01 and A. auratusDHJ03 are represented by single specimens but are further supported by having characteristic ITS1 and 28S haplotypes. The three remaining specialists (A. auratusDHJ06, A. auratusDHJ07, and A. auratusDHJ08) each parasitize a different member (and microhabitat) of the nymphalid genus Opisphanes eating different food plants: broadleaf monocots, rainforest palms, and dry forest palms, respectively. Although these three parasitoids of Opisphanes are not variable within 28S, each bears a distinctive haplotype within the more variable ITS1 gene region (see SI Appendix).

• “Siphosturmia rafaeli”: This morphospecies splits into four with barcoding. Each parasitizes a different taxonomic and ecological group of hesperiid butterfly caterpillars.

• “Lepesia parviteres”: One morphospecies from 178 rearings barcodes into four specialists, each using a different family of caterpillars.

• “Eucalatoria armigera”: One morphospecies barcodes into eight, and each provisional species parasitizes a different species or closely related small group of species.

• “Drino rhoeo”: One morphospecies barcodes into three specialists on different species of Manduca; these specialists are ≈2% different in their CO1 barcodes.

• “Drino piceiventris”: One morphospecies splits into 12 host-specific lineages, each of which specializes on different groups of Sphingidae. One (D. piceiventrisDHJ05) is a specialist on seven species of Manduca and a few other large similar macroglossine sphingid caterpillars, whereas three others (D. piceiventrisDHJ07, D. piceiventrisDHJ10, and D. piceiventrisDHJ13) specialize on different sphingid caterpillars that feed on Dilleniaceae. Many of the barcode divisions within “D. piceiventris” are very slight but consistent, sometimes as little as a single base pair divergence (a value of note because it is within sequencing error). Members of the “D. piceiventris” species complex were also sequenced for ITS1 and 28S (see SI Appendix). These gene regions did not contain haplotypes that provide independent support for the lesser barcode divergences (D. piceiventrisDHJ09, D. piceiventrisDHJ10, and D. piceiventrisDHJ11) but did support the greater CO1 divergences (e.g., between D. piceiventrisDHJ03 and D. piceiventrisDHJ06). On the basis of these results, in a certain sense we have maintained the recognition of these slightly separate provisional species more on the basis of ecological information than based strictly on barcode data.

Discussion

CO1 DNA barcoding has shown that what were thought to be 16 morphospecies of apparently generalist tachinid fly parasitoids are in fact a complex of at least 73 species, and that except for two (and potentially several more within “B. albicauda”) all can be identified by their barcodes. 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 instances in which seemingly small variation in morphology reflects distinguishing traits of cryptic lineages. We say this because 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.

Additionally, in this understudied group of tropical insects of expected high diversity, we encountered just two cases in which we might have overlooked a provisional species if we had used barcodes alone to analyze the 16 species of what appeared initially to be generalist morphospecies. In one case (“B. albicauda”), where ecological information and an independent nuclear marker support very slight CO1 differences, there also are diagnostic CO1 base pair substitutions. In the other case (C. scutellarisDHJ01), where an evident nuclear divergence was shown to be invariant for CO1, most of these specimens tested positive for the bacteria Wolbachia. Because females uninfected by Wolbachia can only breed successfully with uninfected males of the same species, the stage would be set for a sweep of the infected species’ mtDNA through the uninfected species (as discussed in refs. 30 and 31).

CO1 provides an attractive genetic barcode for species identification because of its high copy number, rapid rate of mutation, and ease of amplification/sequencing and alignment for intra- and interspecific comparisons. However, with very young species, or species that can hybridize, a secondary independent molecular marker to solidify or confirm identification may be needed. This is especially true for extraordinarily species-rich and often morphologically very similar groups such as parasitoids, for which alpha taxonomic description based on morphology and behavior alone lags far behind existing diversity. Both of the secondary nuclear markers used here are rRNA and are therefore attractive because of their great abundance and relatively conserved flanking regions, which allows the design of primers of wide utility. However, successfully sequencing from regions with large indels, without cloning and subsequent aligning, is difficult (32). Because of this, we do not use or suggest the rRNA data as a substitute for the CO1 barcode. We apply it here to species complexes or pairs with slight CO1 differentiation in cases where ecological data suggested that the slight divergence was meaningful. It would be computationally and methodologically extremely complex to conduct taxonomically broad sequencing and subsequent comparisons/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. Nuclear sequence divergence correlated with CO1 barcode divergence is also particularly useful for demonstrating that two sympatric CO1
barcode lineages are two separate breeding entities rather than simply two haplotypes in one interbreeding population.

With the finding that 14 of 16 apparently generalist tachinid flies are rich in morphologically cryptic provisional species, these species can now be seen as conforming in large part to the pattern of long specialization displayed by hundreds of other species reared by the ACG caterpillar inventory. In addition to the nearly 5-fold increase in species richness facilitated by DNA barcoding, perhaps five additional species remain to be characterized; these are provisional species that cannot be distinguished from closely related species by simple threshold interpretations of their CO1 barcodes. At the other end of the scale, the 9–10 species that appear to be 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? Are generalists to be thought of as an evolutionary source of specialists (as opposed to specialists evolutionarily begetting specialists)? Some of these questions will disappear if further molecular probing and larger sample sizes expose yet more cryptic diversity invisible to the level of examination we used. It is also possible that the generalists contain extremely rare specialists that have not yet been well represented by collections in the caterpillar inventory. For instance, see “P. xanthura” (Fig. 1 and SI Appendices 1 and 2). In this case, of 337 specimens sequenced, 83% were the generalist P. xanthuraDHJ01, whereas P. xanthuraDHJ02 and P. xanthuraDHJ06 are represented by only one specimen each (4% in the caterpillar inventory to date).

Reviews of the nature of tachinid host use have concluded that, as a whole, the family is generalist, and most variation on this pattern is due to a small subset of even more extremely generalist species (33). However, this conclusion is based almost entirely on host records from temperate regions and on generalist “species” as defined by our morphology. 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 this conclusion. Irrespective of what happens in a tropical–temperate comparison, our results suggest that combining barcoding with morphology and natural history is very likely to increase global estimates of species richness and to expose the tropics as being yet more complex than currently appreciated. Species that are “so different from each other, and dependent on each other in so complex a manner” (34) may result in interactions of tropical species that are even more complex than we have yet realized. Correctly detecting and identifying these species will greatly facilitate our ability to unravel these entangled banks of ecological and evolutionary interactions.

Materials and Methods

All methods (field and molecular biology) were completed as described in the DNA barcoding of Belvosia (12), and slight modifications are detailed in SI Materials and Methods and SI Table 4. Sequences, trace files, and field data are available in the ACG Generalist Tachinidae file in the Completed Projects section of the Barcode of Life Database (BOLD; www.barcodinglife.org). Additional collection information is deposited at http://janzen.sas.upenn.edu, and all sequences have been deposited in the GenBank database (CO1: accession nos. EF180450–EF182583; 28S and ITS1: accession nos. EF183546–EF184019 and EF189688–EF189703 and two representative sequences of C. scutellarisDHJ01 Wolbachia, accession nos. EF192042 and EF192043).

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