Extreme diversity of tropical parasitoid wasps exposed by iterative integration of natural history, DNA barcoding, morphology, and collections

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We DNA barcoded 2,597 parasitoid wasps belonging to 6 microgastrine braconid genera reared from parapatric tropical dry forest, cloud forest, and rain forest in Area de Conservación Guanacaste (ACG) in northwestern Costa Rica and combined these data with records of caterpillar hosts and morphological analyses. We asked whether barcoding and morphology discover the same provisional species and whether the biological entities revealed by our analysis are congruent with wasp host specificity. Morphological analysis revealed 171 provisional species, but barcoding exposed an additional 142 provisional species; 95% of the total is likely to be undescribed. These 313 provisional species are extraordinarily host specific; more than 90% attack only 1 or 2 species of caterpillars out of more than 3,500 species sampled. The most extreme case of overlooked diversity is the morphospecies *Apanteles leucostigmus*. This minute black wasp with a distinctive white wing stigma was thought to parasitize 32 species of ACG hesperiid caterpillars, but barcoding revealed 36 provisional species, each attacking one or a very few closely related species of caterpillars. When host records and/or within-ACG distributions suggested that DNA barcoding had missed a species-pair, or when provisional species were separated only by slight differences in their barcodes, we examined nuclear sequences to test hypotheses of presumptive species boundaries and to further probe host specificity. Our iterative process of combining morphological analysis, ecology, and DNA barcoding and reiteratively using specimens maintained in permanent collections has resulted in a much more fine-scaled understanding of parasitoid diversity and host specificity than any one of these elements could have produced on its own.

Area de Conservación Guanacaste | Costa Rica | caterpillar | Braconidae | host specificity

More than half of all species are likely to be those directly involved in plant/insect/parasitoid dynamics (1). Since insect parasitoids kill their hosts, they have profound effects on the population dynamics of their hosts (2). In this study, their hosts are caterpillars (larval Lepidoptera), which in many ecosystems consume more leaf tissue than all other herbivores combined (3).

Hymenopteran parasitoids are one of the most species-rich groups of animals, potentially accounting for more than 20% of the world's insects (4). The parasitoid wasp subfamily Microgastrinae (Braconidae), exclusively parasitoids of caterpillars, currently includes ≈1,500 described species but is conservatively estimated to include 5,000–10,000 species (5). Identification of specimens within this hyperdiverse group is impossible in the field and difficult in the laboratory, requiring a specialist for a particular genus. With such a small fraction of species described, reasonable hypotheses of species membership, relationships, and ecological impacts are severely impeded (6). A morass of morphologically similar species and a paucity of morphological taxonomists restrict our understanding of host specificity and diversity in microgastrines and in many other parasitoid groups.

A detailed recognition of species in parasitoid communities is necessary because of the pivotal role parasitoids play in food web structure and dynamics. While generalizations about the effects of parasitoids on community diversity are complex (7), a commonplace predictor of the impact of a parasitoid species on local host dynamics is whether the parasitoid is a generalist or specialist. A generalist, especially a mobile one, is viewed as stabilizing food webs (see ref. 8) and may itself have more stable dynamics (9), whereas specialists are viewed as increasing food-web compartmentalization and decreasing connectance (see ref. 10). However, it is impossible to explore such ecological relationships when the species are not recognized or correctly identified. This is especially the case when as few as only 1% of parasitoids may have been described (4, 11).

Efforts to estimate parasitoid host specificity that do not include DNA-based discrimination of the parasitoid species are likely to underestimate host specificity due to the inadvertent labeling of morphologically similar but genetically isolated lineages as being a single species (12, 13). In northwestern Costa Rica, an ongoing 30-year inventory of wild-caught caterpillars and their parasitoids (ref. 3 and http://janzen.sas.upenn.edu) in Area de Conservación Guanacaste (ACG) now includes DNA barcoding (14, 15) in the standard array of analyses performed on the reared parasitoids. This combination of barcode data with morphological data and host records has revealed that most presumptively generalist morphologically defined species of parasitoid flies (Diptera) actually are complexes of taxonomically cryptic host specialists, though a few generalists are present (16, 17).

While only ≈5% of the many hundreds of species of ACG parasitoid flies are generalists, the equally speciose and tiny microgastrine braconid parasitoid wasps (2–3 mm long) are thought to be even more specialized. Here we test this hypothesis by probing host specificity through the combination of morphological traits, ecological traits, and DNA barcodes for the members of 6 microgastrine wasp genera (*Alphomelon*, *Apanteles*, *Cotesia*, *Dolichogenidea*, *Glyptapanteles*, and *Microplitis*) reared from wild-caught caterpillars. These genera were selected because they have many host records in the ACG inventory, each has a taxonomist

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Data Deposition: All sequences have been deposited in GenBank, CO1: DQ492265–DQ492279, EU395928–EU398506; 28S: EU402080–EU402383; and ITS1: EU433961–U433982.

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who has identified them to morphospecies (J.J.R., J.B.W., A.R.D.), and an attempt has been made to barcode a specimen from each rearing. The protocol of independently determining morphospecies, host records, and barcodes and then combining these results iteratively to characterize actual wasp species, is simultaneously *i*) a test of the ability of DNA barcodes to identify specimens and reveal/discover species, and *ii*) an examination of the fit between traditional morphotaxonomic analyses and DNA barcodes. In select cases, supplemental nuclear markers [the D2 region of 28S or the internal transcribed spacer region (ITS1), both rRNA] were further used to explore whether one or more species were present when what seemed to be provisional species were separated by only shallow barcode divergences (e.g., 1 bp) or when taxonomically broad host usage suggested that cytochrome *c* oxidase 1 (CO1) barcoding had overlooked a species-pair.

Results

From 1978 through 2007, nearly 1% of ≈400,000 wild-caught and reared caterpillars were found to be parasitized by a wasp in 1 of the 6 genera of Microgastrinae targeted for analysis. Unless hyperparasitized, each of the 2,978 attacked caterpillars produced 1 wasp or many sibling wasps that invariably belonged to a single morphospecies. The morphotaxonomic processing of these wasps yielded 175 provisional species that ranged in host specificity from attacking a single caterpillar species (often) to occasionally attacking several members of a higher taxon (e.g., members of a subfamily) or a microhabitat (e.g., a subset of the species of leaf-rolling microlepidoptera on 1 species of food plant). This result had come to be expected as the sample size grew over the decades and is portrayed by the interim names based solely on morphology in column 1 of supporting information (SI) Table S1.

A single wasp specimen from each parasitized caterpillar was barcoded and submitted for morphological analysis. Barcodes greater than 500 bp in length were generated for 75% of these wasps, and a shorter CO1 sequence was obtained from an additional 12.3% of the specimens, providing a barcode record for 2,597 of the 2,978 wasps reared over the 30-year interval. Species were provisionally recognized by the formation of a distinct group of barcodes in a neighbor-joining (NJ) tree (e.g., *SI Appendix*, Section 1), a group that also was congruent with morphology, host records, and/or ecological distribution within ACG. Membership in a provisional species was determined using sequences that contained at least 500 bp. This avoids the analytical complications (18) introduced by absentee base-pair information (or lack of sequence overlap). Incomplete barcodes may lack the characteristic 1- or 2-bp differences that may be used to separate species with very similar barcodes (Fig. 1 and *SI Appendix*, Section 1).

This process exposed 313 provisional species among the 171 morphologically defined species, thereby essentially eliminating the ecological category of "generalist." Barcoding never grouped provisional species that had been morphologically identified into the same group, but it did split many morphological taxa into provisional species that subsequently were found to possess minor morphological and/or ecological differences in addition to the variation in species of caterpillars they attacked.

This prospecting for provisional ACG microgastrine wasp species exposed 6 patterns of variation in the morphological, ecological, and barcode data.

Provisional Species with a Single Host Species (or Closely Related Species-Pair). Ninety percent of the 313 provisional species attack only 1, 2, or a narrowly defined group of very closely related species of caterpillars (Figs. 1 and 2, Table S1, Table S2, and Table S3). This level of extreme host specificity of the ACG microgastrine fauna is representative of the group of all other braconid parasitoids of caterpillars reared in ACG. Given that at least 25% of the likely ≈10,000 Lepidoptera species within the ACG caterpillar fauna have been sampled to date, it is unlikely that further caterpillar and parasitoid inventory will change this pattern of extreme host specificity significantly.

Provisional Species with a Narrow Taxonomic/Ecological Range of Host Species. A small number of provisional species parasitize a slightly broader taxonomic range of hosts (i.e., the right tail of the distribution in Fig. 2*A*; see Table S1 and Table S2 for a complete listing of these associations). In each of these cases, however, the host caterpillars occupy similar ecological microhabitats and usually are members of a single family. This kind of host specificity is so strongly correlated with the host's ecology that the wasps cannot be labeled as generalists in any useful sense of the word.

***Example:* Apanteles *attacking* Urbanus.** The species group of *Apanteles* that parasitizes members of the hesperiid genus *Urbanus* (specimens reared from 71 individuals of 10 species of caterpillars) is characterized by quite shallow interspecific barcode divergences (≈0.15% on average, SE = 0.03) (*SI Appendix*, Section 1). Within one of these provisional species, *Apanteles* Rodriguez64 (attacking *Urbanus* doryssusDHJ02), the LepR1 sequence amplifications consistently produced sequencing results that either failed or appeared to have 2 signals. We conclude that the reverse sequencing reaction was preferentially amplifying a nuclear pseudogene while the forward reaction was amplifying the mitochondrial barcode. When we compared the alternative base pairs of the ambiguous positions with those of other members of the same wasp genus (rates of synonymous to non-synonymous substitutions) this conclusion was supported. When these *Urbanus*-attacking wasp species are compared by means of their 500+ bp barcodes, there is unambiguous host specialization. Within 28S sequences there are no characters that differentiate these provisional species. This case suggests a recent radiation onto the various species of *Urbanus* caterpillars.

Provisional Species with CO1 Variation that Fails to Covary with Morphological/Ecological Data. *Examples:* Apanteles *Rodriguez32 and* Glyptapanteles *Whitfield16*. Two of the 313 provisional species showed larger-than-average (> ≈2%) intraspecific barcode variation that was not correlated in any obvious way with variation in host species, food plant for that host, geography, or season. Because independent nuclear markers (28S, ITS1) failed to show any evidence of missed divergence between the 2 CO1 lineages in each species (*SI Appendix*, Sections 2 and 6), we conclude these are cases of high intraspecific CO1 variation within a provisional species. These rare cases may reflect the recent mixing of formerly separated incipient species, naturally large intraspecific variation, or immigration from a different population. We emphasize that the barcodes of these species do not overlap with those of any other species, and there is no evidence that the variants reflect coamplification of a nuclear pseudogene.

Duplication: Different Provisional Species with the Same Host Species. *Example:* Apanteles *attacking the* Astraptes fulgerator *complex (Hesperiidae).* *Apanteles* Rodriguez06 and *Apanteles* Rodriguez20 each parasitize most members of the *Astraptes fulgerator* complex, a butterfly species complex that was revealed using ecology, morphology, and DNA barcoding (20). These *Apanteles* species possess 14% divergence in their CO1 barcodes, have 12 diagnostic differences within the D2 region of 28S (11 substitutions and 1 indel), are 10% divergent within ITS1, and possess distinctive adult and cocoon morphologies. Both nuclear markers lack intraspecific divergence, strongly supporting the hypothesis that the 2 CO1 divergent lineages are distinct species (*SI Appendix*, Section 2). Barcodes for *A.* Rodriguez20 are very similar to those of the *Apanteles* species that parasitize *Urbanus* (see *SI Appendix*, Section 1). Interestingly, neither of these 2 species uses the 2 most divergent species in the *Astraptes fulgerator* complex, *A.* CELT and *A.* TRIGO.

Example:* Apanteles *that parasitize* Stenoma patens *(Elachistidae). Both *Apanteles* Rodriguez69 and *A.* Rodriguez117, members of the *Apanteles* morphospecies01 complex, parasitize caterpillars in the elachistid species complex of *Stenoma patens* (feeding on *Abuta*

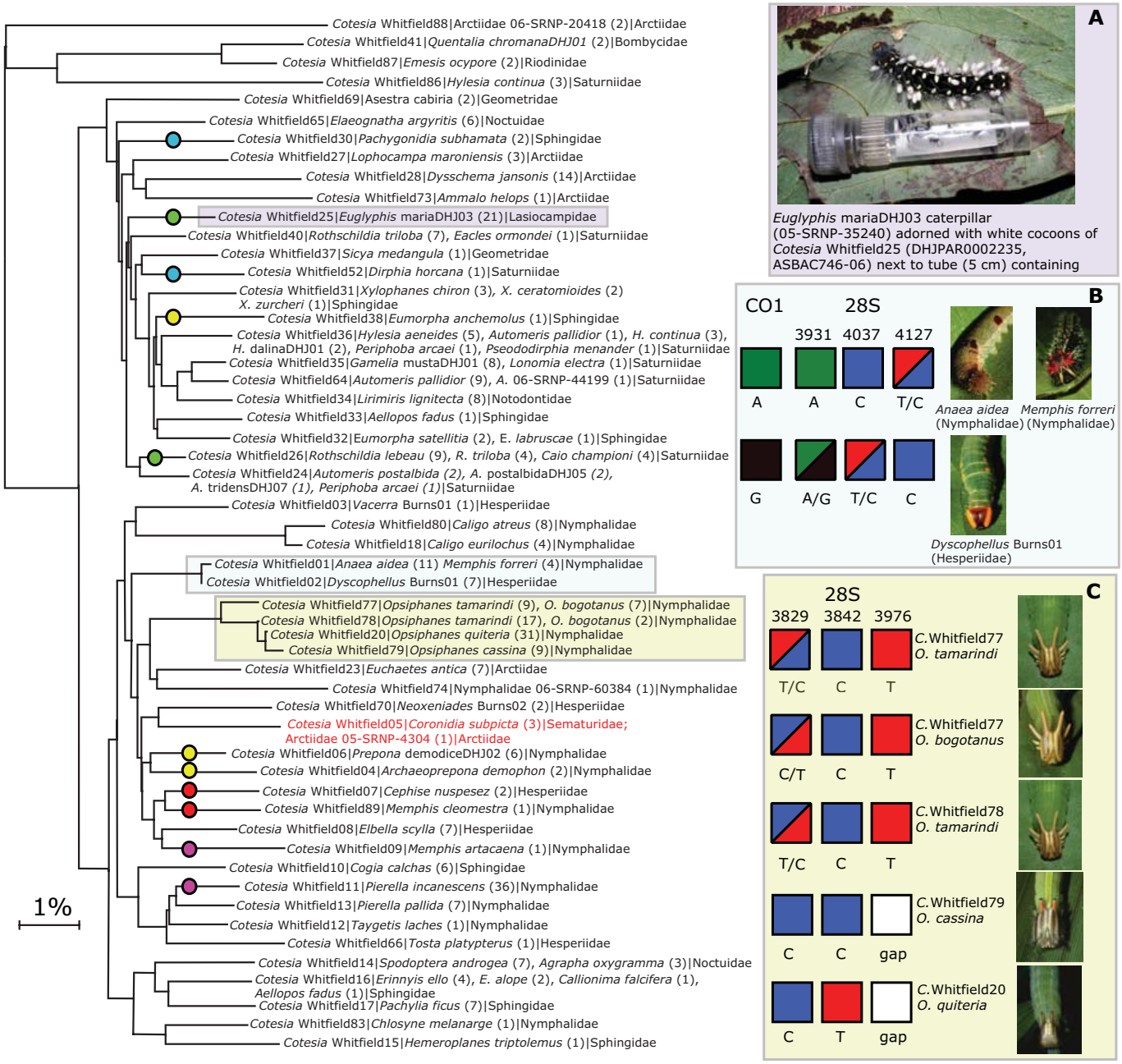

Fig. 1. NJ tree (K2P) with 1 representative for each of the 53 provisional barcode species encountered in the sample of *Cotesia*. Branches are labeled with provisional wasp species name, host species (sample size in parentheses), and host family. Colored circles on the tree link species-pairs or -triplets that would not have been differentiated with confidence using only morphology (See *SI Appendix*, Section 8). Red text flags a species whose host-breadth suggests that it contains further cryptic species that may have been missed by CO1 barcoding; however, no variation in 28S or ITS1 supported this hypothesis, and therefore we treat it as 1 provisional species. (*A*) Representative cocoon structure and usual size for parasitoid and host caterpillar (See *SI Appendix*, Section 4). (*B*) *Cotesia* Whitfield01 hosts (*Top*) and *Cotesia* Whitfield02 host (*Bottom*) are from different families that feed on the same species of plant in the same way, yet their barcodes differ by only 1 bp. Despite the extremely small barcode variation, there are polymorphisms within 28S at 3 loci that agree with host family, barcode, and morphology in supporting the hypothesis of 2 species. Numbers refer to the positions of polymorphic loci when aligned with the complete *Drosophila melanogaster* rRNA gene (M21017). (*C*) Species complex of *Cotesia* parasitizing *Opsiphanes* and having very similar but distinct CO1 barcodes (*SI Appendix*, Section 1). In this example, the combination of barcode, host, and 28S variation support the hypothesis that there are least 4 species of wasp. Within *Cotesia* Whitfield77, there are 28S polymorphisms that co-vary with host use and are suggestive of a species-pair that may have been missed by CO1 barcoding alone (as in *Aphelinus*, 28). Triangles represent polymorphic 28S loci. Base-pair composition is color-coded, and the upper left triangle is the dominant allele. See text for further explanation and *SI Appendix*, Section 4 for specimen and sequence accessions for individuals sequenced and pictured here.

panamensis, Menispermaceae). Their barcodes show more than 14% sequence divergence, and their reproductive isolation is supported by more than 5% divergence of 28S.

Example:* Apanteles *that parasitize* Telemiades *(Hesperiidae). The first 100 specimens reared of *Apanteles* Rodriguez26, parasitizing dry forest *Telemiades fides*, possessed a nearly invariant barcode (average intraspecific divergence = 0.104%). However, 2 additional barcode clusters showing 3% and 4% divergence from the common lineage were encountered with further sampling. If only the host records and wasp morphology had been considered, all would have been

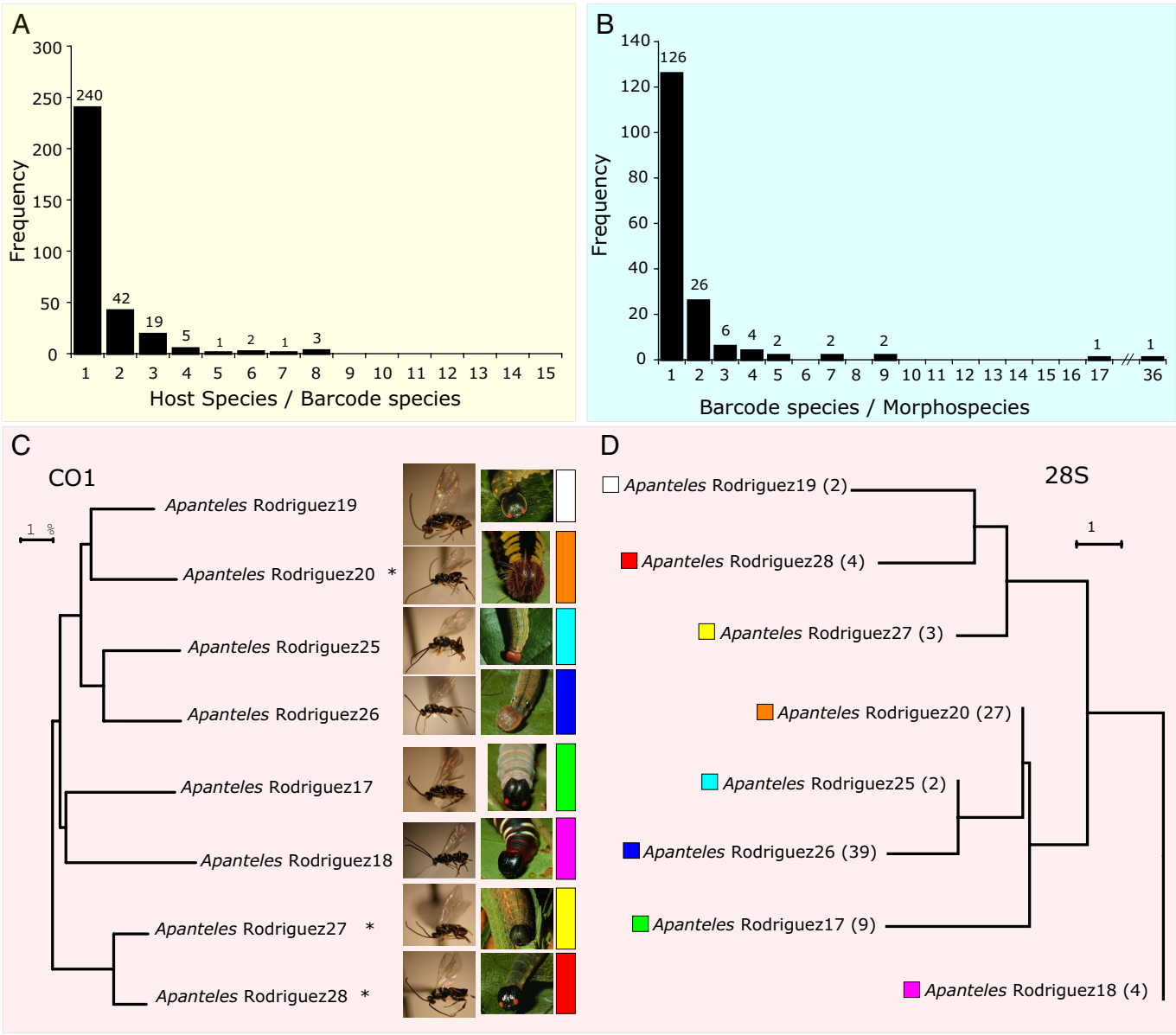

Fig. 2. Host specificity in ACG Microgastrinae. (*A*) Frequency histogram illustrating the number of host species of caterpillars used by each provisional species of wasp. (*B*) The number of provisional species of wasp using barcodes that are contained within each morphospecies of wasp. (*C*) CO1 NJ tree for 8 provisional *Apanteles* wasp species that are morphologically *Apanteles leucostigmus* (species 17 through 28). Accessions for the wasp and host species imaged are detailed in *SI Appendix*, Section 2. The 3 cases marked by an asterisk indicate a wasp whose host records include more than 1 species of host, but these species are all closely related species with nearly identical ecology. (*D*) NJ tree for 28S for same species presented in *C* and using a consensus sequence for each species (the number of identical sequences used is in parentheses).

identified as *Apanteles* Rodriguez26. Within the less divergent cluster (subsequently labeled *A.* Rodriguez155), there is no divergence from *A*. Rodriguez25 within 28S. However, the more barcode-divergent singleton (*A.* Rodriguez156) showed 4 substitutions at 28S that distinguish it from *Apanteles* Rodriguez26. *A.* Rodriguez25 (a species attacking *Telemiades oiclus*) also is invariantly different from *A.* Rodriguez26 in its 28S sequence (*SI Appendix*, Section 2).

These 3 cases reveal reproductively isolated species of parasitoid wasps that attack the same host species without any obvious segregation by microhabitats or ecosystem seasonality.

Invariant COI Barcodes that Encompass Intraspecific Nuclear Variation. ***Example:*** **Cotesia** ***attacking*** **Opsiphanes, *Nymphalidae*.** *Cotesia* Whitfield77 is a provisional barcode species that parasitizes 2 extremely similar species, *Opsiphanes tamarindi* and *O. bogotanus* (Fig. 1). These caterpillars eat the same food plant and occur in the same microhabitat but are easily distinguished morphologically (larvae of the latter have a red stripe down their backs). The barcode divergence within *C.* Whitfield77 is $< 0.5\%$ but 28S sequences of *C.* Whitfield77 show a single substitution that covaries with host species (Fig. 1, *SI Appendix*, Section 4). This pattern suggests a pair of incipient cryptic species within *C.* Whitfield77 that have not yet diverged in their CO1 barcodes. Since it appears that these 2 *Cotesia* wasps can distinguish between these 2 species of caterpillars, they represent the extreme end of host specificity.

Provisional Species Lacking Barcode Variation, but Their Use of Diverse Hosts Suggests that There Are 2 Species. Six (1.9%) of the 313 provisional species delimited by their barcodes were reared from

caterpillar hosts from a wide taxonomic range, often reared from different families (Table S3 and Table S4). The narrow host range of all other provisional species suggests that these cases represent additional species complexes of wasps that are indistinguishable by barcode (Table S3 and Table S4). However, analysis of 28S rRNA sequences revealed no sequence divergence, supporting the conclusion that, in these cases, what appears to be a species-pair actually may reflect ovipositional "errors" by the female wasp, and/or that these 6 provisional species are more generalist than usual in Microgastrinae.

Discussion

Microgastrine parasitoid wasps offer an excellent perspective of the taxonomic impediment. In a survey of fewer than 3,000 of these wasps reared from the ACG's 123,000-hectare conglomeration of tropical dry forest, rain forest, and cloud forest, we encountered 313 largely undescribed provisional species through their morphology, DNA barcodes, and host records. There is no reason to suspect that selective pressures acting on the adults of these tiny wasps invariably will result in species-level morphological diversity that will enable their identification by taxonomists in the field or even in the laboratory. The CO1 barcode was found to unerringly distinguish all of the provisional species that had been identified through morphological analysis. Furthermore, when the divergent barcodes (*SI Appendix*, Section 1) were considered in tandem with the host records, the number of provisional species in the sample was more than doubled. This result indicates that CO1 barcoding can provide a rapid and relatively inexpensive first screen of a large, diverse, and understudied biota in such a way that dramatically improves understanding of species richness and trophic food webs. Such an initial screening also dramatically sharpens the search for whatever correlative diagnostic morphological (and ecological) characters that might exist.

Our results indicate that the ACG microgastrine community is overwhelmingly host specific. Provisional morphospecies identification layered onto host records suggested this conclusion, the DNA barcode filter greatly refined the investigation, and provided independent support that makes us more confident in it. The barcode analysis also revealed cases where one host was parasitized by two morphologically similar but molecularly distinct species of wasps. This result would be missed using morphology and ecology alone. We found no evidence of any microgastrine species that is a generalist parasitoid of caterpillars to the same degree as are the 9 species of parasitoid tachinid flies documented in an earlier analysis of the same caterpillar fauna (16, 17). If this pattern holds for other microgastrine faunas, it will be good news for biological control programs, which often depend on the discovery and introduction of alien, host-specific natural enemy species. However, we note that the smallest interspecific divergence recorded here, 1 bp, is between species that parasitize different families of caterpillars. Such an apparent ability to make a large taxonomic switch is cautionary.

Seventy-four percent of the 171 provisional morphospecies were perfectly congruent with a single species-specific barcode. However, in the remainder of the morphospecies there were 1 or more groups of different barcodes (Fig. 2*B*, Table S1). When these groups are matched with host records, the number of provisional species in our sample more than doubled, as evident in the distribution in Fig. 2*B*. Whereas barcoding identifies the morphospecies (the dominant first bar of Fig. 2*B*), the diversity that morphological analyses misses (the tail of the distribution in Fig. 2*B*) is numerically dense and therefore doubles the species estimates for these 6 genera in aggregate. The most extreme case of overlooked diversity is the morphospecies *Apanteles leucostigmus* (*Apanteles* morphospecies22, and see Table S1). This minute black wasp with a distinctive white wing stigma was thought to parasitize 32 species of ACG hesperiid caterpillars, but barcoding revealed 36 provisional species, each attacking 1 or a very few closely related species of caterpillars.

Our confidence in positing the presence of at least 313 provisional species reflects the congruence of barcoding with host records, morphology, and sequence diversity at additional loci. For 3 genera (*Glyptapanteles*, *Cotesia*, and *Microplitis*), J.B.W. taxonomically characterized the specimens using a genus-specific standard matrix of characters and character states as a basis for comparison with the groupings delineated through barcode and host records. J.J.R. did the same for *Apanteles* and *Dolichogenidea*, and A.R.D. did so for *Alphomelon*. All 3 parasitoid taxonomists feel that, given time and resources, morphology-based alpha-taxonomy would discover many, but most decidedly not all, of the provisional species exposed by the combination of barcoding and host records (e.g., *Cotesia* in Fig. 1 and *Apanteles* and the long tail of the distribution in Fig. 2*A*). In those cases where morphology missed a provisional species-pair, two things were true: the barcodes were unambiguously different (e.g., for *Cotesia* see Fig. 1), and often, additional morphological qualitative character states could be used to identify these species. Inasmuch as time and resources for alpha-taxonomy are limited, a DNA-barcoding first pass through an unknown or understudied taxa or geography will aid species discovery, subsequent identification of specimens, and strategies for subsequent alpha-taxonomy (16, 17, 19).

High host specificity and very narrow host ranges within the parasitoids emerging from wild-caught ACG caterpillars is becoming increasingly expected. The highly host-specific patterns of parasitism uncovered in the Tachinidae (16, 17) are multiplied here by a factor of 3: the emergence of 36 provisional species from 1 morphospecies that had been recognized before barcoding (*Apanteles leucostigmus*) nearly triples the highest total from the tachinids, 12 species from 1 (17). It also more than triples the record for ACG Lepidoptera, 10 species from 1 (20) and surpasses the extraordinary diversity of cryptic amphipods, 33 species from 1 (21). A recent meta-analysis hypothesized that rates of cryptic species discovery will not vary based on taxa or region (22). Extending our results conservatively within 1 subfamily of braconid wasps across taxa and latitude would set the stage for actively reconsidering the upper boundaries for the total number of undescribed, cryptic species remaining in the world.

One pair of species worth special attention is *Cotesia* Whitfield01 and *C.*Whitfield02. Their barcodes differ by only 1 bp (Fig. 1). The variable and diagnostic locus is not included in the 5′ region from which many of the short barcode sequences have been generated, and this draws attention to the issue of species identification accuracy with respect to region selection and amplicon size, as has been noted previously within fishes (23), wasps (18), and Lepidoptera (24). Morphology and ecology expose this pair of species, giving us confidence that a single-bp difference in the barcodes can also reveal a real species-level difference. Even with such a shallow CO1 divergence, there is a predictable 28S-character state change between the 2 species: polymorphism versus homozygosity at 3 bp positions within 28S separates *C.* Whitfield01 from *C.* Whitfield02 (Fig. 1*B*) and *C.* Whitfield78 from *C.* Whitfield20. Why does the *C* Whitfield01/02 species-pair have such small CO1 divergences associated with such a large host difference as well as an aggregate 3bp difference within 28S? Is this a recent jump to *Dyscophellus* Burns01 (Hesperiidae) from an ancestral charaxine (Nymphalidae) host? We expect so, because they feed on the same species of plant and have very similar nesting behavior. Or is this a case of exceptionally slow mitochondrial molecular change? This wasp species pair exemplifies the most shallow barcode divergence that we used to differentiate provisional species, and such a low level of divergence was only used to distinguish among species when it was congruent with other traits.

Only this synergistic, iterative, team-based collaboration between field ecology, standard morphological alpha-taxonomy, and molecular biology could produce the understanding of the ACG microgastrine biodiversity portrayed here. It is illustrative to compare the contrasting scenario represented by shallow barcode separation of

Cotesia Whitfield01 and *Cotesia* Whitfield02 with the barcode separations within *Apanteles* Rodriguez32, a provisional morphospecies with upwards of 3% intraspecific variation within its CO1 sequence (but no overlap with other species). Without the benefit of host-rearing records, and based on the barcodes alone, the *Cotesia* would have been combined as 1 species, and *Apanteles* would have been split into several. Comparing the host records with the barcode divergences reveals exemplars of both low interspecific and high intraspecific variation. In the absence of the rearing records (as in field studies based on Malaise- or yellow pan-trapped microgastrines), following a barcode first pass analysis with a standardized nuclear marker can be an efficient strategy to reject or corroborate provisional species hypotheses erected using the barcode data.

Globally, and especially in the tropics, species discovery cannot afford to wait to collect all of the complementary data desired for the erection of seemingly finalized species hypotheses. In the few cases where there is more than the usual collateral information, proof of principle for use of DNA barcodes in species discovery is feasible. The ACG caterpillar and parasitoid inventory is such a dataset. The barcoded specimens are vouchered in permanent collections for repeated iterative study and are linked through publicly accessible databases of host records and associated metadata. This approach is fully integrative with collections-based alpha taxonomic and faunistic efforts.

Previously, we used CO1 DNA barcoding to demonstrate that the presumably generalist species of Tachinidae are largely arrays of specialists containing many fewer somewhat generalist species (16, 17). Here we have extended this protocol to 6 genera of parasitic Hymenoptera. Barcoding ACG morphospecies of skipper butterflies (Hesperiidae, Lepidoptera) increased their species richness by 10% (23), but barcoding microgastrine wasps of these 6 genera has increased their species richness by more than 70%. This iterative process of discovery, hypothesis generation, and hypothesis testing is a powerful component of this accelerated and integrated program of collaborative taxonomy (25).

Conservation biology is a discipline with a deadline (25, 26), and it is the taxonomists and ecologists of the world who are tasked with identifying the priority species and spaces to ensure that we beat the deadline. Unfortunately, the taxonomy underpinning the conservation effort has been chronically underfunded (27), and funding for the long-term ecological studies that allow the collection of complementary data essential for forming species hypotheses is notoriously difficult to maintain. Barcoding has demonstrated, in an increasingly large number of taxa, that it can offer society and science the acceleration of both species identification and discovery (16, 17) at reasonable costs.

Materials and Methods

Our methods are essentially as described previously (16, 17) and are covered in detail in SI *Material and Methods*. For *Apanteles*, *Microplitis*, *Alphomelon*, and *Dolichogenidea*, which are being studied and revised more broadly by J.J.R., J.B.W., and A.R.D., respectively, morphospecies were determined by comparison to other available specimens by using characters found to be diagnostic from previous studies. For *Cotesia* and *Glyptapanteles*, assignment of morphospecies has less surrounding context and followed the J.B.W. protocol (SI *Material and Methods* and SI *Appendix*, Section 8). In each case, we attempted to delineate morphospecies *a priori* independently of the barcode data and host relationships so that we could determine what the latter 2 traits add to biological species delimitation, rather than to supply a phylogenetic classification. All collection information is deposited at http://janzen.sas.upenn.edu and is available on a specimen-by-specimen basis by search on the DHJPARxxxxxxx specimen voucher codes. Sequences, trace files, and field data are available in the Published Projects section of the BOLD website (www.barcodinglife.org, ACG Microgastrinae). All sequences have been deposited in GenBank (Table S2). Wasp vouchers are organized and maintained in 4°C ethanol storage or dry point-mounted associated with the Illinois Natural History Survey/University of Illinois, Urbana–Champaign and North Carolina State University Insect Museum. For a list of primers used to amplify DNA sequences used in this study, see Table S5.

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