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OPINION

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Overcoming life stage-centric biases illuminates arthropod diversity, systematics and biology

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Abstract

Synthetic studies of arthropod systematics and biodiversity are hindered by overreliance on 'preferred' semaphoronts, those life stages (typically adult males) that provide the most taxonomically distinctive characters. However, modern sequence-based methods for inventory have no such limitations and permit incorporation of any and all representatives of a species. Here, we briefly review the growth and potential of these approaches to faunistic and systematic studies and share results from our own recent work that illustrate the value that other morphs, immature stages and females added to these studies.

KEYWORDS
arthropod biodiversity, dimorphism, larva, megabarcoding, semaphoront

INTRODUCTION

Identification of arthropods is an essential activity in biodiversity inventory, ecology, pest control and ecosystem management. Arthropods play countless ecological roles in natural and human-managed systems, from foundational niches such as soil formation to apex predators and parasitoids, keeping other species' populations in check. It is widely reported that taxonomy as a whole, especially within the hyperdiverse Arthropoda, is suffering from a lack, indeed an ongoing loss (Löbl et al., 2023; Wheeler et al., 2004) of expertise, and that this will have consequences for those endeavours that rely on accurate identifications. Less well reported is the fact that even at its best, traditional insect identification, and in many other groups of Arthropoda, is limited in its capabilities. Even where up-to-date taxonomic resources exist, the vast majority of arthropod species can only be morphologically identified to the species level (and occasionally more inclusive levels) using adult specimens, and often only adult male specimens at that. Immature stages and females are often identifiable only to the family or genus levels.

Considering the whole span of arthropod life, the German entomologist Willi Hennig popularized (if briefly) the 'semaphoront' concept, defined by him as an organism at a particular time in its life history, from conception to death (Hennig, 1965). A species constitutes a collection of more or less distinct semaphoronts, where all of them are essential aspects of its biology. In metamorphic organisms, which include the overwhelming diversity of animals, highly distinctive egg, larval, pupal and adult semaphoronts, in both sexes, may embody largely distinct sets of characters.

The emphasis in systematics on one semaphoront has limited, whether by design or by necessity, biodiversity inventory efforts to focusing preferentially on the most identifiable specimens, ignoring or regarding others as uninformative, or as an unavoidable nuisance (Domènech et al., 2022). For instance, in insects, the most widely publicized data associated with reports of overall declines have been entirely based on flying (therefore adult) insects collected by light and Malaise traps (Hallmann et al., 2017; Lister & Garcia, 2018; Mata et al., 2021), and many more general inventory attempts focus mainly on flight-trapped insects, mostly excluding other semaphoronts (Chimeno et al., 2023; Kaczmarek et al., 2022; Li et al., 2023; Montgomery et al., 2021; Srivathsan et al., 2023; Steinke et al., 2021). However, many of the critical life-history niches occupied by insects are populated by their immature stages, the portion of life during which most of their growth and development occurs, and this is equally true, if less well-appreciated, in other arthropod groups (e.g., oribatid mites; Norton & Ermilov, 2014). Further, when sampling is for whatever reason limited in time, many life stages whose period of activity is brief, synchronized or limited to a particular temporal window will be missed (Rodriguez-Soto et al., 2021). Similarly, males and females of many species may differ considerably in abundances, activity period, behaviour and longevity (Cordellier et al., 2020; Kirkendall, 1993; Sielezniew et al., 2020; Smith et al., 2019; Williams et al., 2022). By omitting unidentifiable immature stages or sexes in such cases, we lose much that is of interest to the community under study, including accurate

estimates of species richness, abundance, biomass, community structure and functional diversity (Fikáček et al., 2023; Marcos-García et al., 2012; Seniczak et al., 2022; Silveira & de Araújo, 2021).

At the same time, studies of arthropod phylogeny, taxonomy and evolution are greatly strengthened by inclusion of characters from all the forms a species and its genome may manifest (Faria et al., 2021; Lawrence et al., 2011; Meier et al., 2016; Norton & Ermilov, 2014; Yeo et al., 2018). While for phylogenetic purposes we would wish to concatenate data from distinct semphoronts to represent a species in an analysis (Sharma et al., 2017), in practice, this requires that those associations are known, which would be little more than hypothetical for the bulk of such species. Connecting semaphoronts definitively would provide rich new data for higher level analyses of relationships and natural history evolution.

The potential for molecular identification methods to help address these issues, allowing non-typical semaphoronts to contribute to studies in biodiversity inventory and systematics, has been recognized since DNA sequences first became readily accessible (Caterino et al., 2000; Stern et al., 1997). Associations of larvae through DNA sequencing have become routine (Ahrens et al., 2007; Caterino & Tishechkin, 2006; Curiel & Morrone, 2012; Jin et al., 2022; Miller et al., 2005; Sinclair et al., 2022; Zhou et al., 2007). A number of authors have also demonstrated successful associations of sexes in dimorphic species (Corley & Ferreira, 2017; Ekrem et al., 2010; Ferreira & Ivie, 2022; Gariepy et al., 2022). In addition, biodiversity inventories that have explicitly included immature stages in molecular assessments report significantly greater results (Blagoev et al., 2013; Ding et al., 2023; Domènech et al., 2022; Köhler et al., 2022), outside of entomology as well as within (Garcia-Vazquez et al., 2021; Kimmerling et al., 2017; Lira et al., 2022). Nonetheless, it seems to us that the broad-scale integration of all semaphoronts into biodiversity surveys and systematic studies remains to be openly and widely embraced.

Modern molecular tools, in particular community-level barcoding, or 'megabarcoding' (Chua et al., 2023), are particularly well suited to more comprehensive documentation of arthropod semaphoront diversity. So long as the samples entrained into such pipelines strive for more representative coverage of life history diversity, the resulting sequence data can characterize community richness more accurately, as well as effectively unite semaphoronts from across a species' spectrum. In addition, such intentional efforts stand to illuminate the life histories of the species involved, broadening our understanding of their phenologies, behaviours, stadium-specific niche associations and intraspecific diversities. In this paper, we call for further attention to this potential and illustrate the promise as revealed by associations discovered through a broad survey of litter arthropods in southern Appalachia, where we allowed any recognizeable semaphoront to stand as its own morphospecies in sample processing.

METHODS

Effectively field sampling a broader selection of life stages depends on the methods applied, and in many cases, it may be appropriate to combine complementary approaches to sample immature members of the arthropod community. Leaf litter sampling is perhaps the foremost of these, where larval specimens may be similar in numbers to adults (Coleoptera), or even vastly outnumbering them where most adults are winged and find their primary habitat elsewhere, as in the case of Diptera and Lepidoptera. This is the technique we employ here. However, other bulk sampling methods that may capture significant numbers of larval specimens include aquatic kicknet sampling, where most insects are represented by immatures (Kilian et al., 2022), pitfall trapping (Barney & Pass, 1986; Sabu et al., 2011), and soil trapping (Jowett et al., 2021), as well as beating and sweeping for plant-associated herbivores, predators and fungivores.

Our methods are described in detail elsewhere (Caterino & Recuero, 2023; Recuero et al., 2023), but briefly, we sifted leaf litter at more than 30 upper elevation sites in southern Appalachia, taking three or more ~1 m^2 samples at each site, and extracted all arthropods using Berlese funnels. The arthropod specimens were sorted first to major group, typically the Order level, and then, each set of samples from a site visit were sorted to morphospecies, explicitly considering all larval (rarely pupal), adult male and adult female specimens (where dimorphism precluded certainty as to identity) as distinct morphospecies. Each site was visited in two distinct seasons, typically spring and fall, performing complete morphospecies sorting of each independently. One individual of each morphospecies from every site and both site visits were extracted and processed through either Illumina (MiSeq) or Oxford Nanopore MinION barcoding pipelines. In either case, each voucher specimen was photographed prior to extraction (voucher photos available here: https://www.flickr.com/photos/183480085@N02/albums) and punctured or, for large specimens, subdivided for digestion and DNA purification using Omega BioTek's MagBind HDQ Blood and Tissue magnetic bead-based extraction kit. Most voucher specimens were retained (some tiny specimens were lost to pipetting) and are housed in the Clemson University Arthropod Collection. A 421 bp 'minibarcode' was amplified using primers BF2-BR2 (Elbrecht & Leese, 2017), each polymerase chain reaction tagged with a unique combination of 9 bp indexes, with the resulting PCR products combined, purified and prepared for sequencing according to each sequencing platform's requirements. Sequencing reads were filtered and demultiplexed (many with the ONTbarcoder program; Srivathsan et al., 2021), and aligned with MAFFT v7 online (Katoh et al., 2017).

For the present paper, we assess 'species' as estimated using Assemble Species by Automatic Partitioning (ASAP; Puillandre et al., 2021), for several major arthropod taxa, including Isopoda, Araneae, Coleoptera, Lepidoptera, Diptera and selected Acari, all of which are represented by substantial numbers of specimens of multiple semaphoronts. Each major group was analysed separately (allowing the optimal barcoding gap to be estimated for each). In a few cases (Isopoda and some Coleoptera genera), we refined the species delimitation results by considering morphological or other molecular characters. For each of these groups, we examined the numbers of ASAP-delimited species represented by immature or otherwise not-morphologically identifiable specimens, and considered their contributions to our study and to systematic

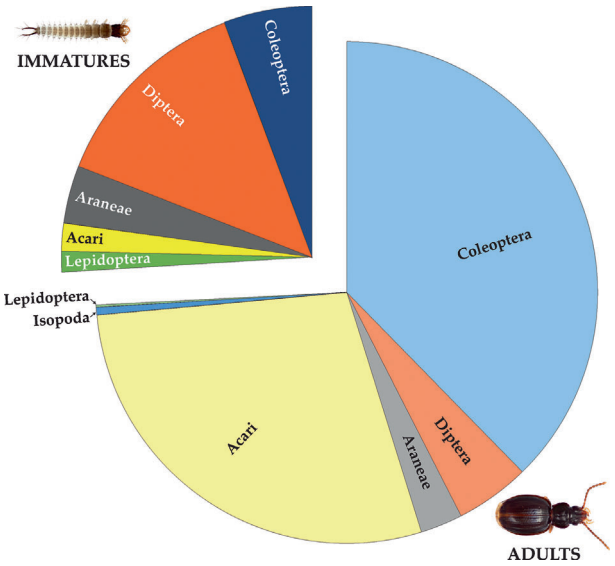

FIGURE 1 Pie chart of species representation of major groups by immatures only versus adults; of 1622 total species, 420 were represented by immatures only.

entomology more broadly. Thus, a species-level 'association' of semaphoronts was inferred if individuals were hypothesized to represent the same putative species.

RESULTS

The main focal taxa in this assessment were represented by 4977 sequences (2609 Coleoptera, 650 Diptera, 52 Lepidoptera, 571 Araneae, 965 Acari and 130 Isopoda). These were estimated to represent 1622 species (703, 295, 23, 104, 489 and 8, respectively; see Figure 1). In aggregate, 447 of these species (28%) were represented only by immature (420) or unidentifiable female specimens (27; this includes only Coleoptera and Diptera, where sexing of adults was unambiguous; Araneae was specifically not included because adult females of spider species are typically identifiable by characters of the epigynum). Furthermore, 1141 unique locality records were provided only by morphologically unidentifiable specimens.

Where adult species identities could be confirmed by morphological assessment (predominantly Coleoptera) or through GenBank/BOLD identification (most Lepidoptera, some Araneae), we searched the literature to determine what larval and female semaphoronts had been previously associated. The most noteworthy results were novel associations of 63 beetle species larvae. In at least seven of these cases (where the literature explicitly states that the larvae for a genus remain unknown; perhaps six others where it does not), these represent the first associations of larvae of their respective genera. In Lepidoptera, nine larval associations (out of 23 species) appear to be novel. In 13 additional species (Diptera and Lepidoptera), larvae and adults were associated, but the species identities were not determinable, so their significance remains unclear, though many are likely to be novel. In one Dipteran (*Molophilus hirtipennis* Osten Sacken), larvae, pupae and adults were all associated by barcodes (larvae, at least, were previously known). Winged males and wingless females were associated with two Dipteran species (one Sciaridae and one Cecidomyiidae, neither identified beyond family by morphology or DNA), both likely to be novel associations. In the Hymenopteran parasitoid *Dipara trilineata* (Yoshimoto) (Diparidae), we discovered that winged males and wingless females had previously been misassociated and were able to correct that mistake (Caterino & Arey, 2023).

DISCUSSION

Approaching this particular biodiversity inventory project without bias toward a particular, preferred semaphoront enriched the results substantially. At the broadest scale, one fourth of the species encountered were represented only by specimens that would have traditionally been considered unidentifiable, and either overly lumped or discounted altogether. The vast majority of these were larval specimens of the major holometabolous insect groups Coleoptera, Diptera and Lepidoptera. Morphological identifications of such larvae are notoriously difficult, laborious at least and impossible in many cases. Juvenile Araneae, Acari and Isopoda also each contributed significant numbers to the total. These inclusive results helped resolve a considerably richer leaf-litter fauna in the southern high Appalachian Mountains than an 'adult males only' approach would have suggested.

Such potential seems to have been appreciated most acutely by arachnologists. When explicitly incorporating immatures, Domènech et al. (2022) found that not only were juvenile spiders the most abundant individuals across several sampling techniques and areas, they contributed nearly 30% to the final species total. Similar results were found in spider surveys by Ding et al. (2023) and Blagoev et al. (2013). Perhaps the only hexapod taxon that has attracted similar attention is the Lepidoptera, where larval sampling has been a principal (e.g., Janzen et al., 2005) or conspicuous secondary (e.g., Strutzenberger et al., 2011) focus for larger scale inventory, although the strongly complementary data provided by larval specimens have also been highlighted in a Diptera (Syrphidae) inventory (Marcos-García et al., 2012). In aquatic systems, biomonitoring based on larvae, not only of Ephemeroptera, Plecoptera and Trichoptera but of Odonata, Diptera, Megaloptera and Coleoptera as well, is more or less the standard (Morse et al., 2007; Mendes et al., 2017), though even there surveys that focus on aerial adults versus benthic larvae may give varying results (Houghton et al., 2011), and important challenges remain in distinguishing species of larvae that vary in ecological tolerances (Morse et al., 2007; Resh & Unzicker, 1975; Zhou et al., 2007).

Considering our data at a finer spatial scale, the contribution of over 1000 unique localities by immatures has broadened our understanding of species distributions considerably, filling in some seeming disjunctions and extending some ranges at their edges. These have important implications for the management of any species that might merit conservation focus. Fuller distributional maps will also permit more accurate inferences of ecological requirements and tolerances

through record-based niche modelling (Taboada et al., 2013). Although our study lumped slightly separated samples from a site into one larger site-based species pool, even finer scale resolution might reveal microhabitat preferences more clearly, if abundances of adults and immatures vary significantly among samples.

Our lab's focus on Coleoptera allowed particularly fine-grained taxonomic resolution of these samples. Thus, we can confidently point to 63 novel species-larval associations. These larvae will be (and have already been) figured in detailed descriptions and represent the beginnings of comparative studies among known larvae of some soil and litter beetle taxa (Caterino & Harden, 2022). They are also allowing integrative descriptions of new species that include larval and adult characters simultaneously (Caterino, 2023; Haberski & Caterino, in press; Harden & Caterino, in prep.), a relatively uncommon opportunity. In some cases, these include multiple or even all larval instars, revealing ontogenetic series and developmental progressions of important character systems such as cuticular chaetotaxy. Particularly exciting are several cases where multiple congeneric species are now represented by larvae (Staphylinidae: *Geostiba* Thomson, *Lathrobium* Gravenhorst; Carabidae: *Trechus* Clairville, *Anillinus* Casey), allowing detailed comparisons of closely related species. Several newly associated larvae represent novel associations for their respective genera (several genera of Scydmaenine and Aleocharine Staphylinidae). These will yield particularly valuable phylogenetic data for resolving relationships in those subfamilies (Beutel & Leschen, 2005; Newton, 1990). In general, in those groups where taxonomic resolution was good (also including Araneae and Lepidoptera), valuable insights into immature habitat preferences, dispersal abilities and morphology are attainable. For example, in the newly associated larva of the crambid moth *Scoparia biplagialis* Walker, six records indicate a preference for spruce–fir litter. Other *Scoparia* spp. feed on mosses or grasses (Heckford, 2011), either of which might host *S. biplagialis* in the litter, but knowing the general habitat will allow the primary association to be sought more productively.

As has been reported elsewhere (e.g., Fikáček et al., 2023), a substantial number of higher Coleopteran taxa were represented almost entirely by larvae. Of 16 species of Cantharidae, only 3 were represented by a single adult each (two of which were female). Elateridae, Lampyridae and Tenebrionidae showed similar biases, with 31 of 34 species, 8 of 9 species and 9 of 12 species, respectively, represented only by larvae in our sampling. Some families were not represented by any adults, including Lycidae, Melandryidae, Melyridae and Ptilodactylidae. In all of these cases, in addition to the taxonomic richness that these larvae provided to the survey, it must be assumed that several of these will reveal novel microhabitat associations for species whose adults' preferences are found elsewhere, with critical implications for their conservation management.

Even where taxonomic resolution was lower, however, our results are encouraging. Diptera were represented predominantly by larvae (525 of 650 specimens and 217 of 295 species). These alone represent a sizeable portion of the total litter fauna and one of generally high abundance and probably high ecological significance (Frouz, 1999). Were flight traps deployed in the same areas, many or most of these species would likely have been documented, but as good fliers, it would have been impossible to know what and where their larval substrates may have been, that is, to what functional degree were they really members of the spruce–fir litter community? Clearly establishing the presence of larvae in the litter answers this question with some certainty. Dipteran diversity was high at the most readily identifiable levels, family and genus for most, with Tipuloidea, Chironomidae, Ceratopogonidae, Cecidomyiidae and Psychodidae together constituting more than half the specimens (both in species richness and abundance), with numerous other groups like Empidoidea, Mycetophilidae and various Muscoidea regular but less prominent. These gross levels should allow coarse-scale assessment about functional roles played by these conspicuous members of the fauna.

Acari represent a Pandora's Box of litter arthropod diversity and generally suffer in biodiversity inventories from denial and avoidance as much as from more specific taxonomic shortfalls. DNA barcoding in general makes their integration more realistic, although sheer diversity and abundance will continue to challenge such efforts (Young et al., 2021). In fact, substantial work has been done to resolve developmental series of mites, particularly in the surprisingly metamorphic Oribatida, and their larval and nymphal stages often are identifiable to genus (not only in spectacular immatures such as those of Compactozetidae, Figure 2v,w), but in the ptyctimous Phthiracaroidea and Euphthiracaroidea, and other common litter families like Nothridae, Nanhermanniidae and Pelopiidae (Norton & Ermilov, 2014). Still, within families, well-documented immatures are relatively few, and doubtless many new species associations will result from any broad survey in a new area, many perhaps for species themselves yet to be described. In those cases where multiple congeneric nymphs have been available, species differences are typically evident (Seniczak et al., 2021) and useful in systematic studies (Seniczak & Seniczak, 2022). Outside of Oribatida, there was considerable disparity in immature/adult morphology in several other mite taxa, including Zerconidae, Sejidae, Uropodidae (Figure 2q–s), Trachytidae, Labidostommatidae and others. While some of these are fairly obvious, at least with congeneric adults in the same samples, and at least minimally documented (e.g., Sikora, 2014 for Zerconidae), others are not, particularly, again, at the species level.

In Coleoptera, morphologically unidentifiable female specimens also contributed substantially to the taxonomic and geographic representation of our survey results. The best example is in the pselaphine Staphylinid genus *Eutyphlus* LeConte, with six described, broadly sympatric species in southern Appalachia (Owens & Carlton, 2016). Like many Pselaphinae, species are only identifiable by genitalic and secondary sexual characteristics of adult males. Species delimitations based on 56 cytochrome oxidase subunit I sequences suggest that there are as many as 25 species. Because sex ratios in the field appear highly biased, 42 of these sequences are females, and 16 of the species are represented only by females. With most localities appearing to host two or three sympatric species, molecular data from the females were critical to accurately assessing their actual richness. This is only the most extreme example of a common complication in arthropod inventory, where taxonomy is based heavily or entirely on adult male morphological characters. Streamlining the incorporation of female specimens will enrich inventories (Ekrem et al., 2010), and documenting definitive associations of neglected females will also

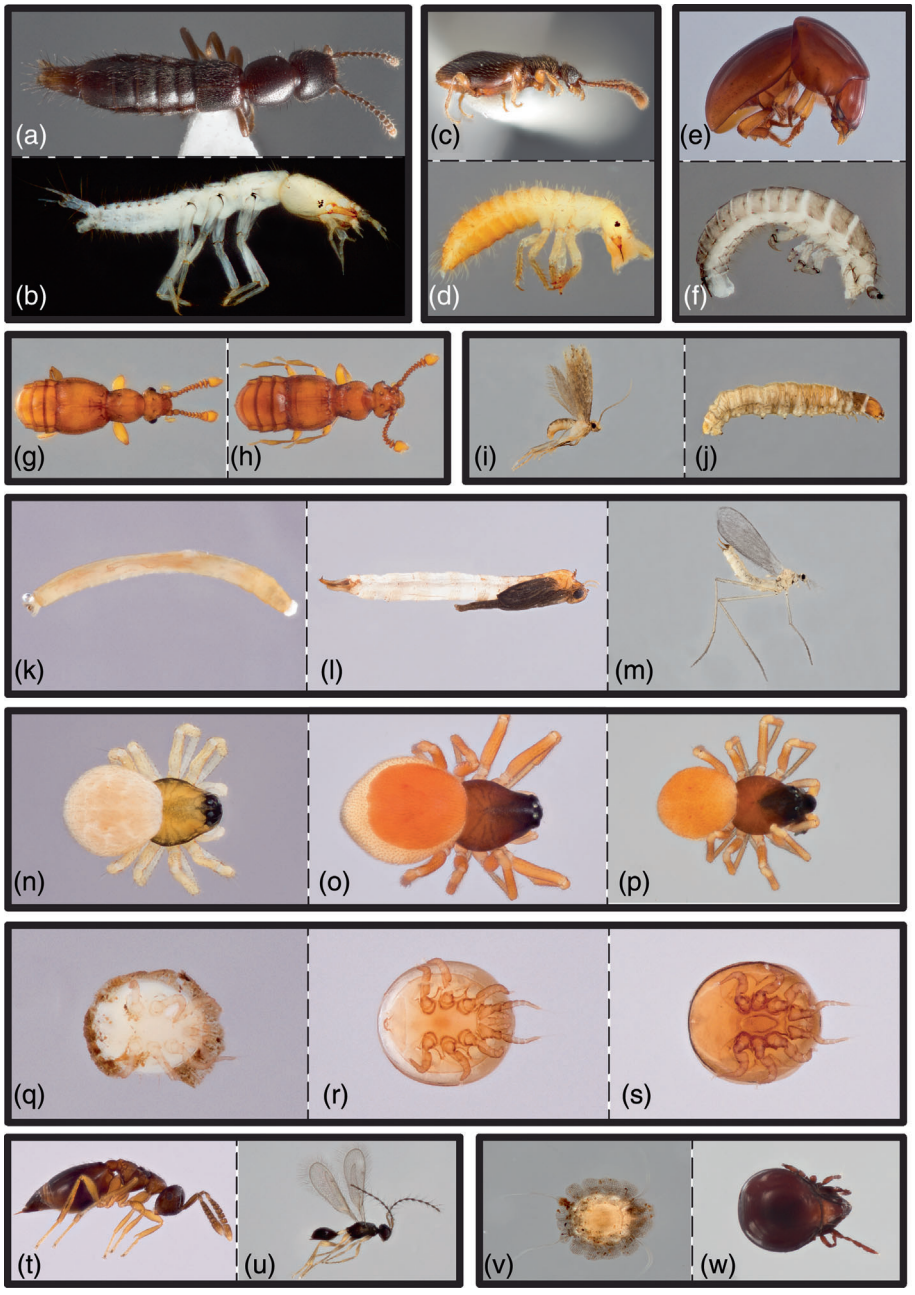

FIGURE 2 Examples of taxa associated using megabarcode sequencing. (a, b) Adult male and larval *Medon icarus* Caterino (Coleoptera: Staphylinidae), respectively. (c, d) Adult male and larval *Euconnus megalops* Caterino (Coleoptera: Staphylinidae). (e, f) Adult male and larval *Agathidium kimberlae* Miller & Wheeler (Coleoptera: Leiodidae). (g, h) Adult male and adult female *Eutyphlus prominens* Casey (Coleoptera: Staphylinidae). (i, j) Adult and larval *Tinea mandarinella* Dietz (Lepidoptera: Tineidae). (k–m) Larval, pupal, and adult male *Molophilus hirtipennis* Osten Sacken (Diptera: Limoniidae). (n–p) Immature, adult female, adult male *Ceraticelus fissiceps* (O. Pickard-Cambridge) (Araneae: Linyphiidae). (q–s) Two immature instars and adult male Uropodina (Acari). (t, u) Adult female and adult male *Dipara trilineata* (Yoshimoto) (Hymenoptera: Diparidae). (v, w) Nymphal and adult Compactozetidae (Acari).

permit more systematic surveys for female characters informative at the species level.

A similar case is observed among the isopod genus *Ligidium* Brandt (Oniscidea: Ligidiidae), with several species morphologically unidentifiable in the absence of adult males. In this case, we found an additional challenge, as we observed sympatry of different species in several localities in the southern Appalchians, frequently observed through molecular identification of a few female or immature specimens from large series (Recuero & Caterino, in press). The assumption in such cases that all females represent whatever species were collected as males is not always supported.

A reversal from the norm is found in the strongly dimorphic hymenopteran genus *Dipara* (Chalcidoidea: Diparidae). In these parasitoid wasps, the wingless, litter-associated females form the basis of

the species-level taxonomy, with the fully winged males of many species unknown. Where both sexes are known, many have been described in different genera, or even families (Braun & Peters, 2021; Desjardins, 2007). *Dipara trilineata* (Yoshimoto) is a common leaf-litter inhabitant in eastern North America, distributed from Louisiana to eastern Canada, described only from female specimens (Yoshimoto, 1977). A male association was asserted when *Trimicrops bilineatus* (Yoshimoto), which was described from both sexes, was synonymized with *D. trilineata* (Bouček, 1993). However, our sequences reveal that male to have been misattributed and that the correct male was presumed to represent a third species, a mistake that we can now correct (Caterino & Arey, 2023). Similar issues may plague other strongly dimorphic taxa. The sciarid (Diptera) genera *Epidapus* Haliday and *Pnyxia* Johannsen have flightless females, some of which have been named as separate species from their respective males (Mohrig et al., 2013). We have collected males and females of one or both of these genera, but despite both being nominally represented in BOLD, none cluster closely with any of the species currently represented by sequence data.

All of the above considered, we join a still-sparse chorus of voices advocating the inclusion of all life stages in biodiversity inventory. To some degree, this is principally a plea for deeper sampling in large-scale barcoding studies. However, there is also a measure of chauvinism in favour of the most easily identifiable semaphoronts in biodiversity studies of all kinds. We argue that there are great benefits to overcoming this bias and that megabarcoding studies offer a particularly useful way to mitigate it. As we navigate the ongoing decline in arthropod biodiversity, a more careful assessment of specific impacts and causes will require consideration of all life stages, and focused studies of immature susceptibility and survivorship will undoubtedly yield key insights.

One seemingly suitable response to the problem would be a greater emphasis on methods that are totally blind to the specimens involved. Surveys of eDNA have shown promise for arthropod inventories both in terrestrial (Kirse et al., 2021; Thomsen & Sigsgaard, 2019) and freshwater (Aunins et al., 2023) habitats. The potential for such methods to sidestep most sampling biases and taxonomic obstacles is attractive, despite its challenges and limitations (Rishan et al., 2023). However, it cannot be forgotten that they do not allow for downstream systematic applications like morphological character coding and diagnostics. They may offer a rapid, inexpensive snapshot of diversity, and if replicated reveal important spatial and temporal trends. However, their applicability to a broader range of systematic studies is minimal.

Looking further ahead, one of the developing frontiers of megabarcoding-based surveys is the potential to reveal interspecies interactions (Roslin & Majaneva, 2016). Such potential has already been realized in focused studies of host–parasite interactions (Hrcek et al., 2011; Sow et al., 2019; Varennes et al., 2014), plant-pollinator symbioses (Casanelles-Abella et al., 2022), predator–prey interactions (Krehenwinkel et al., 2017; Paula et al., 2016), plant-herbivore relationships (García-Robledo et al., 2013; Pitteloud et al., 2021) and microbiomes (Gibson et al., 2014). Broader scale biodiversity inventory could simultaneously reveal considerable ecological network structure through careful informatics. Most current sequencing methods would co-purify DNA of focal arthropods and their gut contents, body cavity 'guests', ectoparasites and phoretics, and internal and external microbiomes. Yet these connections are easily lost, through bulk laboratory methods that cannot identify the sequences originating from any single specimen, or through informatic analyses that assume a single 'correct' sequence for any indexed input specimen, filtering out the 'minority' contributors. Therefore, again, the time and care to segregate individual 'voucher' input specimens, which has been portrayed as an objectionable upfront cost to large-scale barcoding studies, can pay dividends later in the power of the resulting data.

In summary, the promise of molecular tools to overcome various impediments to the global characterization of arthropod biodiversity remains very strong. Here, our goal is to amplify a few related points, mostly that others have made along the way, yet which have not achieved sufficient consensus. Above all, keeping the longer term applicability of any given study's data at the forefront is critical from the outset. With a little more planning and effort in the early stages of a project—employing diverse sampling methods, utilizing all semaphoronts for analysis, and segregating, documenting and preserving individual vouchers that can be connected to indexed sequence pools—similar raw materials and data can serve far more diverse downstream purposes. We entered our current project with this hope as a guiding principle, and although we have not yet explored our data to its fullest extent, have been amply rewarded by the results to date.

AUTHOR CONTRIBUTIONS

Michael S. Caterino: Conceptualization; investigation; funding acquisition; writing – original draft; methodology; visualization; writing – review and editing; formal analysis; project administration; data curation; supervision. **Ernesto Recuero:** Conceptualization; investigation; writing – review and editing; methodology; formal analysis; data curation.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Sequence data referenced in this study are available on GenBank (accession numbers OP021206-OP021420, OP028109-OP028116, OP779406-OP779516, OR169027-OR174759, and OR803728-OR803736). The aligned sequence data are also available on Dryad: https://doi.org/10.5061/dryad.x0k6djhq0.

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