COMMENT / COMMENTAIRE

Comment on "Identifying spiders through DNA barcodes"[1]

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Abstract: R.D.H. Barrett and P.D.N Hebert have demonstrated that it is possible to identify members of a mostly local spider fauna using a short fragment of the mitochondrial gene coding for cytochrome *c* oxidase I. There are instances where DNA-based identification may be very useful, e.g., in identifying juvenile life stages of groups in which adults are required for morphological identification, or matching morphologically different sexes or life stages when those associations are unknown. DNA-based identification may be the easiest and most cost-effective way, or even the only feasible way, to address some of these questions. However, these are also the least challenging problems in taxonomy, and their solution is unlikely to relieve the "taxonomic impediment". Furthermore, to promote the utility of DNA barcoding as a global identification system, these authors must demonstrate that their approach works for distinguishing all the members of a speciose clade, wherever in the world they occur. Much of diversity occurs allopatrically and neither the study by R.D.H. Barrett and P.D.N. Hebert, nor any other presented to date, even begins to address the feasibility of DNA-based identification at this level of detail.

Résumé : R.D.H. Barrett et P.D.N. Hebert ont démontré qu'il était possible d'identifier les composantes d'une faune surtout locale d'araignées à partir d'un court segment type du gène mitochondrial de la cytochrome *c* oxydase I. Il y a des occasions où l'identification à partir de l'ADN peut s'avérer très utile, par exemple pour reconnaître les stades immatures chez les groupes où l'identification morphologique exige des spécimens adultes ou pour apparier des spécimens de sexe ou de stade différents qui ont des morphologies distinctes lorsque ces associations sont inconnues. L'identification à partir de l'ADN peut s'avérer être la méthode la plus facile et la moins coûteuse, et quelquefois la seule, pour résoudre ces questions. Cependant, il s'agit là de problèmes de taxonomie qui sont loin de poser un défi majeur et leur solution ne viendra vraisemblablement pas atténuer « l'obstacle taxonomique ». De plus, pour promouvoir l'utilisation des codes-barres d'ADN comme système global d'identification, les auteurs devront démonter que leur méthode permet de distinguer tous les membres d'un clade riche en espèces, où qu'ils se trouvent dans le monde. Une partie importante de la diversité se retrouve en situations d'allopatrie; ni l'étude de R.D.H. Barrett et de P.D.N. Hebert, ni aucune autre publiée jusqu'à maintenant, n'abordent, même de façon préliminaire, l'applicabilité de l'identification à partir de l'ADN à ce niveau de détails.

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Introduction

Barrett and Hebert (2005) extend DNA barcoding, a version of the "DNA taxonomy" paradigm (Tautz et al. 2002, 2003; Blaxter 2003, 2004; Blaxter and Floyd 2003; Hebert et al. 2003*a*, 2003*b*, 2004*a*, 2004*b*; Hogg and Hebert 2004), to arachnids and show that it is possible to identify members of a mostly local spider fauna using a short fragment of the mitochondrial gene coding for cytochrome *c* oxidase I (*COI*). At the core of Barrett and Hebert's (2005) paper is the question: should DNA taxonomy complement morphology-based taxonomy or replace it altogether? Despite criticisms, the latter idea continues to gather support, as some of the articles listed on the following Web sites attest: http://www.barcodinglife.org; http://barcoding.si.edu; http://phe.rockefeller.edu/BarcodeConference; http://www. nhm.ac.uk/science/BOL. It is therefore important to show that the current proposal of DNA taxonomy as a solution to the "taxonomic impediment" represents a misunderstanding of what taxonomy entails. Furthermore, it oversimplifies the challenges involved in the aspect of taxonomy where DNA is potentially most useful, i.e., species identification. DNA barcoding, and DNA taxonomy more generally, is just "one more tool in the box" of techniques available for species

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identification that may be useful in particular situations. The general utility of this technique as a global identification system remains undemonstrated.

Taxonomy, identification, and DNA

It is important to define at the outset what taxonomy is, and what it is not. Many recent criticisms of taxonomy (e.g., Godfray 2002*a*; Hebert et al. 2003*a*, 2003*b*; Tautz et al. 2003; Barrett and Hebert 2005) demonstrate a misunderstanding of its intellectual content (Lipscomb et al. 2003; Wheeler 2003, 2004; Will and Rubinoff 2004). The raison d'être of taxonomy is the definition, diagnosis, description, and naming of taxa, particularly species. Taxonomy is not "descriptive" and does not equate to species identification (Thiele and Yeates 2002; Lipscomb et al. 2003; May 2004; Wheeler 2004; Wilson 2004). Rather, it is a complex, dynamic science, not a simple (albeit technically difficult) collation of facts like the nucleotide sequences of a genome survey (Thiele and Yeates 2002; Scotland et al. 2003*a*). Taxonomists routinely filter numerous observations through an extensive knowledge base to decide that a group of specimens or taxa constitute a new taxon. Other taxonomists using the same knowledge base may validly arrive at different conclusions, and it may take time for thorough testing of the alternative concepts to achieve consensus, itself subject to future challenge and refinement. Reducing taxonomy, laden as it is with theory and knowledge, to a high-tech identification service industry denies its many levels of rigorous hypothesis-testing, from characters to species to clades, and impoverishes the wider information base that is crucial for describing biodiversity (Lipscomb et al. 2003; Wheeler 2003, 2004; Godfray and Knapp 2004; Scoble 2004; Wheeler et al. 2004; Wilson 2004). Just as technology is no substitute for science, DNA is no panacea for the woes of taxonomy. There are no short-cuts to comparing, analysing, and describing the diversity of organisms (Knapp et al. 2002; Scotland et al. 2003*a*; Seberg et al. 2003; Atkinson 2004; Holmes 2004; Scoble 2004; Wheeler 2004; Wheeler et al. 2004; Will and Rubinoff 2004).

A distinction must be recognised between the potential of DNA for defining or delimiting species versus its potential for identifying species. Despite opinions to the contrary (Wiens and Penkrot 2002; Blaxter 2003, 2004; Blaxter and Floyd 2003; Hebert et al. 2003*a*, 2003*b*, 2004*a*), DNA sequences used in isolation are widely considered inadequate for species delimitation, at least in eukaryotes (Ferguson 2002; Tautz et al. 2002, 2003; Mallet and Willmott 2003; Sites and Marshall 2003; Moritz and Cicero 2004; Wheeler 2004; Wheeler et al. 2004). Like other molecular markers, from allozymes to randomly amplified polymorphic DNAs, DNA sequences may well assist in species discovery, by suggesting candidates for further study in all aspects of their biology (Hebert et al. 2004*a*, 2004*b*; Moritz and Cicero 2004). DNA barcodes should be encouraged as a supplement to species description and diagnosis (e.g., see Brown et al. 2003), but should not replace morphological data (Mallet and Willmott 2003; Proudlove and Wood 2003; Scotland et al. 2003*a*; Seberg et al. 2003; Sperling 2003; Atkinson 2004; Knapp et al. 2004; Moritz and Cicero 2004). Many problematic complexes of cryptic species may be resolved only with the combined use of DNA and morphological data (Proudlove and Wood 2003; Godfray and Knapp 2004; Hebert et al. 2004*a*), but DNA need not be the first or only appropriate source of data in these cases either (Dunn 2003; Sperling 2003; Will and Rubinoff 2004). Even in groups where DNA sequences represent a primary source of data for defining species, e.g., prokaryotes (Theron and Cloete 2000; Nee 2003), protists (Finlay 2004), and nematodes (Floyd et al. 2002), there is no scientifically legitimate reason to exclude other available data a priori (Lipscomb et al. 2003; Wheeler 2003, 2004; Will and Rubinoff 2004). Species identification, rather than species delimitation, is the primary role for DNA taxonomy (Wheeler 2003, 2004; Scoble 2004; Wheeler et al. 2004).

Methodological limitations of DNA taxonomy

DNA has singular merit for diagnosing morphologically different sexes and life stages of local faunas, identifying parasites and their invertebrate disease vectors, DNA-surveillance, forensics, and various other applications (Baker et al. 2003; Besansky et al. 2003; Stoeckle 2003; Hebert et al. 2004*b*; Stoeckle et al. 2004; Whiteman et al. 2004). However, DNA-based species identification has many of the same limitations as DNA-based species delimitation. Both depend on sequence divergence percentages to distinguish intraspecific from interspecific variation, although the ranges of such divergences are still mostly unknown and will certainly vary among groups and across gene loci (Johns and Avise 1998; Ferguson 2002; Seberg et al. 2003; Stoeckle 2003; Dunn 2003; Moritz and Cicero 2004). DNA sequences between closely related, recently diverged, hybrid, or polyploid species, the very cases for which identification may be most crucial (Sperling 2003; Will and Rubinoff 2004), will often be too similar to allow their discrimination. There are at least two reasons (Besansky et al. 2003; Hebert et al. 2003*b*, 2004*b*; Mallet and Willmott 2003; Scotland et al. 2003*a*; Stoeckle 2003; Tautz et al. 2003; Atkinson 2004; Moritz and Cicero 2004). (1) Ancestral polymorphisms may persist long after speciation, while novel mutations may be slow to accumulate. (2) Genes may introgress between closely related species long after intraspecific coalescence would otherwise have fixed divergent alleles. There are numerous examples of identical or near-identical sequences in related species, even among higher animals and flowering plants (Besansky et al. 2003; Mallet and Willmott 2003; Moritz and Cicero 2004). It also remains to be determined if DNA barcodes will resolve identifications among taxa with deep sequence divergences, often those with low vagility or geographically structured populations (Sperling 2003; Wahlberg et al. 2003; Will and Rubinoff 2004). Most test cases presented to date, including the araneomorph spiders studied by Barrett and Hebert (2005), focus on vagile taxa, less likely to show substantial geographic variation than sedentary taxa (Atkinson 2004).

It is simplistic to assume that all species can be distinguished by a single fragment of one gene in the mitochondrial genome (Lipscomb et al. 2003; Mallet and Willmott 2003; Seberg et al. 2003; Tautz et al. 2003; Moritz and Cicero 2004). There is little reason to assume that there is a

universal barcode gene because no single molecular marker is sufficiently conserved to be amplified with universal primers in all domains of life, yet sufficiently divergent to separate closely related species (Blaxter 2003; Dunn 2003; Lipscomb et al. 2003; Stoeckle 2003; Godfray and Knapp 2004; Moritz and Cicero 2004). DNA-based identifications using maternally inherited mitochondrial markers may fail because of male-biased gene flow, divergent selection, and high rates of horizontal gene transfer from the mitochondrial to the nuclear genome (Mallet and Willmott 2003; Scotland et al. 2003*a*; Tautz et al. 2003; Moritz and Cicero 2004; but see Hebert et al. 2003*b*, 2004*b*; Stoeckle and Hebert 2004). *COI*, though widely applicable in Metazoa, fails to discriminate among closely related species of Cnidaria (Hebert et al. 2003*b*; Whitfield 2003), and its "universal" primer-binding sites are missing from other taxa, e.g., orders of tardigrades and nematodes (Blaxter 2004). Ribosomal genes, also in widespread use (Floyd et al. 2002; Blaxter 2003, 2004; Tautz et al. 2003), have profound alignment problems and are subject to different degrees of concerted evolution (Hebert et al. 2003*a*; Seberg et al. 2003; Finlay 2004). A multigene approach is required to distinguish closely related species, where identification is most difficult but often also most important (Blaxter 2003, 2004; Blaxter and Floyd 2003; Mallet and Willmott 2003; Sperling 2003; Tautz et al. 2003; Atkinson 2004; Moritz and Cicero 2004).

Irrespective of whether sequence divergence thresholds can be used to define species, the identity of species cannot be determined using divergence values alone. To derive identifications, sequences must be matched with those of conspecifics, one approach to which involves determining their placement in a reference phylogeny or "DNA profile" (Hebert et al. 2003*a*, 2003*b*, 2004*a*, 2004*b*; Hogg and Hebert 2004; Barrett and Hebert 2005). Correct identification thus depends on adequate taxon sampling (reference sequences from samples that have also been reliably identified) and, in the case of tree-based approaches, a robust phylogeny, neither of which have been satisfied in any test cases presented to date (Sperling 2003; Moritz and Cicero 2004; Will and Rubinoff 2004). For example, Barrett and Hebert (2005) sampled 111 sequences downloaded from GenBank, along with 216 newly generated sequences from a selection of north-temperate taxa. Sequences were included for only 3 of the 11 arachnid orders (Acari, Araneae, and Scorpiones), the third barely so (two sequences). Major arachnid orders, such as Opiliones and Pseudoscorpiones, were omitted altogether. Parasitiformes were grossly overrepresented, compared with Acariformes, despite their much lower diversity within the Acari (Coddington et al. 2004). The sample of spiders collectively represented less than 0.005% of the species (according to counts in Platnick 2004) in approximately 2% of the genera, 16% of the families, and only one of the three suborders, and was biased towards North America (and particularly, Canada). A rigorous test of the ability of barcodes to precisely assign individuals to species requires the inclusion of all members of major monophyletic groups, across the spread of cladistic diversity and geographical distribution (Sperling 2003; Moritz and Cicero 2004; Will and Rubinoff 2004).

Barrett and Hebert's (2005) study exemplifies concerns, long held by taxonomists working with other sources of data, that using one or a few specimens as representatives of species provides little information about their intraspecific variation (Funk and Omland 2003; Scotland et al. 2003*a*; Seberg et al. 2003; Sperling 2003). The problem is not solved by sequencing multiple individuals per species, as samples originating from the same population may group together simply because their sequences are very similar, providing a weak test of DNA-based identification (Sperling 2003). The grouping of samples from multiple allopatric populations would constitute a stronger test and confirmation. Barrett and Hebert (2005, p. 490) acknowledge that "much of this diversity occurs allopatrically" but neither their study, nor any other presented to date, even begins to address the feasibility of DNA-based identification at this level of detail. As long as DNA-based identification depends on reference databases of DNA sequences (e.g., GenBank) and these databases are neither exhaustive nor representative, taxonomically or geographically, the approach will be severely handicapped. Multiple haplotypes, from as many geographically isolated populations as possible, are required for every species in the database, to derive identifications with confidence (Sperling 2003; Holmes 2004; Will and Rubinoff 2004).

A related problem is that differences in divergence between putatively conspecific sequences obtained from such databases cannot be properly evaluated because there are little to no data regarding their geographical origins. Existing databases of DNA sequences are also replete with misidentifications that can only be solved by inspection of the vouchers from which DNA was isolated in the first place (Knapp et al. 2002; Seberg et al. 2003; Sperling 2003). The challenge of matching existing Linnaean names with DNA sequences is immense because expert taxonomists are scarce or nonexistent for many groups. Proposals to replace existing types with neotypes tied to barcodes (Tautz et al. 2003) are hazardous, and destructive sampling of type specimens to extract their DNA are potentially short-sighted, in light of future needs (Seberg et al. 2003; Will and Rubinoff 2004).

Considering the problems associated with obtaining correct identifications for every DNA sequence in the reference database, it seems naïve to suggest that distance-based analysis of test sequences as conducted by Barrett and Hebert (2005), Hebert et al. (2003*a*, 2003*b*, 2004*a*, 2004*b*), Hogg and Hebert (2004), and others (Tautz et al. 2003; Blaxter 2004) will confidently place every new sequence acquired or even a significant fraction thereof (Sperling 2003). Problems of aligning sequences of different length, distinguishing paralogs from orthologs, and even the application of different tree-building algorithms will compound the unreliability of identifications (Lipscomb et al. 2003; Seberg et al. 2003; Will and Rubinoff 2004).

Criticisms of the analysis conducted by Barrett and Hebert (2005) apply to most other recent studies using DNA taxonomy (see Holmes 2004; Will and Rubinoff 2004). Neighbor-joining, a phenetic method, widely considered philosophically inappropriate for phylogenetic reconstruction (Farris 1981, 1985, 1986, 1990; Swofford 1981; Penny 1982; Steel et al. 1988; Siebert 1992; Farris et al. 1996; Hillis 1996; Swofford et al. 1996), was used on the grounds that it recovers trees "at least as good as those generated by alternate methods" (Barrett and Hebert 2005, p. 483). Neighbor-

joining produces a single, fully resolved tree, regardless of how weak the signal is in the data. The absence of bootstraps or decay indices supporting the branches of Barrett and Hebert's (2005) tree, as well as the extremely short branch lengths, suggest that many are weakly supported. Weak support is echoed in the topology, which is dubious when compared with detailed analyses by others (Platnick et al. 1991; Hormiga 1994; Griswold et al. 1998, 1999; Coddington et al. 2004). Barrett and Hebert (2005, p. 488) euphemistically state that "there was a moderate level of association at the family level" when several major families and widely accepted monophyletic higher taxa were not retrieved: Amaurobiidae, Araneoidea, Dysderidae, Entelegynae, Gnaphosidae, Hypochilidae, Linyphiidae, Lycosidae (genus *Pirata* Sundevall, 1833 is a lycosid, not a pisaurid), and Salticidae. There can be little confidence in the correct placement (and consequent identification) of species in a tree with weak support and questionable groupings of higher taxa. A cladistic (synapomorphy-based) method of analysis would at least offer a hypothesis of phylogenetic relatedness even when a new sequence did not exactly match a previously identified sequence (Will and Rubinoff 2004). Beyond that, there seems no particular advantage of a tree-based approach to identification. Attaching species identifications, or nearest approximations, to sequences might be achieved as effectively by searching the reference database for exact, or closest, matches based on overall similarity or distance, much like the BLAST algorithm of GenBank. Similarly, it seems illogical to refer to barcodes as "unique identifiers", at least in their current formulation. Distances are arbitrary divisions of a continuum and there is no necessary requirement for an exact identification when a list of close matches, subject to further scrutiny with additional data, might be almost as useful.

Philosophical limitations of DNA taxonomy

Contrary to recent suggestions (e.g., Scotland et al. 2003*b*), there is no reason to give DNA greater stature than any other class of characters. DNA sequences are simply characters like any other (Lipscomb et al. 2003; Seberg et al. 2003; Wheeler 2004). Classifications based on single-character systems are bound to fail because other informative data are ignored. Descriptions and phylogenies based on a broad range of data make broad predictions about the distributions of attributes among organisms, and are more interesting than those based on a narrow range of data. When nothing is known about organisms except their DNA, there are no evolutionarily interesting patterns to explain, only repetitive patterns of sequence similarity (Lipscomb et al. 2003; Wheeler 2004; Will and Rubinoff 2004). Exclusive recognition of species as molecular operational taxonomic units (MOTUs), clusters of sequences divided by an arbitrary percent divergence threshold (Floyd et al. 2002; Blaxter 2003, 2004; Blaxter and Floyd 2003), would divest us of knowledge about the natural world with its richness of morphology, behaviour, and ecology (Sperling 2003; Wheeler 2003, 2004; Holmes 2004; Godfray and Knapp 2004; Will and Rubinoff 2004), and reduce the *Encyclopedia of Life* (Wilson 2003, 2004) to an impoverished shadow (Scotland et al. 2003*b*). Morphological and other phenotypic data remain relevant in a molecular millenium (Baker and Gatesy 2002; Freudenstein et al. 2003; Jenner 2004; Wiens 2004).

Arguments justifying the need for DNA on the basis of "problematic morphology" (e.g., Godfray 2002*a*, Hebert et al. 2003*a*, 2003*b*; Tautz et al. 2003; Barrett and Hebert 2005) are misleading. Many of the problems cited by Barrett and Hebert (2005) reflect an earlier time when taxonomy was conducted with fewer specimens and less was known about ontogenetic or sexual variation. Such problems disappear when revisions are based on adequate series of specimens and a limited knowledge of the biology of the taxa in question. Furthermore, new technology has given taxonomists access to additional morphological character systems, with the potential for identifying both sexes at all life stages (Coddington et al. 2004). Recent technological advances for three-dimensional reconstruction of morphology, notably confocal laser scanning microscopy (Klaus et al. 2003; Schawaroch et al. 2005) and microcomputer tomography (Wirkner and Richter 2004), are accelerating the pace at which morphological characters are discovered and documented, while a parallel "revolution" in online infrastructure is transforming the rate at which they are disseminated (Agosti and Johnson 2002; Anonymous 2002; Bisby et al. 2002; Gewin 2002; Godfray 2002*a*, 2002*b*; Knapp et al. 2002; Lee 2002; Agosti 2003; Scotland et al. 2003*a*; Wheeler 2003, 2004; Godfray and Knapp 2004; Scoble 2004; Wheeler et al. 2004; Wilson 2003, 2004). There is no reason to abandon morphological data at a time when technology facilitates its rapid documentation, distribution, and interpretation (Wheeler 2003, 2004).

Some authors (e.g., Tautz et al. 2002, 2003) have suggested that DNA-based taxonomy will reduce the instability caused by nomenclatural changes but this is unlikely. Species are hypotheses, not facts (Thiele and Yeates 2002), and most name changes arise from changing concepts of taxa rather than from confusion over the names assigned to type specimens. Names would continue to change in a DNA-based taxonomy because the group of organisms circumscribed by a name tied to a DNA sequence would remain a matter of opinion (Mallet and Willmott 2003; Seberg et al. 2003; Lipscomb et al. 2003), even if there were "rules of thumb" for associating MOTUs with biological entities (e.g., Blaxter 2003, 2004). A DNA barcode could only ameliorate such confusion if the sequence used were constant among all members of a species but different in all others, and any other character meeting these criteria could also be used for that purpose (Lipscomb et al. 2003).

Sociological limitations of DNA taxonomy

As currently proposed, DNA taxonomy is unlikely to relieve the "taxonomic impediment", especially in the developing world. Rather, it threatens to retard taxonomic activity and usurp the very resources needed for it to survive (Dunn 2003; Lipscomb et al. 2003; Scotland et al. 2003*a*; Seberg et al. 2003; Sperling 2003; Wheeler 2003, 2004; Holmes 2004; Will and Rubinoff 2004). The validity of DNA-based identification depends on establishing and maintaining a database of reference sequences from specimens that have been reliably identified. This process requires cooperation among di-

verse scientists and institutions (Stoeckle 2003; Barrett and Hebert 2005), not to mention considerable time and money that might be invested in training and supporting more taxonomists, on one hand (Mallet and Willmott 2003; Rodman and Cody 2003; Wheeler 2003, 2004), and continuing biodiversity inventories in the world's many understudied ecosystems, on the other (Ronquist and Gärdenfors 2003; Scotland et al. 2003*a*; May 2004; Raven 2004).

Although DNA sequencing is increasingly easier and less expensive (Godfray 2002*a*; Tautz et al. 2002, 2003; Godfray and Knapp 2004), morphology-based taxonomy remains cost-effective and widely accessible (Dunn 2003; Scotland et al. 2003*a*; Seberg et al. 2003; Sperling 2003; Will and Rubinoff 2004). DNA-based taxonomy could disenfranchise many taxonomists who have limited access to sequencing technology (Knapp et al. 2002; Seberg et al. 2003; Godfray and Knapp 2004; Scoble 2004; Will and Rubinoff 2004). Regardless of the intuitive appeal of a portable *Star Trek* style "tricorder" (Pennisi 2003; Blaxter 2004; Godfray and Knapp 2004; Holmes 2004; Janzen 2004; Stoeckle et al. 2004), field biologists and naturalists are unlikely to reject traditional methods of identifying and classifying species based on visual examination and interpretation of morphological traits (Dunn 2003; Sperling 2003; Whitfield 2003; Godfray and Knapp 2004).

Conclusions

DNA-based methods are not demonstrably more objective, accurate, or useful than morphology or other sources of phenotypic data for species identification or other taxonomic purposes. DNA barcoding as presented by Barrett and Hebert (2005), and DNA taxonomy more generally, is just another technique for species identification that may be useful in particular situations but for which the general utility as a global identification system remains undemonstrated.

Ultimately, nothing will substitute for the observations and collections made by naturalists in the field (Scotland et al. 2003*a*; May 2004; Raven 2004). No modern technology will alleviate the biodiversity crisis unless there are numerous people who can recognise organisms and find them in their natural habitats. As such, the continued training of students, parataxonomists, volunteers, and others involved in taxonomy and conservation is essential and must remain grounded in morphology (Dunn 2003; Rodman and Cody 2003; Sperling 2003; Wheeler 2003, 2004; Holmes 2004; Will and Rubinoff 2004). It is difficult to envisage DNA aiding students to learn a flora or fauna, identify living or preserved specimens, or conduct fieldwork, without first training them to know the organisms, understand their features, and identify them visually.

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