Ask the ecologist

Are genetic barcodes the magic tools we need to achieve a precise estimation of biodiversity in tropical ecosystems? What are the advantages and problems associated with this methodological proposal?

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Molecular genetics has long provided useful diagnostic tools in medicine, forensic science, and evolution. Mitochondrial DNA (mtDNA) was often used because, before the polymerase chain reaction (PCR) was invented, it was the most accessible DNA available for study.

More recently, ease of PCR, sequencing and alignment, as well as rapid evolution, haploidy, and low effective population size of organelle DNA has led to the proposal that mtDNA could be used as a "barcode" to identify species of all organisms, by analogy with supermarket barcodes. Small sequences (primers) were readily found which are highly conserved, but which allow mtDNA from a wide range of organisms to be PCR amplifed. The originators of the DNA barcode idea, particularly Paul Hebert of Canada, proposed that a ~600-800 base fragment of the *Cytochrome oxidase I* mitochondrial gene (*CoI*) would enable sequence-based identification of most species across the living world.

Since then, DNA barcodes have become a major talking point in the systematics and biodiversity science community. There has been strong promotion of barcoding, particularly for the neotropical region by Paul D.N. Hebert and Daniel H. Janzen. The promotion effort has successfully brought in major grant funds. An organization, The Consortium for the Barcode of Life (COBOL, www.barcoding.si.edu), now oversees the barcoding community, and there is also an associated online data repository, Barcode of Life Data Systems (BOLD, www.barcodinglife.org). Some scientific journals (e.g. *Molecular Ecology Resources*) now require submission of mtDNA barcode data to BOLD. The movement has not been without critics, however. Two criticisms, and their possible solutions are discussed below.

Problem 1: CoI doesn't work as a barcode in all groups of organisms, particularly plants. In these groups, mitochondrial DNA may have lower substitution rates than in the animals studied by Hebert. Therefore, *CoI* in flowering plants can be inefficient for identifying species. *Solution:* Use a different (e.g. chloroplast DNA) sequence in flowering plants or other organisms for which *CoI* is not useful, even though this makes barcoding somewhat less universal. Barcoders have agreed on a 2-gene cpDNA barcode, and 3 genes are useful for neotropical trees[1].

Problem 2: Divergent *CoI* clusters don't necessarily correspond to species. Traditional morphology work and species description will take too long to document biodiversity on our planet before the current extinction crisis happens. It was therefore suggested that barcoding should be used in place of traditional taxonomy. However, many disagreed. For example, in my lab we have recently found problems for the common neotropical (and Venezuelan) butterfly genus *Mechanitis*[2]. There is a clear correlation *CoI* and nuclear genes, morphological and ecological differences. So *CoI* is useful. On the other hand, we have some equally major *CoI* splits (2-3% mtDNA divergence) *within* two of our species, *M. polymnia* and *M. messenoides*. These splits are not even associated with geography, let alone ecology, morphology, or nuclear sequences. In another genus, *Melinaea*, a number of good species are indistinguishable via barcodes.[3] DNA barcoding therefore finds some species (but not all) that are real, and some 'species' that are not. *Solutions*: (a) Accept some slop in the identification and discovery of species, or (b) continue using traditional methods and accept that not all species are going to be describable, or (c) both. Most now agree that *CoI* clusters alone are not a good way to delimit species, even though there is fairly good correlation between *CoI* clusters and what we mean by species in nature. In my opinion, traditional biology-based species studies are still enormously worthwhile. Finding a diversity of DNA tells us little about interesting functional aspects of diversity; morphology, ecology and behaviour. DNA taxonomy shouldn't make museum taxonomy extinct, and this is now the view of most barcoders as well.

In conclusion, barcoding is useful. It can help in rapid biodiversity surveys if we have more molecular biology expertise than taxonomic expertise. It may be essential when organisms are hard or impossible to identify by morphology (e.g. microbes, roots or seedlings of plants, immature stages of insects). But we should always bear in mind that DNA barcodes are not the same as supermarket barcodes, each of which uniquely identifies a single 'species' of food product. DNA barcodes are a lot messier.

1. Kress W.J. &c. (2009). Plant DNA barcodes and a community phylogeny of a tropical forest dynamics plot in Panama. Proc. Natl. Acad. Sci. USA (online).

2. Dasmahapatra, K.K. &c (2009). Mitochondrial DNA barcoding detects some species that are real, and some that are not. Molec. Ecol. Resources(online).

3. Whinnett, A., &c (2005). Strikingly variable divergence times inferred across an Amazonian butterfly 'suture zone'. Proc Roy. Soc. B 272: 2525-2533.