Study finds DNA barcoding requires caution without closer examination

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The goal of DNA barcoding is to find a simple, cheap, and rapid DNA assay that can be converted to a readily accessible technical skill that bypasses the need to rely on highly trained taxonomic specialists for identifications of the world's biota. This is driven by a desire to open taxonomic identifications to all user groups and by the short supply of taxonomists that do not even exist in many groups.

Although DNA barcoding is being rapidly accepted in the scientific literature and popular press, some scientists warn that we are being too hasty in wholeheartedly embracing this technique. Dr. David Spooner, a researcher with the USDA and an expert in the potato and tomato family (*Solanaceae*), offers just such a cautionary note against accepting this technique without closer examination in his recent article, "DNA Barcoding will Frequently Fail in Complicated Groups: An example in Wild Potatoes" in the June 2009 issue of the American Journal of Botany.

One of the critical issues surrounding the DNA barcoding debate is that using a section of DNA may not adequately distinguish among closely related species or complex groups. Moreover, in plants, there is still much debate over which gene sequence region should be used and its reliability. In animals, the 5' segment of mitochondrial cytochrome oxidase subunit I (COI) is relatively established as a barcoding marker, but Spooner highlights many groups where COI fails to distinguish species.

The COI region fails completely for plants because it evolves at a slower rate in plants and has a much more variable sequence. The search for alternative barcoding regions in plants is especially problematic. Although several gene sequences have been proposed for plants, none of them serves as a universal barcode marker. Regions that have been proposed for plants include a section of the nuclear ribosomal DNA: the internal non-transcribed spacer region (ITS); and various plastid regions to include the trnH-psbA intergenic spacer and the plastid genes rpoC1, rpoB, and matK.

Spooner tested the utility of barcoding in a well-studied but complex plant group, wild and cultivated potatoes (*Solanum* section *Petota*). Section *Petota* includes over 200 species and is widespread throughout the Americas. In his study, 63 ingroup species and 9 outgroup species (in the genera *Solanum*, *Capsicum*, and *Datura*) were used. DNA was extracted from young leaves of single plants. Spooner tested the most frequently suggested DNA barcoding regions for plants: ITS, trnH-psbA, and matK. He found that none of these regions were very accurate at distinguishing or serving as markers for species boundaries in the section *Petota*.

There was too much intraspecific variation in the nuclear ITS region, and the plastid markers did not have enough variation and thus failed to group together some well-supported species. Section *Petota* is a complex group because, among other things, there is much hybridization among species; some of the species have multiple divergent copies of their DNA arising from past hybridization (allopolyploidy); there is a mixture of sexual and asexual reproduction; and there is possible recent species divergence. Such complications are not uncommon in many plant groups. Spooner concludes that a variety of morphological and molecular approaches are needed, and we cannot rely on a DNA barcode alone to distinguish among species in complex groups such as section *Petota*.

Spooner extrapolates from the taxonomic difficulties of section *Petota* to many other groups possessing similar biological traits, and points out that DNA barcoding needs to be accepted with great caution as these groups have not been tested with the technique. He also urges caution against limiting the identification, or in some cases even the definition of a species to a small sequence of DNA. He suggests that the search for a DNA barcoding marker or limited set of such markers that reliably identify the majority of life forms will be a continuously elusive goal.

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