

# Utility of Mitochondrial DNA Barcodes in Species Conservation

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**Abstract:** *Molecular tools are a standard part of many conservation studies and can be informative at many different levels of analysis, although there are inherent limitations and strengths of different genes or parts of genes to inform specific questions. Animal DNA barcodes, 600- to 800-base-pair segments of the mitochondrial gene cytochrome oxidase I, have been proposed as a means to quantify global biodiversity. Although mitochondrial (mt) DNA has a long history of use at the species level, recent analyses suggest that the use of a single gene, particularly mitochondrial, is unlikely to yield data that are balanced, universally acceptable, or sufficient in taxonomic scope to recognize many species lineages. Mitochondrial and nuclear genomes have different patterns of evolution and modes of inheritance, which can result in very different assessments of biodiversity. The ramifications of choosing a particular definition of species (species concept) need to be carefully considered because current efforts have designated DNA barcodes as the universal species concept without demonstrating its superiority over preexisting concepts. The results of such a barcoding paradigm may include a failure to recognize significant portions of biodiversity or nuclear/mitochondrial mixed lineages and could spuriously focus conservation resources on populations with relatively minor mtDNA divergence. DNA barcodes are most likely to provide potentially useful information for groups that are already well studied, and such taxa do not constitute the majority of biodiversity or those in most need of research attention. DNA barcode-length sequences are an important source of data but, when used alone or out of context, may offer only a fraction of the information needed to characterize species while taking resources from broader studies that could produce information essential to robust and informed conservation decisions.*

**Key Words:** biodiversity, conservation planning, conservation prioritization, introgression, species concepts

Utilidad de los Códigos de Barras de ADN Mitocondrial en la Conservación de Especies

**Resumen:** *Las herramientas moleculares son una parte estándar de muchos estudios de conservación y pueden ser informativas en varios niveles de análisis, aunque hay limitaciones y fortalezas inherentes a diferentes genes o sus partes para informar sobre preguntas específicas. Los códigos de barras de ADN animal, 600- a 800- segmentos de pares de bases del gene mitocondrial citocromo oxidasa I, han sido propuestos como medios para la cuantificación de la biodiversidad global. Aunque el ADN mitocondrial (mt) tiene una larga historia de uso al nivel de especie, análisis recientes sugieren que es poco probable que el uso de un solo gene, particularmente mitocondrial, proporcione datos balanceados, universalmente aceptables o suficientes en su alcance taxonómico para reconocer linajes de muchas especies. Los genomas mitocondriales y nucleares tienen formas hereditarias y patrones evolutivos distintos, lo que puede resultar en evaluaciones de biodiversidad muy diferentes. Se deben considerar cuidadosamente las consecuencias de elegir una definición particular de especie (concepto de especie) porque los esfuerzos actuales han designado a los códigos de barra de ADN como el concepto universal de especie sin demostrar su superioridad sobre conceptos preexistentes. Los resultados del paradigma de los códigos de barra pueden incluir la falla en el reconocimiento de porciones significativas de linajes de biodiversidad o nucleares/mitocondriales mixtos y, artificialmente, enfocar recursos de conservación en poblaciones con menor divergencia de ADNmt relativamente. Es más probable que los códigos de barra de ADN proporcionen información potencialmente útil para grupos que ya están bien estudiados, y tales taxa*

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no constituyen la mayor parte de la biodiversidad ni los que requieren mayor atención. Las secuencias de los códigos de barras de ADN son una fuente importante de datos pero, cuando son utilizados individualmente o fuera de contexto, pueden ofrecer solo una fracción de la información requerida para caracterizar especies al tiempo que utilizan recursos de estudios más amplios que podrían proporcionar información esencial para la toma de decisiones de conservación robustas e informadas.

**Palabras Clave:** biodiversidad, conceptos de especie, introgresión, planificación de la conservación, prioridades de conservación

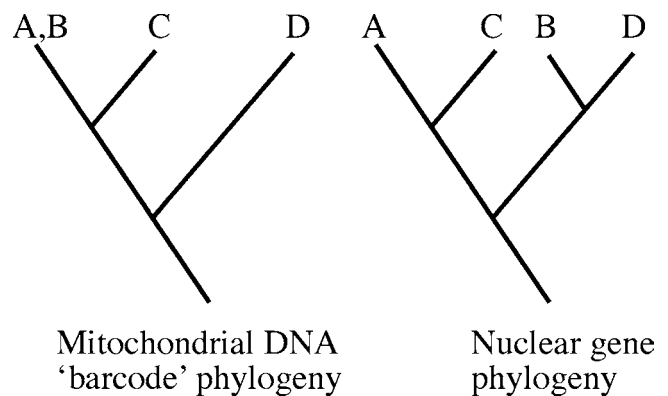
## Molecular Tools, DNA Barcoding, and Conservation

Molecular tools have become an indispensable part of innumerable systematic and conservation-based studies (Hillis et al. 1996), providing information across a large scale of research, ranging from differential heritage of genes within the same individual (Avice et al. 1983; Avice & Saunders 1984; Avice 2004) to population biology and species-level relationships (Holland & Hadfield 2002; Rubinoff & Sperling 2002) and studies that track ancient divergences between basal lineages (Deep Green: <http://ucjeps.berkeley.edu/bryolab/GPphylo/>). For conservation biology specifically, DNA data contribute to research as diverse as fine-scale management of fish stocks through the assignment of individual fish to one of several populations in the same watershed or fishery (Hansen et al. 2001; Ruzzante et al. 2004), to cryptic and invasive species recognition (Holland et al. 2004), identification of appropriate source populations for local reintroduction (Ludwig et al. 2003), and even tracking the postharvest use of sensitive species through forensic identification of animal parts (Shivji et al. 2002).

Despite the broad utility of DNA, the length and combination of markers best suited for any particular question is highly variable. Different genes, or parts of genes, often evolve at different rates, making the same genetic marker indispensable for one level of analysis but uninformative or even misleading at an older level of divergence (Reed & Sperling 1999; Rubinoff & Sperling 2002). For example, because it is haploid with a maternal inheritance pattern, mtDNA has an effective population size one-quarter that of the nuclear genome and reflects relatively rapid rates of substitution (Moore 1995), which makes mtDNA especially informative for species-level questions particularly when close phylogenetic relationships for the taxa in the analysis already have been ascertained based on other sources of data. However, for older divergences, those same rapid rates of substitution lead to saturation of base-pair changes that cause homoplasy (similarity not through descent) because phylogenetic signal is diminished (e.g., Hillis et al. 1996; Reed & Sperling 1999; Caterino et al. 2000; Rubinoff & Sperling 2002; Holland et al. 2004; McCracken & Sorenson 2005). Additionally, because different genes may reflect different evolutionary histories (Fig. 1) (Avice 2004), use of multiple genetic markers is

often necessary even for many intra- and interspecific studies to provide an accurate perspective on an organism's evolutionary history and systematic/taxonomic relationships (Funk & Omland 2003). Although the length of sequence needed from a particular gene and the optimal choice of genes to be included in an analysis are contingent upon the taxa and phylogenetic level of the study at hand (Baverstock & Moritz 1996), the use of a short sequence from a single marker is not offered as an alternative.

A central tenet of conservation biology is the preservation and management of global biodiversity. Two of the major impediments to such an endeavor are the difficulty of developing an assessment of this diversity for prioritization of hotspots of species richness (Dobson et al. 1997; Dunn et al. 1997) and the identification of lineages particularly worthy, or in need, of preservation (Daugherty



*Figure 1. Hypothetical phylogenies for species A, B, C, and D illustrating introgression as a source of potential disagreement between mitochondrial and nuclear DNA. Due to introgression, species A and B share an mtDNA haplotype. But based on nuclear DNA, species B is distinct and not even the sister species to A. Based on the nuclear DNA phylogeny, species A and B likely would be treated as separate conservation units. This inconsistency between the genomes is not uncommon (Shaw 2003; Funk & Omland 2003) and would lead to literally millions of misidentifications if DNA barcodes were implemented broadly.*

et al. 1990; May 1990; Vane-Wright et al. 1991; Faith 1994; Crozier 1997; Haig 1998; Soltis & Gitzendanner 1999; Moritz 2002). Understudied taxa are disproportionately vulnerable to extinction (McKinney 1999), suggesting there is a conservation penalty for our ignorance.

Even conservative estimates project millions of unidentified and unknown species (Novotny et al. 2002). To counter this dearth of knowledge, DNA barcodes, segments of approximately 600 base pairs of the mitochondrial gene cytochrome oxidase I (COI), have been proposed as a fast, efficient, and inexpensive way to catalogue all biodiversity (Hebert et al. 2003b; Stoeckle 2003; Stoeckle et al. 2003; Hebert et al. 2004). Barcoding is the use of universal polymerase chain reaction (PCR) primers to amplify and sequence an approximately 600-base-pair fragment of the COI gene (see Hebert et al. 2003a for more details). That portion of sequence is then compared using distance-based algorithms (which have their own suite of problems for barcodes; Will & Rubinoff 2004), with an existing database of "known" sequences from specimens previously identified by taxonomists. If the new sequence meets an arbitrary similarity criterion, recently set at 3% for all insects but 2% for birds and mammals (Hebert et al. 2003a), then the sample is "identified." If the dissimilarity is >3%, the sample remains unidentified and must be examined by a taxonomist. Animal mtDNA may be the most widely used and important genome for answering species-level questions (Caterino et al. 2000; Avise 2004), but most of such studies seek to answer specific questions in predefined groups for which other information has already been gathered. Therefore, the efforts are based on hypothesis-driven, directed research questions posed within a predefined experimental framework that seek specific answers, whereas true barcoding consists of broad, essentially blind and random surveys of communities with little or no background information.

Proponents of the barcode methodology have received extensive media attention including coverage in over a dozen newspapers and science journals in North America and Europe (available at: <http://www.barcodinglife.com/static/media/mediareponse.html>), and private donors such as the Moore and Sloane foundations recently committed more than US\$3 million to this effort, although the U.S. National Science Foundation and other public agencies remain uncommitted to the concept. The number of taxonomists is inadequate to tackle the job of assessing this biodiversity (Hebert et al. 2003a). In addition, the number of trained taxonomists capable of describing biodiversity incorporating morphological characters is in decline, although there have been efforts to change this trend (Lipscomb et al. 2003). Although many authors argue against barcoding (Lipscomb et al. 2003; Sperling 2003; Wahlberg et al. 2003; Wahlberg 2003; Will & Rubinoff 2004; Rubinoff & Holland 2005), here I focus on

a few points that are especially relevant to the impact a barcoding regime could have on the field of conservation biology.

Superficially, given the daunting challenge of identifying and preserving biodiversity for which science seems currently unprepared (Herbert et al. 2003), DNA barcodes from a small portion of the mitochondrial genome might seem like an effective and rapid way to assess at least some, perhaps minimal, level of biodiversity. And for groups that are already relatively well known, especially birds and mammals, molecular studies based on barcode-sized sequences have revealed cryptic DNA lineages and may be helpful (Herbert et al. 2004). However, the use of DNA to survey already studied groups or test hypotheses is not new (Moritz & Cicero 2004) and is not truly barcoding as defined in Hebert et al. (2003a). Identification of all life on Earth, not detailed studies on the best-known groups, is the stated incentive for committing the several billion dollars needed for global barcoding (Hebert et al. 2003a). I discuss how and why the barcode paradigm may not be the boon to conservation biology that supporters would like it to be. Shortcomings of barcoding for conservation include technical problems with the use of a single gene, particularly a mitochondrial gene, theoretical issues regarding barcode species concepts, a lack of broader context and utility for the final product, and practical problems specific to the implementation of a barcode-based conservation effort.

### Problems with mtDNA as a Sole Source of Data

Mitochondrial DNA can be a powerful tool in the effort to identify species, their relationships to each other, and threatened or endangered populations with divergent haplotypes worthy of conservation attention (e.g., Moritz 1994, 2002; Avise 2004; Holland & Hadfield 2002). But inheritance of the mitochondrial genome is not always predictable, and problems with heteroplasmy (Gryzbowski et al. 2003) may disrupt the typical "simple" inheritance pattern. In addition, the mitochondrial genome is usually maternally inherited, and patterns of nuclear genetic relationships in organisms can be quite different (Shaw 2002; Avise 2004). Phenomena such as hybridization and incomplete lineage sorting (see Ballard & Whitlock 2004 for a complete review; McCracken & Sorenson 2005) can lead to different species boundaries and levels of recognition between nuclear and mitochondrial genes for the same set of individuals (Fig. 1), resulting in (single) "gene tree" versus "species-level tree" disagreements. Funk and Omland (2003) found that in over 20% of the studies they surveyed the mitochondrial genome suggested a different pattern of relationships between taxa than that given by the nuclear genome, and they hypothesized that the actual number is higher but suppressed in results due to limited

sampling by researchers. For many years, results of studies have shown a wide range of functional and inheritance-based inconsistencies between mitochondrial and nuclear genes in many organisms that would lead to very different conclusions regarding the species' status, rarity, and conservation importance of taxa (Patton & Smith 1994; Ballard & Whitlock 2004; Rubinoff & Sperling 2004) (I do not address the technical aspects here). Because conservation priority is awarded based on taxonomic divergence (Faith 1994; Crozier 1997), the DNA barcode's inability to recognize any nuclear-based differences (or uniqueness) puts taxa that share mitochondrial but not nuclear DNA in peril (Karl & Bowen 1999).

Therefore, by focusing solely on the mitochondrial genome, mtDNA barcodes do not reference a larger and important genomic portion of the evolutionary history of the organisms that DNA barcodes are supposed to be identifying. I do not advocate avoiding mtDNA in systematic research, although others have suggested it (Ballard & Whitlock 2004). Rather, I suggest that an integrated approach that uses mtDNA and nuclear DNA, usually in conjunction with morphology and ecology, is better able to access different avenues of inheritance, producing more accurate results that are essential when assessing and managing biodiversity.

## Problems with DNA Barcodes and Species

Species are the principal currency of biodiversity and usually the focal taxonomic unit of conservation biology. The majority of conservation programs and legislation are focused on saving species (U.S. Endangered Species Act [ESA], World Conservation Union [IUCN] Red List of Threatened Species, Convention on International Trade of Endangered Species [CITES]). To make reliable and consistent conservation and fisheries management decisions, accurate, unambiguous, and robust species identifications are needed. DNA barcodes are supposed to increase our ability and efficiency in identifying new species. The most obvious flaw with such an effort is the assumption of what constitutes a species. On a practical level, only an individual "representative" sample from identified species will be used in analyses (Hebert et al. 2003a), and an individual is unlikely to regularly reflect the diversity and complexity inherent in the genetic makeup of a species as a whole. This can lead to an inaccurate characterization of what constitutes a species caused by localized or unexpected patterns of variation. On a theoretical level, dozens of (occasionally contradictory) species concepts exist (Wilson 1999; Wheeler & Meier 2000; Coyne & Orr 2004), with none able to claim supremacy over the others. DNA barcode "species" are an unexceptional addition to this pantheon. Species concepts are so contentious because none fully explain the

diversity of life or adequately capture the dynamic and varied processes that result in speciation.

The number of species recognized, and even regions of highest species richness (hotspots), changes depending on the species concept used (Peterson & Navarro-Sigüenza 1999). Considering the diversity of life and range of selective forces on its evolution, such inconsistency should be expected and incorporated but, if DNA barcodes are adopted, a species concept based solely on a small piece of DNA from one genome will take precedence *de facto* rather than due to any philosophical superiority or general agreement among scientists. This is because barcoding could relatively quickly "identify" large portions of biodiversity according to a barcode species concept. Thus, a barcode concept of what defines species will, by brute force in the form of sheer numerical dominance become the most prevalent species concept. But mtDNA, on which barcodes are based, fails to consistently reflect patterns found in other sources of data (Funk & Omland 2003) and therefore is an unwise candidate for the most prevalent species concept with which to identify and guide conservation of biodiversity.

Barcoding is not meant to and does not provide evolutionary information about taxa; rather, it is intended only as a means of "yes" or "no" identification based on predetermined units. But species come from a dynamic evolutionary process, and the failure of barcoding to incorporate evolution prevents it from accurately modeling evolutionary products. An illustration of the problems with using barcodes for species designations are the challenges pertaining to the association of any particular, predefined level of genetic divergence with the species boundary without the benefit of an ecological, morphological, or behavioral context. What percent divergence between two mtDNA barcodes constitutes a species? A figure of 3% for invertebrates and 2% for mammals and birds is proposed (Hebert et al. 2003a). This inconsistency between classes is arbitrary and places a lower value on the same level of genetic diversity for invertebrates. In addition, using a strict figure such as 3% promotes other problems. Does a 3% rule mean taxa that are 2.8% diverged (and therefore not species) should not be considered separate from a conservation perspective? What about well-studied subspecies that are >3% diverged but retained as subspecies (Wake 1997; Rubinoff & Sperling 2004) or otherwise distinct species that share haplotypes, especially over small sample sizes (Peters et al. 2005)? Although barcode proponents might suggest further investigation in those cases, this would be impractical given the selling points of speed and universal applicability and efficiency.

Because speciation is a dynamic, continuous process, taxa that do not neatly fit such 3% or 2% divergence rules will be the norm, not the exception. What if <3% divergence entails a major ecological difference (Crandall et

al. 2000)? If two taxa share mtDNA but not nuclear DNA, are they conspecific (Fig. 1)? Such a question cannot be considered because barcoding does not use nuclear data, but hybridization, or divergent selection, on mitochondrial and nuclear DNA can cause such inconsistent patterns of inheritance (Funk & Omland 2003). Advocates of barcoding have not adequately addressed these issues. Our inability to concisely model the complex process of speciation is not a validation for using an oversimplified species concept that fails to recognize more than one level of diversity.

Many examples of the complexity of speciation exist. Two that perhaps best demonstrate the inconsistency between species delineations and mtDNA divergence are the African cichlid fish radiations and California's *Ensatina eschscholtzii* salamanders (Avice 2004). Meyer et al. (1990) surveyed 14 species of cichlids (of over 200 recognized) in nine genera from Lake Victoria and found <1% mtDNA divergence among any of them, despite vast morphological and ecological variation so profound that the system is commonly used as a case study of speciation. In sharp contrast, the salamander *Ensatina eschscholtzii* forms a ring species complex some of whose members diverged more than 5 million years ago, with many subspecies having >5% mtDNA divergence from each other. Yet, because of population connectivity based on intensive ecological research, this complex is considered one species (Moritz et al. 1992; Wake 1997). Mitochondrial DNA barcodes would not hint at the fundamental mechanisms of evolution revealed by these classic studies. Rigid, molecular divergence rules enforcing species boundaries could obfuscate, not simplify, the problems of identifying units for conservation. Furthermore, because mtDNA barcodes cannot provide enough information about taxa to help make decisions regarding species status, and the technicians doing the barcoding will not have the expertise with all the new taxa they sequence; rigid rules for species boundaries based on prescribed divergence levels would be a necessary part of the procedure and a serious drawback to barcoding.

## Problems with Application

The success of conservation biology, as a field, is highly dependent on public support. Legislation authorizes protection of habitat and allocates funding for research. Economic demand for endangered species, their products, and the resources on which they depend fuels their demise. Local groups may provide crucial support for, or opposition to, conservation initiatives. The sociological component of conservation biology is evidenced by frequent articles evaluating the public's perceptions and support for specific conservation efforts and columns in journals such as *Conservation Biology* devoted to "Conservation Education" and "Conservation in Practice."

There has always been a contingent of the public that opposes saving habitat and species because the innate value of species is not universally recognized. This opposition is especially pronounced when conservation leads to economic deprivation. DNA barcoding could weaken the position of conservation biologists to fight for public support. If barcoding is adopted, conservation biologists could be forced, or at least distracted, into defending a species definition based on a small portion of one genome because we will be ignorant of almost all other characters for most species. Convincing the public to support such barcoded species when they can see little difference or value in their preservation will take more time and effort and undermine the credibility of the field. On a purely aesthetic level, broad-based mtDNA barcoding may short change species in terms of making them compelling to the public for conservation. Communities may lose interest in the conservation of many species because such conservation would be based wholly on DNA—something they do not understand or perceive and therefore probably do not appreciate. Conservation efforts that attempt to apply barcodes could be seen as unreliable, inconsistent, and perhaps even dishonest when otherwise indistinct species are championed based solely on barcoded segments of mtDNA. Barcoding protagonists are unlikely to share this challenge or be penalized by the erosion of the public's confidence because barcoding is simply a system of identification. The ramifications of its application will fall to those in more applied fields such as conservation.

Furthermore, if there is a lack of mtDNA variation between two otherwise distinct populations (a common result of introgression/hybridization between distinct species [Shaw 2002; Kulikova et al. 2004; Peters et al. 2005]), will conservationists willingly let a recognizably distinct population disappear without attempting to protect it? If not, the public may feel manipulated if the criteria for saving species changes from barcoding distance to another criterion when barcoding fails to recognize an entity of predetermined conservation value. Legislation protecting rare and threatened species is already under intense scrutiny and any erosion of remaining public support or perceived decrease in the integrity of conservationists could lead to weakened legal protection. The empiricism and simplicity of a standard measure such as an mtDNA barcode is appealing and initially easy to sell and explain. But application of an oversimplified standard is risky because it will be widely applied and essentially inflexible and therefore—when based on insufficient data—could be ultimately counterproductive.

## Conclusion

Because DNA barcoding enlists the biodiversity moniker, there may be some appeal to include it as an inherent part

of the conservation biology ethos. Supporters of barcoding expect the identification of all of life to have some application to preserving biodiversity, but such putative identifications can be questionable, incomplete, and frequently misleading. A possible caveat to the futility of barcoding is, in fact, hollow. This caveat is that barcodes could identify where cryptic mtDNA lineages exist in well-studied groups. In such cases it is not a "primary" barcode pushing frontiers and revealing raw biodiversity; rather, a secondary scan is covering already studied and identified taxa (Hebert et al. 2004). This is neither the crux of the problem nor the source of the biodiversity identification crisis. Most undescribed biodiversity lies in poorly known groups, particularly the invertebrates, especially the insects (Novotny et al. 2002). For such groups, the standard of knowledge is much lower than for vertebrates, and discovery of new species is commonplace. Even new orders have been recently discovered (Klass et al. 2002). Therefore, for most biodiversity, a DNA barcode will not help distinguish between cryptic, overlooked taxa in otherwise well-known groups; rather, barcodes will be the first, and almost certainly only, source of data for the identification of a new species, genus, or family.

Ultimately, mtDNA barcodes are unlikely to make a significant contribution and will siphon financial resources and energy away from poorly known groups that are in need of basic research based on more complete data sets (Lipscomb et al. 2003). Because barcodes will provide little data of conservation value, we must consider whether it is better to spend the US\$2 billion estimated cost of barcoding a portion of the world's species as they go extinct or use that funding to save as many of them as possible for future integrated research.

If DNA barcodes become the standard for identifying life on Earth, we may ultimately save only that fraction of diversity that corresponds to mtDNA divisions, learn little about the fraction we do save, and likely lose crucial support from a public that will be disillusioned with results based on a small subset of the genome that they cannot see. Conservation biologists must carefully consider the ramifications of using DNA barcode species as they become available. Despite the superficial and immediate convenience, such barcode species may be ultimately counterproductive because any studies or decisions based on barcodes could be questionable from their inception. There are no biodiversity assessment shortcuts. Diversity can only be measured by using a diversity of tools and characters. Morphology (Wiens 2004), ecology (Crandall et al. 2000), adaptive differences (*sensu* Waples 1991), and genetic data from the mitochondrial and nuclear genomes may tell different, equally valid, stories about the evolution, placement, and importance of a taxon.

Although it is obviously not always practical to use all possible sources of data for the vast task of identifying

biodiversity, it is essential to acknowledge the shortcomings of not doing so. Even just using two of the above-mentioned sources of data instead of one could enhance a study. This article should not be seen as an argument for nDNA barcoding, which would be fraught with its own set of shortcomings, some of them specific to the nuclear gene chosen. Instead, this is a call for the use of data from more than one source to account for the shortcomings inherent in the use of any small subset of the vast array of characters that together constitute an organism's identity. Proponents might argue that at least barcoding makes some information available for taxonomic decisions, but taxonomy based on insufficient data may not be better than no taxonomy (May 1990). At least with the latter there is no false sense of confidence when making conservation decisions.

If DNA barcoding is adopted it may decrease the stability of implementation and credibility of species identifications and leave conservationists with an exaggerated sense of confidence about the taxonomic units they manage while offering too little information to make informed decisions about most of the planet's biodiversity.

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