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DNA barcodes find four new bird species

Short stretch of DNA sequence fast, accurate method for identifying species

The task of identifying Earth's estimated 10 million species has daunted biologists for centuries — fewer than two million have been named. Using a technique called DNA barcoding, researchers at Rockefeller University and two Canadian institutions have uncovered four new species of North American birds. The findings are reported in the September 28 issue of *Public Library of Science (PLoS) Biology*.

The result is an important step toward proving that the sequence of a short stretch of DNA — a so-called DNA barcode — can be used genetically to identify known species and to find new ones.

"A uniform system to use DNA to identify all plants and animals would allow many more people — from environmental regulators to nature lovers — to identify organisms," says Mark Y. Stoeckle, M.D., guest investigator in the Program for the Human Environment at Rockefeller University.

"For humans, birds are probably the easiest species to identify. They're big, they're colored differently, and they sing different songs. Yet even in that easy to identify group, there are hidden species," says Stoeckle.

As the cost of DNA sequencing goes down, Stoeckle and other proponents of DNA barcoding envision developing a hand-held device that amateur naturalists and others could take outdoors for species identification.

"New species won't be determined by DNA analysis alone," says Stoeckle. "Morphology, behavior, and vocalization, for example, will still need to be accounted for in determining whether something is a species. But barcoding will enable rapid screening of large numbers of organisms and highlight those with novel barcodes that are likely to be new species."

Identifying Species by DNA

Taxonomists traditionally have classified organisms on the basis of their physical characteristics. They use DNA too, but current techniques are labor intensive and difficult to compare.

Zoologist Paul Hebert, Ph.D., at the University of Guelph, the first author on the *PLoS Biology* paper, proposed that a short DNA sequence from a gene found in

all animals can be used to identify species because in each species the sequence varies slightly. He coined the term DNA barcode for this idea, analogous to the supermarket barcodes that allow quick identification of millions of items.

The technique depends on analyzing a portion of a gene called cytochrome *c* oxidase I (COI) that is found in the power sources of cells of all animals. Most DNA is found in the nucleus of a cell. However, the mitochondria, the organelles within cells that are responsible for energy production, also contain DNA. Mitochondrial DNA (mtDNA) is known to accumulate mutations three to five times faster than DNA in the nucleus. As a result, the mtDNA of closely related species differs more than the nuclear DNA of those species and can be used to tell them apart.

In earlier studies, Hebert and colleagues at the University of Guelph showed that the COI gene was easy to isolate from a variety of animals, and that species in a broad range of animal life, from flatworms to vertebrates, have distinct COI sequences.

"The genetic barcoding technique developed by Paul Hebert and used in the *PLoS Biology* paper is the latest tool to be added to the taxonomist's toolkit, and it has enormous potential for broad impact," says David Schindel, Ph.D., executive director of the International Consortium for the Barcode of Life, based at the Smithsonian Institution in Washington, D.C. "The technique used to identify bird species by Hebert, Stoeckle and their colleagues could allow non-specialists to make these same species identifications using a relatively simple and inexpensive genetic test."

A DNA Barcode for Birds

The new research reported in *PLoS Biology* puts the COI barcode to a more discriminating test by measuring whether the technique could distinguish closely related species as well as very different ones. Stoeckle and Hebert, along with Hebert's Guelph colleague Tyler S. Zemlak and Charles M. Francis at the Canadian Wildlife Service's National Wildlife Research Centre, applied it to birds, one of the largest and best-studied vertebrate groups. Taking samples mainly from specimens held at the Royal Ontario Museum in Toronto, Canada, they analyzed COI sequences from 260 bird species that breed in North America.

The researchers first measured how much the COI barcode varied within members of the same species. Then they compared this variation with the degree of variation among birds of different species.

They found that each of the 260 species tested had a distinct COI sequence. For 130 of these species, the researchers looked at the DNA of two or more individuals. They found that, in most cases, the variation in sequences between species — even closely related ones — was on average 18 times higher than the variation among individuals within the same species.

"The ratio of 'interspecific' to 'intraspecific' variation offers a new provisional definition of what is a species," says Stoeckle.

New Species Discovered

In the course of their research, the scientists found two distinct COI barcodes within each of four species — solitary sandpiper, eastern meadowlark, marsh wren and warbling vireo. This discovery indicated that what had been identified as one species, in these cases, was actually two.

Based on traditional species identification methods such as morphology and behavior, some taxonomists had already suspected that these species should be split. The DNA barcode data confirmed the suspicions.

In the future many more species will be discovered both by splitting currently described species and by identifying the millions of unknown organisms yet to be collected and analyzed by taxonomists. Although most unknown species live in tropical climates, even in a place as well trodden as New York City's Central Park a previously unknown species of centipede was discovered two years ago.

"DNA barcodes will be the key for the naturalist's field guide for the new millennium," says Stoeckle.

A Public Library of Species

With the results reported today, scientists took an important step toward broadly applying the DNA barcode technique. "The first step toward a library is to show scientifically that a uniform approach can work. Then museums, ecologists and others can adopt it as a standard," says Stoeckle.

In fact, earlier this year an International Consortium for the Barcode of Life was formed and includes among its members natural history museums, herbaria, biological repositories and biodiversity inventory sites, as well as academic and commercial experts in genomics, electronics, taxonomy and computer science. The group's goal is to accelerate the compilation of DNA barcodes of known and newly discovered plant and animal species, and to establish a public online database of DNA barcode sequences.

The consortium arose from two conferences on DNA and taxonomy organized in 2003 with leadership from Rockefeller Professor Emeritus Norton Zinder, Ph.D., the first chair of the Human Genome Project, and Jesse Ausubel, director of the Rockefeller Program for the Human Environment and also a program director for the Alfred P. Sloan Foundation, which is supporting the consortium.

An important aspect of the database is that each barcode is linked to an actual museum specimen. "Linking sequences to physical specimens makes the results believable, repeatable and open to revision," says Stoeckle.

“Once you build a library, then many people can take advantage of it,” adds Stoeckle.

The barcode technique has several advantages that complement traditional methods of identifying species. It requires only a small sample of tissue so that wildlife biologists could use it to identify the stomach contents of animals and reconstruct food cycles. Other uses include identifying birds that fly into airplane engines and testing for protected fish species, for example, that sometimes make their way to market. It also works for identifying organisms at different stages of life, such as the eggs and larvae of insects. And it can easily distinguish between species that look alike.

For further information on DNA barcoding and the International Consortium for the Barcode of Life, go to:

http://barcoding.si.edu/

http://www.barcodinglife.org

http://phe.rockefeller.edu/BarcodeConference/

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