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explorations

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MARINE BIOLOGISTS EMPLOY GENETIC BARCODING IN THE QUEST TO POSITIVELY IDENTIFY FISH SPECIES

NOT LONG AGO, A PAIR OF HIGH SCHOOL STUDENTS in Manhattan conducted a science project to check the authenticity of fish sold at New York seafood and sushi restaurants.

Of the 60 samples they evaluated, one-fourth that were identified were mislabeled. A high-priced piece of sushi being sold as white tuna, for example, was instead Mozambique tilapia, a much less expensive fish. Seven of nine red snapper samples turned out to be something else.

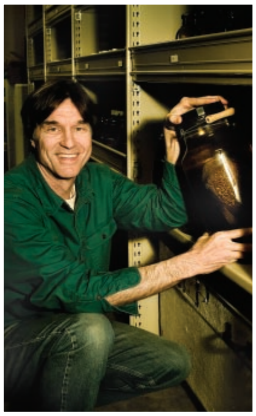
The students' revelations, detailed in a *New York Times* feature last summer, are hardly unique. Bait-and-switch practices are widely known in the seafood business, but because so many fish species look alike, even seasoned marine biology experts have a tough time identifying marine creatures by sight irrefutably.

What made the high schoolers' exposé possible was a tool that scientists at Scripps Institution of Oceanography at UC San Diego are also using to end false fish species identification. Like the students in the fish project, the scientists are tapping into a burgeoning technology that combines DNA identification with barcoding, the same striped-box technology used at checkout stands. Instead of tracking loaves of bread or containers of milk, biological barcodes match animal DNA to specific species.

life behind bars

By Mario C. Aguilera

Above and Right, DNA "barcodes" could provide a foolproof way to reveal the true identities of fishes served as sushi and often incorrectly billed as red snapper in fish markets.



Above, *Scripps Marine Vertebrate Collection Curator Phil Hastings is the North American coordinator for the Fish Barcode of Life project.* **Left**, *Since 1993, collection manager H.J. Walker has archived tissue samples that are now used for DNA barcoding.*

Scripps marine biologists Ron Burton and Phil Hastings are part of a blossoming movement building a public library of fish barcodes, not unlike the FBI's database of criminal fingerprints.

Burton and Hastings have concentrated on barcode identification for California marine fish species, an effort feeding into broader programs working to produce barcodes for all of the world's fishes. That effort, in turn, is part of an all-encompassing project aiming to do the same for all living organisms on the planet, the ambitious "International Barcode of Life Project."

Burton and Hastings say their project and other fish barcoding efforts will help resolve longstanding debates in marine science and bolster studies of evolutionary processes. Fish barcoding also promises to reveal new information about the health and future conservation of fish populations and identification of threatened species.

"Barcoding gives people confidence," said Mark Stoeckle, a bird biologist at Rockefeller University, an early proponent of DNA barcoding, and father of Kate Stoeckle, one of the students in the Manhattan fish project. "People need to know that what restaurants and fish markets are selling is what they say they are selling."

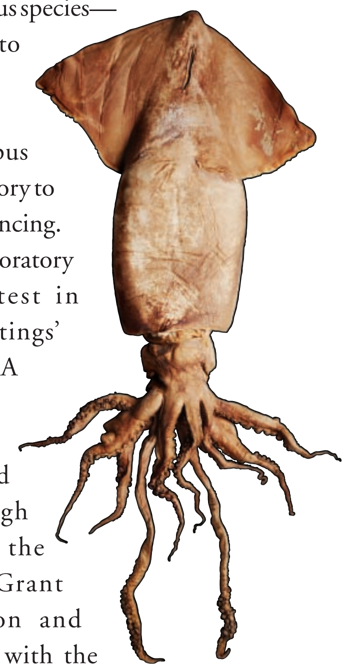
Researchers envision a not-too-distant future in which hand-held barcode devices are able to instantly identify a range of plant and animal life by matching genetic samples from the field against reference information in online databases. This new age of species identification, they say, will bring the natural world to our fingertips, and closer than ever before.

IDs WILL BE CHECKED

Lani Gleason held a pair of forceps up to the light, then checked and double-checked her instructions. The fourth-year UC San Diego undergraduate student, who was at her first day in a hands-on biology course taught by Burton, wanted to make absolutely certain that the miniscule amount of tissue she had just extracted from a megamouth shark would be adequate for DNA sequencing.

"Yes," Burton assured her, the crumb of fleshy innards would be more than enough to do the job.

Earlier in the day at the Scripps Marine Vertebrate Collection, Gleason collected two shoebox-sized containers of tissue vials of various species—from groupers to grunts—before walking across the Scripps campus to Burton's laboratory to begin DNA sequencing.

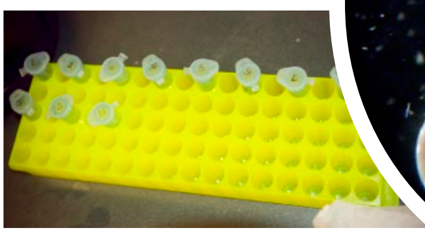


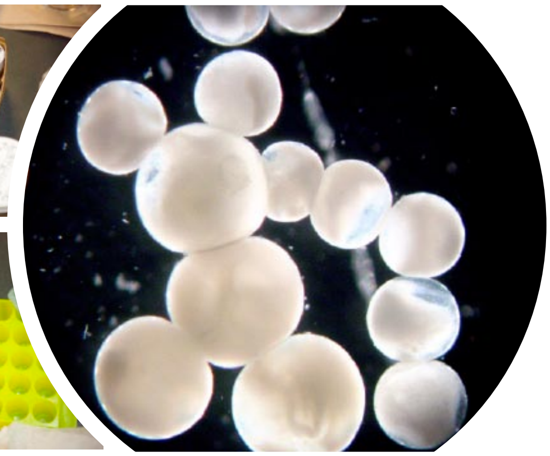
Gleason's laboratory work is the latest in Burton and Hastings' expanding DNA library of fishes.

When the project started in 2004 through funding from the California Sea Grant Program, Burton and Hastings set out with the modest goal of establishing a DNA database of marine fish inhabiting the waters off California. It wasn't until much later that they discovered their effort folded so neatly into broader efforts to barcode fish and other animals around the world.

"Every group of biologists has wanted to be able to tell species apart," said Burton. "They've all been doing this DNA work on their own for years.

Right and Above Right, *DNA barcoding projects include a broad range of fish and in the future will include animals such as Humboldt (jumbo) squid found in the Scripps Pelagic Invertebrate Collection.*



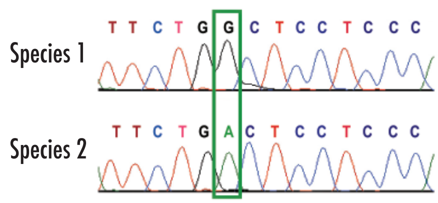


***Left and below left**, UCSD student Lani Gleason and Scripps Professor Ron Burton prepare fish specimen tissue for DNA sequencing as part of the Scripps barcoding project. **Below**, Fish eggs of varying sizes are difficult to distinguish by even the most seasoned biologists. **Bottom**, A DNA sequence, or "barcode," illustrates the slight difference between two copepod species.*

Barcoding is a new twist on an old problem of wanting to identify species and determine evolutionary relationships."

The Burton-Hastings database and countless others across the planet might have continued independently were in not for the efforts of Paul Hebert, an evolutionary biologist at the University of Guelph in Canada. In 2003 Hebert set the global standard for biological barcoding. Rather than haphazardly extracting DNA samples from diverse areas of animal specimens, Hebert spearheaded a consensus among researchers calling for all DNA extractions from one common animal gene, thus lassoing all of the individual efforts into one unified DNA library.

He set the target site as "cytochrome c oxidase subunit 1," otherwise known as CO1, which resides in a section of mitochondrial DNA—the area of cells responsible for energy production. And so the baseline of comparison was set across all animals—whether fish or monkeys or moths—and a sort of Dewey Decimal System for biological species identification was born.



"When we first started we realized that humanity needs to start to identify organisms but there wasn't a system in place," said Hebert. "There are so many applications in which species identification is required. Now that we have a system we're seeing that this one tiny piece of DNA can be quite revealing."

Inside Burton's laboratory, Gleason soaked each sample in water to remove ethanol, the liquid used to preserve tissue samples inside the Marine Vertebrate Collection.

She then extracted DNA from the tissue and sent the samples through a process known as polymerase chain reaction, or PCR, an automated technique that takes small, targeted pieces of DNA and amplifies them into millions of copies. The end result is a long, unique sequence of DNA—a fingerprint, more or less—characteristic of that species and none other.

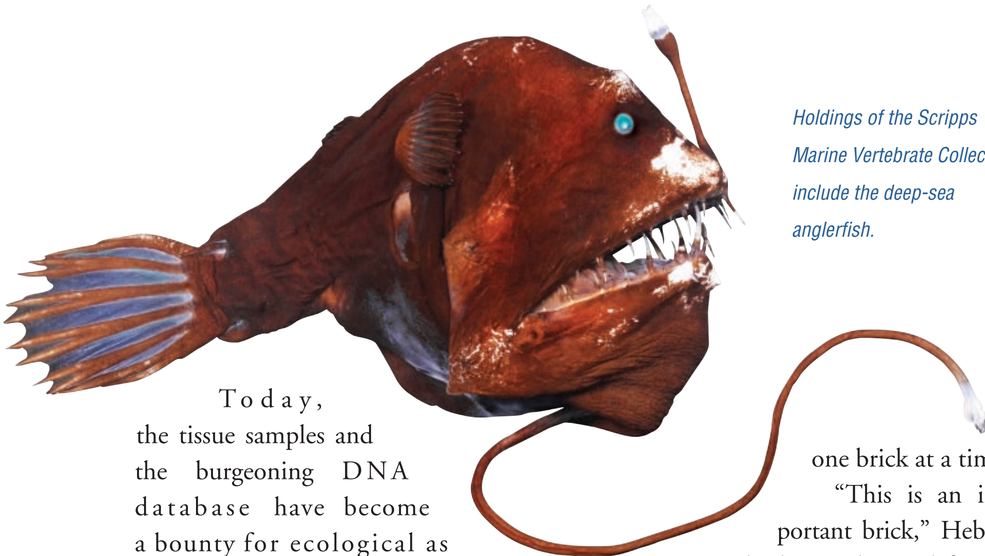
"That sequence," said Burton, "is, in fact, the DNA barcode."

BUILDING THE LIBRARY OF FISHES

Although the California fish barcoding project started at Scripps in 2004, its unofficial launch was more than a decade earlier inside the Scripps Marine Vertebrate Collection. Among the vast rows of fish specimens, roughly two million in all, collection manager H.J. Walker began archiving fish tissues in ethanol—rather than formalin—and maintaining all-important voucher specimens so that identifications could be confirmed.

Formalin, a solution of formaldehyde in water, is generally the best substance for preserving the shape of biological specimens. But formalin exposure causes a variety of changes in DNA, making formalin-preserved specimens a poor source of DNA for genetic studies. Student researchers posed this problem to Walker and in 1993 he began building up a cache of tissue samples preserved in ethanol, a liquid that keeps DNA intact.

***Above**, Greg Rouse, curator of the Scripps Benthic Invertebrate Collection, has barcoded hundreds of specimens, including this spionid worm.*



Holdings of the Scripps Marine Vertebrate Collection include the deep-sea anglerfish.

Today, the tissue samples and the burgeoning DNA database have become a bounty for ecological as well as evolutionary scientific investigations.

One example is the identification of fish eggs and larvae, key indicators of the health and prosperity of fish populations. Many fish eggs appear mind-bogglingly similar, so it's a boon to fisheries managers to know what kind of fish are spawning, and where. Barcoding can provide the answer without ambiguity.

"If we see two fish, why do we care if it's one species or two?" Burton asks. "The answer is that fish stock assessments and fisheries quotas are built upon knowing whether we're talking about one or more species. If you fish one threatened species out of Northern California waters, can fish in Southern California repopulate it? Not if they are a different species."

DNA barcoding can be used in examining and identifying the mangled contents inside predatory fish stomachs to determine what and possibly where they were feeding.

Barcoding and DNA identification have become so reliable that Burton and Hastings have been pulled into legal proceedings to aid agencies such as the California Department of Fish and Game. A chunk of mollusk recently residing in a jar in Burton's laboratory was a key piece of evidence in determining whether an abalone catcher ran afoul of the law.

Such applications are exclusive to the marine environment, but they are fundamentally vital to the global barcoding initiative, says Hebert, because the barcode library of all life is being built one brick at a time.

"This is an important brick," Hebert said about the California fish identification project. "To build this library we all need to start in our backyard. I started with moths in my backyard."

BARCODE SEAL OF APPROVAL

Hastings, curator of the Scripps Marine Vertebrate Collection and North American coordinator for the Fish Barcode of Life project, believes barcoding can enlighten and inform debates about seafood and future sustainability.

"If scientists are saying red snapper is overfished and yet people all over the country are seeing red snapper in the market, then it's fair for them to ask, 'How rare can it be?'"

He envisions a day when fish are sold with a sticker bearing a barcode seal of approval, which will give consumers peace of mind in knowing that red snapper is indeed red snapper, not vermillion snapper, lane snapper, or some other species.

As Burton and Hastings approach 500 barcoded species in their California fish project, they are now expanding the regional focus of their database by including fishes off Mexico.

Mark Stoeckle, who helped guide his daughter's Manhattan fish project, touts the practical applications of DNA barcoding, especially in vital areas such as public heath, including testing food shipments for invasive species and other threats. He's now involved in another high school project, this time with something he describes as "CSI for the house." He and his student investigators will test the authenticity of anything inside a home that can be traced to an animal product, be they refrigerator items or dog food.

Stoeckle and Hebert, the father of biological barcoding, believe the future is enticing, especially as barcoding devices shrink to be hand-held. They envision Star Trek-type "tricorder" devices capable of identifying plant and animal life by simply scanning over an organism and linking to online databases full of information about that species.

Such a future is now in the works. In February Hebert participated in a meeting in Toronto in which technologists met with biologists to plot a course for DNA barcoding device miniaturization. He envisions a table-top device for animal barcode readings being introduced within five years. A hand-held device? Perhaps 10 years, he believes.

"In the future every kid gets one of these in their Christmas stocking," said Hebert. "It's really a global positioning system for life. It can get people and children excited about the natural world. Just walking through the woods and seeing an animal, they can read and understand everything that humanity has learned about that organism."

View the complete multimedia presentation of this story at explorations.ucsd.edu.

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