Biological Barcodes

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Vast numbers of species are still waiting to be discovered. Although nothing can replace the work of classical taxonomists, biologists now want to speed up and simplify the identification of species by creating catalogs of genetic barcodes

In the mid 18th century, Swedish naturalist Carol Linnaeus's work *Systema Naturae* introduced the binomial (two-part) system of biological nomenclature, set forth descriptions of some 7700 plant and 4000 animal species, and thereby laid the foundations of modern taxonomy. In the 230 years since them, researchers have cataloged a total of some 1.7 million species of eukaryotes, or organisms whose cells possess clear cellular nuclei (a group that includes plants, fungi, and animals). Still, that figure is not high when one considers that the number of living eukaryote species is estimated at least 10 million!

Taxonomists wanted

It might seem that the identification of millions more species is just a matter of time. But the pace is hampered by the fact that there are simply not enough taxonomist biologists in the world (and their numbers are growing fewer) and that not many researchers are capable of identifying more than 1,000 species. Moreover, species identification on the basis of morphological traits can be a time-consuming and arduous endeavor. Fortunately, the development of effective methods for extracting, purifying, and analyzing DNA, the ever-greater computing power offered by computers, and the emergence of the field of bioinformatics are nowadays making it possible to rapidly identify species on the basis of DNA sequences alone.

In 2003 Paul Hebert from the Biodiversity Institute in Guelph in Canada proposed a method for identifying

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The idea of genetic barcoding has enabled many new animal species to be identified, such as this Alpine long-eared bat (*Plecotus macrobullaris*)

species which he dubbed "DNA barcoding." The barcodes widely used to identify products on sale in retail stores are made up of 13 elements. DNA, in turn, is comprised of four nucleotides arranged in a specific order into long chains. Within the animal kingdom, a segment of the cytochrome c oxidase subunit I gene (COI) that is approx. 650 nucleotides long was chosen to serve as a DNA barcode. This mitochondrial gene is characterized by low variance within a species but high variance between species, and can therefore serve as an excellent biological marker.

Not just new species

Advanced genetic barcoding has since led to a breakthrough in molecular methods of species identification. It has become possible not only to identify new species, but also to distinguish between species that are hard to differentiate by traditional methods. Although new species are often discovered among "relatively unattractive" animals, discoveries of truly large species do occur (a dolphin or whale). COI sequence analysis within a group of "forest" butterflies in Costa Rica previously considered to constitute a single species (*Astraptes fulgerator*) showed that in reality they belong to 10 different species. A kind of reverse example can be found in the reduction, nearly by half (from 300 to 170), of the number of "morphological"

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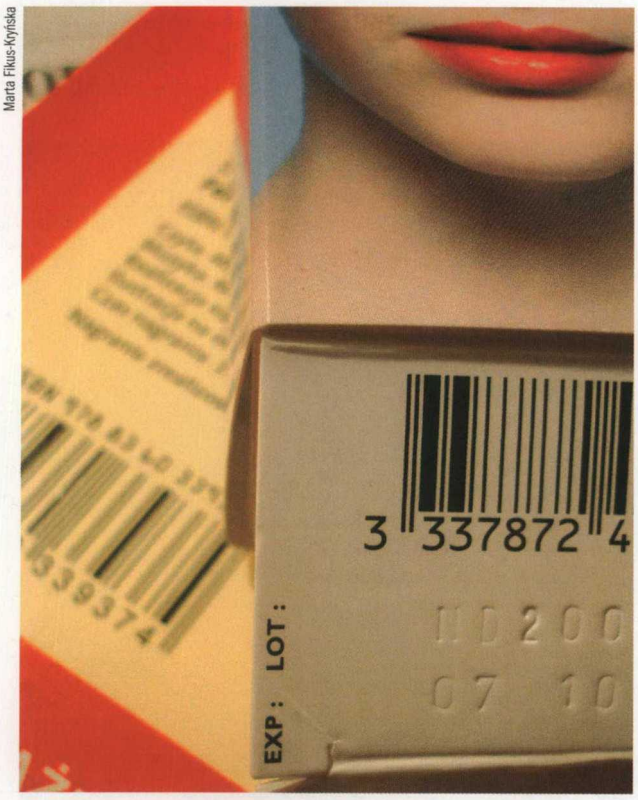


Retail stores in Europe usually use a 13-element barcode to mark products for sale. "Genetic barcoding," in turn, identifies species using a COI gene fragment that is 650 nucleotides long

species of coral from the *Acropora* genus, inhabiting the Indonesian coast.

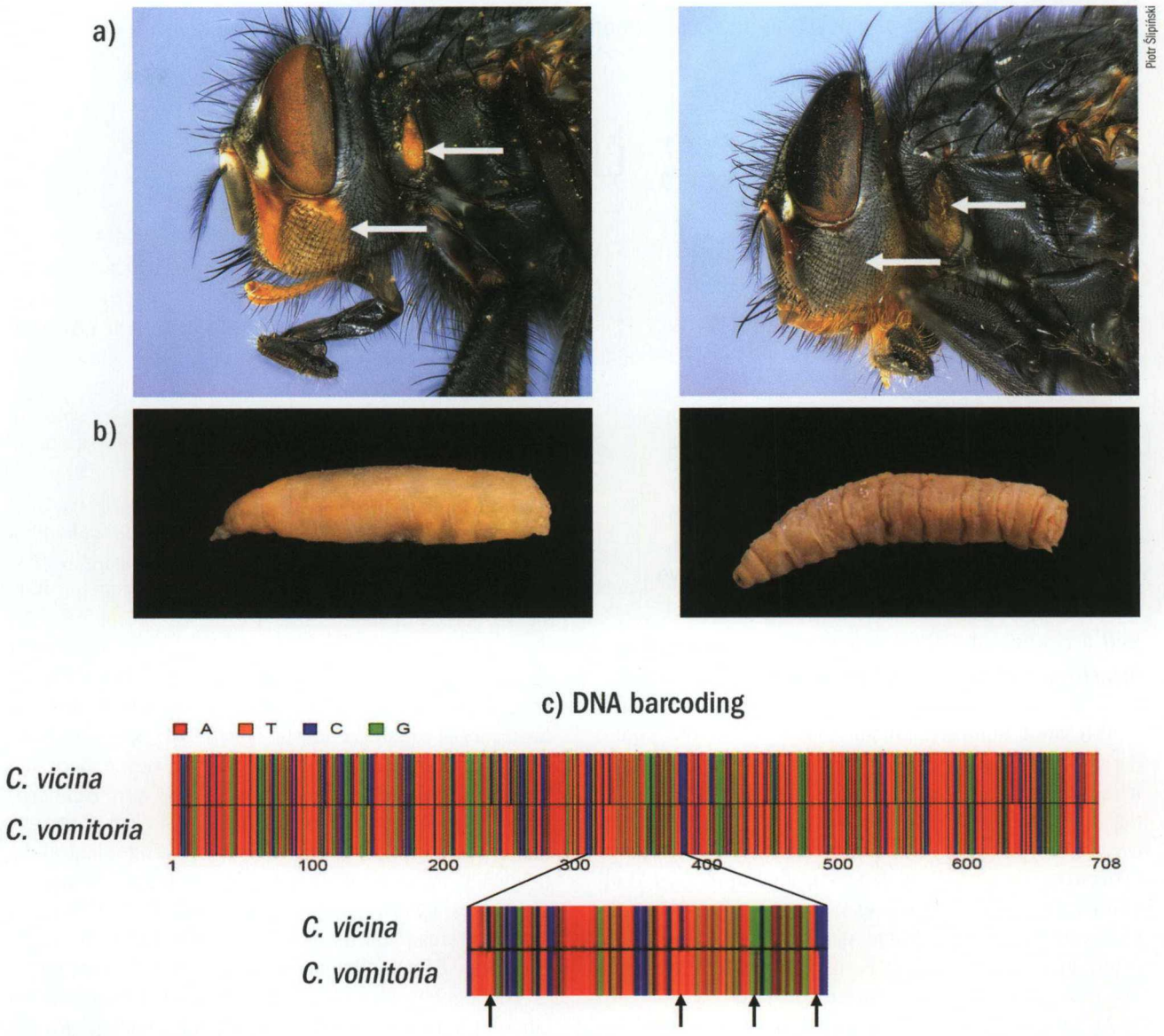
Most such discoveries are being made in the tropics, but they also occur closer to home. On the basis of DNA research, the existence of several new bat species has been discovered or confirmed in Europe alone, including the soprano pipistrelle (*Pipistrellus pygmaeus*), Alcathoe's whiskered bat (*Myotis alcathoe*) and several long-eared bats: the Sardinian (*Plecotus sardus*), Balkan (*P. kolombatovici*) and Alpine (*P. macrobullaris*) long-eared bats.

One great advantage of the DNA barcoding method is that it can accurately identify organisms in stages of development that are difficult to identify by traditional methods (e.g. larvae, seeds, seedlings). Moreover, a species can be identified purely on the basis of fragmentary samples, such as individual feathers, bone fragments, or dried leaves. New species identified by DNA analysis are also being found in such strange habitats as town market squares.

DNA barcoding has also been finding practical applications - one in studying the impact of various types of human pressure and its consequences (e.g. climate warming) on biodiversity on a regional and global scale. Studying the phylogenetic links between species enables us to better predict and curb invasions of alien species. DNA barcoding is also assisting in the study of human allergies, monitoring the state of the environment (e.g. soil condition), boosting effective control over trading in wild plants and animals, and identifying species important for forensic medicine.

Flies go to court

Work carried out at our institute, where we study 15 necrophagous blow-fly species of the family Calliphoridae, is proving useful in the latter case, i.e. in forensic medicine. Before human bodies are discovered by investigators, they are most often first colonized by insects that lay their eggs on them. This biological marker makes it possible to precisely identify how much time has passed between death and when the body was found, which is one of the most important tasks of forensic medicine. In a moderate climate, blow-flies initiate start the process of decomposition of the remains (appearing only 2-3 hours after the moment of death) and take part in its two first stages: (1) the initiation of lytic processes (i.e. the brown blowfly *Calliphora vicina* and the blue bottle fly *C. vomitoria*), (2) initiating putrefactive processes. Given information about such factors as temperature, light, and moisture, the pace of development of these flies is well-known. Moreover, certain blowflies, such as the brown blowfly, can lay eggs throughout the day or night, but the flies of the genus *Lucilia* can only do so in the daytime. *C. vicina* is the most common blowfly occurring on human remains especially in urban areas, while *C. vomitoria* occurs more commonly in rural regions. Pinpointing the time and sometimes also



Distinguishing between two blowfly species, *Calliphora vicina* and *C. vomitoria*, on the basis of morphological traits (a) of adult specimens, (b) larvae, and (c) DNA barcoding. Adult specimens can quite easily be distinguished morphologically, yet the same cannot be said for their larvae. The DNA code shown below allows the species to be unequivocally identified at any stage of development: in egg, larva, chrysalis, or adult form. Differences in adult (imago) morphology (cheek color and hair, coloring of spiracles) and in the DNA barcodes are highlighted with arrows

the location of death is therefore possible by precisely identifying the species of larvae and their age. Here we come to the heart of the matter: as is easy to imagine by looking at the pictures shown here, identifying the species of larvae purely on the basis of morphological traits is not simple. In this case, only DNA barcoding can offer a clear-cut answer.

The whole world is now working on cataloging DNA barcodes. The Consortium for the Barcode of Life (CBOL) includes 157 organizations from 45 countries, acting as patron over programs for sequencing COIs for all species of butterflies (All-Leps), fish (Fish Barcode of Life), birds (All Birds Barcoding Initiative) and everything that lives in polar regions (Polar Barcode of Life Initiative). In Poland, one of the places where research using DNA barcodes is being pursued is in connection with the National Plant, Fungi, and Animal DNA Bank coordinated by the Museum and Institute of Zoology PAS in Warsaw. It therefore seems that universal barcoding of the animal kingdom is just a question of time. However, using the technique to identify plants and fungi encounters significantly more difficulties... but that is a topic for another article. ■

Further reading:

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