

# Barcoding Life Highlights 2013

*BARCODE OF LIFE: a short DNA sequence, from a uniform location on the genome, used for identifying species*



Snowberry clearwing (*Hemaris diffinis*)

Butterfly bush (*Buddleja davidii*)



DNA barcoding, first developed in 2003, is a standardized approach to identifying species by DNA. Here we focus on recent highlights since the Fourth International Barcode of Life Conference, Adelaide, Australia, 2011.

### *Products need barcoding*

Barcoding uncovers continued international mislabeling of fish products, with costs to consumers and threats to protected species, and reveals common mislabeling of other foods and herbal products.<sup>1-3</sup> Barcoding helps detail the enormous illicit global trade in timber and in threatened and endangered animals and plants, including products such as “bushmeat,” meat from wild animals especially in Africa and Asia, which carries human diseases.<sup>4-6</sup> Barcoding documents invasive species at ports of entry and crop pests in agricultural goods.<sup>7,8</sup>

For these and other applications, certified, affordable, and rapid barcode testing services promise great benefits. Simple devices for rapid DNA extraction and amplification, and overnight sequencing services are already described or commercially available, indicating technical feasibility.

### *Ecology yields insights, next-gen helps*

Barcoding of food fragments in guts and excreta is helping answer previously intractable questions about diets that fuel complex ecosystems.<sup>9-11</sup> Next-generation sequencing improves accuracy of “meta-barcoding,” i.e., analysis of pooled environment samples and other mixtures, including commercial products.<sup>12,13</sup>

### *Answering early worries*

Is the minimalist barcode approach of a short standard sequence best? In the near future it may be possible to generate large amounts of sequence data quickly and inexpensively, perhaps even whole genomes, from biological specimens. However, results continue to indicate that more sequence data rarely identify species better than a standard minimum barcode. Recent work has dispelled some other early worries such as whether the widespread insect bacterial parasite *Wolbachia* blocks insect barcoding (no), how much geographic sampling is enough (in some cases a lot), and how accurate are public databases (sequence error is low and overlooked pseudogenes are uncommon).<sup>14-16</sup>

### *Educational use growing*

The required technology is relatively simple and widely applicable, allowing students and others to design diverse projects and make real discoveries. Recent U.S. National Science Foundation awards are supporting DNA barcoding efforts by high school students at Coastal Marine Biolabs in Ventura, California, and citizen scientists at Mount Desert Island Laboratory, Maine.<sup>17,18</sup> Following on the hooves of the horsemeat-in-beef scandal in Europe, the

Gates Foundation funded high schoolers in Danville, Illinois, and five other U.S. cities to barcode meat products—so far, no adulteration has been found.<sup>19</sup> Cold Spring Harbor Laboratory’s Urban Barcode Project had a second successful year, in which the 2013 first prize was awarded to students documenting ant diversity in a park adjacent to their school.<sup>20</sup> University of Guelph ran an insect DNA barcoding program with 2,000 students at 60 high schools.<sup>21</sup> BioLabs now offers an educational fish DNA barcoding kit.<sup>22</sup> An online course and a published compilation of protocols should help disseminate barcoding expertise.<sup>23,24</sup>

### *Diverse support*

Recent papers and news reports indicate government and private funding in dozens of countries around the world. In the U.S., a \$3 million Google Global Impact Award to the Consortium for the Barcode of Life (CBOL) is helping establish an endangered species barcode database and train biodiversity enforcers in Nigeria, South Africa, Kenya, Mexico, and Brazil.<sup>25</sup> The Chinese Kunming Institute is sponsoring Cold Code, an international effort to barcode all “cold-blooded” vertebrates, namely, amphibians and non-avian reptiles, and providing no-cost sequencing for contributed specimens.<sup>26</sup> In Brazil, nearly a dozen government-supported projects are investigating terrestrial and aquatic biodiversity with barcoding, and in the Dominican Republic, a government grant will aid local scientists documenting biodiversity in an urban wildlife refuge.<sup>27,28</sup> Perhaps a harbinger, a citizen science DNA barcoding project in Alaska raised funds through crowd-sourcing.<sup>29</sup>

### *Dealing with dark taxa*

The challenge of specimens without species names continues.<sup>30,31</sup> Many incompletely identified specimens (e.g., Lepidoptera spp.), some likely new species, have been barcoded. How to best make use of such data? For animal COI barcodes, Barcode of Life Datasystems has instituted an automated Barcode Index Number (BIN) system that sorts sequences into numbered clusters approximating species, which should help researchers amalgamate knowledge about similar specimens.<sup>32</sup> The underlying difficulty is the sheer number of living species: only 15% of the 2 million so far described among the 8 million likely existing have been barcoded.<sup>33</sup> Museums and herbaria have shown a promising way forward, establishing barcodes for specimens already archived and named and making barcoding part of the collections process.<sup>34,35</sup> A wiki approach may help improve reference databases.<sup>36</sup>

### *Looking ahead*

The past decade answered the question, Does barcoding work? with an emphatic yes. For the next decade, while continuing to build libraries and apply barcoding, we can also ask *why*. More exactly, why does evolution yield limited





variation within, and larger differences among, animal species, and why are plants different?

Thanks to the efforts of many, DNA barcoding is established as a valuable resource for society and science.

Continued work will improve open access to knowledge about the amazing multitude of life on Earth, a value for the public and professionals alike.

## ***The first decade of the Barcode of Life***

In 2003 at the University of Guelph, Paul Hebert and colleagues discovered that a short mitochondrial gene sequence could identify most animal species.<sup>37</sup> A standard 650 base pair region of mitochondrial cytochrome c oxidase I (COI), recoverable from diverse animal phyla using a single primer pair, varied little within species but generally differed among even closely related species, making it straightforward to match sequences to species names.<sup>38</sup>

By analogy with the Uniform Product Code (UPC) for commercial goods, Hebert and colleagues called this biological identifier a “DNA barcode of life” and proposed a universal barcode library. Potential advantages of such a standard were immediately apparent: organisms in any life stage could be named from bits and pieces, by more people, and at relatively low cost.

Workshops at Cold Spring Harbor Laboratory in 2003 discussed the value and feasibility of an organized effort. These led to CBOL, inaugurated in April 2004 with support from the Alfred P. Sloan Foundation.

Barcoding has flourished in its first decade. CBOL agreed on standard loci for major taxonomic groups [animals, 5' COI (2005); plants, *rbcL*/*matK* (2009); and fungi, ITS

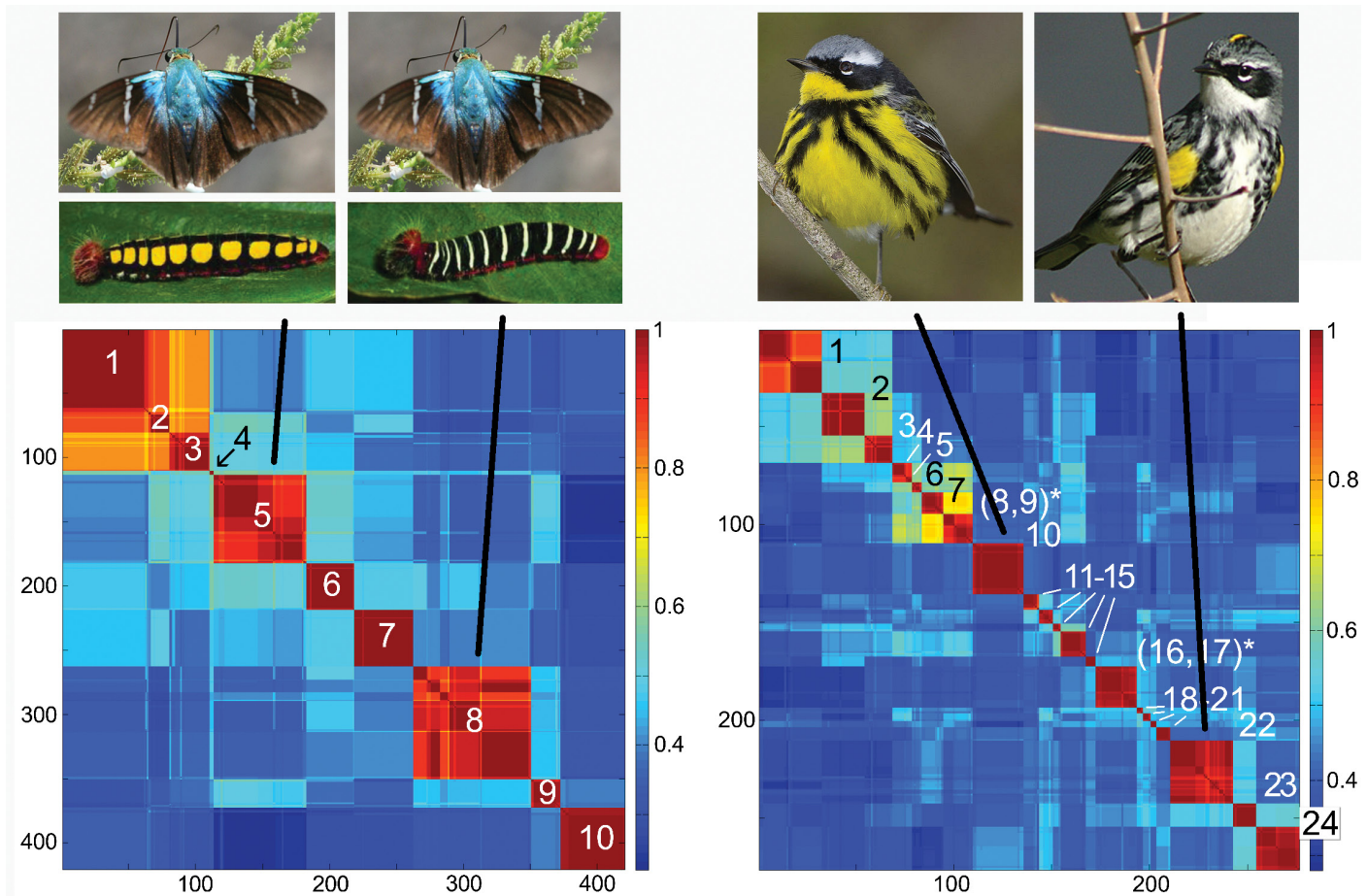


**Beginnings.** Paul Hebert, Winnie Hallwachs, Dan Janzen, and John Marchioni discuss the potential for a DNA barcoding initiative at the Cold Spring Harbor Laboratory workshop in 2003.

**Photo:** Cold Spring Harbor Laboratory

(2012)].<sup>39-41</sup> Inspectors, educators, and scientists adopted barcoding. A web-based database and workbench, Barcode of Life Datasystems (BOLD) was built, and libraries of open access barcode records grew.<sup>42</sup> To speed library building, the International Barcode of Life (iBOL) was launched in 2010 with major support from Genome Canada and Ontario Genomics Institute.<sup>43</sup> National and regional networks and taxon and ecosystem campaigns emerged, coordinating and stimulating barcoding efforts.

The essential elements are (1) agreed-upon standard gene region(s), and (2) high quality sequence records from documented specimens. A decade of effort now shows the fundamental premise is sound. New DNA sequencing technologies may supplant current methods, but the need will continue for a reference library of standard loci sequences derived from museum or herbaria specimens with authentic species names.



**Visualizing species as DNA barcode clusters.** New software helps generate Klee “heat map” diagrams of skipper butterfly *Astraptes fulgerator* complex (left) and *Setophaga* warbler (right) DNA barcodes.<sup>44</sup>

Each numbered block indicates a cluster of similar barcodes corresponding to a species, examples of which are shown above. Block sizes reflect the number of sequences per species. This approach also shows barcode clustering at higher taxonomic levels, which may help understanding of evolution.

A Klee diagram is a colorized indicator vector correlation matrix that depicts similarities and differences among a set of sequences. Colors indicate correlation between sequences; identical sequences have a correlation of 1 and appear dark red; dissimilar sequences have lower correlation and are dark blue. Small differences within species create red blocks of high correlation along the diagonal.

**Photo:** *A. fulgerator* adult, Ann Toal; larvae<sup>45</sup>; Magnolia warbler, William H. Majoros; Yellow-rumped warbler, John Harrison

**Improving methods.** Redesigned broad-range PCR primers amplified COI barcodes from 70% of 12,000 marine metazoan specimens representing 13 marine phyla, compared to 40% with standard Folmer primers.<sup>46</sup> Red letters indicate differences in new primers.

#### Forward

Folmer primer LC01490

5' –GGTCAACAAATCATAAAGATATTGG–3'

Redesigned primer jgLC01490

5' –**T****I****T****C****I****A****C****I****A****A****Y****C****A****Y****A****R****G****A****Y****A****T****T****G****G**–3'

#### Reverse

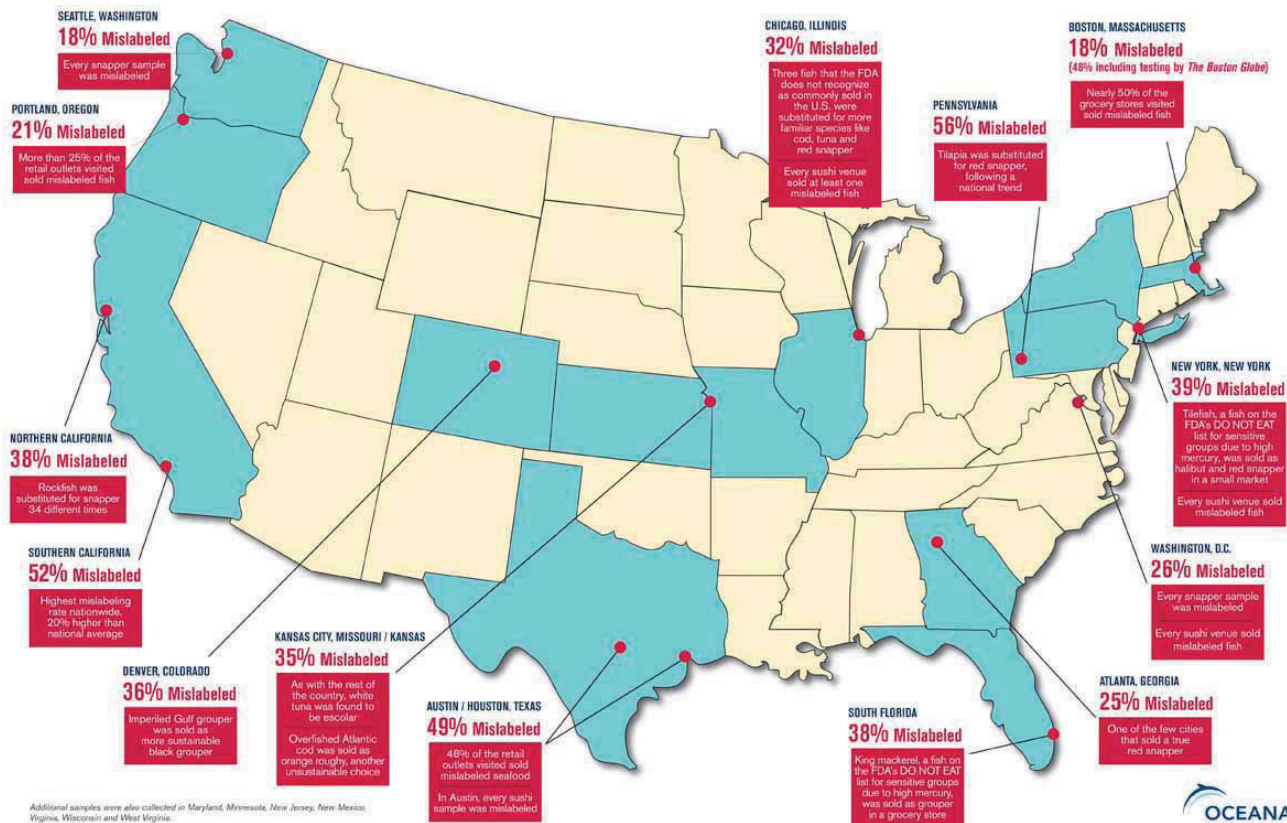
Folmer primer HCO2198

5' –TAACTTTCAGGGTGACCAAAAAATCA–3'

Redesigned primer jgHCO2198

5' –**T****A****I****A****C****Y****T****C****I****G****G****R****T****G****I****C****C****R****A****A****R****A****Y****C****A**–3'





**Fish fail testing.** In the largest study of fish products to date, barcoding revealed that about one-third of items in the U.S. are mislabeled.<sup>1</sup>

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**eBOL** education & the barcode of life

Getting Started BOLD-SDP DNA Barcoding Assistant A Message From IBOL

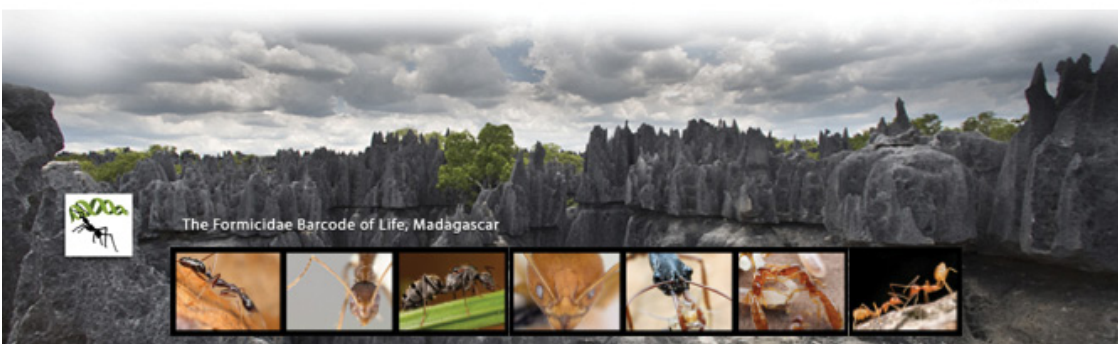
**The eBOL Community Web Portal**

The eBOL Community Web Portal establishes a vital link between biodiversity genomics research and science education. Designed in collaboration with IBOL researchers, bioinformaticians, science media and communications experts, and scientists committed to innovating life science education, eBOL provides the resources and infrastructure needed to bridge the biodiversity knowledge gap and advance DNA barcoding as an interdisciplinary teaching and learning tool. [read more...](#)

Explore other habitats representing major IBOL barcoding campaigns

Major funding provided by the National Science Foundation DRL-1030983

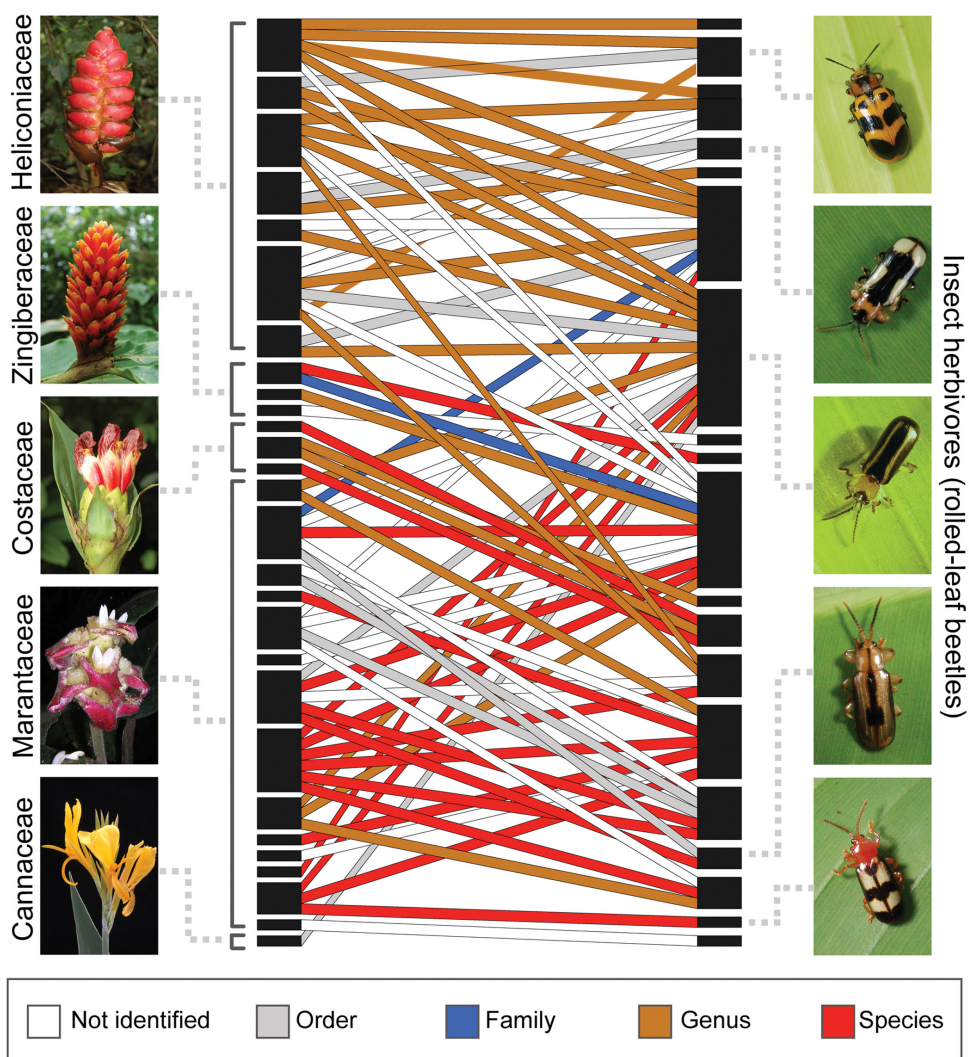
Sponsors



**Barcoding attracts students.** Dedicated websites help high school students and other citizen scientists organize barcoding projects and contribute reference sequences to public databases.<sup>47,48</sup>

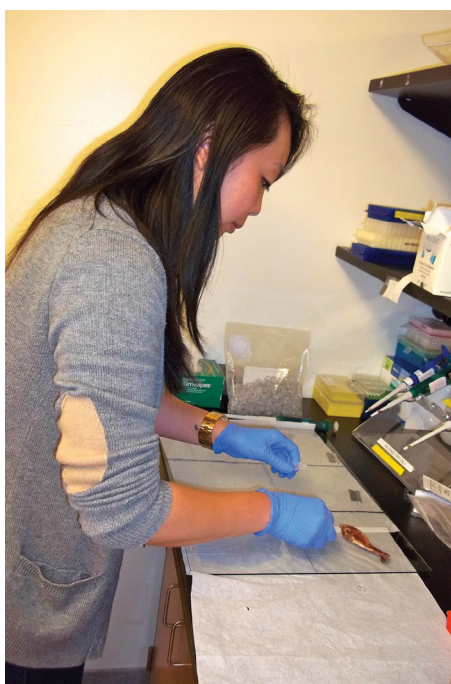


**What eats what.** Barcoding plant remnants in beetle guts accurately, efficiently, and comprehensively documents plant-herbivore networks.<sup>9</sup>



**Aiding enforcement.** Barcode ID of seized products enables enforcement of regulations on protected species.<sup>5</sup>

**Photo:** AMNH



**Truth-in-labeling.** Barcoding reveals that herbal products commonly lack active ingredients.<sup>3</sup>







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## ***Links to learn more and join in***

Fifth International Barcode of Life Conference, Kunming China

<http://www.dnabarcodes2013.org/dct/page/1>

### ***International organizations***

Consortium for the Barcode of Life	<a href="http://www.barcodeoflife.org/">http://www.barcodeoflife.org/</a>
What is DNA barcoding?	<a href="http://www.barcodeoflife.org/content/about/what-dna-barcoding">http://www.barcodeoflife.org/content/about/what-dna-barcoding</a>
Social Network Connect Barcode of Life	<a href="http://connect.barcodeoflife.net/">http://connect.barcodeoflife.net/</a>
International Barcode of Life (iBOL)	<a href="http://ibol.org/">http://ibol.org/</a>
Partner nations	<a href="http://ibol.org/about-us/partner-nations/">http://ibol.org/about-us/partner-nations/</a>
Campaigns (e.g., FishBOL)	<a href="http://ibol.org/about-us/campaigns/">http://ibol.org/about-us/campaigns/</a>

### ***Selected regional and national organizations***

European Consortium for the Barcode of Life	<a href="http://www.ecbol.org/">http://www.ecbol.org/</a>
Australian Barcode of Life Network	<a href="http://www.ala.org.au/about-the-atlas/our-data-providers/abon/">http://www.ala.org.au/about-the-atlas/our-data-providers/abon/</a>
Brazilian Barcode of Life (BrBOL)	<a href="http://www.brbol.org/content/brbol-brazilian-barcode-life">http://www.brbol.org/content/brbol-brazilian-barcode-life</a>
Finnish Barcode of Life Network (FinBOL)	<a href="http://www.finbol.org/">http://www.finbol.org/</a>
French Bibliothèque de Vivant	<a href="http://bdv.ups-tlse.fr/">http://bdv.ups-tlse.fr/</a>
German Barcode of Life (GBOL)	<a href="https://www.bolgermany.de/">https://www.bolgermany.de/</a>
Korea Barcode of Life (KBOL)	<a href="http://koreabarcode.org/">http://koreabarcode.org/</a>
Mexican Barcode of Life (MexBOL)	<a href="http://www.mexbol.org/index.php">http://www.mexbol.org/index.php</a>
Netherlands Barcode of Life	<a href="http://dnabarcoding.nl/">http://dnabarcoding.nl/</a>
Norwegian Barcode of Life Network (NorBOL)	<a href="http://www.norbol.org/">http://www.norbol.org/</a>
Russian Barcode of Life (RusBOL)	<a href="http://www.wimb.dvo.ru/misc/barcoding/index.htm">http://www.wimb.dvo.ru/misc/barcoding/index.htm</a>

### ***Public databases***

Barcode of Life Datasystems (BOLD)	<a href="http://www.boldsystems.org/">http://www.boldsystems.org/</a>
Taxonomy browser	<a href="http://www.boldsystems.org/index.php/TaxBrowser_Home">http://www.boldsystems.org/index.php/TaxBrowser_Home</a>
Identification engine	<a href="http://www.boldsystems.org/index.php/IDS_OpenIdEngine">http://www.boldsystems.org/index.php/IDS_OpenIdEngine</a>
NCBI GenBank	
Keyword BARCODE records	<a href="http://www.ncbi.nlm.nih.gov/nuccore/?term=BARCODE[keyword]">http://www.ncbi.nlm.nih.gov/nuccore/?term=BARCODE[keyword]</a>
Barcode submission tool	<a href="http://www.ncbi.nlm.nih.gov/WebSub/index.cgi?tool=barcode">http://www.ncbi.nlm.nih.gov/WebSub/index.cgi?tool=barcode</a>

### ***Barcoding in education***

Barcoding Life's Matrix	<a href="http://studentdnabarcoding.org/">http://studentdnabarcoding.org/</a>
Education and Barcode of Life (eBOL)	<a href="http://www.educationandbarcoding.org/">http://www.educationandbarcoding.org/</a>
Rockefeller University Barcoding Site	<a href="http://phe.rockefeller.edu/barcode/index.php">http://phe.rockefeller.edu/barcode/index.php</a>
U Guelph Online DNA Barcoding Course	<a href="http://dnabarcodingcourses.com/">http://dnabarcodingcourses.com/</a>
Urban Barcode Project	<a href="http://www.urbanbarcodeproject.org/">http://www.urbanbarcodeproject.org/</a>

### ***More***

American Museum of Natural History	<a href="http://research.amnh.org/genomics/Programs/ConGen/ConGen-Research/DNA-Barcoding-Initiative-Conservation">http://research.amnh.org/genomics/Programs/ConGen/ConGen-Research/DNA-Barcoding-Initiative-Conservation</a>
Biodiversity Institute of Ontario	<a href="http://www.biodiversity.uoguelph.ca/">http://www.biodiversity.uoguelph.ca/</a>
DNA Barcoding Blog, University of Guelph	<a href="http://dna-barcoding.blogspot.ca/">http://dna-barcoding.blogspot.ca/</a>
Google Global Impact Awards	<a href="http://www.google.com/giving/impact-awards.html">http://www.google.com/giving/impact-awards.html</a>
International Barcode of Life Conferences	
Adelaide, Australia, 2011	<a href="http://www.dnabarcodes2011.org/">http://www.dnabarcodes2011.org/</a>
Mexico City, Mexico, 2009	<a href="http://www.ploscollections.org/article/browse/issue/info%3Adoi%2F10.1371%2Fissue.pcol.v02.i14">http://www.ploscollections.org/article/browse/issue/info%3Adoi%2F10.1371%2Fissue.pcol.v02.i14</a>
Taipei, Taiwan, 2007	<a href="http://barcoding.si.edu/cbol_taipei/">http://barcoding.si.edu/cbol_taipei/</a>
London, England 2005	<a href="http://www.barcoding.si.edu/London.html">http://www.barcoding.si.edu/London.html</a>
New York Botanical Garden	<a href="http://www.nybg.org/science/scientist_profile.php?id_scientist=29">http://www.nybg.org/science/scientist_profile.php?id_scientist=29</a>
Toyota Enviro Challenge, South Africa	<a href="http://www.toyotaoutreach.com/">http://www.toyotaoutreach.com/</a>
2012 IBOL Targets and Milestones Report	<a href="http://tinyurl.com/lopayqd">http://tinyurl.com/lopayqd</a>

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*Barcoding Life Highlights 2013* was designed and written by Mark Stoeckle with assistance from Paul Waggoner and Jesse Ausubel.

Cover photo: Mark Stoeckle. Colorized barcodes generated by BOLD [adenine (green), cytosine (blue), guanine (black), thymine (red)].

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