

Barcoding for Endangered Species Conservation

Report of a Planning Meeting hosted by the
Center for Conservation and Research for Endangered Species
San Diego Zoological Society
13-14 January 2006

Executive Summary

DNA barcoding is being used as a research tool for refining our understanding of biological diversity, and as a system for assigning biological samples to their species of origin. The Steering Committee for a proposed initiative, Barcoding for Endangered Species Conservation (BESC), explored ways in which DNA barcoding could be put to use for the protection of threatened species, especially those species under pressure from illegal bushmeat hunting and trade. DNA barcodes from mitochondrial or chloroplast gene regions are not well-suited as legal evidence because they only reflect the species identity of one parent. Nevertheless, barcodes can be very useful for many purposes that will promote species protection: preliminary legal investigations, customs/taxation inspection, import/export control, and documenting the scope of the bushmeat problem to raise awareness among policymakers and the public. The Steering Committee recommends that the Consortium for the Barcode of Life establish a standing committee that will promote the use of DNA barcoding for the protection of endangered species, and to launch focused barcoding projects devoted to pressing conservation problems such as African bushmeat and Asian turtles.

Background

The Consortium for the Barcode of Life (CBOL) convened a Steering Committee (see Appendix 1) for the purpose of obtaining advice concerning the possible use of DNA barcoding for species conservation. CBOL approached Dr. Oliver Ryder, Head of the Genetics Division of the Center for Conservation and Research for Endangered Species (CRES), Zoological Society of San Diego, and requested that he assemble experts in this field and organize a planning meeting. The 1½ day meeting was held on Friday and Saturday, 13-14 January 2006 at the Arnold and Mabel Beckman Center for Conservation Research, Escondido, CA (see Appendix 2). The participants in the meeting were:

- Dr. Oliver Ryder;
- Dr. Robert Hanner, University of Guelph and Chair of CBOL's Database Working Group;
- Dr. Janette Wallis, ABTI-American University of Nigeria and representative of the Bushmeat Crisis Task Force and the American Society of Primatologists;
- Dr. Robert Martin, Field Museum of Natural History, Chicago;
- Dr. Ken Goddard, Director the US Fish and Wildlife Service's Forensic Laboratory, Ashland, OR (present only on Friday, 13 January);
- Dr. James Moore, Anthropology Department, Univ. California San Diego (present only on Friday, 13 January);
- Dr. Eduardo Eizirik, Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS), Porto Alegre, Brazil (participated by telephone for several hours on Saturday, 14 January; and
- Dr. David Schindel, Executive Secretary, CBOL

Meeting Content

Participants were provided with a series of presentations on the San Diego Zoological Society and CRES (by O. Ryder), DNA barcoding and barcode data on primates (R. Hanner), and CBOL and the barcoding “campaigns” it has started on birds and fish (D. Schindel). Following these introductory presentations, the Steering Committee explored a series of topics associated with the use of DNA barcode data for species conservation.

The application of DNA barcode data to rare and endangered species

Participants in the meeting then discussed the ways in which DNA barcoding could be most useful to research and enforcement related to species conservation. The rarity of specimens in endangered species and the special needs of conservation biologists may require approaches that are different from other barcoding projects.

CBOL has already launched several large “campaigns” aimed at obtaining reference barcodes for all species in a taxonomic group, such as birds and fish. Once completed, the reference barcode libraries generated by these campaigns can be used to assign any unidentified bird or fish to its correct species. For species in which geographic races are well-established and gene flow is limited, barcode data may even be able to assign specimens to their geographic region of origin.

Participants agreed that it would be desirable to have good reference barcodes for endangered species, but in some cases the limited availability of identified “voucher” specimens may be an obstacle. In some applications of barcoding, it may not be necessary to be able to assign an unidentified to its correct species. Small sample sizes may provide sufficient reference data, even if they cannot resolve individual species. For example:

- It may be sufficient in some cases to determine if a specimen is NOT a member of a particular endangered species or a set of endangered species. This will often be the case for inspection of suspected contraband. In these cases, it will not matter to what species a confiscated item represents, as long as it is not from an endangered species.
- In some cases it will only be necessary to determine if a specimen represents a species in a particular species cluster. For example, if all species of a genus of African ungulates are protected, it would be valuable to know if a confiscated item came from within that genus, regardless of which species it represents.

DNA barcodes and law enforcement

Dr. Goddard provided an overview of the uses of molecular data for enforcement and prosecution of illegal hunting and trafficking of endangered species. Biochemical and genetic data (e.g., spectrographic data from hemoglobin, nuclear gene sequences) are being used increasingly to identify contraband and in prosecution. However, legal protection extends only to members of listed species, and does not extend to interspecific hybrids, even if both parents belong to endangered species. Defense attorneys have learned to challenge the species membership of confiscated plants and animals, and specifically to claim that the specimens are interspecific hybrids. The burden of proof falls on the prosecution in these cases. Mitochondrial and plastid gene data are useless by themselves because they are inherited maternally. Defense attorneys can and do claim that the father belonged to another species, and mitochondrial and plastid gene data cannot refute this claim.

Dr. Goddard stressed that at the present time, the legislation that protects endangered species does not contain legal definitions of species boundaries or of the basis on which species identity can or must be proven. The Fish and Wildlife Service (FWS) has been providing morphological, behavioral, genetic and hemoglobin data since the early 1990s that have led to convictions. From the FWS perspective, it could be counter-productive to introduce a strict and restrictive legal definition of species based on gene sequence data. Basing species definitions on molecular data would make prosecutions more difficult because narrower definitions are more easily countered.

Even though barcode data cannot be used by themselves in court cases, Dr. Goddard agreed that barcode data could be very valuable if used in two ways:

- For preliminary identification of contraband as members of endangered species, to which additional molecular data could be added to build strong legal arguments, and
- To establish probable cause for the purpose of justifying continued investigation into possible crimes.

Areas of application beyond law enforcement

Participants in the meeting explored additional ways in which DNA barcode data could be used to protect endangered species, beyond law enforcement. In discussing these applications, participants noted the risk of inadvertently supporting illegal hunting and trade by buying suspected bushmeat products for barcoding analysis, thereby creating market demand for banned products.

The Bushmeat Crisis Task Force (BCTF) has an IMAP Project (<http://www.bushmeat.org/IMAP/index.html>) which displays conservation projects and monitoring programs in Africa. BCTF is in the process of establishing an online database of bushmeat confiscation events, but there are currently no sources for reliable data on the scope of the bushmeat problem. Dr. Goddard shared information on the relatively few confiscations of bushmeat in the U.S., which could lead to the impression that this is not a serious problem. In reality, the low number of seizures reflects the difficulty of infiltrating trade pipelines and testing for illegal products.

Participants agreed that one of the most important and potentially high-impact uses of barcode data would be to document better the scope of illegal hunting and trade, thereby raising public awareness. They compiled the following list of organizations that are active in this area and could be interested in exploring the use of DNA barcodes for documenting bushmeat hunting and trade:

- BCTF IMAP (Andy Tobiason)
- Conservation International/CABS (which will probably become host to the BCTF/IMAP database of bushmeat seizures)
- TRAFFIC (Ginette Hemley)
- American Zoological Association
- American Society of Primatologists and the International Primate Society
- IUCN Primate Specialist Group, Species Survival Commission (Holly Dublin, John Hutton), Sustainable Use Specialist Group
- Fauna and Flora International
- International Fund for Animal Welfare
- Born Free

- Ape Alliance
- Dian Fossey Fund
- Orangutan Foundation International
- Jane Goodall Institute
- Save the Tiger Fund
- Save the Whales
- Airlines wanting to refuse shipments of live animals
- IATA – Air transport regulators, working groups on cargo, safety, environment
- CDC, other health agencies
- FAO Species Identification and Data Program (SIDP)
- UNEP Great Apes Program (GRASP, Ian Redmond)
- Great Ape Conservation Fund (with Fish and Wildlife Service, International Affairs, Richard Rugario)

The meeting participants discussed other forms of regulation, other than legal prosecution, that are devoted to species protection and could benefit from DNA barcoding. These include:

- Enforcement of CITES regulations at border inspection stations (John Sellar is the US official to be contacted);
- Customs services that need to identify imported/exported items for the purpose of taxation;
- State Fish and Game Departments responsible for hunting and fishing regulations; and
- Regulatory controls on the import and export of exotic species in the pet and horticulture trade

Wildlife management is another area of application in which DNA barcoding could be beneficial. Park and sanctuary managers are frequently called upon to identify the remains of animal prey (fecal droppings, pieces of fur and hide, fragmentary remains of carcasses). The presence of extremely rare species (Ivory-billed woodpecker feces, Ivory Coast *colobus* monkeys) can also be documented from feces, fur and feathers that are found in the field. The species and regions of origin need to be established as accurately as possible before confiscated animals and the offspring of captive breeding programs are re-introduced into the wild.

Zoological parks, botanical gardens, cell culture collections and other biological resource centers could also make excellent use of DNA barcoding as a tool for managing their living collections. Barcodes provide a way to verify species identities and therefore serve as a Quality Assurance/Quality Control (QA/QC) system. CBOL has involved AZA in the Database Working Group and its discussions of barcode data standards because ZIMS will be an important repository for voucher specimens.

Barcoding is becoming an important and commonly-used research tool in taxonomy and systematic biology. These disciplines are essential foundations of species conservation. Several other research fields are also relevant to the protection of endangered species. Ecological studies and conservation genetics are fundamental to species conservation, and barcoding has the potential to be a standard tool in these fields. Samples from archeological and anthropological studies, especially tools and pots, can bear the residue of animals and plants that were used as food sources. Barcoding can help to identify

these species, providing useful data on the history of human consumption.

During a conference call with Eduardo Eizirik, the meeting participants discussed the following potential uses of DNA barcoding for species identification Brazil:

- The Brazilian FBI has started to use DNA data to identify seized animal pelts, natural products, and specimens of animals in the pet trade, especially birds (including eggs), amphibians, spiders, plants, snakes;
- The Ministry of the Environment (IBAMA) is concerned about the overfishing of sharks and may be interested in using barcodes for sustainability studies;
- Border inspection for control of exports and confiscation;
- Rescue centers are responsible for the repatriation and management of seized live animals. They often requires data that can help to establish provenance;
- Biodiversity surveys need using non-invasive techniques such as identifying species from DNA in fecal droppings;
- Planning mammal (or just carnivore) barcode project; and
- Regulating the local consumption of turtle species

Logistical issues

Meeting participants discussed the special problems that would be associated with barcoding initiatives on endangered species. The first phase in any barcoding initiative is creating a reference library of barcode sequences using tissue from identified individuals and tied to voucher specimens. For obvious reasons, representatives of endangered species cannot be sacrificed to create voucher specimens, so a more flexible definition of “voucher specimen” might be needed (e.g., digital images plus tissue samples). The participants identified the following sources of identified specimens from which DNA barcodes could be obtained:

- Frozen Zoo, San Diego Zoological Society;
- Museum frozen tissue collections;
- Culture collections;
- Live zoo specimens;
- Wildlife sanctuaries, conservation centers, and confiscated live specimens sent to rescue centers; and
- Wildlife biologists/ecologists, field projects,

Museum specimens (including dried study skins and formalin-fixed individuals), can be valuable vouchers (especially type specimens) but it can be much more difficult to obtain the full barcode sequence from them, owing to degradation of their DNA. In such cases, it could be very valuable to link short sub-sequences of the barcode region from museum vouchers to full-length barcode sequences from frozen tissue, thereby creating “proxy vouchers”.

Once a credible library of reference barcode sequences has been established and made available to the research community, researchers and others located anywhere could obtain barcodes and identify them using the reference sequences. Some, but not all, of the organizations devoted to species conservation have access to biological material that needs to be identified. The meeting participants agreed that the following would prove to be the most important sources of unidentified material in need of identification through barcoding:

- Customs inspections and confiscations
- Covert operations leading to seizure of suspected contraband
- Airline baggage suspected of containing animal remains (especially bloody bags coming from certain African airports)
- World Wildlife Fund's bushmeat recording activity
- Ecological surveys
- Sanctuaries, such as the Pan-African Sanctuary Alliance (PASA)
- Wildlife health surveys (e.g., fecal samples)
- Public health officials
- Food inspectors
- Educational field courses (high school, university)

Findings, Recommendations, and Action Items

Participants agreed that DNA barcoding could have significant impact on the fate of endangered species, and that the Barcode Initiative and CBOL should be proactive in tackling this urgent societal problem.

Recommendation 1. CBOL should have a standing activity devoted to species conservation. This activity could explore and promote the use of barcoding for the protection of endangered species, and could raise general awareness of how barcoding can be employed for this purpose. CBOL could work with zoos and conservation which would increase the general visibility of DNA barcoding. Barcoding for conservation would be an excellent topic around which CBOL could conduct its public education and outreach efforts.

Recommendation 2. CBOL should catalyze the formation of specific barcoding projects devoted to particular endangered species in particular geographic regions. African bushmeat and Asian turtles are two excellent candidate projects.

Participants agreed that ideally, confident identifications will require reference barcodes not just for the species of interest in an area (e.g., African ungulates). Representative barcodes must also be obtained from all members of the monophyletic group in which the species of interest are found. Without these other reference barcode sequences, identifications would be based on sometimes tenuous assumptions about the amount of genetic variability in a species. Identifications in a clade for which only a portion of the barcodes are known will be subject to criticism.

Nevertheless, participants agreed that barcoding all the species in a clade within a restricted geographic area (e.g., Madagascar lemurs) can be valuable for conservation. Barcodes can be used to great advantage while the reference database is being populated with representatives from other taxa and regions.

Action Item 1. Raise awareness of barcoding in conservation community. CBOL should approach the conservation organizations listed above for the purpose of:

- Organizing barcoding outreach sessions at their meetings (especially through IUCN, WWF and Conservation International);
- Organize barcode presentations or a barcoding session at the International Primatological Society meeting in Uganda, June 2006;
- Circulate the report of this meeting report along with a short introduction to

barcoding; and

- Solicit ideas for barcoding projects related to conservation and other potential uses of barcoding through a survey questionnaire and personal interviews.

Action Item 2. Plan a workshop on Barcoding for Species Conservation. CBOL should approach the Sloan Foundation for an “officer grant” of \$45K to support an international meeting. Participants would include taxonomists who work on threatened groups and representatives of conservation organizations and government agencies. African bushmeat and/or Asian turtles could be used as model projects that would illustrate the uses, costs, limitations of barcoding for conservation.

Action Item 3. CBOL should catalyze the creation of a barcoding project on African bushmeat. The need for this project is clear and immediate, as describe above. Good taxonomic compilations are available for African primates (Groves) and artiodactyls (Metzler and Species2000). The Frozen Zoo at the San Diego Zoo and the frozen tissue collections at the Field Museum would provide samples for a significant portion of the species of interest.

Participants outlined three next steps:

- Create a “union catalog” of frozen tissue collections, starting with the Frozen Zoo and the Field Museum, that integrates information on potential sources of reference barcodes. This would form the nucleus of a growing database of reference material of African species that are subject to bushmeat hunting. The holdings of the Smithsonian, Natural History Museum of London, and major zoos should be added to this core database.
- In addition to developing the “supply” side of a bushmeat barcoding project, CBOL should explore and promote interest on the “demand” side by approaching potential users of barcode data such as WWF, TRAFFIC, and the other potential users listed in this report. CRES at the San Diego Zoo is willing to receive and analyze unidentified specimens from the sources listed above, for the purpose of monitoring the effectiveness of the barcode reference library as it grows.
- CBOL should organize and hold a small workshop on bushmeat barcoding that will bring together potential, curators of critical collections, and other stakeholders in the conservation community.

APPENDIX 1. Participant List

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APPENDIX 2. Meeting Agenda

Bushmeat Barcoding:

Developing tools for species identification as part of international efforts for maintaining sustainable wildlife populations

CRES – Beckman Center
San Diego Wild Animal Park
Zoological Society of San Diego
Escondido, CA

Friday, January 13, 2006

1:30 PM – Founder’s Room – Arnold and Mabel Beckman Center for Conservation Research

Welcome and overview (O. Ryder)

Background:

- DNA barcoding (R. Hanner)
- Consortium for the Barcode of Life (D. Schindel)
- Bushmeat crisis (O. Ryder)
- Barcoding of primates and other mammals to date (R. Hanner)
- Other barcoding initiatives (D. Schindel)
- Forensic evidence in species conservation (K. Goddard)

Goals for the Steering Committee Meeting (D. Schindel)

- Identifying potential uses of bushmeat barcodes (inventorying biodiversity, border control, identification of unknowns, screening against control list, identifying country/region of origin, etc.)
- Identifying potential users of bushmeat barcodes
- Determining overall scale of project (just bushmeat or other threats, such as pet trade, CITES lists, etc.)
- Determining taxonomic/geographic scope: How completely must clades be barcoded to address users’ needs?
- Setting procedures and quality standards for bushmeat barcode data (e.g., sample sizes, voucher specimens, data submissions)
- Identifying potential sources of reference specimens
- Identifying potential lab facilities for barcoding
- Setting timeline, goals, stages of project

Overview of logistical issues (R. Hanner)

- Sources of samples
- Tissue and DNA archiving
- Lab capacity
- Permits for collection and specimen transfer
- Data management
- Ownership and sharing of data
- Publication policy
- Management of project, distribution of labor

Funding

5:00 adjourn for dinner off-site

Saturday, January 14, 2006

8:30 AM Founder's Room – Arnold and Mabel Beckman Center for Conservation
Research

Project scope (establishing remit): reference database

Taxa to be included

Zoogeographical context

Access to samples and criteria

Molecular characterization issues

Lunch on-site

Bushmeat monitoring: preparations for utilization of reference database

Research projects

Existing enforcement structures and agreements

Opportunities to link barcoding results to assessment and monitoring

Assignments and follow-up

Cocktails and dinner at Mombassa Pavilion, San Diego Wild Animal Park