DNA Barcoding for Conservation of Mesoamerican Orchids

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This project aims at surveying orchid diversity, establishing long-term monitoring sites and undertaking a pilot study on DNA barcoding for conservation and trade surveillance in Costa Rica. Most projects on DNA barcoding of plants have focused on taxa (e.g. several taxonomic groups from around the world), whereas our work concentrates on a defined geographical area, Costa Rica, for one taxonomic group: the hyper-diverse family Orchidaceae (orchids). Hence, among other activities of this project, we are currently working on the development of a DNA barcode for Mesoamerican orchids, in particular Costa Rican species. The project is funded by the Darwin Initiative for the Survival of Species (14-001)

Costa Rica has one of the richest orchid floras in the world, with over 1300 species of orchids on a relatively small territory of 51,000 km2.

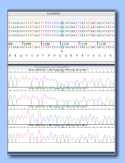
In spite of the fact that this country has a well-developed network of protected areas, the orchid flora remain under constant threat from factors such as deforestation and illegal trade.

Furthermore, orchids are well known to be difficult to identify, particularly when they are sterile.

DNA barcoding is a diagnostic technique for **identifying** species using a short DNA sequence from a standardized and agreed-upon region in the genome; such DNA barcode sequences are very short relative to the entire genome and they can be obtained reasonably **quickly** and **cheaply**.

Fieldwork is being conducted throughout Costa Rica, but especially in three locations (Tapanti National Park, Monteverde area and Coco Island National Park) that we will use as long-term monitoring sites.

Specimens are collected in the wild and living plants are accessed in the collection at Lankester Botanical Garden (Costa Rica) for further taxonomic identification and as an *ex-situ* repository. The orchid silica collection, often with more than one specimen per species, is duplicated at Kew where most of the DNA extractions and sequencing take place.

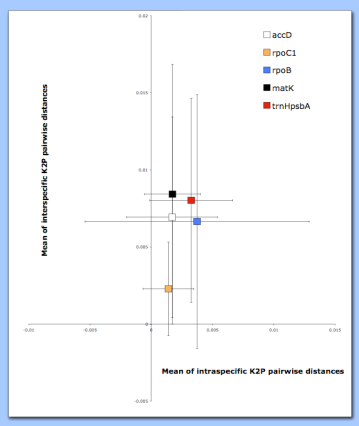


The six plastid loci being studied are those under consideration by the Plant Working Group of CBOL for the standard DNA barcode for land plants.

accD, rpoC1, rpoB, ndhJ, matK, trnH-psbA

We evaluated the inter- and intra-specific variation from a genetic distance matrix constructed using pairwise Kimura 2 parameter (K2P). We compared phylogenetic trees constructed using Neighbour Joining and Parsimony methods. From this we are able to evaluate the potential of each plastid region for building unique groups of individuals from the same species.

Over 600 orchid specimens (about 400 species) have been collected so far, and over 700 putative DNA barcodes have been produced.



Means with standard deviations of pairwise K2P distances for Costa Rican Orchids calculated between congeneric species for interspecific diversity and between representatives of the same species for intraspecific diversity for 5 potential DNA barcodes (*ndhJ* has been removed from the graph because of a low PCR/sequencing success).

With the highest inter-specific variation and the lowest intra-specific variation, *matK* appears to provide the greatest resolution, grouping over 50% of the species with replicates into unique groups.

It is clear that no single region will be sufficient as an efficient and universal barcode for orchids. A multi-locus barcode, based on two or three plastid regions, seems to be the most realistic solution.

So far we recommend combining *matK* with a non-coding region like the *trnH-psbA* spacer. This would provide the most effective DNA barcode for orchids and probably most other angiosperms, although further tests are needed.

Visit: http://www.jardinbotanicolankester.org/ing/project_a.html

Our geographical approach on DNA barcoding, dedicated to a Mesoamerican hotspot and a hyper-diverse family, will contribute to the international initiative laid by the Plant Working of the CBOL.

The use of a **standardized identification tool** could provide many potential uses and applications, for example: identification of different life stages, identification of fragments of plant material, forensics, verification of herbal medicines/foodstuffs, biosecurity and trade in controlled species, inventories and ecological surveys.

http://www.barcoding.si.edu/

CONSORTIUM FOR THE BARCODE OF LIFE

The Darwin Initiative (DI) for the Survival of Species promotes biodiversity conservation and sustainable use of resources around the world (http://www.darwin.gov.uk). It is funded and administered by the UK Department for Environment, Food and Rural Affairs, (DEFRA).

The main goal of the DI is to assist countries rich in biodiversity but poor in resources with the conservation of biological diversity and implementation of the Biodiversity Convention. Projects funded under the DI are collaborative, involving either local institutions or communities in the host country in collaboration with a British institution.

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