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DNA barcoding for identification of Cephalotaxus and the discovery of new species

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Abstracts

one for a genus; highly diverse insect orders have yet to be studied. We collected 12 763 specimens of the class Insecta using Malaise traps in three different types of vegetation (a perturbed zone, a mature recovered one, and a mangrove) during 159 days of sampling. Results: Our preliminary results include the identification of 16 orders and 76 families of insect. Nevertheless, the major orders Diptera, Hemiptera, and Lepidoptera have only 22 families identified, which is a small number in comparison with the percentage of specimens in these orders (69.78%). Specimens were distributed among the major orders as follows: Diptera (57.06%), Hymenoptera (15.34%), Coleoptera (11.93%), Hemiptera (7.09%), and Lepidoptera (5.63%). Preliminary results for 155 successfully sequenced specimens show 114 BINs, 61 of which were not previously registered in BOLD. Of the 114 Bin's, we identified 47 to the ordinal level, 53 to family, and 14 to generic level. The preliminary results show greater diversity in the mangrove zone than in the other sites. Significance: This is the first time this kind of sampling has been undertaken in this region. We linked some immature stages with adults and some worker ants with the reproductive stages of the same species. We also identified species distribution patterns by comparing our data with published records in BOLD. We found species that have not yet been sequenced in geographically close and well-studied regions such as Costa Rica. A library of insect barcodes for this region is just in the starting phase.

DNA barcoding for identification of *Cephalotaxus* and the discovery of new species

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The genus Cephalotaxus (Taxaceae) is comprised of eight species and two varieties distributed in East Asia as understory trees in temperate montane forest. As there is a lack of clear-cut morphological differences among species, taxonomy of this genus is difficult and controversial. In this study, all species and varieties of Cephalotaxus were collected from the distribution range, with 2-11 individuals per taxon, for species delimitation using six candidate DNA barcodes (ITS, rbcL, Atpf, trnH-psbA, trnL-F, and psbK-psbI). Among the six DNA barcodes, ITS showed the highest species discrimination rate at 36.4%, followed by Atpf and trnL-F (27.3%), while rbcL and trnH-psbA exhibited the lowest rate (9.1%), by using tree-based (NJ) analysis. Combinations of all six DNA barcodes can significantly improve the discriminatory power (63.6%) for Cephalotaxus species identification. Based on DNA barcoding analysis, seven species, including a new species, were identified in Cephalotaxus, which correspond well with its distribution. Recent taxonomic revisions of Cephalotaxus were not supported by our analysis. DNA barcoding is an efficient tool for new species discovery and taxonomic revision.

Collection data of black flies, mosquitoes, and sand flies of Mexico for further DNA barcode study

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Background: The dipterans, dubbed true flies, are one of the largest insects orders, accounting for an estimated 120 000 species. Many species of flies of this order are bloodsuckers and disease vectors of public health concern. Accurate taxonomic identification of vectors is of paramount importance for control programs and scientific research. For example, the morphotaxonomy of black flies is extremely difficult because of phenotypic plasticity, genetic variability, cryptic diversity, and the presence of different life-cycle stages and sexual dimorphism. Thus, the DNA barcode cytochrome c oxidase subunit I (COI) gene for the discovery of cryptic diversity and species complexes in black flies is highly useful. **Results:**

The present study updates the collection data of 257 specimens of dipterans, including important disease vectors such as black flies, mosquitoes, and sand flies, collected from several localities in Mexico from 2012 through to 2015. In total 84 morphospecies were identified: two species belong to Psychodidae (genus *Lutzomyia*), four species to Simuliidae (genus *Simulium*), and 78 species to Culicidae (genera *Aedeomyia*, *Aedes, Anopheles, Coquillettidia, Culex, Culiseta, Deinocerites, Haemagogus, Howardina, Limatus, Lutzia, Mansonia, Psorophora, Sabethes, Shannoniana, Toxorhynchites, Trichoprosopon, Uranotaenia,* and Wyeomyia). **Significance:** All 257 specimens are currently processed using the standard COI barcode protocol for further analysis and study. Several vector species belonging to common genera, such as *Aedes, Culex, Lutzomya,* and *Simulium,* have now been DNA barcorded which might facilitate their identification.

Identifying Malva species in Libya through DNA barcodes techniques, using four candidate DNA barcoding markers

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The approach of DNA barcoding has been used to distinguish and identify the *Malva* species of Libya. This study has been conducted using both official and novel DNA barcode regions tested on herbarium-derived DNA samples. Twenty-three specimens representing eight *Malva* species were collected from Libyan herbaria (Benghazi University, Omar Mukhtar University, and Tripoli University) and University of Reading herbarium (RNG). DNA fragments of regions rbcL, *psbA*–*trnH*, Atpf, and ITS were used as DNA barcodes to test their ability to distinguish species of *Malva*. Taxon DNA analysis and tree-based methods were used. The average intra- and interspecific distances were calculated, and DNA barcoding gaps were used to investigate the molecular identification ability of the chosen markers. The results showed that the best single barcode region was *psbA*–*trnH*. It is considered as a good candidate for use as a DNA barcode for identifying *Malva*, showing 100% identification efficiency.

Global perspectives on participating in the International Barcode of Life Project

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Background: As an international effort to create a publicly accessible database, the International Barcode of Life Project (iBOL) is vulnerable to cultural differences and social dilemmas that may discourage individuals from participating. We apply the Institutional Analysis and Development (IAD) Framework, which is a tool used to study types of "commons" (shared resources), to understand participation in iBOL iBOL commons include barcode databases and the repositories that store voucher specimens. Collectively, the barcodes and specimens are genetic resources, governed by national laws that implement the Nagoya Protocol to the Convention on Biological Diversity (CBD). Concern over the use of genetic resources differs between researchers in the Global North and the resource-poor, but biodiversity-rich Global South. These cultural, societal, and legal differences can create divide in the global barcoding community over how the shared resources should be managed. The success of iBOL is contingent on promoting use of the resource as well as recontribution of value-added data, while remaining sensitive to the divide in interests of researchers. Accomplishing this balance requires a set of rules that coordinates the behaviours of the different actors that comprise the international barcoding community. **Results:** We completed 44 semi-structured interviews with members of the iBOL community, funding agencies, and external stakeholders. Interviewees discussed many topics including research collaborations, genetic resource collection, data release, and knowledge about the CBD. Through applying the IAD