through the generation and analysis of DNA barcodes, the diversity of ants of Iguazú National Park (INP) as part of a major project that aims to barcode the ants of Argentina. The INP constitutes a biodiversity hotspot located in northeastern Argentina with over 200 species of ants and represents one of the biggest remnants of the Atlantic Forest, which is a priority for conservation as it harbors 7% of world's diversity. **Results:** We obtained 178 COI sequences from 332 individuals belonging to more than 100 species from over 30 genera. Unidentified species were not included. The mean intraspecific sequence divergence was slightly over 0.70%, which was 23 times lower than the mean interspecific divergence (16.6%). After removing a few species with strikingly deep intraspecific divergence, the mean intraspecific divergence fell to 0.30%. There were more BINs (73) than species (67), with no BIN being shared by different species. Seven species showed two clearly differentiated barcode clusters, suggesting the possible presence of cryptic biodiversity at INP. The existence of these divergent intraspecific lineages was supported by high node support values in complementary phylogenetic analyses. **Significance:** Taxonomic keys for Neotropical ants are mostly incomplete and based almost exclusively on workers, precluding the identification of males and queens of most species. Increasing the reference barcode library for the ants of INP (and Argentina) will certainly help to identify these castes and to register morphological variability of Neotropical ants, which may eventually lead to the generation of new taxonomic keys. Lastly, our results support the biodiversity hotspot status of the Atlantic Forest and suggest that ant diversity in INP is currently being underestimated.

Deep intraspecific barcode splits: cryptic species, *Wolbachia* or something else?

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Background: Many studies of DNA barcodes have revealed extraordinary variation in mitochondrial DNA in some species. Sometimes this variation is highly clustered so that a species shows a deep intraspecific split in mtDNA with no intermediates. While building a complete reference library for ~2600 species of Finnish Lepidoptera, dozens of such deep splits were detected. In order to investigate the presence of cryptic species, we sequenced six taxonomically informative nuclear genes (*EF-1a*, *MDH*, *CAF*, *IDH*, *RpS5*, and *wingless*) in 29 lepidopteran species, each having a deep (>2%) sympatric intraspecific split in the DNA barcode region. **Results:** The results suggest that some, but only a minority of splits, are due to the presence of a previously undetected morphologically cryptic species. Some cases may be attributed to historical polymorphism. Several splits have likely resulted from introgression, i.e., transfer of the mitochondrial genome from one species to another. *Wolbachia* infection was detected in many cases, and some splits may be explained by *Wolbachia*-driven spread of alien mitochondrial haplotypes in the population after an introgression event. **Significance:** Overall, our results suggest that DNA barcodes have great potential in revealing new species even among well-investigated groups and areas. We also show that other biological processes may result in mitochondrial polymorphisms that may occasionally impede the usefulness of DNA barcodes in species identification.

Balancing sensitivity and specificity in primer design for eDNA studies using ePRIMER

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Background: The detection of species-specific environmental DNA (eDNA) via real-time polymerase chain reaction (qPCR) is emerging as an important monitoring technique for rare and invasive species. To be effective, a qPCR assay must be sensitive enough to detect the small amounts of target DNA that may be present in the environment, but specific enough to avoid detection of non-target DNA. We developed a program (ePRIMER) that uses one or more target sequences and one or more non-target sequences to produce a list of potential primers ranked by relative efficiency and specificity, allowing the user to select a primer set with a desirable balance between the two. To illustrate the utility of ePRIMER, we designed and tested an eDNA detection assay for the watersnake (*Nerodia sipedon*) in Ontario, with the queensnake (*Regina septemvittata*) as a non-target species. **Results:** Among 11 226 potential primer and probe sets produced by ePRIMER, the most specific (with 11 base-pair mismatches between species) was the 7572nd most efficient. Using tissue-derived watersnake DNA (with initial quantities ranging from 7.1×10^{-4} to 20 ng) and the most specific primer and probe set, the efficiency of qPCR (as determined by 10-fold serial dilution tests) was 68%. The assay did not amplify queensnake DNA. **Significance:** Our study demonstrates that sensitivity and specificity may require a trade-off in primer design, and with ePRIMER we provide a platform for assessment of this trade-off in the development of qPCR assays for eDNA detection.

Calibrating the taxonomy of a megadiverse family on BOLD: 2700 geometrid moth types barcoded (Geometridae, Lepidoptera)

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Background: One of the major challenges in creating a global database like BOLD is warranting the correct identification of the voucher specimens. The strict BOLD policy to require images, indication of specimen deposition, and accurate geo-referencing for all submitted datasets is extremely helpful to control doubtful data and potential misidentifications. Nevertheless, there are still many incomplete identifications (to genus or subfamily level), interim names, or even misidentifications on BOLD, mainly for species from tropical regions. Unfortunately, experts are lacking for many problematic groups and regions, and even when there are experts, they usually are not available for correcting the taxonomy of large amounts of data due to time constraints. **Results:** The best way to reliably calibrate the system is to barcode the original type specimens. In recent years, the challenge of sequencing up to 250-year-old museum specimens has been overcome by improved techniques and protocols developed by the Canadian Centre for DNA Barcoding. These innovations allowed for the generation of barcode sequences for ~2700 geometrid type specimens, which represent 2150 species corresponding to about 9% of the 23 000 described species worldwide. **Significance:** Here, we present case studies to show the efficiency, reliability, and sustainability of this approach as well as promising strategies to complete the calibration of the reference library within a reasonable amount of time.

Using DNA metabarcoding to investigate the medicinal properties of honey

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Background: Honey possesses therapeutic properties that are the result of a range of factors including high sugar content, low pH, hydrogen