all other dimensions of biodiversity. DNA barcoding constitutes an efficient tool to assess the evolutionary history of communities and understand the assembly rules that sustain biodiversity.

Barcoding as a useful tool for South American wild bee systematics

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Background: The bee genera *Corynura* and *Halictillus* (Hymenoptera: Halictidae) contain species that are very abundant in Chile and Argentinean Patagonia, and which are key elements in southern South American ecosystems. For instance, *C. chloris* is among the major pollinators of crops such as raspberry, Chilean hazel, and buckwheat as well as native wildflowers. These bee species are very difficult to identify due to close morphological similarity among species and extreme sexual dimorphism. To assess this, we analyzed the barcodes of 15 species of *Corynura*, as part of a revision of the genus, as well as four species of *Halictillus*. **Results:** We obtained 170 barcode-compliant sequences. Barcodes were useful to confirm gender associations and to detect two cryptic species previously considered as one. The genera showed a median interspecific distance (13.5%–14.3%), considerably higher than among other closely related halictid genera. The sequence divergence between specimens of the same species was up to 0.9%, with a few exceptions. The barcodes of five specimens differed from the rest by more than 2%, although these individuals were not morphologically different from the others, nor collected in distant areas. In contrast, *C. patagonica* showed a distance of 4.2% when any of the specimens from Chile was compared to those from Argentina. The only morphological difference found between the barcode clusters was the colour of the metasomal terga of the female. BIN analysis was useful to identify the species that showed high intraspecific variation. **Significance:** Species delimitation and identification is particularly important in halictine bees, which are renowned for being morphologically monotonous. The results suggest that *Corynura* and *Halictillus* species can be identified through DNA barcodes, although some species showed a high intraspecific variation which requires further study. We conclude that *C. patagonica* is probably two cryptic species.

DNA barcoding reveals a possible cryptic species complex of *Mycalesis mineus*: a case study from Sri Lanka

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Background: The lepidopteran genus *Mycalesis* consists of over a hundred species spread throughout the Oriental and Australasian regions. Of these, *M. perseus*, *M. patnia*, *M. mineus*, *M. subdita*, and *M. rama* are found in Sri Lanka, with the latter two being endemic to the island. The Sri Lankan populations of *M. perseus*, *M. mineus*, and *M. patnia* are closely related to those of the Oriental region but are divergent enough to support subspecies status as *M. perseus typhlus*, *M. mineus polydecta*, and *M. patnia patnia*, respectively. The aim of this study was to investigate whether DNA barcoding can achieve unambiguous species identification and delineation of *Mycalesis* species in Sri Lanka. This group was selected for DNA barcoding analysis because these species display close morphological similarity with each other and also contain examples of high morphological variation within species. **Results:** We analysed the genetic divergence in the cytochrome c oxidase subunit I (COI) gene of *M. perseus*, *M. patnia*, *M. mineus*, and *M. subdita* in Sri Lanka, supplemented with sequence data from GenBank. It was possible to unambiguously distinguish *M. perseus* and *M. patina* from the *M. mineus* and *M. subdita* cluster in neighbour-joining, maximum likelihood, and Bayesian tree analyses. Sri Lankan *M. mineus* and *M. subdita* appear relatively closely related, while the regional *mineus* group formed a separate cluster from the Sri Lankan *M. mineus* with strong bootstrap support (>90%). These clear barcode clusters may provide evidence for a possible cryptic species complex within the currently recognised *M. mineus*. **Significance:** These barcode results provide evidence for the presence of a genetically diverged *M. mineus* population in Sri Lanka and highlight the necessity for detailed morphological and ecological investigations to reveal any overlooked species within the *Mycalesis* subspecies present in the island.

The application of next-generation sequencing barcoding in identifying mixed-pollen samples from a historic bee collection

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Background: Increasingly, studies are employing DNA barcoding and next-generation sequencing (NGS) approaches to identify different organisms in environmental samples. However, this approach in pollination biology is still lacking, with available barcoding data only assessing fresh pollen. NGS provides an easier, faster way to generate large volumes of data on pollen sampled directly from bees, eliminating the need for separating the pollen grains by taxon prior to sequencing. In this study, DNA barcoding is combined with Illumina NGS to provide taxonomic classification for pollen sampled from one species of indigenous, solitary bee that was collected across South Africa over a 93-year period. Three genomic regions were studied: the internal transcribed spacer regions 1 and 2 (ITS1 and ITS2) and the rbcL gene. A bioinformatic workflow using custom-made databases for the entire internal transcribed spacer region and rbcL was developed. **Results:** In total, 22 mixed-origin pollen samples were assessed. Samples represent eight decades, spanning over 90 years, of bee collection. Samples from as far back as 1910 were successfully sequenced and classified. Species-level delimitation of pollen was possible for all genomic regions, with higher confidence at family level. **Significance:** This is the first time that samples from a historic insect collection have been used in studying pollen origins using NGS and DNA barcoding techniques. This approach provides a historical perspective on how floral choice in indigenous bees changed over time and insights into the effects that land use and climate change have on bee–plant interactions in regions with high levels of oligolectic bees species.

Integrative approach and molecular barcoding of dagger and needle nematodes infesting grapevine soils in Portugal

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Background: Dagger (*Xiphinema* spp.) and needle (*Longidorus* spp., *Paralongidorus* spp.) nematodes are two of the main groups of parasitic nematodes in grapevines worldwide, causing severe damage to plants by their direct feeding; in addition, some species may transmit plant viruses. Some of these nematode species are included in the list of quarantine organisms in many countries. Grapevine fanleaf virus (GFLV) is specifically transmitted by *Xiphinema index*, and it is one of

the most harmful viruses to vineyards wordwide. Despite their phytopathological importance, this research area has been deserted for over fifteen years in Portugal. In recent years, plant health of the Portuguese vineyards has declined, characterized mainly by reduced vigor in plants. Thus, the main objective is to determine the ocurrence and distribution of longidorids infesting vineyards from Portugal. Nematode surveys have been conducted since 2015 on 30 commercial vineyards of the main Controlled Denomination of Origin (DOC) zones of Portugal. **Results:** The use of an integrative strategy, based on the combination of morphometric and morphological characterizations with molecular analysis using barcode regions such as the ribosomal DNA segments (rDNA) (D2–D3 regions of the 28S gene, ITS1 region, and 18S gene), has allowed the identification of collected longidorid species, associated with severe infestations found in grapevine soils in the studied DOC areas. We emphasize the successful identification and detection of *Xiphinema index* due to its phytopathological importance. The most important longidorid nematodes detected, in order of decreasing frequency of total soil infestation, were *Xiphinema pachtaicum*, *X. index*, *X. santos*, *Xiphinema* sp., and *X. italiae*. **Significance:** Our study highlights the validity of using an integrative approach based on the combination of morphological data and molecular barcodes for the correct and timely identification of this group of nematode species characterized by high morphological similarity and phenotypic plasticity. The high prevalence of *X. index* makes this species a severe threat to grapevine production in Portugal.

Potato cyst nematodes infesting potato fields in Ecuador: integrative diagnosis and molecular phylogeny

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Background: Potato cyst nematodes (PCN), *Globodera rostochiensis* and *G. pallida*, are serious pests of potatoes worldwide, with some species included in the quarantine lists of many countries. Their accurate and timely identification is a prerequisite in designing effective management strategies. In Ecuador, potato is one of the main crops, and PCN represent an important pest. This situation led to a national survey where a total of 85 soil samples were collected in 2013 from the major potato-growing regions. The main objectives were to determine the prevalence of PCN and to establish a method for the integrative diagnosis of species based on the combination of morphological data with molecular analysis using rRNA regions. PCR-based techniques for species-specific amplification of the ITS region and for sequencing the D2–D3 expansion segments of the 28S rRNA gene were also developed. **Results:** *Globodera pallida* was the only PCN species found in the potato fields, but it was widespread (55% frequency of infestation). Moderate to high *G. pallida* population densities were found. The results from the molecular methods were congruent with the morphological data. Interspecific divergence of the D2–D3 region of PCN is low, and it is not possible to discriminate between *G. pallida* and *G. rostrochiensis* based upon this molecular region, but it is useful for the distinction of other species. Phylogenetic analysis yielded two major well-defined and supported clades, where Ecuadorian sequences grouped with sequences of *G. pallida* and *G. rostochiensis* populations deposited in the GenBank database. **Significance:** The prevalence of *G. pallida* makes it a severe threat to potato production in Ecuador. Moreover, management practices such as the short term of crop rotations and the use of the "super-chola" susceptible cultivar could be worsening the problem. A strategy based on the integration of morphological data and molecular analysis is useful for identifying PCN species. In addition, other molecular barcodes are currently being characterized (ITS-rRNA, COI-mitDNA).

Recovery of nucleic acids from microhymenopterans with four non-destructive methodologies and considerations for museum slides preparations

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Background: Microhymenopterans are used in biological control programs of insect pests as an ecological alternative to chemical toxicants. These minute wasps require particular protocols and skills for preparing the slides that allow a correct taxonomic identification. DNA barcoding offers taxonomists the opportunity to improve the identification of tiny hymenopterans. The molecular approach may be of better use if, after DNA extraction, permanent slides of such specimens are prepared for comparative analyses with taxonomic keys. We evaluated four non-destructive methods for DNA extraction: (*a*) DNeasy Blood & Tissue Kit, (*b*) Protocol with $CaCl_2$ buffer, (*c*) Method HotSHOT, and (*d*) Phire Tissue Direct PCR master mix. Using PCR to amplify a ~680-bp sequence of the mitochondrial gene cytochrome c oxidase subunit I, we demonstrated the efficiency of these methods on insects maintained dry or in ethanol along a time course of conservation (i.e., 23 years, 12 years, 3 years, 4 months, 1 day). **Results:** Two techniques (*a*, *b*) yielded DNA extracts that were successfully PCR amplified for all samples, while technique (*c*) amplified the last four samples in a faster, cheaper, and easier way than (*a*) and (*b*). The last technique (*d*) amplified only the two more recent samples, but it was the fastest one that did not produce non-specific PCR products, as detected by observing multiple bands on an agarose gel. Moreover, we adapted the traditional methodology of permanent slides preparation in Canada balsam for every technique after DNA extraction, including the discoloration step. **Significance:** The results reported here allow combining the utilization of classic and molecular biology methodologies for taxonomic studies of microhymenopterans. The most significant result was the recovery of DNA from 23-year-old insects, allowing the description of additional biological traits for old museum specimens.

Evolving the concept, and use, of DNA barcode libraries

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Background: DNA barcode library concepts and construction methods are diverse. As a result, they stand as important, but isolated resources toward a coordinated mapping effort for a first draft of eukaryotic life using DNA barcodes. In particular, annotating the biological and community relevance of DNA-barcoded specimens for comparative identification in a reference library is a current frontier for the use of DNA barcode data. **Results & Significance:** Global consortium science methods have arisen as efficient ways to coordinate annotation and knowledge development of large-scale genomic and microbial data. These platforms stand as comparative templates to advance eukaryotic biodiversity informatics via DNA barcodes. This poster will present the structural elements of successful consortium science methods toward promoting discussion of the complimentary use of public, common-source DNA barcode libraries for identification and discovery.