A special issue on DNA barcoding edited by the Belgian Network for DNA Barcoding (BeBoL)

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Background: The Belgian Network for DNA Barcoding (BeBoL) was launched in 2011. It provides a multidisciplinary platform for 25 institutes active in DNA barcoding and molecular systematics. Between 2011 and 2013, collaborative projects of BeBoL resulted in about 45 ISI publications on DNA barcoding, integrative taxonomy, phylogenetics, phylogeography, identification of cryptic species, biosecurity, and conservation genetics. BeBoL activities also included organization of congresses (Third European Conference for the Barcode of Life in 2012, DNA in Forensics 2014), symposia, workshops, and training activities. More information about the Network is available on the BeBoL website (http://bebol. myspecies. info). **Results:** In December 2013, the Network edited a special issue of ZooKeys (issue 365) entitled "DNA barcoding: a practical tool for fundamental and applied biodiversity research". This open-access publication (http://zookeys.pensoft.net/browse_journal_issue_documents?issue_id=377) comprised 21 research papers and included, among others, contributions of BeBoL partners and participants of the ECBOL3 conference. Contributions focused on many aspects of the DNA Barcode of Life initiative: (*i*) technical challenges (DNA barcoding museum or processed biological material, biobanking); (*ii*) testing the utility of COI and alternative markers for species identifications of flatworms, molluscs, insects, birds, and plants; (*iii*) validation of DNA barcoding identification pipelines (for fish landings and forensic entomology); and (*iv*) applications in biodiversity monitoring (marine mammals, forest resources). **Significance:** As a supplement to large-scale DNA barcoding campaigns, this collaborative initiative is providing methodological resources together with reference DNA barcodes for specific purposes (e.g., monitoring fish landings, forensics, medicinal plants, etc.).

Large-scale DNA barcoding of ants from Ecuador

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Background: Ecuador has a great diversity of habitat, rough topography, and high species richness. Unfortunately, its ant fauna is poorly understood. Some taxa are morphologically difficult to identify (caste polymorphism, overlap between intraspecific variation and interspecific differentiation, uniform worker morphology, lack of recent taxonomic revisions, etc.). To facilitate species identification, a joint project of three Ecuadorian universities and two Belgian research institutes was launched in 2015. It aims at creating a reference collection of DNA barcodes for every Ecuadorian ant species or morphospecies. **Results:** A first focus of this project was on the genus *Leptanilloides* (Formicidae: Dorylinae) from Central and northern South America that is rarely collected due to its subterranean foraging habits. Seven workers and one male were collected, and two putative unknown species were recognized based on the worker morphology and COI barcode sequences (p-distances ranging from 18.8% to 21.7%). These also differed (18% to 21.8%) from the barcodes of the two *Leptanilloides* species available in BOLD (6 specimens). **Significance:** Our results show that DNA barcoding can distinguish among morphospecies of ants of the genus *Leptanilloides*, and thus can complement morphology for species identification. Unfortunately, *Leptanilloides* species are currently known from a limited number of workers and incomplete DNA barcode libraries. Therefore, we cannot compare the new sequences with those of all other *Leptanilloides* species. This case study illustrates the importance of large-scale sampling in order to investigate a highly diverse insect fauna, document intra- and interspecific variation, and build a comprehensive reference library of DNA barcodes.

High-throughput sequencing of PCR amplicons: a test to barcode a bee species complex (Hymenoptera: Apoidea: Halictidae) and survey *Wolbachia* infections

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Background: High-throughput sequencing of PCR amplicons, also called targeted amplicon sequencing (TAS), combines the flexibility of PCR amplification with next-generation sequencing (NGS) technologies. In comparison with Sanger sequencing, NGS potentially improves the sequencing success rate and the detection of heteroplasmy, heterozygosity in nuclear markers, and endosymbionts. Here, we applied TAS to simultaneously sequence the COI barcode region, three nuclear markers (wingless, white gene, and HOG7036-02), and a fragment of the *Wolbachia* surface protein (wsp) in 24 museum bee specimens of *Halictus* (*Seladonia*). This bee genus is frequently infected by *Wolbachia*, and one of the species, *Halictus smaragdulus* Vachal, 1895, is suspected to be a species complex on the basis of the morphological variation in the male genitalia. Results obtained for the DNA barcode fragment were compared to those obtained by Sanger sequencing, using the same specimens and DNA extracts. **Results:** Sequencing of COI was more successful with NGS (21/24 specimens) than with Sanger sequencing (18/24 specimens). COI haplotypes obtained from both approaches were identical and showed divergences that were congruent with the male genitalia differentiation. These results suggest that *H. smaragdulus* comprises more than one species. No signs of heteroplasmy were observed. Nuclear markers were successfully sequenced for 15-20 (62%–83%) of the specimens, and *Wolbachia* was detected in ~50% of the individuals. **Significance:** By sequencing standard DNA barcodes and specific DNA markers (including DNA fragments from *Wolbachia*), we produced a dataset that allows a better taxonomic interpretation of the species complex.

Barcoding plant hotspots in Patagonian Monte Desert

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Background: Under the current biodiversity crisis, an approach used to prioritize areas for biological conservation is the identification of "biodi-