

DNA Barcodes for Evolution and Biodiversity

Stephan Koblmüller 

Institute of Biology, University of Graz, Universitätsplatz 2, 8010 Graz, Austria; stephan.koblmueeller@uni-graz.at

Questions centered around how biological diversity is being generated and maintained, as well as how this biodiversity can be conserved/protected, are being frequently asked in basic and applied evolutionary biological and biodiversity research. However, identifying the entities of biodiversity, i.e., the species, by means of traditional morphological methods is often anything but trivial and is time-consuming. Our ability to identify and assess biodiversity has been enhanced by the establishment of DNA barcoding, which had, has, and will continue to have a great impact on many fields of basic and applied research.

DNA barcoding is a method used for identifying specimens (ideally to species level) and involves employing an expert-based reference system (an open-access database) that drastically increases the number of people who are able to identify organisms down to the species level and reduce the number of misidentifications among morphologically similar taxa. Specifically, DNA barcoding is a standardized approach used for identifying organisms based on specific sections of their DNA [1]. Depending on the taxonomic group, different genes have been established as the standard DNA barcoding markers, even though also other genes may be used for certain applications or taxa. Consequently, DNA barcodes should (in most cases) allow for an unambiguous specimen identification, as well as of morphologically unidentifiable life stages/sexes or parts of organisms, once a reliable DNA barcode reference database is available. Thus, DNA barcoding has become an important tool in basic and applied biodiversity and evolutionary biology research. Indeed, since the onset of large-scale DNA barcoding initiatives, researchers have aimed to increase the time and cost-efficiency of this method [2–5], obtain reference data from samples with suboptimal DNA quality (e.g., from older museum specimens) [6,7], provide comprehensive reference DNA barcode libraries for certain taxa and regions [8–11], or characterize entire communities via (eDNA-)metabarcoding [12,13].

This Special Issue includes a collection of 14 papers that use DNA barcodes to answer questions in basic and applied biodiversity and evolutionary biology research. Many of the key aspects of DNA barcoding are addressed by these studies which provide some important new insights in their respective fields of research.

Typically, in any biological study, species identification and delimitation is the first and often most important step. For a long time, this has solely been based on morphological characteristics, but with the establishment of molecular genetic methods, and especially with the advent of DNA barcoding, DNA data have been increasingly used for this purpose within an integrative taxonomical framework. DNA-based methods are a particularly useful supplement for delimiting species in taxa that comprise several phenotypically similar or indistinguishable species (=cryptic species complexes) (e.g., [14–16]). Five papers in this Special Issue focus on species delimitation among, in part, morphologically very similar taxa [17–21]. The first paper explores species diversity in dogfish sharks (genus *Squalus*) from the Pacific and western Atlantic Oceans, a taxon notorious for its conserved morphology, by means of DNA barcodes and a variety of molecular species delimitation methods [17]. The study shows that all samples analyzed represent species that are already known. The presence of obviously misidentified samples in databases, however, makes drawing inferences on the real distribution and diversity of species that belong to this genus difficult. The second paper [18] characterizes the diversity of invertebrates in Croatian



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olive orchards and vineyards by means of DNA barcoding and by comparing the obtained DNA barcodes with available reference data. With their protocol, which uses standard barcoding primers for animals (LCO1490/HCO2198 [22]), the authors managed to obtain data for only slightly more than half of their samples, a finding that is in line with other studies that show that these standard primers do not work well for all taxa. A finding that is particularly interesting and relevant for many taxa, especially within Collembola and Oligochaeta, which are considered major players in (soil) ecosystems, is that many samples cannot be assigned to particular species, which indicates that there is a lack of reference data in the two largest databases (BOLD and GenBank). The third paper [19] establishes a DNA barcode library for mealybug species from Espírito Santo, a major coffee-producing region in Brazil, by combining newly generated DNA barcodes with barcodes available on BOLD. The study shows that, in principle, molecular species delimitation works well in the relevant taxa, if obviously misidentified samples on BOLD are taken into account/excluded. The fourth paper [20] focuses on the diversity of Lepidoptera from Greece. DNA barcodes of ~600 morphospecies were generated and assigned to molecular taxonomic units (MOTUs) on BOLD. A large number of these could be assigned to MOTUs/species that are also present in other parts of Europe (including new records for Greece). However, about one-sixth of the identified MOTUs had no references in BOLD, despite the generally good coverage of European Lepidoptera on BOLD (e.g., [10]). Hence, these MOTUs may be restricted to Greece (or southeastern Europe) and may potentially include a large number of undescribed species. The fifth paper [21] focuses on the agaricoid mushroom genus *Cortinarius* s.l. in Romania. By means of an integrative taxonomical approach, morphological analyses and DNA barcoding data were combined. Of the 109 *Cortinarius* s.l. species identified in this study, only 43 were previously reported for Romania, while 66 species were new to the country. Collectively, these five papers show the potential of DNA barcoding for species delimitation, species discovery, and general biodiversity assessment, but also highlight obvious problems/difficulties associated with erroneous species identification for some samples on BOLD and a lack of reference data for some important taxa, which makes it difficult for taxonomic laymen (one of the alleged huge advantages of DNA barcoding used for species identification) to characterize their (local) diversity.

Two further studies [23,24] used DNA barcodes to identify samples to species level. The first study [23] used DNA barcoding to identify a cichlid fish population from a freshwater reservoir in the Ouémé Basi, Benin, as an invasive and primarily estuarine/brackish species that is only rarely found further upstream in pure freshwater habitats. Whether this represents a case of natural range expansion or human introduction remains unclear. The second study [24] used DNA barcoding to identify species of single-drug herbal powders collected from markets in Tamil Nadu, India. As herbal powder is more prone to adulteration than intact plant parts, its authentication is essential to ensure the safety and efficacy of herbal drugs. The study shows that of the 107 herbal powders analyzed, a surprisingly large portion of samples (46%) were adulterant. In 59% of these adulterant samples, the authentic species were entirely replaced with taxonomically unrelated, but sometimes phenotypically similar, species. This low rate of authentic plants in the investigated herbal powders is alarming and calls for thorough training centered around the correct identification of relevant plants and routine validation, e.g., by means of DNA barcoding, to minimize potential health risks for consumers.

High taxonomic coverage is crucial for the applicability of reference DNA barcode databases like BOLD. Although there is comprehensive coverage for certain taxa and regions [8–11], this is not the case for other taxa and regions. These are typically understudied taxa and regions. In this Special Issue, one paper [25] targets the monogenean fish parasites of gobies in Greece. By conducting morphological analysis combined with the sequencing of three genes, including the DNA barcoding region, the authors provide the first record of *Xenoligophoroides cobitis* (Monogenea: Dactylogyridae) for Greece and

the first DNA barcode of this monotypic genus. In addition, the authors proposed some hypotheses regarding the evolution of this monotypic genus.

A combination of DNA barcoding, the sequencing of additional genes, and phenotypic data analysis was also used in another paper included in this Special Issue [26] to characterize population of the invasive colonial ascidian *Botrylloides niger* in the northeastern Mediterranean Sea. Several distinct morphotypes were found, but DNA-based species delimitation methods suggest that these all belong to *B. niger*. In addition, this study provides important information on population dynamics, demographic history, and intraspecific genetic diversity for this invasive species.

The intraspecific diversity of the crambid moth *Maruca vitrata* in India was the focus of another study [27]. This species is one of the most destructive pests of grain legumes across the subtropical and tropical regions of the world, and hence knowledge on intraspecific diversity is important for its management. Based on DNA barcoding data, very little intraspecific variation was inferred, indicating the presence of a panmictic population in India. Furthermore, the data show clear signatures of recent population growth. Thus, the study provides very important baseline data for the future management of this pest species.

Recent advances in (eDNA-)metabarcoding methods have resulted in a range of technologies that now can be applied to monitor the occurrence and abundance of diversity in different environments [13,28–30]. A review paper included in the Special Issue [31] focuses on how eDNA-based methods are used in the biodiversity monitoring of protected areas. Specifically, the advantages (and disadvantages), as well as the challenges and limitations, of potential applications are discussed. The paper provides useful information on the use of eDNA approaches in protected areas and also explicitly states what is needed to increase applicability and comparability. Thus, this review may serve as a guideline for where to focus in the future development/improvement of eDNA approaches to be applied for monitoring-associated research and answering explicit management questions (not only) in protected areas.

One study included in this Special Issue [32] used metabarcoding of bulk samples to assess the species composition of ichthyoplankton in the Oujiang River estuary in China. The authors compared the performance of 12S and *cytb* as metabarcoding markers and found that 12S consistently performed better, both in terms of species coverage and detection rates. In total, 145 taxa were identified. This study makes an important contribution to our knowledge about fish diversity in Chinese river estuaries.

The monitoring of pathogens and parasites to identify high-risk-infection areas, enabling disease control, is also facilitated by eDNA methods [33,34]. A study included in this Special Issue [35] provides reference DNA barcodes for the Austrian avian schistosomes of the genus *Trichobilharzia*. Based on these data, an eDNA-based PCR assay was developed to identify *Trichobilharzia* in water samples. Though these parasites typically use birds as final hosts, they may also infect humans as accidental hosts, causing dermatitis symptoms. Thus, these trematodes are of human medical relevance, suggesting that the assay developed in this study will be of great use for the routine monitoring of waterbodies.

Finally, one opinion paper included in this Special Issue [36] presents the reflected opinions of early-career biodiversity researchers regarding questions related to the future of DNA barcoding and whether the currently employed standard barcoding, i.e., the sequencing of short standardized fragments of DNA, will also remain the method of choice for rapid and reliable species identification in the future. From their reflections, it seems to be clear that DNA (meta-)barcoding will also continue to impact biological sciences and environmental management in the future, as long as a focus on data quality is prioritized and the methodological and technological advancements remain aligned.

Conflicts of Interest: The author declares no conflict of interest.

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