NRC Research Press

INTRODUCTION

From Barcodes to Biomes: Special Issues from the 6th International Barcode of Life Conference

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The two special issues—Barcodes to Biomes—mark the one-year anniversary of the 6th International Barcode of Life Conference held in Guelph, Canada. The 6th Conference brought together 601 delegates from 51 nations. The diversity of talks and poster presentations revealed that the global DNA barcoding community has expanded from its original focus on building libraries of reference sequences to a more diversified community, presenting research on themes ranging from systematics to food web ecology to wildlife forensics. Based on a comparison with an earlier conference, marked trends were observed in the increasing breadth of topics and in the pronounced rise of contributions employing DNA barcoding for diverse socio-economically important applications, including pest management, invasive species detection, and monitoring of environmental health (Adamowicz 2015*a*, 2015*b*).

One year ago, *Genome* published a special open-access issue containing the abstracts from the 6th Conference (Adamowicz 2015*b*); that compilation highlighted the breadth of research topics and leading-edge research directions. The current open-access special issues feature 24 full articles. Part 1 includes two reviews that expand upon conference presentations and themes and 10 additional research articles associated with conference presentations, four of which are first authored by winners of a postdoctoral or graduate student prize at the 6th Conference (Beet et al. 2016; Mark et al. 2016; Saitoh et al. 2016; Sing et al. 2016). Part 2 includes 12 additional articles—three reviews, eight research articles, and one opinion piece—that cover topics ranging from a review of the field of fungal DNA barcoding (Xu 2016) to applications of DNA barcoding for biosecurity (Hodgetts et al. 2016) to multiple novel methods of species detection (discussed below). A large virtual special issue to be published online in 2017 will collate all conference-associated papers published across multiple issues of *Genome*. These special issues of *Genome* complement a special collection of 16 articles in *Philosophical Transactions of the Royal Society B* (Hebert et al. 2016). Together, these issues showcase the progress that has been made in applying DNA barcoding in diverse ways and outline exciting future prospects for discovery.

Innovating to generate reference DNA barcodes

Although primer design efforts and complementary marker testing are ongoing for some taxonomic groups

Received 8 August 2016. Accepted 8 August 2016.

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Published at www.nrcresearchpress.com/gen on 9 September 2016.

(e.g., Xu 2016), methods for specimen-by-specimen DNA barcoding have now become well established for animals as well as for many plants and fungi. Steinke et al. (2016) exemplify a dedicated effort to collect fresh specimens and use standardized molecular methods to generate a large DNA barcode reference library for the marine fishes of South Africa. When combined with other publicly available sequences, the reference library for marine fish species of that region was sufficiently complete that they could identify nearly 90% of all collected immature specimens using DNA barcodes. While such works remain vital to the development of DNA barcoding, many research teams are now developing and applying high-throughput sequencing (HTS) approaches, also known as next-generation sequencing, primarily to study mixed-species assemblages (metabarcoding). Indeed, nearly one-fifth of all submitted abstracts for the 6th Conference related to using or developing HTS methodologies (Adamowicz 2015*a*, 2015*b*). Despite this community-level expansion of focus, conference participants continued to stress the importance of building more complete libraries of identified reference sequences. Two novel contributions in the first special issue combine these dual mandates of expanding the usage of HTS by the DNA barcoding community and also improving the libraries of reference sequences.

Hausmann et al. (2016) present a method employing HTS to gather reference DNA barcodes from museum type specimens. The authors first highlight that the veracity of Linnaean identifications of reference sequences is important for many applications of DNA barcoding; however, species-level identifications can be hampered by many challenges, including the existence of morphologically similar or cryptic species as well as significant undescribed diversity, particularly in tropical regions. The authors present a graduated approach whereby type specimens of varying ages are subjected to different barcoding procedures, including standard DNA barcoding and the sequencing of multiple short, overlapping fragments via HTS. Using these methods, they were able to obtain a high level of success in sequence recovery as well as repeatability of results, including for type specimens a century old. In addition to methods development that will open the door to further such efforts in natural history collections, their library of DNA barcodes for 3000 type specimens will serve as a foundation for a high-quality reference dataset for a family of Lepidoptera, whereby Linnaean names can be confidently associated with specific sequences.

Similarly, Mark et al. (2016) use HTS to generate reference sequences for a challenging taxonomic group, the lichens. Through a series of thoughtful analyses and comparisons, the authors were able to generate convincing reference sequences for lichens while largely overcoming the challenges they encountered arising from sequencing error associated with the 454 platform, intra-individual genetic variability in the fungal barcode region (ITS, internal transcribed spacer), and samples consisting of mixtures of lichen-forming fungal species. Their study generated reference sequences for common lichen species of central Europe, while their overall approach, which may be adapted for other sequencing platforms, can contribute to library generation for lichens globally. Such libraries will contribute to the ability of other researchers to interpret their own data, such as in studies of mixed-species fungal assemblages and in investigations of soil environmental DNA (eDNA) (Xu 2016).

Transitioning from barcodes to biomes

The subtitle for the 6th International Barcode of Life Conference was Barcodes to Biomes. Five papers in the first special issue especially exemplify this theme and the remarkable expansion in the scale of research that has been enabled through DNA barcoding approaches. Three contributions focus upon obtaining a more comprehensive understanding of species diversity in specific geographic regions, while two works focus upon species interactions.

Janzen and Hallwachs (2016) outline a large-scale effort to prepare a complete inventory of the Lepidoptera biodiversity in a hyper-diverse tropical biome: the Area de Conservación Guanacaste in Costa Rica. Involving a large team of collaborators, this project has inventoried more than 11 000 species of Lepidoptera to date. The authors highlight their methodology and explain how DNA barcoding has played a crucial role in enabling this inventory. The project is also making important steps in revealing species interactions through the barcoding of insect parasitoids hatched out of caterpillars. Geiger et al. (2016) outline a large campaign to build a DNA barcode reference library for an entire temperate nation. Phase one involved generating a reference library for about one-third of the plant and animal species residing in Germany, providing a resource for future identifications due to their commitment to specimen deposition in museums and data deposition in online repositories. The authors also share several insights about their practices that would be valuable for other such ambitious national-level campaigns. In the marine realm, Al-Rshaidat et al. (2016) present a significant step forward in elucidating invertebrate species diversity inhabiting reef environments in the Red Sea, using standardized sampling and HTS. A substantial portion of the molecular operational taxonomic units (MOTUs) they detected did not match to sequences in reference databases (75% having no match for those macroinvertebrates over 2 mm and 95% for microinvertebrates). Their results thus reveal a substantial marine biodiversity awaiting further study. Even while most of these MOTUs currently lack Linnaean identifications, the authors' usage of standardized field collection and molecular methods enabled them to compare diversity among sites. The deployment of standardized Autonomous Reef Monitoring Structures (ARMS) by Al-Rshaidat et al. (2016) and other researchers parallels the original call for standardization in molecular markers for DNA barcoding,

thus enabling comparison of results across time, space, and research teams.

Ecosystem functioning depends on species interactions. This fact has emerged as an important contemporary issue in light of pollinator declines. Bell et al. (2016) review methodological progress and prospects for the DNA barcoding and metabarcoding of pollen for diverse applications, including studying plant–pollinator interactions, investigating the origins and safety of honey products, monitoring for allergens, and applying DNA barcoding in forensic palynology. It is clear that standardized genetic approaches can contribute significantly to these important areas of study. The review by Roslin and Majaneva (2016) similarly focuses upon species interactions, but in the context of elucidating entire food webs. With the addition of DNA barcode data, nodes (species units) and links among nodes (species interactions) become better resolved than when using traditional methods alone for identifying specimens and, especially, their gut contents. Among the multiple case studies that they highlight (see references therein), Roslin and Majaneva (2016) review how a focal ecosystem in Greenland—whose plant and animal species have been comprehensively DNA barcoded—has been shown to be more complex and more densely linked than would be concluded with traditional methods. These interesting findings stress the importance of expanding this line of work; applying the same methods elsewhere would permit exploration of the generality of these patterns among taxa and habitat types and to test for potential large-scale trends, such as latitudinal gradients in food web structure.

The generation of large datasets of standardized DNA barcodes for focal habitats and geographic regions is also enabling other lines of enquiry, integrating phylogeography, biogeography, speciation, and molecular evolution (Barreira et al. 2016; Mitterboeck et al. 2016). Barreira et al. (2016) review the multiple avian studies that have gone beyond specimen identification and analyzed the evolution of birds using DNA barcodes. Birds are an excellent model for the study of evolution because their taxonomy and systematics are very well known, and around 60% of bird species have already been DNA barcoded. Analyses performed so far include different taxonomic and geographic scales and also combine the use of cytochrome *c* oxidase subunit I with other genetic loci, morphology, and behaviour to tackle different aspects of avian evolutionary history. While the percentage barcode coverage is much lower for insects, the class is numerically the most-barcoded taxon to date, providing a rich data source for evolutionary study. Mitterboeck et al. (2016) use a sister lineage comparative approach to investigate the rates and patterns of molecular evolution in terrestrial versus freshwater insects. The increasing availability of geo-referenced DNA barcode records through the Barcode of Life Data (BOLD) Systems (Ratnasingham and Hebert 2007) will continue to increase the utility of DNA barcoding for diverse research directions, including macroecology, modeling and prediction of species distributions, and biosurveillance.

Advances in quantification

For diverse questions in biology, it is invaluable to be able to quantify the abundance of organisms, in addition to generating a species list for a given area. However, quantification of organismal abundance via metabarcoding and HTS has remained an underdeveloped area of methodological study. In this special issue, Saitoh et al. (2016) present a novel protocol employing HTS for the quantification of springtail (Collembola) communities, which form an important component of the soil biota. Using natural and artificially constructed test communities, the authors find that reliable quantification is possible under standardized molecular conditions involving the inclusion of a known species as an internal control. This work may open the door for further applications of metabarcoding for ecology and biomonitoring. More generally, this work displays the careful methodological research required for developing quantitative protocols. Finally, as with several works above, the results of this study emphasize that the standardization of methods will be important for comparisons across sites.

The importance of being consistent is also stressed by Clare et al. (2016), who quantify the number of MOTU in the diet of two cohabiting vertebrate species of Mauritius. They found that bioinformatics decisions yielded dramatic differences in MOTU richness, while a metric of dietary overlap between skinks and shrews was relatively consistent across analysis settings. Such results further reinforce the value of consistency when comparing sites and species; this includes bioinformatics pipelines for analyzing metabarcoding data.

Monitoring biodiversity in nature and from field to marketplace

DNA barcodes are increasingly being used by members of the global research community for diverse socioeconomically important applications (Adamowicz 2015*a*, 2015*b*). Across the two-part special issue, 10 contributions represent this major trend, with a focus upon conservation, the detection of invasive species and agricultural pests, and the identification of marketplace products.

Three contributions apply DNA barcoding methods in unique study systems. Beet et al. (2016) use DNA barcoding to characterize the diversity and distributions of springtail species (Collembola) in a region of Antarctica. Their work reveals historical genetic structuring and establishes a baseline for future biomonitoring in this remote region, which may be influenced by climate change and other impacts. Focussing on urbanization, Sing et al. (2016) ask whether city parks can provide valuable habitat for butterflies. They use DNA barcoding to conduct an efficient survey of butterflies in four habitat types within 10 parks of the megacity of Shenzhen in China. They find that urban parks harbour substantial butterfly diversity and that unman-

aged microhabitats are particularly useful for conservation. It would be interesting to expand this line of research to additional taxonomic groups, which may be less dispersive than butterflies, for urban planning and conservation purposes. Thirdly, Williamson et al. (2016) present an enlightening study on the cycads—an evolutionarily unique and highly endangered group of plants—which are being collected from the wild and traded at traditional medicine markets in South Africa. Their study emphasizes the value of DNA barcoding for the identification of organismal fragments and for distinguishing closely related species, which can have different conservation status. Their findings also underscore the utility of well-curated reference sequences for the identification of specimens belonging to taxonomic groups of conservation concern.

Additional special issue contributions focus upon methodological development. Sirianni et al. (2016) outline their unique method termed Closed-Tube Barcoding, which may provide a rapid, affordable alternative to sequencing, especially for projects involving numerous samples. This approach also could be a step towards developing a portable barcoding device, with diverse applications such as monitoring mosquito populations. Researchers are also diversifying the methods available for obtaining source DNA; this is especially advantageous for rare, threatened, invasive, or hard-to-sample organisms. Lee et al. (2016) compare multiple methods of surveying tropical forest mammals in two reserves in Malaysia, revealing that sequencing blowfly-derived mammal DNA using HTS is a promising tool. Applying both metabarcoding via HTS and quantitative PCR (qPCR), Lacoursière-Roussel et al. (2016) develop methods for the detection of amphibians and reptiles using aquatic environmental DNA (eDNA). With these groups being particularly threatened, it is important to be able to study their distributions and population health efficiently. Such methods can also be applied for detecting aquatic invasive species. While there is emerging usage of DNA barcoding and associated methods in the governmental sector (Adamowicz 2015*a*, 2015*b*), Thomas et al. (2016) call for DNA-based identification techniques to be extensively implemented and formally incorporated into federal and provincial policies and laws relating to the detection and management of invasive species in Canada. Studies that calibrate and compare multiple survey methods will be valuable foundations for practitioners seeking to apply the most suitable method for a given context.

Finally, a group of papers in part 2 highlights the utility of DNA barcoding and associated approaches in the agricultural sector. A review by Littlefair and Clare (2016) provides a context for appreciating the utility of DNA barcoding and related methods for protecting the entire food supply chain, from field to table. Xu (2016) highlights DNA barcoding efforts for plant pathogenic fungi that are exotic and under quarantine in various countries and regions. Focusing on arthropod pests, Ashfaq and Hebert (2016) review progress to date in the DNA barcoding of agricultural pests and quarantine species. Moreover, they draw attention to the frequent presence of cryptic species complexes within single Linnaean species names, underscoring the utility of molecular approaches for monitoring species distributions and managing pests. Hodgetts et al. (2016) provide several case studies demonstrating how DNA barcoding methods are being used for plant protection in the United Kingdom. One such case involved the rapid identification of juvenile nematodes, leading to the successful deployment of hygiene methods in the affected agricultural field. Such cases of "real-world" applications of DNA barcoding may be expected to increase.

International collaboration and looking forward

Geary et al. (2016) provide a unique piece on the media coverage of DNA barcoding. Their findings indicate that the media has covered many aspects of DNA barcoding, including scientific discovery, applications for the conservation of biodiversity and the detection of invasive species, and controversies, such as relating to food fraud. However, media coverage tends not to consider certain socially important topics, such as governance structures and international collaboration models, including access and benefits sharing. Despite this, DNA barcoding is an increasingly international endeavour, with numerous countries participating and with multi-country collaboration teams being prevalent in the DNA barcoding community (Adamowicz 2015*a*, 2015*b*; Adamowicz and Steinke 2015). The documenting of biodiversity via DNA barcoding on a global scale is important, for reasons ranging from the study of macroecology to the international management of agricultural pests and invasive species. For the practical purpose of access to molecular infrastructure and for scientific collaboration, DNA barcoding can productively involve the transfer of biological materials across borders. The piece by Geary et al. (2016) may be considered when researchers prepare press releases and for opening thoughtful dialogue about modes of international collaboration.

Together, the two special issues showcase research being conducted on all seven continents and in the oceans. These papers are representative of a diverse and evolving research community that is making strong contributions to scientific discovery, enabling new applications through methodological innovation, and contributing to the protection of natural resources.

Acknowledgements

We sincerely thank Ann McCain Evans and Chris Evans for their generosity in defraying the open-access fees for this special issue of *Genome*. We are also grateful to Editor Melania Cristescu for the invitation to prepare this special issue and for many helpful discussions. We also thank Editorial Assistant Lori Cayer for her significant assistance throughout the preparation of the special issue as well as the many reviewers for sharing their insights.

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