

International Barcode of Life: Focus on big biodiversity in South Africa

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Abstract: Participants in the 7th International Barcode of Life Conference (Kruger National Park, South Africa, 20–24 November 2017) share the latest findings in DNA barcoding research and its increasingly diversified applications. Here, we review prevailing trends synthesized from among 429 invited and contributed abstracts, which are collated in this open-access special issue of *Genome*. Hosted for the first time on the African continent, the 7th Conference places special emphasis on the evolutionary origins, biogeography, and conservation of African flora and fauna. Within Africa and elsewhere, DNA barcoding and related techniques are being increasingly used for wildlife forensics and for the validation of commercial products, such as medicinal plants and seafood species. A striking trend of the conference is the dramatic rise of studies on environmental DNA (eDNA) and on diverse uses of high-throughput sequencing techniques. Emerging techniques in these areas are opening new avenues for environmental biomonitoring, managing species-at-risk and invasive species, and revealing species interaction networks in unprecedented detail. Contributors call for the development of validated community standards for high-throughput sequence data generation and analysis, to enable the full potential of these methods to be realized for understanding and managing biodiversity on a global scale.

Key words: DNA barcoding, research trends, ecology, evolution, pollination, community phylogenetics, biomes, environmental DNA, next-generation sequencing, high-throughput sequencing, genomics, genome skimming, bioinformatics.

Résumé : Les participants à la 7e Conférence internationale sur le codage à barres (Parc national Kruger, Afrique du Sud, 20 au 24 novembre 2017) ont partagé les plus récentes découvertes en recherche portant sur le codage à barres de l'ADN et ses applications de plus en plus diverses. Dans cet article, les auteurs passent en revue les grandes tendances émanant de 429 résumés de contributions sollicitées ou soumises, lesquelles sont colligées au sein de ce numéro spécial de *Génome libre d'accès*. Tenue pour la première fois sur le continent africain, la 7e Conférence a placé une emphase particulière sur les origines évolutives, la biogéographie et la conservation de la flore et de la faune africaine. En Afrique et ailleurs, le codage à barres de l'ADN et autres techniques apparentées sont de plus en plus employées en forensie environnementale et pour valider des produits commerciaux, comme des plantes médicinales et des espèces de fruits de mer. Une tendance marquante de la conférence est la croissance fulgurante des études sur l'ADN environnemental (eDNA) et les divers usages des techniques de séquençage à haut débit. Les plus récentes techniques dans ces domaines ouvrent de nouvelles avenues pour la biosurveillance, la gestion des espèces menacées ou envahissantes, et révèlent des réseaux d'interaction entre espèces avec un niveau de détail sans précédent. Les contributeurs appellent au développement de standards communautaires validés pour la production et l'analyse de données de séquençage à haut débit, afin de réaliser le plein potentiel de ces méthodes en matière d'une meilleure connaissance et gestion de la biodiversité à l'échelle globale. [Traduit par la Rédaction]

Mots-clés : codage à barres de l'ADN, tendances en recherche, écologie, évolution, pollinisation, phylogénétique des communautés, biomes, ADN environnemental, séquençage de prochaine génération, séquençage à grand débit, génomique, écrémage génomique, bioinformatique.

Introduction

The 7th International Barcode of Life Conference is jointly hosted by the African Centre for DNA Barcoding (University of Johannesburg, South Africa) and the Department of Environmental Affairs from 20–24 November 2017. The biennial conference series—previously held at London, UK (2005); Taipei, Taiwan (2007); Mexico City, Mexico (2009); Adelaide, Australia (2011); Kunming, China (2013); and Guelph, Canada (2015)—is thus hosted in Africa

for the first time. With Kruger National Park serving as the conference venue, African biodiversity and African-focused conservation topics naturally take centre stage in both the conference setting and the scientific program. The global nature of this meeting also remains evident, with contributions by researchers working on all seven continents as well as in the world's five oceans.

Here, we synthesize eight salient trends from among the 429 accepted conference abstracts by ~1500 total authors from

Received 10 October 2017. Accepted 10 October 2017.

Corresponding Editor: M.E. Cristescu.

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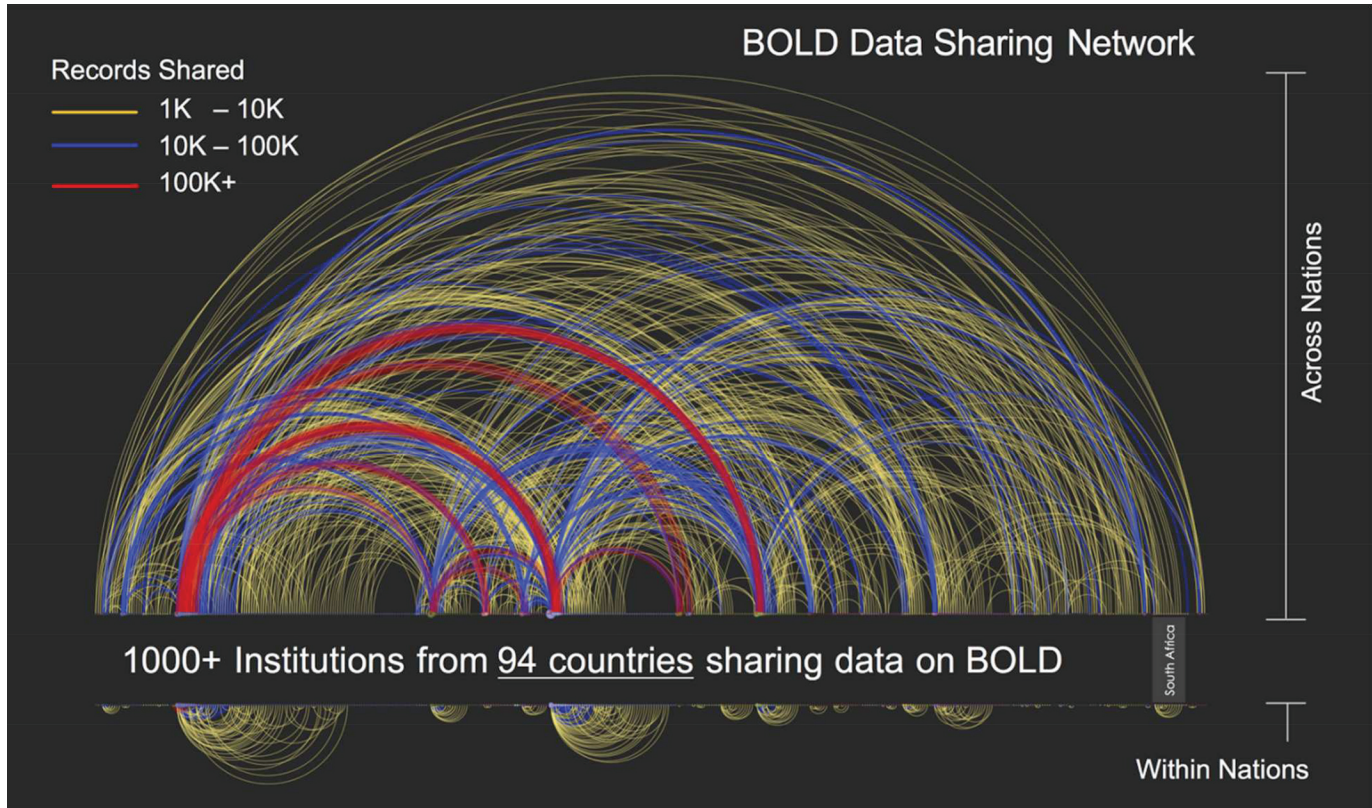
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Fig. 1. Network diagram of data shared between institutions on the Barcode of Life Data (BOLD) System workbench, where at least 1K records are shared between two institutions from August, 2015, with South Africa highlighted. Each line represents the sharing of data between two institutions (yellow, 1K to 10K records; green, 10K to 100K records; red, more than 100K records). The volume and incidence of data sharing across nations greatly exceeds sharing within nations, reflecting collaboration patterns in the International Barcode of Life community.



73 nations. Abstract coauthorship trends mirror international collaboration patterns within this research community (Fig. 1; Adamowicz and Steinke 2015). Key scientific trends at the 7th Conference include a focus on African biodiversity, the increasing usage of DNA barcoding for wildlife forensics and product validation, and a momentous rise in studies of environmental DNA (eDNA). It is apparent that the next generations of sequencing technologies have been adopted, as high-throughput sequencing (HTS) techniques are pervasive throughout the scientific program. Applications of HTS range from recovering reference barcodes from museum specimens, to biomonitoring via mixed-sample metabarcoding and eDNA metabarcoding, and to constructing detailed species interaction networks. We conclude by looking ahead to the next steps for this dynamic international research community.

African flora and fauna: origins and future

The African continent has a special place in the study of human genetic diversity, being the evolutionary cradle of humankind. In addition to recently evolved species such as ours, Africa harbours many deeply-unique lineages of the tree of life, owing to its diverse habitats and climates as well as to its complex geological history of connection and isolation from other continents. As the first conference in this series to be held in Africa, the meeting prominently features studies of the evolutionary history, biodiversity and biogeography, and conservation concerns of the biota of Africa.

The focus upon African biodiversity is apparent from the opening day of the program, with contributions addressing the history of the savanna biome (Bond et al.) and its influence upon African biogeography (Tolley) as well as the macroevolutionary history of

monocot plant diversity in southern Africa (Bouchenak-Khelladi). Additional presentations seek to understand the formation of whole communities of interacting species (e.g., Hardy et al.) as well as the evolutionary origins of diversity. For example, a great hotspot of endemic freshwater biodiversity in Africa, the Great Rift Lake Tanganyika, is the subject of a genomics-based investigation of evolutionary radiation in cichlid fishes (Salzburger).

Other researchers look to the future and to the protection of the unique biota of Africa. For example, Khayota et al., Mwale et al., and Williamson et al. describe how DNA barcoding and associated techniques are being used in the aid of wildlife forensics, for the identification of animal and plant fragments, and in the service of conservation goals. Additionally, DNA barcoding techniques have proven useful in identifying freshwater invasive plant species in South Africa (e.g., Bezeng et al.; Niemann et al.). The overall conference program reveals that DNA barcoding is being increasingly used in regulatory frameworks, such as for the identification of pest species and the investigation of wildlife crime, and it is recognized as a valuable tool for the protection of threatened species of both plants and animals.

Plants: diversification and authentication

For more than a decade, South African researchers have been among the global leaders in the DNA barcoding, molecular systematics, and community phylogenetics of plants (Kyalangalilwa et al. 2013; Charles-Dominique et al. 2016; Daru et al. 2016; Bezeng et al. 2017). This strength is again apparent at the 7th Conference, in which abstracts about plants of Africa feature prominently, alongside other plant-focused contributions from around the globe. Together, the botanical contributions range from reference library building and systematic studies on target taxa (Kabongo et al.; Bester

et al.) to large-scale studies on biogeography (Bello et al., on legumes) and phylogenetic community structure (Hardy et al.; Davies and Carvajal Endara).

The DNA barcoding of plants is based upon multiple genetic markers, generally 2–4 plastid genes (Hollingsworth et al. 2016), while many authors incorporate additional or complementary markers to increase species-level resolution. Therefore, plant barcodes are particularly amenable to phylogenetic analysis, contributing to higher-level systematics (e.g., Kabongo et al.) and to the understanding of macroevolutionary patterns of diversification (Bello et al., on Asteracea). Moreover, comprehensive regional-scale phylogenies enable research into phylogenetic community structure, revealing patterns of species coexistence and community assembly (e.g., Worthy et al.).

Another topic of special interest at the 7th Conference is the authentication of medicinal plant species, and other marketplace products such as seafoods and ground meats (Hanner), using DNA barcoding and related approaches. Viljoen provides an overview of how molecular methods can contribute to realizing the opportunities and mitigating the challenges associated with increasing global use of traditional African medicinal plants. Additional contributions highlight how DNA barcoding is being used to study medicinal plant products traded at markets in Tanzania (Veldman et al.) and South Africa (Lekganayane et al.). Others highlight the importance of genomics and metabarcoding approaches for plant species authentication (Forest et al.; Manzanilla et al.; Raclariu et al.) and for the sequencing of products containing mixtures of species (Xin et al.; Ravikanth et al.; Xu et al.). Yang et al. present a striking case whereby DNA barcoding of ginseng remnants helped to solve a murder case. Conference contributions on plants thus showcase a broad variety of important applications of DNA barcoding and associated molecular approaches in domains ranging from conservation to marketplace surveillance to forensics.

Lepidoptera: a campaign comes of age

Lepidoptera, the moths and butterflies—one of the “big four” orders of insect biodiversity—have featured prominently since the inception of the Barcode of Life endeavour (Hebert et al. 2003a, 2003b). Concerted DNA barcoding efforts are being conducted for nations (e.g., Lavinia et al.; Lees et al.; Ferreira et al.; Hausmann; Rougerie et al.) and even continents (Dinca et al.; D’Ercole et al.). These data are being used for continental-scale investigations of biogeography and comparative phylogeography (Huemer and Hebert; Lait and Hebert). In addition to such geographically-broad studies, several teams of researchers are also conducting specific case studies for elucidating systematics (Talavera et al.), the prevalence of hybridization (Jasso-Martinez et al.), and mechanisms of diversification (e.g., Huemer and Hebert).

While such intriguing investigations of macroecology and, increasingly, larger sections of the genome are going forward, the case of Lepidoptera also may inspire the community to continue to pursue library-building efforts. This intensively barcoded order is now represented by more than 1M public barcode records on the Barcode of Life Data System (BOLD; Ratnasingham and Hebert 2007; accessed 23 August 2017), representing 55K species and 111K Barcode Index Numbers (BIN; Ratnasingham and Hebert 2013). With approximately 180K described species, and many more undoubtedly awaiting discovery and description, the task of building the barcode library remains to be completed even for this most barcoded of taxonomic groups. However, strong species coverage for some geographic regions opens new opportunities for studies of systematics, comparative phylogeography, macroecology, and macroevolution. Increasing global species coverage to a near-complete level would provide a singular resource for scientific discovery.

The cutting edge of DNA barcoding methods

Diverse taxa have been included among the targets for novel methodological developments presented at the conference. For example, Forest et al. argue for the use of genomic approaches for plant species authentication, helping to improve species-level resolution and confidence in DNA-based identifications. In addition to applications of genomics and other multi-marker approaches for plants (e.g., Lammers et al.; Merkel et al.; Manzanilla et al.; Nevill et al.; Suyama et al.), the use of larger segments of the genome is also advocated for Protista (Amaral-Zettler) and for deeper understanding of species boundaries in species complexes of animals (e.g., Lijtmaer et al.; Mutanen et al.; Ivanov et al.). Techniques such as genome skimming (Braukmann et al.; Coissac et al.; Taberlet et al.; Zhou; Zeng et al.), whole plastome sequencing (Chen et al.), and hybrid capture (Hollingsworth) make available larger portions of the genome than traditional barcoding while also being more affordable than whole-genome sequencing. Despite their power, extended DNA barcoding approaches still present researchers with tradeoffs to consider (Coissac et al. 2016). The extent of genome coverage versus scalability across species may be differentially weighted depending upon the specific research goal.

Complementary to those research teams working at the interface of DNA barcoding and genomics, several other abstracts highlight how HTS techniques can contribute to sequencing specimens and products containing degraded DNA, such as confiscated wildlife products. For example, Prosser et al. and Arulandhu et al. describe the development of HTS methods suitable for the identification of highly processed, mixed-species products derived from animal and plant wildlife. As well, HTS is contributing to the generation of reference barcodes from old museum specimens of Lepidoptera (Hausmann) as well as plant specimens housed in herbaria (Zeng et al.). Such techniques will contribute to building high-quality barcode libraries, because type specimens bear special importance for solving taxonomic conundrums and for authoritatively associating formal taxon names with DNA barcodes.

The contribution by Hebert reasserts the continued value of minimalism at the genetic level for the study of global biodiversity. As DNA barcoding using standard sets of genes generally separates most animal species, Hebert advances the position that high-throughput sequencing power can be channeled in the service of understanding global trends in biodiversity. By sequencing small segments of the genome from billions to trillions of specimens—rather than hundreds to thousands as is the current practice in many studies—we can attain a new understanding of the extent of global diversity and the responses of biodiversity to global environmental change.

Big biodiversity and ecological interactions

A shift to analyzing large segments of biodiversity, rather than narrow taxonomic groups, is also apparent in the sessions on biodiversity surveys and ecological interactions. Several conference contributions characterize entire microbiomes and mycobionemes in gut communities (e.g., Castaneda and Verdugo; Chellappan et al.) as well as in soils (e.g., Wall; McGee et al.; Ritter et al.). Others focus upon barcoding large segments of biodiversity, such as regional or national floras and faunas (Bezeng et al.; Davies and Endara; Ekrem et al.). Such reference library-building efforts serve diverse research projects, such as regional-scale ecological investigations, as well as provide a baseline of knowledge for managing biodiversity and identifying specimens of marketplace products. Methodological developments are also presented for applying HTS for conducting standardized biodiversity assessments (Braukmann et al.).

In addition to quantifying biodiversity, metabarcoding approaches are also being used to understand species interactions in

exceptional detail. Pollen metabarcoding (Bell; Fowler et al.; Jones et al.; Lucas et al.; Swenson et al.; Yu) enables researchers to characterize the plant community that is carried by specific pollinator species, a topic of special contemporary concern in light of pollinator declines. HTS is also applied to dietary studies (Clever and Preziosi; Pansu et al.; Shehzad et al.) and for building comprehensive food webs (Clare; Kankaanpää et al.). Recent methodological innovations outlined by Kankaanpää et al. result in substantial cost savings and permit large numbers of insect parasitoid samples to be analyzed, opening new avenues for food web reconstruction.

Evolution: genes to communities

Scaling up is also a theme among the conference contributions focusing on evolutionary biology. At the gene level, studies are including thousands of species to understand the environmental and biological correlates of rates of molecular evolution (e.g., Adamowicz et al.; May et al.; Orton et al.). Kekkonen et al. have combined a large DNA barcode dataset of insects with mitochondrial and nuclear genome sequence data to examine how the transition to haplodiploidy influences the evolution of the genome more broadly. Other contributions outline large programs in comparative phylogeography, permitting researchers to ask whether specific geographical barriers and climatic or oceanographic events have promoted genetic diversification across taxa (Huemer and Hebert; Lait and Hebert; Moura et al.).

DNA barcoding and higher-level molecular systematics are frequently pursued in tandem in plant studies, which typically sequence multi-marker barcodes (see above); in animals, DNA barcoding and molecular phylogenetics are also now being pursued together. Using simulated and empirical datasets of butterflies, Tavalera et al. outline an approach for molecular phylogenetics that combines broad DNA barcoding of species with partial multilocus coverage to yield robust phylogenies. Due to extensive species coverage efforts, DNA barcode campaigns are permitting more complete phylogenies to be built, which are being used for studies of macroevolutionary dynamics and timeframes (Bello et al.; Bond et al.; Bouchenak-Khelladi; Tolley) and to understand the role of evolutionary history in shaping community assembly (Davies and Endara; Roslin et al.).

Environmental DNA and metabarcoding

A dominant trend of this conference is the surge in contributions focused upon sequencing environmental DNA (eDNA), with seven of the parallel sessions of oral presentations focused upon eDNA, together with the metabarcoding of bulk samples. Environmental DNA refers to DNA extracted not directly from organisms (nor from discernible organismal fragments) but from diverse types of environmental samples, such as from freshwaters (e.g., Beentjes et al.; Deiner et al.; Evans et al.; Krol et al.; Kuzmina et al.; Macher and Leese; MacIsaac; Majaneva et al.; Sales et al.), marine waters (Bakker et al.; Lacoursiere-Roussel et al.; Stat et al.; van der Hoorn et al.), soils (e.g., Kirse et al.), lake sediments (Alsos et al.), intertidal sediments (Fais et al.), and from organismal storage media, including ethanol from Malaise-trapped insect samples (Kirse et al.).

The intensification of research in this area reflects the exciting possibilities as well as the diverse sources of demand for these novel methods. For example, regular and affordable biomonitoring of biological communities will enable the early detection of invasive species (Callander et al.; MacIsaac; Trebitz et al. 2017), substantially increasing prospects for the elimination or mitigation of invasive species and their harmful effects. Other areas of interest include the need to gain a better understanding of the distributions of threatened species, using more efficient as well as non-invasive field sampling methods. For example, Farrell et al. investigated eDNA metabarcoding of watering holes as a method

for detecting mammals in Kruger National Park, South Africa, and verified the visitations detected by comparing with camera trapping.

Important validation work remains to be done in this innovative research domain, such as quantifying rates of false positives and the limits of species detection (MacIsaac) under varying field conditions and species attributes. Additionally, comparison of results across sites and research teams would benefit from attention towards developing validated best practices and community standards (Cristescu). We expect that the maturation of this research field will be a compelling area for engagement over the coming years.

Bioinformatics: challenges and opportunities

Together with flourishing research activities on eDNA and metabarcoding, there comes a deluge of data, especially from the use of HTS technologies. Diverse research teams presenting at the 7th Conference have employed varying solutions for managing and analyzing these data, with a particular focus in much of the recent literature upon using custom analysis scripts as well as protocols adapted from the prokaryote molecular biodiversity community, which primarily uses 16S as the target gene. The diversification of custom analytics solutions plays an important role in enabling research novelty. However, the large diversity of decisions that have been made for the generation, filtering, and analysis of sequence data also poses challenges for comparing results across projects, time periods, research teams, and sites, thus reducing the value of a growing repository of datasets.

While a variety of abstracts mention custom bioinformatics approaches, several presenters call for validated analytics solutions for the community (e.g., Cristescu; MacIsaac). Meanwhile, while several contributions specifically draw attention to a new informatics tool. For example, Arulandhu et al. highlight their development of a new bioinformatics pipeline, with a web interface for users, to analyze Illumina MiSeq data for the identification of mixed-species samples, and Chen and Wang present a novel pipeline for mito-genome assembly. Others have focused on tools for effective use of reference libraries with HTS data. For example, Yu et al. discuss the use of PROTAX to increase identification confidence, Porter and Hajibabaei present a naive Bayesian classifier for the identification of animal COI metabarcodes, and Rulik et al. present a pipeline, named TaxCI, to identify taxonomic inconsistencies in reference libraries.

First released a decade ago, BOLD (Ratnasingham and Hebert 2007) is now used as a data repository and as a workbench for analysis and collaboration (Fig. 1) by tens of thousands of users, demonstrating the value of community-focused data repositories and analytical platforms. At the 7th Conference, Ratnasingham presents a new bioinformatics platform, entitled mBRAVE, which will help researchers to enter into the domain of metabarcoding and that will standardize metabarcoding analyses across research groups. This is complemented by the introduction of FENNEC, by Ankenbrand et al., a trait database supporting metabarcoding.

Concluding remarks

The global International Barcode of Life community remains dynamic, regularly adapting technological advances from related disciplines, such as genomics. The usage of HTS techniques continues to expand, in the service of diverse goals from barcode reference library generation to studies of whole communities and interaction networks using eDNA and bulk-sample metabarcoding. At the same time, several previously noted hallmarks of this community remain apparent for the 7th Conference, such as a large diversity of nations being represented (51 among presenting authors, 73 nations among all coauthors) and the continued propensity for collaboration teams to traverse national and continental boundaries (see Adamowicz 2015; Adamowicz and Steinke

2015; Fig. 1). South Africa, the host nation of the 7th Conference, is at the epicentre of one such set of international research networks and is taking a lead role in several research areas of global importance, such as wildlife forensics and plant DNA barcoding. Collaboration across international lines will only increase in importance as we aspire to understand, protect, and sustainably use global biodiversity in the face of environmental change.

Most citations in this article are to abstracts contained within this special issue. Additional references are below.

Acknowledgements

We sincerely thank the following individuals for their help with abstract reviewing and (or) editing: Inger Greve Alsos, Muhammad Ashfaq, Bezeng Simeon Bezeng, James Stephen Boatwright, Alex Borisenko, Thomas Braukmann, Kevin Burgess, Filipe Costa, Pedro Crous, Jonathan Davies, Natasha deVere, Jeremy deWaard, Torbjorn Ekrem, Robert Hanner, Axel Hausmann, Mehrdad Hajibabaei, Paul Hebert, Ian Hogg, Natalia Ivanova, Riana Jacobs-Venter, Ronny Kabongo, Mari Kekkonen, Linda Lait, Eddie Ma, Anthony Magee, Scott Miller, Megan Milton, Muthama Muasya, Marko Mutanen, Monica Mwale, Marko Nieminen, Mikko Pentinsaari, Adriana Radulovici, Ryan Rattray, Rodolphe Rougerie, Dirk Steinke, Pablo Tubaro, Sandi Willows-Munro, Kowiyou Yessoufou, and Evgeny Zakharov. We also greatly appreciate the support of Megan Milton and Chris Ho for managing abstracts and Dean Chan and Catherine Wei for programming of the abstract management system. We thank Ryan Rattray, Dorcas Lekganyane, Sanele Shiba, and Khanyisile Shabangu for conference organization. We also thank Melania Cristescu for helpful comments on this article and Scott Bryant for his work on improving the quality of the special issues. We are grateful to *Genome* for support for the special issues and student prizes.

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