



# Next-generation technologies applied to age-old challenges in Madagascar

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## Abstract

Madagascar is a biodiversity hotspot that is facing rapid rates of deforestation, habitat destruction, and poverty. Urgent action is required to document the status of biodiversity to facilitate efficacious conservation plans. With the recent advent of portable and affordable genetic technologies, it is now possible to take genomic approaches out of the lab and into the field. Mobile genetics labs can produce scientifically reproducible data under field conditions, dramatically minimizing the time between sample collection and data analysis. Here, we show “proof of concept” by deploying miniPCR bio’s miniaturized thermal cycler alongside Oxford Nanopore’s MinION DNA sequencer in Madagascar. Specifically, we deployed this technology at Anjajavy, northwestern Madagascar for rapid biodiversity assessment. We successfully extracted mouse lemur DNA, amplified and sequenced a phylogenetically informative mitochondrial gene (cytochrome-b; *cytb*), and thereby confirmed the presence of Danfoss’ mouse lemur (*M. danfossi*) within the Anjajavy Reserve. We show that a mobile genetics lab can provide expeditious results, and allow scientists to conduct genetic analyses, potentially allowing for rapid interventions under emergency conditions in situ. Additionally, mobile labs offer powerful training opportunities for in-country scientists for whom training opportunities were previously confined to ex-situ locations. By bringing genomic technologies to Madagascar and other economically challenged and biodiverse regions of the world, the next generation of scientists and conservationists can more fully implement their leadership roles. Local laboratory and training facilities are changing the polarity of research programs in Madagascar and empowering national researchers to take charge of environmental stewardship.

**Keywords** Anjajavy · Field genomics · MinION · miniPCR · Mouse lemurs · Nanopore sequencer

## Introduction

Madagascar, one of Earth’s most threatened biodiversity hotspots, is challenged to preserve its endemic wildlife in the face of rampant habitat loss and degradation, extreme poverty, and one of the fastest population growth rates worldwide (Gardner et al. 2018; UNFPA 2018). There is an increasing sense of urgency to accurately assess the island’s true species richness and region-specific conservation risks and to translate these data into policy action. Somewhat paradoxically, the quantification of biodiversity levels in Madagascar continues to rise, despite high deforestation rates. This is largely due to new biological surveys targeting poorly known areas, the integration of genetic analyses with more traditional morphological assessments, and DNA barcoding approaches used in species identification. It is becoming typical that as new species are formally described in the literature, they are immediately classified as a ‘threatened’

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category under International Union for Conservation of Nature (IUCN) regulations due to restricted distributions and highly fragmented habitats.

Despite economic hardship, Madagascar is revamping its academic and research programs. Since the seventeenth century, Madagascar has been a land of fascination for foreign explorers and naturalists (Anderson 2018; de Flacourt 1658). The first detailed and illustrated accounts of Madagascar's biodiversity were variably undertaken by foreign commercial envoys (primarily from Europe), amateur naturalists, and missionaries alike. These works were predominantly written in French and were inaccessible to the Malagasy people, with some exceptions (Anderson 2018; Andriamialisoa and Langrand 2003).

At the end of the XIX century (1896), Madagascar was officially annexed as a French colony. One of the first colonial Institutions was the Académie Malgache, aimed at merging the efforts of French, Malagasy and other international researchers (Andriamialisoa and Langrand 2003). In practice, however, Malagasy people did not have many opportunities for formal training. Even well into the twentieth century, most scientific “discoveries” on the island were conducted by non-Malagasy researchers collecting specimens in situ, who then analyzed the resulting data and published abroad.

National academic programs were only begun in 1961, a year after Madagascar's independence from France, with the establishment of the University of Antananarivo, the major academic institution in the country (Hayward and Rasoanampoizina 2015). The academic program, however, was modeled after the French system, which continued to permeate formal educational Institutions. In the 1980s, nature conservation institutions (e.g., World Wildlife Fund for Nature, Conservation International and Wildlife Conservancy Society) began to play an important role in research and education programs aimed at training national scientists.

Beginning in 2007, a renewed interest in addressing the education crisis led the Malagasy government to implement changes, including the transition from a French system to an Anglophone model (Hayward 2008). Today, there are at least five regional academic centers and more than a dozen private higher education institutions across the country. That a new generation of Malagasy professionals is emerging to take leadership and enter the competitive world of academia is undisputable, though progress has continued to depend on commitment from external funding and international assistance (Hayward 2008). That assistance, though very well intended, often means that Malagasy students conduct field work and sample collection, but do not participate in data generation and analysis, or play major roles in developing scientific publications or grant writing.

Madagascar is now at a scientific, academic, and technological crossroads: Malagasy researchers and professors

are establishing labs and/or developing competitive research programs in-country, at a time when new DNA sequencing technologies are revolutionizing the fields of conservation genetics and genomics, and expanding applications worldwide through the creation of miniaturized devices. These new technological products are relatively affordable and user friendly, and have the potential to sequence even whole genomes in real time (Tyler et al. 2018). At the forefront of these developments are devices released from Oxford Nanopore Technologies (ONT) and miniPCR bio. These companies have created the MinION (ONT; a portable nanopore-based DNA sequencer platform) and the miniPCR (a miniaturized thermal cycler). These technologies have already been tested under field conditions in a variety of projects around the world (e.g. Johnson et al. 2017; Pomerantz et al. 2018; Quick et al. 2016), and even in outer space (Castro-Wallace et al. 2017). As these tools become increasingly available, there is also an increasing need for operational workflows that streamline analyses and reduce laboratory costs, while facilitating surveys at remote sites and producing expedited and reliable results (Maestri et al. 2019). These technologies are proving to be excellent platforms for engaging in-country students and scientists in the fast-moving area of field genetics and genomics (Watsa et al. 2019, 2020).

In the present article, we provide two case studies to illustrate the power of mobile-technology deployment in Madagascar. In the first case, “bringing the lab to the field” we demonstrate that a fully equipped “mobile laboratory” can provide rapid and accurate species assignment of endemic wildlife under various field conditions. In the second case, “bringing the students to the lab”, we demonstrate the potential for mobile technology to provide hands-on learning experiences for Malagasy students across educational stages and academic institutions. We hope these case studies spark further discussion regarding the respective roles for, and benefits gained by, international collaborations and in-country genetics research in Madagascar.

### Case 1: bringing the lab to the field

Madagascar's iconic lemurs have undergone significant taxonomic revision since the advent of genetic sequencing. As “cryptic” species, mouse lemurs (*Microcebus*) have undergone one of the most dramatic taxonomic expansions within the lemuriform primates, and the number of recognized species has increased from a few to 24 in the last decades (Hotaling et al. 2016). Though there has been tentative evidence of species hybridization occurring in sympatry (Hapke et al. 2011), there are additional sites where two or more species occur while maintaining lineage specificity (e.g., Poelstra et al. 2020; Rakotondranary and Ganzhorn

2011). For species diagnostics, researchers have relied primarily on a handful of mitochondrial marker genes, most notably, cytochrome-b (cytb) (Yoder et al. 2016). Amplification protocols for cytb are well established for these primates and online repositories contain hundreds of consensus sequences. When generated, sequences from new subjects can thus provide rapid and preliminary species assignments, rendering this gene a prime candidate for diagnostic in situ DNA barcoding via mobile technology.

We tested this possibility by deploying a fully equipped mobile laboratory to provide the first genetic species confirmation of the mouse lemurs in the Anjajavy forest. This site was an ideal test site, because it has reliable electricity and an unusually high density of mouse lemurs awaiting genetic confirmation.

### Lab implementation in Madagascar

Our mobile genetics lab included the equipment, reagents and supplies needed to process samples from DNA extraction to sequencing (Supporting Information), and the computational power and bioinformatics software to perform preliminary species assignments. In particular, we collated a cytb reference library containing over 300 consensus sequences from online repositories and from in-house sequences generated in the lab of ADY. We tested all lab components at Duke University, North Carolina and upon initial arrival in Madagascar in May, 2018 (Table 1).

In early July 2018, MBB and LKG deployed the lab at the Anjajavy Lodge and Reserve in northwestern

Madagascar. Anjajavy (S14.99025 E47.22958) encompasses ~ 1200 ha of private Reserve, and ~ 8000 ha of newly protected area, predominantly dry deciduous forest. We conducted our field mission between July 9 and 29, 2018. We first captured and obtained tissue samples from 12 mouse lemurs, using previously described methods (Blanco et al. 2017). Secondly, we extracted all tissue samples and chose a high-quality extraction to sequence. The process from DNA extraction to sequencing took ~ 8 h and was partitioned over two days (Supporting Information).

Following deployment at Anjajavy, we conducted a third test of the mobile lab in the capital city of Antananarivo in August, 2018, using DNA collected and extracted the previous month at Anjajavy. This test served to demonstrate that (1) our methods produced reliable and repeatable results across conditions; and (2) future projects could successfully perform sample extractions in the field, followed by more temperature-sensitive sequencing and computational analyses at a centralized location.

In total, we successfully sequenced the cytb marker gene from four mouse lemurs using the mobile genetics lab in three locations across Madagascar. Two mouse lemur samples were sequenced in Sambava, one sample was sequenced in situ at the Anjajavy field site, and one sample was sequenced in Antananarivo (Fig. 1, Table 1). Cytb sequences from all mouse lemur samples grouped with those derived from verified populations of *Microcebus danfossi*: We thus genetically confirmed the presence of this species at Anjajavy (Fig. 2). Consensus sequences generated in this study were stored in GenBank under accession numbers MT765748-MT765751.

**Table 1** Procedures by location, describing conditions, sequenced samples (S.S.), major challenges and broader impact activities (B.I.)

| Location and species  | Conditions                 | Procedures   | # S.S. | Challenges   | B.I.                                      |
|---|----------------------------|--|--------|--|---|
| Duke University & Duke Lemur Center, NC, USA<br><i>M. murinus</i> | Standard lab               | Protocol adaptation to mobile lab: primer selection, troubleshooting wet lab procedures, collate reference library, test all equipment | 1      | Unable to simulate field conditions  | PAL trained LKG and MBB                   |
| SAVA region, NE Madagascar<br><i>M. danfossi</i> <sup>a</sup>     | Hot, humid<br>Office space | Protocol adaptation to local conditions: optimize PCR, test all equipment following international travel                               | 2      | Keeping reagents cold and MinION cool enough to run  | LKG and MBB trained LA; workshop at CURSA |
| Anjajavy Reserve, NW Madagascar<br><i>M. danfossi</i>             | Hot, dry<br>Field location | Sequenced field collected sample using optimized protocols   | 1      | No internet for online base calling, keeping MinION cool enough to run, flow cell lifespan | N/a                                       |
| Antananarivo, capital Madagascar<br><i>M. danfossi</i>            | Cool office space          | Sequenced field collected and field extracted sample using optimized protocols   | 1      | N/a  | Workshop at Vahatra                       |

<sup>a</sup>Sample collected at Anjajavy during prior research mission

**Fig. 1** Locations for the use of the genetics lab. 1. Sambava: **a** Loading gel in the DLC/SAVA office, **b** Loading MinION prior to sequencing; 2. Anjajavy: **a** deciduous forest, **b** lab setting near forest, **c** Danfoss' mouse lemur; 3. Antananarivo: lab setting at Vahatra office

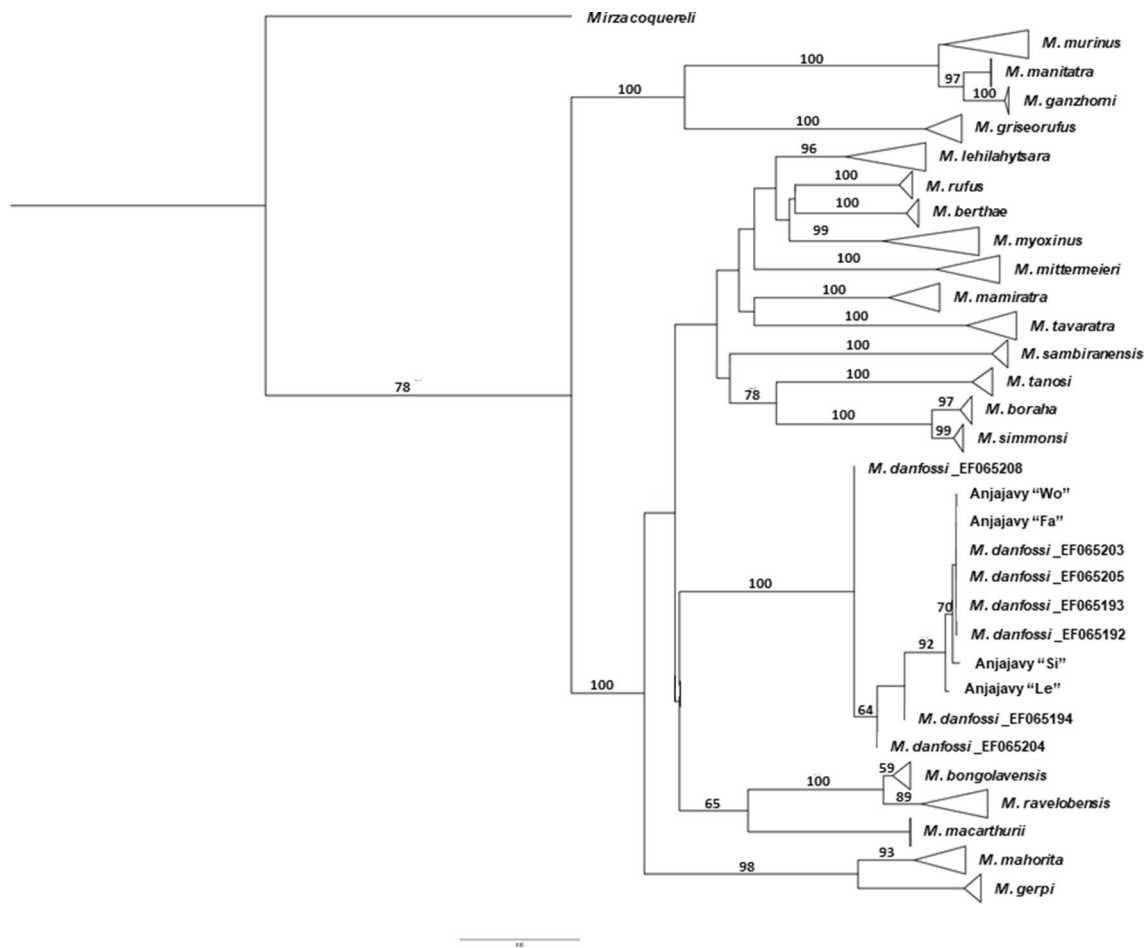


## Case study 2: bringing the students to the lab

Though *ex situ* training opportunities have allowed for a transfer of knowledge from academic institutions in wealthy countries over the past several decades, the absence of reliable and accessible laboratory and research facilities has severely constrained opportunities for Malagasy students in the natural sciences to learn molecular methods critical to applied conservation research. Mobile labs offer a powerful mechanism for changing this dynamic. They can be built and deployed to fulfill pedagogical and research goals, from reaching a handful of students coming to the field, to training many students in large classrooms (Watsa et al. 2020). Over 2 years, members of our team organized a suite of workshops and hands-on learning experiences for Malagasy students.

Building off the deployment of the mobile lab, we hosted two-day workshops in collaboration with academic

institutions in Madagascar. Our first workshop was conducted by MBB, LKG, and LA in June 2018, at the Centre Universitaire Régional de la SAVA (CURSA) in the town of Antalaha, a local branch of the University of Antsirana in northeastern Madagascar. A total of 66 students enrolled in the Environmental Sciences program attended this workshop, which was conducted in Malagasy. During the workshop, the students actively participated in hands-on molecular techniques such as pipetting, melting and setting the agarose gel and loading samples using the Bluegel DNA electrophoresis kit. Our second workshop was conducted by MBB and LKG in August 2018 at the Vahatra Association office, in Antananarivo (Fig. 3). Vahatra is the leading Malagasy NGO promoting capacity building and scientific research in Madagascar whose director, Achille Raselimanana, has been a long-term collaborator of ADY (e.g., Yoder et al. 2005; Raselimanana et al. 2009; Chan et al. 2012). With logistical assistance from Vahatra staff, we recruited university students and researchers interested in genetics. A



**Fig. 2** *Microcebus* phylogeny based on cytb sequences. Branches were collapsed for practical purposes, except for the *M. danfossi* lineage, to highlight consensus sequences from Anjajavy; node support

above 50% is shown. GenBank accession numbers for Anjajavy samples are listed in the main text, those for published sequences of Danfoss’ mouse lemurs are listed next to the species name

total of 25 students (the maximum room capacity) attended the workshop, which was conducted in English. Workshop participants engaged in all lab activities as well as discussions about potential implementation of this technology in a variety of research topics. During the workshop at Vahatra Association, we sequenced a mouse lemur sample from our recent field mission at Anjajavy.

Going forward, we anticipate increased integration with emerging scientific infrastructure in Madagascar. Long-term research development and capacity building in-country require permanent, year-round functional facilities. Two of us (FR and ET) founded Mahaliana Labs, a research and training center focusing on molecular biology based in Antananarivo, Madagascar. Mahaliana Labs offers mobile technology, as well as more sophisticated wet-lab capabilities, rendering it a unique place to integrate field work and diagnostics. At Mahaliana, we aim to provide local students and scientists with the training, guidance and tools they need to ask their own questions and lead conservation

science research in Madagascar. In its first year of existence, Mahaliana Labs organized training workshops in which 50 Malagasy students were familiarized with the molecular techniques commonly used in laboratories such as DNA extraction, PCR, DNA sequencing and an introduction to bioinformatics.

The majority of workshop participants stated that lack of access to laboratory equipment and hands-on training in basic molecular techniques were the biggest barriers to incorporating such techniques in their research. Attendees came from different backgrounds, ranging from graduate and undergraduate students in biology, veterinary medicine, as well as scientists from conservation-oriented, non-governmental organizations who wish to incorporate or explore the potential for using these techniques into their research projects. For example, using techniques learned at these workshops, a veterinary student is studying the prevalence of tick-borne parasites in dogs and cats from villages neighboring protected areas and is assessing the risks of disease

**Fig. 3** Workshops in Madagascar: **a, b** at CURSA in Antalaha; **c, d** at Vahatra office in Antananarivo; **e, f** at Mahaliana Labs in Antananarivo



spillover from introduced to native species. Another participant is focusing on the ecological integrity of a river basin and its benthic macroinvertebrates community and, following the workshop, will explore the use of eDNA techniques to complement her taxonomic identifications. Finally, science educators, also workshop participants, are planning to include the basics of molecular techniques in their teachings at the high-school level.

In addition to the formal trainings, a select group of biology and veterinary students are mentored throughout their various research projects by Mahaliana Labs staff and collaborators to produce scientific publications that are Malagasy-led, bridging the gap between western and Malagasy scientific contributions (Waeber et al. 2016). By providing hands-on experience coupled with the necessary theoretical information, we hope to demystify the methodology sections of current scientific publications that seem inaccessible to many local students.

## Discussion

Portable and affordable technologies in the field of genetics have made it feasible for researchers across disciplines to produce scientifically robust data under field conditions. We confirmed species assignment for one of the lemurs at Anjajavy within a week, from animal capture to tissue collection, to phylogenetic analysis. Our results are consistent with the known distribution of *M. danfossi*, between the Sofia and Maevarano rivers, NW Madagascar (Olivieri et al. 2007). Anjajavy's mouse lemurs had been tentatively assigned as *M. danfossi* on the basis of morphological assessments (Randrianambinina et al. 2010), but we provide the first genetic confirmation of Danfoss' mouse lemurs at this site.

Implementation of the mobile lab in Madagascar, however, did not happen without challenges. One of the major logistical hurdles was keeping reagents and flow cells cool while travelling between sites, as the ice packs quickly melted during long hours under hot conditions. Moreover, a lack of internet access restricted our sequencing capabilities in the field, as well as our ability to check for online protocols. Financial considerations are also important for

those interested in using a mobile genetics lab. The major components of the lab, e.g., equipment, including computer and associated software, and the sequencer kit, cost around USD 15,000 in 2018. Though the equipment can be re-used, reagents and flow cells need to be purchased primarily on a project-by-project basis and are costly (e.g., R10 flow cell unit USD ~900). However, recent upgrades to the ONT MinION system (e.g., Flongle flow cell USD ~80 ea) and expanded molecular barcoding options are reducing nanopore sequencing costs. These supplies are also problematic to transport across international borders: most companies do not directly ship to biodiversity hotspots, like Madagascar, and researchers generally rely on a network of volunteers to hand carry temperature-sensitive chemicals and other supplies.

Despite these logistical obstacles, we showed “proof of concept” that this technology can be deployed in remote sites, that results can be obtained in a timely manner, and that training sessions can prepare advanced students with the skills and means to conduct genetic analysis *in situ*. There is an exciting and growing literature on the use of mobile genetics technology in biodiversity hotspots (e.g., Krehenwinkel et al. 2019; Pomerantz et al. 2018), showing a promising- but still unfolding- path for national researchers and management workforce to benefit from these advanced technologies. This is in stark contrast with a long-standing practice of “helicopter science” where foreign researchers collect and export samples with little participation of nationals in data gathering or analysis (Olufadewa et al. 2020). More affordable technologies and training opportunities have the potential to contribute towards a more widespread “democratization” of science and biodiversity.

Biodiversity hotspots are natural targets for the implementation of advanced genetics technology to address urgent conservation concerns in real time. Conservation crises range from the need to perform rapid biodiversity assessments in threatened habitats, to dealing with wildlife die-off situations or disease-related cases that require immediate intervention (e.g., Carver 2018). Moreover, given the undeniable reality that habitats—and thus entire populations of plants and animals—are under immediate threat of local extirpation, we have a limited timeframe within which to document their genomic diversity. It is therefore critically important to establish protocols for generating reference genomes for as many species as possible, from which point whole-genome resequencing (WGR) methods can be applied at the population level. A WGR approach to characterizing population genomics can be applied on the ground and in real time, thus allowing insights into mechanisms of speciation, signatures of selection and local adaptation, and other fundamental aspects of the evolutionary process (Fuentes-Pardo and Ruzzante 2017). However, the extent to which the power of these technologies can be realized will depend on

bridging the “conservation genetics gap” (Britt et al. 2018; Hoban et al. 2013; Hogg et al. 2018; Taylor et al. 2017), i.e., the concern that technological and scientific findings are not easily translatable to management and policy action. Whether the disconnect between science and action plans stems from a lack of funding, a lack of communication, or other gaps, scientists and non-academic conservationists must find common ground to intervene in long-standing and arising environmental problems.

Although international collaborations continue to play a critical role in providing financial and academic support to research enterprises in low income countries, a shift towards national leadership is warranted, one that may bring different perspectives for approaching and coping with the conservation dilemmas of today. In this context, miniaturized and more affordable technologies have the potential to help solve environmental crises at remote settings, while also shaping the careers of scientists for whom professional advancement is thwarted by economic challenges and resource limitation.

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**Data availability** Relevant data are within the manuscript and its supplementary materials file.

## Compliance with ethical standards

**Conflicts of interest** The authors declared that no conflict of interest or competing interest exist.

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