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PRACTICE AND POLICY

# Using miniaturized laboratory equipment and DNA barcoding to improve conservation genetics training and identify illegally traded species

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## Abstract

Illegal wildlife trade (IWT) is one of the largest global illegal activities, and it negatively affects biodiversity and sustainable development worldwide. DNA barcoding coupled with high-throughput sequencing (i.e., metabarcoding) is useful in identifying taxa affected by IWT and has been used routinely for decades. However, for countries lacking laboratory infrastructure, sequencing units, and trained staff, the application of DNA barcoding tools in conservation is limited and depends on slow sample transport processes and molecular analyses carried out abroad. Guinea-Bissau, on the West African coast, has one of the lowest human development indices in the world and is a biodiversity hotspot threatened by IWT. We explored the potential use of inexpensive and portable miniaturized laboratory equipment (MLE) and DNA barcoding tools to improve training in conservation genetics and identification of traded species. We tested these technologies in tissue samples collected at different times and contexts in Guinea-Bissau and used 3 primer pairs amplifying mitochondrial DNA fragments. We successfully identified 33 tissue samples to the species level; thus, MLE may accelerate the use of DNA and metabarcoding methods in countries that have low research funding and limited infrastructure. The use of these technologies has the potential to advance the discipline of conservation genetics in Guinea-Bissau and other countries and to train students and employees of government agencies dedicated to investigating environmental crimes.

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## INTRODUCTION

The illegal wildlife trade (IWT) involves smuggling, poaching, capture, collection, and sale of endangered species or their parts or derivatives (South & Wyatt, 2011). Taxa are sold alive or dead, whole or processed, and the resulting products or specimens may be used for pharmaceutical, food, pets, ornamental, or traditional medicinal purposes (Nellemann et al., 2014; Stringham et al., 2021). IWT may lead to overexploitation of natural resources, which is a primary driver of biodiversity loss (Maxwell et al., 2016), and affect society in direct and indirect ways (Mozer & Prost, 2023). More than 10 years ago, illegal global trade was dominated by narcotics, human trafficking, counterfeit products, and wildlife and wildlife products (Rosen & Smith, 2010), and its annual value has likely increased substantially since then (Nellemann et al., 2016). Importantly, IWT often affects low-income countries with biodiversity hotspots (UNODC, 2016).

Actions to tackle IWT (Nellemann et al., 2014) are based on a good understanding of the phenomenon at national and international extents, which requires accurate identification of species. DNA barcoding tools have been widely and effectively used to identify taxa affected by IWT (Staats et al., 2016) and are particularly important in cases of impaired visual identification, such as with processed specimens (e.g., Eaton et al., 2010; Gaubert et al., 2015; Minhós, Wallace et al., 2013; Olayemi et al., 2011). These techniques require the amplification by polymerase chain reaction (PCR) of short (e.g., <200–600 base pairs [bp]) and taxonomically informative DNA sequences, which typically are regions of mitochondrial DNA (mtDNA) (Gaubert et al., 2015; Hebert et al., 2003). Genetic information obtained from a specimen is compared with vouchers deposited in reference databases (e.g., Benson et al., 2013; Gaubert et al., 2015; Ratnasingham & Hebert, 2007) to obtain a molecular identification. High-throughput sequencing (HTS) technologies have been used in IWT contexts (Staats et al., 2016) and allow multiple DNA barcode templates and samples to be sequenced in parallel.

Despite the existing technologies and a recognized need to integrate molecular tools to inform conservation actions, there is a mismatch between the availability of genetic tools (and data) and their application in conservation and incorporation in policy (Bertola et al., 2024; Garner et al., 2016; Holderegger et al., 2020; Klütsch & Laikre, 2021). This mismatch could be due to a lack of knowledge on how to initiate, apply, or use these tools or difficulty in interpreting results (Holderegger et al., 2020); prohibitive costs; unavailability of laboratory facilities with HTS equipment, high-performance computing clusters, and university-trained technicians able to perform the necessary procedures (Klütsch & Laikre, 2021; e.g., in sub-Saharan Africa: Helmy et al., 2016); and delays in transportation of sample to countries equipped to conduct the analyses due to international regulations (e.g., Convention on International Trade in Endangered Species [CITES]) or difficulties in obtaining permits to carry out genetic sampling by foreign teams (e.g., associated with the Nagoya Protocol on Access and Benefit Sharing [https://www.cbd.int/abs/]) (Bertola et al., 2024).

The use of miniaturized laboratory equipment (MLE) in DNA barcoding could resolve some of these challenges. Such equipment (benchtop centrifuges, thermocyclers, gel electrophoresis systems, and portable sequencing devices, such as the MinION) is portable and inexpensive and is used for DNA extraction, PCR, and sequencing (e.g., Pomerantz et al., 2022). DNA and metabarcoding (DNA barcoding of mixed, community, or environmental samples) protocols in which MLE is used have been developed specifically for nonstandard contexts, including ones in which electricity and internet access are inconsistent, personnel have little experience in bioinformatic methods, and equipment is limited to personal laptops (Pomerantz et al., 2018, 2022; Srivathsan et al., 2018). Pomerantz et al. (2018) obtained highly accurate consensus sequences that allowed the identification of species, including rare ones, 24 h after collection of samples in the Ecuadorian Chocó rainforest.

A key piece of equipment for complete processing of DNA barcode data in situ is the MinION, an inexpensive sequencing platform from Oxford Nanopore Technologies (ONT). Nanopore-based technology allows the sequencer to be very small (10 × 3.2 × 2 cm and 90 g) compared with other HTS platforms (https://nanoporetech.com/). It works reliably for in situ molecular analyses (e.g., Blanco et al., 2020; Menegon et al., 2017; Pomerantz et al., 2018; Srivathsan et al., 2021), for education (e.g., Salazar et al., 2020; Watsa et al., 2020), and for medical applications (e.g., Quick et al., 2017). The use of ONT's MinION sequencing platform coupled with DNA barcoding analysis software (NGSpeciesID, Sahlin et al., 2021) produced reliable genetic data admissible in court (wildlife forensic application) (Vasijevic et al., 2021; reviewed in Ogden et al. [2021]).

Guinea-Bissau on the West African coast (Figure 1) (36,125 km$^2$, population 2.08 million in 2022 [CIA, 2024; https://stat-guinebissau.com/]) is an important biodiversity hotspot and a regional stronghold for iconic and threatened species (Bersacola et al., 2018; Brugiére et al., 2005, 2006; Ferreira da Silva et al. 2025; Palma et al., 2023) (Figure 1). Nine protected areas, covering almost 26.3% of the country's area, were formally designated to manage the conservation of biodiversity (Figure 1) (https://ibapgbissau.org/areas-protegidas/). Coastal and marine ecosystems of the Bijagós Archipelago were nominated as a UNESCO World Heritage site in July 2025 (https://whc.unesco.org/en/list/1431/).

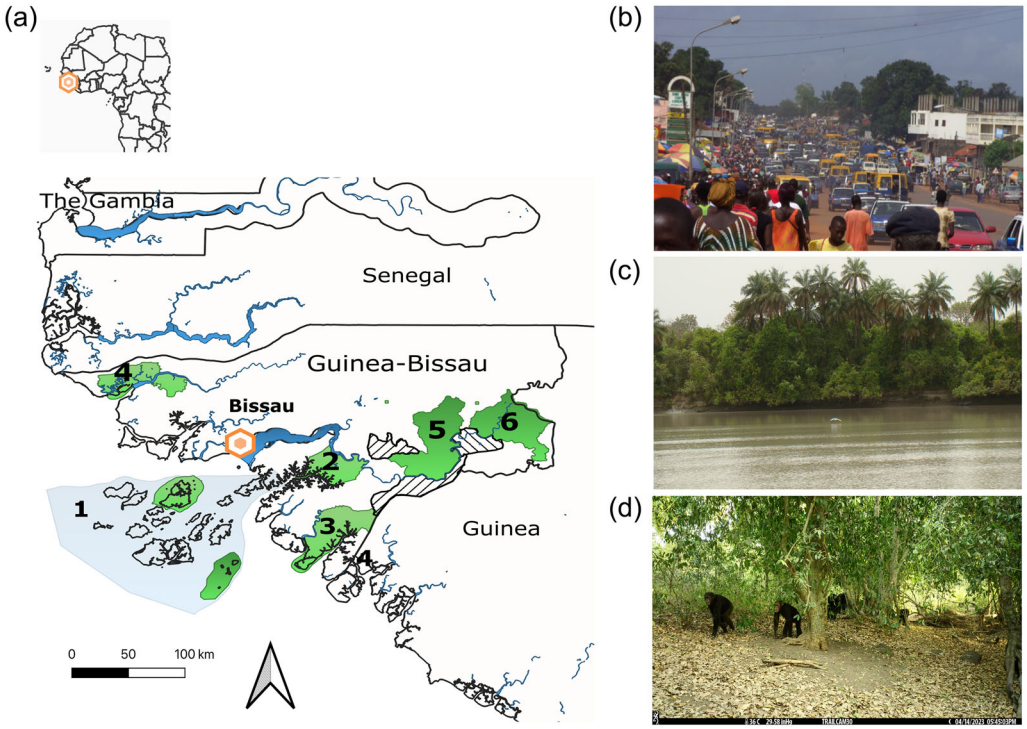


**FIGURE 1** (a) Location of Guinea-Bissau on the West African coast (hexagon, Bissau, the capital city; 1, Bolama Bijagós Archipelago Biosfere Reserve; 2, Cacheu River Mangroves Natural Park; 3, Cufada Lagoons Natural Park; 4, Cantanhez National Park; 5, Dulombi National Park; 6, Boé National Park; hashing, ecological corridors [https://ibapgbissau.org/areas-protegidas/]), (b) Bissau (photo by T.M.), (c) Corubal River, the main waterbody of the country (photo by M.J.F.S.), and (d) regional stronghold for the western chimpanzee (camera trap photo, supplied by Luis Palma, Research Center in Biodiversity and Genetic Resources).

Guinea-Bissau is a low-income country, where more than 60% of the population lives below the poverty line, and there is a high level of inequality in income distribution (CIA, 2024; World Bank, 2020). The stagnation of the national economy is related to an acute shortage of skilled workers due to a poor educational system (World Bank, 2020) and high political instability (Sangreman, 2019).

Wildlife in Guinea-Bissau is threatened by commercial hunting. Medium to large mammals are hunted for trade in meat, skins, and body parts and for the national and international pet trade (Ferreira da Silva & Regalla, 2025; Ferreira da Silva, Minhós, et al., 2021; Minhós, Wallace, et al., 2013; Sá et al., 2012) (Figure 2). There is little information on targeted species, but DNA barcoding has been used to determine the species being traded (e.g., Minhós, Wallace, et al., 2013). The results from these studies suggest that primate species are commercially traded, including species classified as threatened by the International Union for Conservation of Nature (e.g., the western red colobus monkey [*Piliocolobus badius temminckii*] and the king colobus [*Colobus polykomos*]) (Minhós, Wallace, et al., 2013).

The molecular identification of tissue samples collected in IWT contexts in Guinea-Bissau was done abroad because the country lacks molecular laboratory infrastructures dedicated to conservation genetic applications. This is a common challenge among West African countries (Helmy et al., 2016; Kanteh et al., 2022). Moving biological samples abroad for molecular identification has 2 important drawbacks: exportation permits related to CITES issued by national focal authorities are slow and have delayed the production of data and understanding of the IWT phenomena, and staff of agencies responsible for law enforcement and conservation (i.e., Instituto para a Biodiversidade e Áreas Protegidas, IBAP) are not trained in genetic or genomic technologies (Ferreira da Silva & Regalla, 2025) and thus miss important learning opportunities offered by these studies.

We aimed to test the use of MLE and ONT's MinION sequencer and a published bioinformatic protocol (Pomerantz et al., 2022; Sahlin et al., 2021) in the molecular identification of samples collected from traded carcasses in Guinea-Bissau. We also considered and explored its potential for local capacity building and training. We analyzed animal tissue samples collected across different periods and contexts, representing a range of situations encountered by researchers investigating IWT and DNA preservation conditions. The samples were obtained from 2010 to 2022 from partly smoked carcasses and pieces of uncooked meat at urban wild meat markets, in bars and restaurants, and along the trade route from hunting sites to final consumers. Our overarching aims were to raise awareness of the gap between the need and inability to apply conservation genetics technologies in Guinea-Bissau and possibly in other countries lacking sufficient research funding, adequate laboratory infrastructures, and sequencing units and to propose MLE as a way to accelerate the use of DNA barcoding-based technologies in efforts to establish conservation genetics as a


**FIGURE 2** In Guinea-Bissau, West Africa, (a) wild meat market in Bissau, the capital city (photo by Dawn Starin, 2010), (b) heads of Guinea baboons being prepared for consumption (photo by T.C.), (c) meal containing primate meat served in a restaurant in Guinea-Bissau (photo by T.C.), (d) animal body parts, including leopard skins and parts of western chimpanzee and Guinea baboon for sale in Bandim market, the largest generalist market in Bissau (photo by R.S.), and (e) chimpanzee living as a pet before it was confiscated by local authorities (photo by Lara Espirito Santo).

discipline in countries with emerging economies and to improve understanding of IWT by local organizations.

## METHODS

### Study area and sample collection

We used 33 tissue samples collected as part of other studies that investigated hunting, trade, and consumption of wildlife in several locations (Figure 2). Here, we analyzed 11 samples collected in Bissau in 2010 in 2 meat markets for which the species identity was lost, 12 samples collected as part of a 2015–2017 study in which the consumption of wild meat at dedicated restaurants was monitored, and 10 tissue samples collected in 2022 from an island of the Bijagós Archipelago.

Tissue samples were placed in 2-mL Eppendorf tubes containing 98% ethanol, and these tubes were placed in prelabeled zip-lock plastic bags. We collected tissue from unburned areas of smoked carcasses at urban markets and from uncooked pieces of meat at bars and restaurants (Figure 2).

### DNA extraction, amplification, and sequencing

We extracted DNA with ThermoFisher's MagMAX DNA Multi-Sample Ultra 2.0 Kit on the ThermoFisher Kingfisher Apex DNA extraction platform; default parameters were

used. For the PCR amplification, we used primers designed by Gaubert et al. (2015) for the identification of bushmeat samples that amplify the first 402 bp of *cyt b* (primer sequences: GVL14724 50 GATATGAAAAACCATCGTTG and H15149 CTCAGAATGATATTTGTCCTCA) and 390 bp of the 12S region (primer sequences: bush-12SF GGGATTAGATACCCCACTATGC and 12SR GTGACGGGCGGTGTGT) and approximately 250 bp of the 16S rRNA gene (primer sequences: Vert-16S-eDNA-F1 AGACGAGAAGACCCYDTGGAGCT and Vert-16S-eDNA-R1 GATCCAACATCGAGGTCGTAA) from Vences et al. (2016) that were designed to amplify vertebrate DNA from environmental DNA samples. Fragments were amplified by singleplex PCR (with only one primer pair per PCR reaction) in a 30-μL total volume reaction with 15 μL of Qiagen Multiplex PCR Mix (Qiagen, GER), 0.6 μM of forward and reverse primers, and 1 μL of DNA. Cycling conditions were as follows: 94°C for 3 min, followed by 35 cycles at 92°C for 30 s and at primer-specific annealing temperatures for 30 s (*cytb*—48°C, 12S—56°C, and 16S—59°C), and 72°C for 30 s, followed by a final extension of 72°C for 15 min. A template-free PCR (i.e., PCR blank) was included in each amplification to control for potential contamination. The amplification was conducted using the portable MiniPCR (https://www.minipcr.com/), which can run 16 samples at the same temperature at the same time. We also ran PCRs with conventional machines (Applied Biosystems Veriti and VertiPro 96 well Thermal cyclers and the Bio-Rad T100 Thermal cycler), following the same cycling conditions as described above. Amplification success was checked on 2% agarose gels.

The sequencing was carried out on the ONT's MinION Mk1C platform with the native barcoding kit (ONT, SQK-NBD114.96) according to the manufacturer's manual with minimal exceptions. The 3 markers were pooled for each individual, and 33 mixed pools were processed. In brief, the ends of the DNA amplicons were prepared for index ligation with the NEBNext Ultra II End Repair/dA-tailing module (NEB). We extended the reaction time to 30 min at 20°C and 30 min at 65°C; ligated individual indices with the NEB Blunt/TA Ligase Master Mix (NEB); cleaned the amplicons with AMPure XP Beads (Beckman Coulter), and then pooled the cleaned amplicons in equal ratios. Sequencing adapters were ligated to the pooled and indexed amplicons with the NEBNext Quick Ligation Module (NEB), and the reaction was cleaned with AMPure XP Beads (Beckman Coulter) with ONT's short fragment buffer (SFB). The final, single library was quantified on the Qubit 4 Fluorometer, and about 200 ng was loaded onto an ONT Flongle sequencing flow cell on the MinION MK1C sequencing platform. The flow cell was run for 24 h.

## Bioinformatic protocol and assignment of samples

We followed the bioinformatic protocol described in Pomerantz et al. (2022). First the raw pod5 files were converted to the fastq format and demultiplexed with Dorado 0.5.0 (https://github.com/nanoporetech/dorado). Next, the number of reads and their quality were assessed with NanoPlot (https://github.com/wdecoster/NanoPlot). We retained only reads with a Phred score of at least 17 from NanoFilt (https://github.com/wdecoster/nanofilt). Priming sites were removed from the reads with cutadapt (https://github.com/marcelm/cutadapt/; Martin, 2011), and the consensus sequences were reconstructed with NGSpeciesID (Sahlin et al., 2021). We used NGSpeciesID and ONT's Medaka software (https://github.com/nanoporetech/medaka) for consensus polishing. The consensus sequences and the number of supporting reads for each sequence are publicly available (see "DATA AVAILABILITY STATEMENT").

To identify the sample to the species level, we compared the sequences and data on the National Center for Biotechnology Information database (NCBI) (http://www.ncbi.nlm.nih.gov/) with Nucleotide BLAST (Boratyn et al., 2013). We considered similarities over 98% indicative of species-level identification and inspected percent similarities to the next closely related species to avoid using uninformative DNA barcodes. Next, we verified the species identification with phylogenetic analyses. To do so, we first downloaded sequences of the identified genera (*Cercopithecus* sp., *Erythrocebus* sp., *Chlorocebus* sp., *Papio* sp., and *Piliocolobus* spp.) from NCBI GenBank (Appendix S1). Next, we used MAFFT to align the sequences (Katoh et al., 2009) and reconstructed their phylogenetic relationships with the maximum likelihood approach implemented in IQ-TREE (Nguyen et al., 2015). This allowed automatic substitution model selection, fast bootstrapping (1000 bootstraps), and approximate likelihood ratio branch tests (1000 replicates). The phylogenetic trees were visualized with TreeViewer (Bianchini & Sánchez-Baracaldo, 2024).

## Ethics

Researchers who interacted with people when procuring samples kept the identify of these people and collection locations confidential. Tissue samples were given freely by people in possession of specimens or taken from animals found dead. The study protocol was approved by the ethics committee of Porto University, Portugal (report 127 /CEUP/2022) and Cardiff University, United Kingdom (SREC 22 11-01). Guinea-Bissau authorities approved the use of tissue samples for research and the timeline and were acknowledged in dissemination activities. Institute for Biodiversity and Protected Areas and Direcção Geral de Florestas e Fauna authorized sample collection and issued CITES permits. Instituto para a Conservação da Natureza e Florestas (ICNF, Portugal) issued import CITES permits (*Cercopithecus petaurista*, 18PTLX00592I and 23PTLX00512I; *Cercopithecus campbelli*, 18PTLX00590I; *Erythrocebus patas*, 8PTLX00589I; *Chlorocebus sabaeus*, 18PTLX00586I; *Papio papio*, 18PTLX00585I). Direção Geral de Veterinária (DGV) issued health and veterinary permits to import samples to Portugal. Research was based on fairness, respect, care, and honesty, following the Global Code of Conduct for Research in Resource-Poor Settings (https://www.globalcodeofconduct.org/).

## RESULTS

DNA was successfully extracted from the tissue samples. The PCR and sequencing procedures worked equally well for all DNA extracts. The MinION sequencing run with a Flongle flow cell resulted in 453,993 demultiplexed reads. All analyzed samples, regardless of age and sampling context, were successfully identified to the species level with the 3 mtDNA fragments. The 99 mtDNA sequences obtained (3 markers per sample: *cyth*, 12S, and 16S) had high percent matches with voucher samples deposited in GenBank. The 3 genetic markers consistently identified species in all samples (Table 1). Further verification of identities of samples via phylogenetic analyses showed the same result for the species identification as the BLAST analyses (Appendices S2–S4 show results for each marker, respectively). In all cases, the 3 genetic markers matched the species identification (Table 1).

## DISCUSSION

We found that MLE accurately identified the investigated primate species with DNA barcoding. The application of MLE in Guinea-Bissau and other similar settings could improve knowledge of IWT because its use bypasses the need for sample export and can provide training opportunities in genetic or genomic technologies for university students and officials.

### Wild meat trade and its ecological and societal impacts in Guinea-Bissau

In Guinea-Bissau, primate meat is traded in urban meat markets (Figure 2) and in restaurants and bars scattered in urban and rural areas (Ferreira da Silva, Camará, et al., 2021; Minhós, Wallace, et al., 2013) probably since the 1980s (Ferreira da Silva, Minhós, et al., 2021). The minimum of 1500 traded specimens extrapolated for 6 months in 2 urban markets in Bissau in 2010 is similar to that in larger capitals of West African countries (Minhós, Wallace, et al., 2013). The sale of young chimpanzees in the pet trade may also lead to mortality of adult individuals (Ferreira da Silva & Regalla, 2025). Harvest of wildlife for commercial purposes in the last decades may have contributed to significant demographic alterations in threatened primate populations, which have been identified based on genetic and genomic data (e.g., western red colobus and king colobus [Minhós, Nixon, et al., 2013; Minhós et al., 2016], western chimpanzees [*Pan troglodytes verus*] [Ferreira da Silva et al., 2025], Guinea baboon [*Pa. papio*] [Ferreira da Silva et al., 2014, 2018], and western lesser spot-nosed monkey [*Ce. petaurista buettikoferi*] [Colmonero-Costeira et al., 2025]). There has been a recent decrease in the effective population size of colobus monkeys and chimpanzees to <500 breeding individuals; thus, the risk of extirpation of some populations is high (Ferreira da Silva et al., 2025; Minhós et al., 2016). Commercial hunting likely poses a significant threat to protected wildlife in Guinea-Bissau.

### Identifying species affected by trade in wild meat in Guinea-Bissau

There is still very limited knowledge on which and how many species are targeted for consumption in the IWT and their geographic origin (Ferreira da Silva, Camará, et al., 2021; Ferreira da Silva, Minhós, et al., 2021). This information is key for effective law enforcement and in the design of conservation actions to change behavior and decrease the risk of extinction of hunted species.

Use of DNA barcoding tools has been fundamental to the identification of species affected by commercial hunting in Guinea-Bissau. Before the first study in which DNA barcoding was conducted, it was thought that the species most frequently targeted for the wild meat trade were the Guinea baboon, green monkey (*Ch. sabaeus*), and colobus monkeys (Cá, 2008; Casanova & Sousa, 2007). Minhós, Wallace, et al. (2013) provided molecular evidence that 6 out of the 10 primates in the country were traded in urban meat markets. The suspicion that hunting of primates was widespread was confirmed; however, the species-specific contribution to the trade was unexpected. The green monkey and the Campbell's mona monkey (*Ce. campbelli*) were the most frequently traded species (32.9% and 29.7%, respectively) (Minhós, Wallace, et al., 2013), despite presumably representing a lower financial return in the trade than other larger species, such as the Guinea baboon (Ferreira da Silva, Minhós, et al., 2021). The use of DNA barcoding tools demonstrated that vendors misidentified green and the Campbell's mona monkeys frequently. Carcasses were traded smoked and disemboweled and were generally identified visually based on external features (Figure 2). The green and the Campbell's mona monkeys were frequently misidentified most likely because the 2 species have similar body sizes (Rowe, 2016) and the common names in Guinea-Bissau creole can be used interchangeably (Minhós, Wallace, et al., 2013). By following the information provided by vendors exclusively, the green monkey would be considered the single most traded species, and, more importantly, the trade in Campbell's mona monkeys would be ignored.

Our study suggests that the use of DNA barcoding with MLE could improve investigations of IWT in Guinea-Bissau. We successfully identified 33 tissue samples to the species level with HTS on ONT's MinION sequencer. The samples used were collected in different contexts and times (i.e., 14 to 2 years old) and therefore were subject to various DNA preservation conditions. Our results are consistent with results from studies in which different genetic markers and sanger sequencing (Minhós, Wallace, et al., 2013) and genetic markers in common and standard HTS platforms were used (Ferreira da Silva, Camará, et al., 2021). Furthermore, we identified samples not analyzed previously (i.e., the western lesser spot-nosed monkey [Table 1]) and provided molecular information on the wild meat trade in the Bijagós Archipelago (Colmonero-Costeira et al., 2023).

The use of DNA and metabarcoding and MLE has advantages over methods used by Ferreira da Silva, Camará, et al. (2021) and Minhós, Wallace, et al. (2013) because it allows

**TABLE 1** Results from the use of National Center for Biotechnology Information BLAST for molecular identification of 33 tissue samples from tissue samples collected from wild meat carcasses traded in Guinea-Bissau.

| Sample number | Previous information on species identity | Reference[a] | Species identified with all 3 markers[b] | IUCN category[c] | Species identification (%)[d] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | cytb | | 12S | | 16S | |
| | | | | | Match length | Match similarity | Match length | Match similarity | Match length | Match similarity |
| 224 | Suspected *Cercopithecus petaurista* | | *Ce. petaurista* | NT | 100 | 99.06 | 100 | 99.49 | 100 | 100 |
| 225 | | | *Ce. petaurista* | NT | 100 | 99.29 | 100 | 99.49 | 100 | 100 |
| 276 | | | *Ce. petaurista* | NT | 100 | 99.29 | 100 | 99.50 | 100 | 100 |
| 278 | | | *Ce. petaurista* | NT | 100 | 99.29 | 100 | 99.49 | 100 | 100 |
| 277 | | | *Ce. petaurista* | NT | 100 | 99.29 | 100 | 99.49 | 100 | 100 |
| 280 | | | *Ce. petaurista* | NT | 100 | 99.06 | 100 | 99.49 | 100 | 100 |
| 258 | | | *Ce. petaurista* | NT | 100 | 98.82 | 100 | 99.49 | 100 | 100 |
| 256 | | | *Ce. petaurista* | NT | 100 | 99.29 | 100 | 99.23 | 100 | 100 |
| 254 | | | *Ce. petaurista* | NT | 100 | 99.29 | 100 | 99.23 | 100 | 100 |
| 255 | | | *Ce. petaurista* | NT | 100 | 99.06 | 100 | 99.49 | 100 | 100 |
| B24 | *Papio papio* | Ferreira da Silva, Camará, et al., 2021 | *Pa. papio* | NT | 100 | 100 | 100 | 100 | 100 | 100 |
| B115 | *Papio papio* | | *Pa. papio* | NT | 100 | 100 | 100 | 100 | 100 | 100 |
| B197 | *Papio papio* | | *Pa. papio* | NT | 100 | 100 | 100 | 100 | 100 | 100 |
| B155 | *Papio papio* | | *Pa. papio* | NT | 100 | 100 | 100 | 100 | 100 | 100 |
| B20 | *Erythrocebus patas* | | *E. patas* | NT | 95 | 98.76[e] | 100 | 99.23 | 100 | 99.17 |
| B126 | *Papio papio* | | *Pa. papio* | NT | 100 | 100 | 100 | 100 | 100 | 100 |
| B415 | *Papio papio* | | *Pa. papio* | NT | 100 | 100 | 100 | 100 | 100 | 100 |
| B127 | *Papio papio* | | *Pa. papio* | NT | 100 | 100 | 100 | 100 | 100 | 100 |
| B120 | *Papio papio* | | *Pa. papio* | NT | 100 | 100 | 100 | 100 | 100 | 100 |
| B119 | *Papio papio* | | *Pa. papio* | NT | 100 | 100 | 100 | 100 | 100 | 100 |
| B17 | Lost ID | Minhós et al., 2013 | *Pa. papio* | NT | 100 | 100 | 100 | 100 | 100 | 100 |
| B117 | | | *Pa. papio* | NT | 100 | 100 | 100 | 100 | 100 | 100 |
| 205 | | | *Piliocolobus badius* | EN | 100 | 99.06 | 100 | 99.74 | 100 | 100 |
| 209 | | | *E. patas* | NT | 95 | 98.51[e] | 100 | 99.23 | 100 | 99.17 |
| 206 | | | *Pa. papio* | NT | 100 | 100 | 100 | 99.74 | 100 | 100 |
| 208 | | | *Ch. sabaeus* | LC | 100 | 100 | 100 | 100 | 100 | 99.59 |
| 203 | | | *Cercopithecus campbelli* | NT | 95 | 98.51 | 100 | 98.72 | 100 | 99.17 |
| 202 | | | *Ch. sabaeus* | LC | 100 | 100 | 100 | 100 | 100 | 100 |
| 20 | | | *Pa. papio* | NT | 100 | 100 | 100 | 100 | 100 | 100 |

(Continues)

**TABLE 1** (Continued)

| Sample number | Previous information on species identity | Reference[a] | Species identified with all 3 markers[b] | IUCN category[c] | Species identification (%)[d] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | cytb | | 12S | | 16S | |
| | | | | | Match length | Match similarity | Match length | Match similarity | Match length | Match similarity |
| 201 | | | *Pa. papio* | NT | 100 | 100 | 100 | 100 | 100 | 100 |
| 204 | | | *Pi. badius* | EN | 100 | 99.29 | 100 | 99.75 | 100 | 100 |
| 21 | | | *Ce. campbelli* | NT | 95 | 98.76[e] | 100 | 98.72 | 100 | 98.76 |
| 1 | | | *Ch. sabaeus* | LC | 100 | 100 | 100 | 100 | 100 | 100 |

[a]Publications in which samples were first mentioned.
[b]*Chlorocebus sabaeus* (green monkey), *Cercopithecus campbelli* (Campbell's mona monkey), *Papio papio* (Guinea baboon), *Piliocolobus badius* (red colobus), *Erythrocebus patas* (patas monkey), and *Cercopithecus petaurista* spp. (western lesser spot-nosed monkeys).
[c]International Union for Conservation of Nature conservation status: LC, least concern; NT, near threatened; EN, endangered.
[d]Match similarities >98% were used to assign sequences to the species level.
[e]Match similarity percent was calculated based on the full match length.

a greater number of samples to be analyzed simultaneously, which reduces costs and effort; identification of samples containing highly degraded DNA, namely the ones collected from cooked meat or preprocessed products; identification of multiple species in mixed samples, such as meals with different types of meat (Ferreira da Silva, Camará, et al., 2021; Staats et al., 2016); and bypassing of sample export and acceleration of research by local organizations.

## Advancing molecular biology and conservation genetics in Guinea-Bissau

Due to the civil war from 1998 to 1999, most of the limited infrastructure in Guinea-Bissau was destroyed or severely damaged, including hospitals, education, and research establishments (e.g., National Institute of Studies and Research) and the National Archives. In the aftermath of the war, and in the context of bilateral and multilateral cooperation agreements, education and health in Guinea-Bissau became dependent on the generosity of international partners. The teaching of biology gained emphasis due to cooperation programs with mainly Portuguese nongovernmental organizations, such as the Faith and Cooperation Foundation, which implemented the Program to Support the Reform of the Education System in Guinea-Bissau (2016–2020). This program included the teaching of biology and related subthemes, although only at a theoretical level for some disciplines. Currently, the main challenges are a lack of a network of libraries and adequate textbooks and teacher training programs.

The university is privately owned; thus, it is not dependent on funds from the national budget, and its management is autonomous and independent. This has allowed the university to maintain stability and continuity in educational programs in the face of a troubled social and political context. Two laboratories dedicated to experimental teaching (chemistry and biology) were set up but did not include equipment dedicated to molecular biology because electricity is unstable and cuts in its supply are frequent, which would damage laboratory equipment (R.S. personal observation). Moreover, the regular use of laboratory materials and consumables in educational contexts is expensive (R.S., personal observation).

Nonetheless, the demand from university students for a more empirical and laboratory-based teaching pedagogical model is high. Two science fairs opened to the public demonstrated widespread consensus that proper investment should be made in the training of young people (R.S., personal observation). The expectation among prospective students is that the access to materials, equipment, and training in conservation biology methods will lead to social elevation and changes in attitudes toward the environment (R.S., personal observation; M.J.F.S., personal observation, January 2025).

There is a lack of university-trained technicians working for governmental agencies in themes related to environmental criminology. The training of specialized technicians in environmental criminology and forensics is carried out abroad, and it is common for graduates to not return to Guinea-Bissau. There is a critical need to attract and retain skilled professionals special-

ized in wildlife in Guinea-Bissau to improve the conservation of the country's biodiversity (Ferreira da Silva & Regalla, 2025).

The use of ONT's MinION sequencer has opened new opportunities for hands-on education in different contexts, from field courses (Blanco et al., 2020; Watsa et al., 2020) and classroom settings (Salazar et al., 2020; Zaaijer et al., 2016) and high school (Wünschiers et al., 2024) to master's-level university courses (Prost et al., 2020). Together with inexpensive MLE, portable sequencing devices could provide new opportunities for teaching hands-on molecular biology courses in Guinea-Bissau. The possibility of running the equipment on batteries, which is not possible for most standard tabletop equipment, would circumvent the problem of unreliable electricity and avoid damage to equipment. The availability of many inexpensive and easy DNA extraction and PCR amplification methods makes this equipment affordable and desirable for state-of-the-art molecular biology education.

## Prices and logistics

It is difficult to estimate per sample prices for different countries as product availability and prices can vary. In general, per sample prices depend on several factors. For example, DNA extraction can add substantially to the costs. We used an automated extraction platform because it is fast, but it is rather expensive. Methods, such as Chelex or the alkaline lysis buffer-based HotSHOT DNA extraction (Truett et al., 2000), are highly effective in remote settings and inexpensive to use (e.g., Labrador et al., 2019; Pomerantz et al., 2022; Seah et al., 2020). These methods can be used with MLE. Seah et al. (2020) calculated that a Chelex extraction costs roughly US$0.17 per sample. Another way to reduce costs is the use of relatively inexpensive polymerases. We used Qiagen's Multiplex polymerase, which works well even for difficult and degraded samples. Cheaper alternatives exist that work efficiently for DNA barcoding, especially in case of fresh wild meat samples. We recommend using a hot-start polymerase.

Another consideration is the number of samples to process. In general, several hundred samples can be pooled on a single MinION flow cell (standard or even the much smaller and cheaper Flongle flow cell) because only a few hundred to about 1000 reads per sample are needed to obtain highly accurate consensus sequences (Krehenwinkel et al., 2019; Vasiljevic et al., 2021). This reduces the per sample cost drastically. There are several different library preparation options available for the MinION sequencer. The cheapest option is the addition of indices via a 2-step PCR approach (Pomerantz et al., 2022) and the subsequent sequencing with ONT's Ligation Sequencing Kit (e.g., SQK-LSK114). Another efficient but more expensive option is the addition of the indices from ONT's Native Barcoding Kit (e.g., SQK-NBD114). To reduce costs further, different DNA markers can be pooled for each sample. We applied this method here. Optimizing reagents and pooling will likely allow for the generation of DNA barcodes for <US$3 for small projects and <US$2 for large projects (e.g., Seah et al., 2020; Srivathsan et al., 2018).

Although usually smaller and thus allowing less throughput, MLE is comparatively inexpensive. For example, portable PCR machines usually cost US$800–1000, whereas conventional PCR platforms cost US$5000–20,000. The ONT's MinION sequencer costs around US$3000, and other long-read HTS platforms cost several hundreds of thousands to over a million U.S. dollars.

A common limiting logistical factor is the availability or the time it takes to import reagents and kits. These can vary between countries and must be considered for project planning. Another problem is the availability of reliable cold chains (Pomerantz et al., 2022). Although lyophilized polymerases are available, ligases used for the ONT sequencing library preparation still need cooling.

## Contributions to reducing barriers to equitable access and application

We tested the usability of MLE for the successful identification of wild meat samples collected in different settings and DNA preservation conditions and explored their use in improving the knowledge on ITW and in promoting molecular biology and training of conservation genetics methods in Guinea-Bissau. Relatively inexpensive equipment, such as MLE, could help achieve several United Nations Sustainable Development Goals (SDGs) (https://sdgs.un.org/). For example, it can greatly support investigations into the illegal wild meat trade and thus support SDG15 (improve conservation of biodiversity) and SDG16 (support development of efficient legal prosecution of wildlife crimes) or be used in classroom environments for education and local capacity building, which supports SDG4 (provide quality tertiary education). The reliable use of MLE for DNA barcoding has been shown in many contexts, including in situ molecular analyses and education (e.g., Blanco et al., 2020; Pomerantz et al., 2018; Salazar et al., 2020; Watsa et al., 2020). Furthermore, ONT's inexpensive and portable MinION sequencer has been validated for use in DNA barcoding for wildlife forensic casework (Vasiljevic et al., 2021).

Considering the current limitations for the use of molecular biology protocols in Guinea-Bissau (i.e., unstable electricity supply that may damage standard laboratory equipment, expensive laboratory materials and consumables, and lack of trained personnel), the use of MLE for DNA barcoding is a promising alternative to standard and often expensive molecular biology laboratory equipment. These methods may be used in West African countries lacking large molecular laboratories. In-country training programs on MLE should be actively promoted to enhance local capacity.

## AUTHOR CONTRIBUTIONS

**Maria Joana Ferreira da Silva**: Conceptualization; methodology; validation; formal analysis; investigation; supervision; project administration; funding acquisition; writing—original draft; writing—review and editing. **Ivo Colmonero-Costeira**: Conceptualization; methodology; investigation; visualization; funding acquisition; writing—original draft; writing—review

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## DATA AVAILABILITY STATEMENT

Alignments for the consensus sequences of all 3 markers are available on Dryad: http://datadryad.org/share/nwvzMHCoB7bYkVRZrv_M-JLIhxoq9zBGz1nAF-6jTRY.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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