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Improving Information on Over-looked Generalists: Occurrence and Mitochondrial DNA Diversity of Campbell's (*Cercopithecus campbelli*) and Green Monkeys (*Chlorocebus sabaeus*) in Guinea–Bissau, West Africa

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Abstract

Non-threatened primates are often overlooked in conservation efforts despite their increasing vulnerability to local extirpation. Campbell's (*Cercopithecus campbelli*) and green monkeys (*Chlorocebus sabaeus*) are sympatric medium-sized West African guenons (tribe Cercopithecini) whose intraspecific genetic diversity remains understudied in most of their distribution. Both species are ecological generalists and are globally considered non-threatened. In Guinea–Bissau, *C. campbelli* and *Chl. sabaeus* are considered the most abundant of the ten extant primate species and are the most frequently hunted for meat. Their populations are thought to be decreasing and but up-to-date data on their occurrence in the country hinders their conservation status assessment. We aimed to update occurrence data and estimate the country-wide mitochondrial (mtDNA) genetic diversity for both species in Guinea–Bissau. From 2008–2022, we conducted surveys in four mainland protected areas and on the islands of the Bijagós Archipelago. We identified *C. campbelli* populations outside their known distribution. We found high mtDNA diversity for both species on the mainland and lower diversity in insular populations. Our results show

Badges earned for open practices: Open Data. Experiment materials and data are available in the repository at https://github.com/Colmonero-CI/Campbelli_sabaeus_GB2024.

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significant signals of geographically induced mtDNA differentiation, particularly in *C. campbelli*. In *Chl. Sabaeus*, we found divergent haplotypes at geographically close locations. We identified differentiated haplogroups with an estimated divergence time of 1.53 million years ago (Ma) in *C. campbelli* and 1.16 Ma in *Chl. sabaeus,* possibly linked to Pleistocene climatic fluctuations. Given the local presence of differentiated mtDNA haplogroups across these and other primate species, we suggest that Guinea–Bissau should be considered as an important region for primate conservation in West Africa.

Keywords Guenon · Cercopithecini · Generalist taxa · Widespread taxa · Phylogenetic structure

Introduction

Over half of mainland African non-human primates (hereafter primates) are threatened with extinction, and more than 80% have a declining population trend (Estrada et al., 2017). Considering the scale of the conservation crisis and the urgency to mitigate species extinction, primates classified as Least Concern and Near Threatened by the International Union for Conservation of Nature (IUCN) are usually overlooked in global conservation efforts (Fernández et al., 2022). Primates not classified as threatened by IUCN frequently occupy large ranges, have broad dietary requirements and are thought to be at lower risk of extinction than threatened species (Fernández et al., 2022). However, populations of globally non-endangered primates can undergo local extirpation, particularly if the interplay of several threats undermines their resilience (e.g., Ferreira da Silva et al., 2014).

Genetic data are increasingly used in conservation management and policies to delimitate conservation units, such as differentiated populations, or to describe the patterns of connectivity, hybridization processes, and to assess historical population size (Hoban et al., 2022). Moreover, estimates of genetic diversity are key to evaluate the ability of populations to persist in the face of environmental changes and inform conservation action planning (Bertola et al., 2024; Ferreira da Silva & Bruford, 2017). In comparison to more informative markers that require a greater quantity and quality of host DNA, mitochondrial DNA genes can be used as genetic markers for a large number of opportunistically-collected samples with relative ease (e.g., Bertola et al., 2024; Colmonero-Costeira et al., 2019; Ferreira da Silva et al., 2020). The characterization of mitochondrial genetic diversity and differentiation (e.g., spatial structuring and estimation of divergence times) across all the extant primate species is recognized as important for the future prioritization of areas of conservation interest (Carvalho et al., 2017). Nevertheless, the incorporation of molecular data in management plans remains challenging, particularly for locally threatened species in low- to middle-income countries, where the infrastructures, financial, and

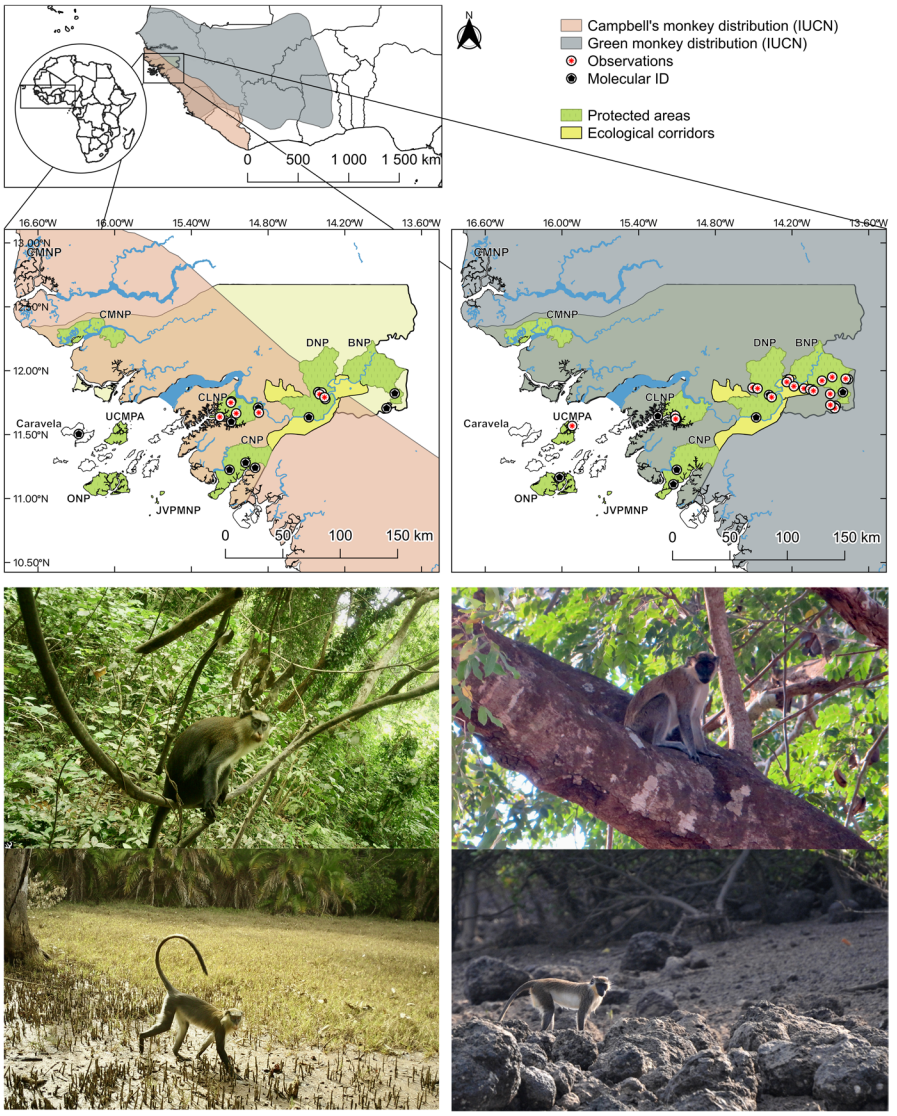

Fig. 1 Distribution of Campbell's monkey (*Cercopithecus campbelli*) (left) and the green monkey (*Chlorocebus sabaeus*) (right) in West Africa and Guinea-Bissau. The figure shows the polygon of the species range by the International Union for Conservation of Nature (IUCN) and the geographic location of observations and molecular records of recent presence (2008–2022). We grouped molecular records in geographically distinct locations (see Methods). Some presence records overlap due to the scale of the map (see Supplementary Material 2 for detailed geographic information for each sample). Also shown: protected areas and ecological corridors (in *green*) on the mainland: CMNP — Cacheu Mangroves Natural Park, CLNP — Cufada Lagoons Natural Park, CNP — Cantanhez National Park, DNP – Dulombi National Park, BNP — Boé National Park; protected areas in Bijagós Archipelago: UCMPA — Urok Communitarian Marine Protected Area (Formosa, Tchedia, and Nago), ONP — Orango National Park (Orango, Ganogo, Menegue, and Orangozinho), JVPMNP — João Vieira and Poilão Marine National Park (João Vieira, Cavalos, Meio and Poilão), and Caravela island. Photographs representative of the taxa in Guinea–Bissau. *C. campbelli* (*bottom left*) (credit L. Palma), and *Chl. sabaeus* (*bottom right*) in Boé National Park, and on the banks of the Buba River, Cufada Lagoons Natural Park (credit F. Gerini, M. J. Ferreira da Silva and P. Huet).

human resources required for such studies are scarce (Bertola et al., 2024; Ferreira da Silva et al., 2024a, 2024b; Helmy et al., 2016).

Campbell's monkey (*Cercopithecus campbelli,* Waterhouse, 1838) and the green monkey (*Chlorocebus sabaeus,* Linnaeus, 1766) are medium-sized African primates that have wide distributions across West Africa (Fig. 1). *C. campbelli* is present from The Gambia to Côte D'Ivoire (Matsuda Goodwin et al., 2020) (Fig. 1). *Chl. sabaeus* is distributed across a larger area, including Mauritania, Mali, Burkina Faso, and Ghana (Gonedelé Bi et al., 2020) and shows high phenotypic variability (Turner et al., 2018) (Fig. 1). Both species have a generalist diet and inhabit a wide range of landscapes (Rowe & Myers, 2016). The two species differ in group composition and dispersal patterns. *C. campbelli* is organized in groups of one adult male and multiple females, in which males are the most frequent dispersing sex, and females are philopatric (Rowe & Myers, 2016). Meanwhile, groups of *Chl. sabaeus* are formed of multiple males and females (Rowe & Myers, 2016) and their social organization is flexible and dependent on environmental factors (e.g., food availability, Galat & Galat-Luong, 1977).

In the most recent IUCN conservation status assessment, *Cercopithecus campbelli* was classified as Near Threatened and *Chlorocebus sabaeus* as Least Concern (Gonedelé Bi et al., 2020; Matsuda Goodwin et al., 2020). Both species are assumed to have a decreasing population trend due to habitat loss and hunting for meat throughout their geographic ranges (Gonedelé Bi et al., 2020; Matsuda Goodwin et al., 2020). Little is known about the evolutionary history of *C. campbelli*, which, to the best of our knowledge, is the least studied guenon in the *Cercopithecus mona* group. In contrast, the *Chlorocebus* genus is a relatively well-studied phylogeographical model for the evolution of wide-spread savannah species (Dolotovskaya et al., 2017; Gagnon et al., 2022; Haus et al., 2013; Svardal et al., 2017; Warren et al., 2015). However, population-level studies of the westernmost species (*Chl. sabaeus*) are scarce but suggest high intra-specific mtDNA diversity (e.g., Almeida et al., 2024; Haus et al., 2013).

Guinea–Bissau (36,125 km^2), a West African country bordered by Senegal and the Republic of Guinea, comprises one mainland region and the Bijagós Archipelago. Ten primate species have been reported in the country (Gippoliti & Dell'Omo, 2003; Bersacola et al., 2018; Ferreira da Silva et al., 2020; Colmonero-Costeira et al., 2023). Most of these primate populations are threatened with habitat degradation/fragmentation and commercial hunting for meat, which are thought to have intensified in the last three decades (Colmonero-Costeira et al., 2023; Ferreira da Silva et al., 2021; Gippoliti & Dell'Omo, 2003; Minhós et al., 2013b, 2023). A lack of information for most fauna, including primates, hinders effective conservation planning in Guinea–Bissau (Ferreira da Silva et al., 2020; Colmonero-Costeira et al., 2023; Palma et al., 2024). Most surveys documenting the occurrence and the conservation status of local primates are 20 years old (Gippoliti & Dell'Omo, 1996, 2003) or have only considered Endangered taxa, such as the Western chimpanzee (*Pan troglodytes verus*) and colobus monkeys (*Piliocolobus badius temminckii* and *Colobus polykomos*, Casanova & Sousa, 2007). More recent information on primate occurrence may exist in non-digital repositories and unpublished reports, but these are usually difficult to access. There is an urgent need to re-evaluate the local

conservation status and develop action plans for species that were not considered threatened in the past but may have become impacted in the last three decades due to increasing habitat loss and wild meat hunting (Gippoliti & Dell'Omo, 2003; Minhós et al., 2013b). Specifically, there is an important gap in information on the national occurrence and genetic diversity of generalist primates, such as *Cercopithecus campbelli* and *Chlorocebus sabaeus*, which limits the understanding of the degree of threat and thus decision-making regarding conservation efforts.

Cercopithecus campbelli (*santcu mona* or *kankulma* in Guinea–Bissau Kriol) and *Chlorocebus sabaeus* (*santcu di tarrafi* in Guinea–Bissau Kriol) are considered the most abundant primates in Guinea–Bissau (Gippoliti & Dell'Omo, 2003; Karibuhoye, 2004; Bersacola et al., 2018). They are widely distributed throughout the mainland territory of Guinea–Bissau, except in the northeast (Gippoliti & Dell'Omo, 2003). The occurrence of *C. campbelli* is uncertain in the southeast of the country (the Boé sector). In the Bijagós Archipelago, *Chl. sabaeus* is reported in Formosa, Orango, Bubaque, Rubane, Enu, and Carache islands, and *C. campbelli* in the Caravela island (Campredon et al., 2001; Colmonero-Costeira et al., 2019; Gippoliti & Dell'Omo, 2003; Reiner & Simões, 1999) (Fig. 1). *C. campbelli* can be found in riverine and open forests (Bersacola et al., 2018). Although some authors suggest that *Chl. sabaeus* is predominantly associated with mangrove forests in Guinea–Bissau (Gippoliti & Dell'Omo, 1996, 2003), it also uses grassland, woodland, savannah, and cashew orchards (*Anacardium occidentale*) habitats (Bersacola et al., 2018).

A survey of two wild-meat markets in the capital city (Bissau) showed that *Cercopithecus campbelli* and *Chlorocebus sabaeus* were the most traded of all the primates, with approximately 500 individuals of each species sold during the dry season in 2010 (Minhós et al., 2013b). Consumption of *C. campbelli* is also widespread in more rural locations (Ferreira da Silva et al., 2021). Furthermore, the trade in primate infants as pets seems common for both species (Gippoliti & Dell'Omo, 2003; Karibuhoye, 2004) possibly as a by-product of the occasional hunting of lactating females (Colmonero-Costeira et al., 2023; Ferreira da Silva et al., 2021). Farmers from Southern Guinea–Bissau classify the species as two of the most impactful crop-foragers and commonly pursue and kill monkeys (Amador et al., 2015). Overall, these observations suggest that the two species are heavily hunted, populations may be under threat, and that the species may have become rare or extinct in areas where they were previously recorded (e.g., Gippoliti & Dell'Omo, 1996, 2003).

Here, we aimed to update information on the distribution of *Cercopithecus campbelli* and *Chlorocebus sabaeus* and to assess their mitochondrial diversity and structure at a country-wide scale in Guinea–Bissau. Between 2008 and 2022, we recorded direct and indirect geo-referenced presence data and collected non-invasive biological material for molecular analyses for the two species across a large area of Guinea–Bissau, including the four parks in southern mainland Guinea–Bissau and eight of the largest islands of the Bijagós Archipelago (Fig. 1). Our specific objectives were to: i) compile geo-referenced visual and molecular records collected between 2008 and 2022 to confirm species occurrence and update information on distribution, ii) estimate mitochondrial genetic diversity and population structure using non-invasive methods, iii) reconstruct the phylogenetic relations of mtDNA

haplotypes and estimate divergence times between lineages, and iv) identify themes for future research that would improve the national conservation strategy for these primates.

Methods

Study area

The study area encompasses most of the described range of *Cercopithecus campbelli* and *Chlorocebus sabaeus* in Guinea–Bissau (Colmonero-Costeira et al., 2019; Gippoliti & Dell'Omo, 2003) (Fig. 1): Cantanhez National Park, Cufada Lagoons Natural Park, Dulombi National Park, Boé National Park, and Bijagós Archipelago. The study area lies across an ecological transition. In approximately 200 km, from the southwestern regions to the northeast part of the country, the vegetation changes from sub-humid and dry tropical forests to mosaics of dry tropical forest and savanna woodland (Catarino et al., 2001).

Data collection

We carried out surveys from 2015 to 2022 as part of the PRIMACTION project—*Protecting the Western chimpanzee and other primate species from illegal logging and hunting in Guinea–Bissau*. Expeditions aimed to update non-human primate distributions across the country and collect information on species diversity, population structure, and gene flow patterns. We collected geo-referenced observations and biological material in Cufada Lagoons Natural Park (December 2015), Dulombi National Park (February 2016), and Boé National Park (January 2017), and in the largest islands of the Bijagós Archipelago (Galinha, Canhabaque, Canogo, Orango, Uracane, Uno and Caravela, from March to September 2016, and Formosa in 2022) (Fig. 1). We collected presence and genetic data from Cantanhez Forest National Park between 2008 and 2010 (Ferreira da Silva, 2012).

Prior to fieldwork and during expeditions, we requested the help of local guards and villagers to identify areas frequently used by primates. We visited the primates' drinking spots, sleeping sites and foraging areas in croplands, woodland savannah, and gallery, mangrove, primary, and secondary forests. When arriving at these locations, we attempted to observe groups or searched for indirect signs of the species' presence (footprints, vocalizations, and/or fecal samples). We remained at each location for a minimum of 30 min. Using a geographic positioning system (GPS) device we recorded the location of sites where we observed, heard, or photographed primate groups. We also recorded the coordinates for all the collected fecal samples. We collected fecal samples that were fresh (i.e., moist and still holding the expected cylindrical shape) and that were more than 2 m apart, in an attempt to avoid sampling the same individuals repeatedly. To preserve fecal DNA, we used RNAlater™ (Invitrogen™, USA), 99% ethanol (Sigma–Aldrich, USA), or the "two-step" protocol (Roeder

et al., 2004). We obtained tissue samples opportunistically from individuals that had been hunted for meat by local inhabitants (please refer to the Ethical Note section for more details). We preserved tissue samples in 98% ethanol (Sigma–Aldrich, USA) at room temperature until DNA extraction.

DNA extraction

We exported samples to Portugal and processed them in Centro de Investigação em Biodiversidade e Recursos Genéticos, *Research Center for Biodiversity and Genetic Resources*, Porto University, Portugal (CIBIO–InBIO). We extracted total genomic DNA from feces using the QIAamp DNA Stool Mini Kit (Qiagen, Germany) with a few modifications from the manufacturer's protocol to maximize DNA yield (Ferreira da Silva et al., 2014). We took several precautions to avoid contamination from exogenous human DNA or cross-contamination between samples (Colmonero-Costeira et al., 2019; Colmonero-Costeira, 2019). We extracted DNA from tissue samples using the DNeasy Blood & Tissue Kit (Qiagen, Germany) following the manufacturer's protocol.

DNA amplification and sequencing

We used two fragments of mitochondrial DNA (mtDNA) to assign samples to the species level and estimate mitochondrial genetic diversity. We amplified the following mtDNA fragments by polymerase chain reaction (PCR): 1) 402 base pairs (bp) of the *cytochrome b* gene (*cytb*) for both species using primers GVL14724: 5' GAT ATGAAAAACCATCGTTG 3' and H15149: 5' CTCAGAATGATATTTGTCCTCA 3' (Gaubert et al., 2015), and 2) a fragment of the hypervariable region I (HVRI), using primers LCERCOHVRI: 5' CGTGCATTACTGCTAGCCAAC 3', and HCERCOHVRI: 5' GGGATATTGATTTCACGGAGGA 3' (Colmonero-Costeira et al., 2019). PCRs had a total volume of 10 µL and included 1X MyTaq™ Mix (Bioline, UK). *Cytochrome b* amplifications contained 0.1 µm of each primer and 1 µl of DNA extract. HVRI amplifications contained 0.2 µm of each primer and 2 µl of DNA extract. *Cytochrome b* PCRs started with a Taq DNA polymerase activating step of 15 min at 94ºC followed by 35 cycles of denaturing at 92 ºC for 30 s, annealing at 50 ºC for 30 s, extension at 72 ºC for 30 s, and a final extension step at 72 ºC for 15 min. Hypervariable Region I PCRs started with an activating step of 15 min at 95 ºC followed by 40 cycles of denaturing at 94 ºC for 30 s, annealing at 58 ºC for 30 s, extension at 72 ºC for 30 s, and final extension 72 ºC for 15 min. We conducted all the PCRs in a T100™ 96 Well Thermal Cycler (Bio-Rad, USA). To limit DNA cross-contamination between samples and by exogenous DNA, we prepared PCRs in non-invasive DNA PCR preparation rooms. We tested amplification success by electrophoresis at 300 V using 2% agarose gels stained with GelRed™ (Biotium, USA) and visualized using a UV Gel Doc™ XR +Gel Documentation System (Bio-Rad, USA) transilluminator. We purified PCR products using Exonuclease I and FastAP (1 UµL- 1) (Thermo Fisher Scientific™, USA) and sequenced them using a 3130XL

automated sequencer (Applied Biosystems™, USA) at the Center for Molecular Testing at CIBIO–InBIO, Portugal facilities using BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems™, USA).

We confirmed the quality of forward and reverse sequences and the polymorphic positions visually using Geneious v4.8.5 (Kearse et al., 2012). We assigned samples to species using the Basic Local Alignment Search Tool (BLAST; Altschul et al., 1990) in the National Center for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov). We aligned sequences of each fragment separately for each species using Geneious v4.8.5 automatic alignment option and trimmed the alignments to the length of the shortest sequence. We adopted procedures to control for the presence of Nuclear Mitochondrial DNA Segments (i.e., unintended amplification of nuclear insertions of mitochondrial genes; Bensasson et al., 2001). We screened the chromatograms for double electrophoretic peaks and translated *cytb* fragments to the aminoacidic sequences to identify the presence of multiple STOP codons (Bensasson et al., 2001). We took a conservative approach by removing all sequences showing double electrophoretic peaks from the final datasets.

Data analyses

Mapping the current occurrence of *Cercopithecus campbelli* and *Chlorocebus sabaeus*

We entered geo-referenced species records, such as observations, and molecular identifications of fecal samples into geographic information system (GIS) software (QGIS v3.32). Because we did not observe defecation for most samples, we grouped fecal samples located within a radius equal to estimates of each species' daily range (1155 m for *Cercopihecus campbelli* and 11,000 m for *Chlorocebus sabaeus*, Rowe & Myers, 2016) together in the same "geographically distinct location". We overlaid our presence data on the distribution of the two species published by IUCN to identify differences from these distributions.

Estimating mitochondrial genetic diversity and spatial structure

We computed summary mitochondrial genetic diversity statistics for *cytb* and HVRI fragments separately, and for the concatenated alignment using DNAsp v6.12.03 (Rozas et al., 2017). We estimated the number of haplotypes, haplotype diversity (Hd), nucleotide diversity (π) (Nei, 1987), and their standard deviations for the overall genetic dataset for each species and sampling area. We estimated haplotype richness following a rarefication approach to account for the different sample sizes and implemented in *vegan* v2.6–4 R package (Oksanen et al., 2022). We computed a 95% parsimony haplotype network for *cytb* and HVRI fragments separately and for the concatenated alignment using PopART (Leigh & Bryant, 2015) to visualize genetic variation and explore the spatial distribution of the mtDNA haplotypes. To assess whether the observed genetic variation patterns for each species were consistent with a model of neutrally evolving locus under mutation-drift equilibrium, we estimated Tajima's D (Tajima, 1989), Fu's Fs (Fu, 1997), and Ramos-Onsins and

Rozas' R_2 (Ramos-Onsins & Rozas, 2002) summary test statistics for the concatenated alignment using DNAsp v6.12.03 (Rozas et al., 2017). To account for the confounding effects caused by population structure on mutation-drift equilibrium summary test statistics (Moeller et al., 2007; Städler et al., 2009), we estimated these statistics for each differentiated haplogroup within each species.

We tested for spatial structuring and isolation-by-distance by performing distance-based redundancy analyses (db-RDA; Legendre & Anderson, 1999) and Mantel tests on the concatenated alignment of each species. Distance-based redundancy analysis combines an ordination method (multidimensional scaling) with multiple regressions of a trend-surface of the geographic coordinates of sampling locations (Legendre & Legendre, 2012). We generated nine geographic variables based on geographic coordinates (long, lat, long x lat, $long^2$, lat^2, $long^2$ x lat, long x lat^2, $long^3$, lat^3). We used a forward selection procedure to avoid over-fitting the regression models. To account for the increased type-I error rates due to multiple testing in the forward selection procedures (Legendre & Legendre, 2012), we applied a stringent significance level of 0.01 and the adjusted determination coefficient (R^2) as stopping criteria (i.e., stopping the addition of predictors when the R^2 of the partial models exceeds the R^2 of the global model; Blanchet et al., 2008). Subsequently, we estimated the variance inflation factor (VIF) of the model and removed highly collinear variables (VIF > 5) in a stepwise manner. We obtained the statistical significance of the multiple regression models and each of the resulting canonical axes (CAP) using ANOVA-like permutation tests (9999 permutations). To obtain a visual representation of the main spatial structures, we interpolated the fitted site scores of the first significant canonical axis using the inverse distance weighting with power equal to two (more detailed information on the spatial methods and the list of R packages used can be found in Supplementary Material 1). We conducted statistical analysis in R v4.2.2 (R CoreTeam, 2022) coupled with RStudio v2023.06.2 + 561 (Posit team, 2023).

Reconstructing the phylogeny and estimating divergence times between lineages

We concatenated fragments of *cytb* and HVRI and collapsed sequences into unique haplotypes. We used sequences from gelada baboon *Theropithecus gelada*, Guinea baboon *Papio papio*, chacma baboon *Papio ursinus*, rhesus macaque *Macaca mulatta,* and Barbary macaque *Macaca sylvanus* retrieved from GenBank as outgroups (see Supplementary Material 2 for accession numbers). We corrected the final alignment visually and pruned indels and miss-aligned positions using Gb0.91b, allowing for smaller final blocks (http://phylogeny.lirmm.fr/phylo_cgi/one_task.cgi?task_type=gblocks). For phylogenetic tree reconstruction, we used 1) maximum likelihood (ML), implemented in IQ-Tree 1.5.2 (Nguyen et al., 2015; Trifinopoulos et al., 2016), and 2) Bayesian inference, implemented in BEAST2 v2.6.1. (Bouckaert et al., 2014). We set concatenated partitions as *cytb* (1 to 344 bp) and HVRI (345 to 569 bp). To identify the best-fit model of molecular evolution, we used ModelFinder (Kalyaanamoorthy et al., 2017) implemented in IQ-Tree and chose the best-fit model based on BIC. We selected TPM3u + I with empirical base frequencies for the *cytb* and HKY + G4 with empirical base frequencies for HVRI

partitions. We estimated the statistical significance of ML trees reconstructed in IQ-Tree using 9,999 ultrafast bootstrap (BS) replicates (Hoang et al., 2018).

We used a Bayesian approach implemented in BEAST2 v2.6.1 to estimate the divergence time between mitochondrial haplogroups. We applied the best-fit model of molecular evolution to *cytb* and HVRI partitions but assumed an uncorrelated relaxed lognormal clock model and Coalescent Constant Population tree prior model for both. To calibrate the molecular clock, we defined priors of the most recent common ancestor (MRCA) to the splits (1) Papionini and Cercopithecini, (2) Macacina and Papionina, (3) *Papio* and *Theropithecus*, and (4) African and Eurasian Macacina. We retrieved the prior distributions for constraints 1–4 from the most conservative calibration set (set- 2) described in Roos (2019). To calibrate the African and non-African Macacina MRCA, we used hard minimum bounds (based on the youngest possible age of the earliest known unambiguous member of a clade) and soft maximum bounds (based on the oldest possible age of the earliest known unambiguous member of the most closely related sister-taxon; Roos et al., 2019). We set the minimum bound of the MRCA constrain at 5.3 Ma., following the timing estimated for the earliest known exemplars of non-African *Macaca* sp. in Spain (5.9–5.3 Ma.; Köhler et al., 2000) and Italy (5.4–5.3 Ma.; Alba et al., 2014). We set the maximum bound at 7.4 Ma. using the timing of the earliest known members of Papionina (e.g., *Parapapio lothagamensis* in Lothagam, Kenya, 7.4–5.0 Ma.; Jablonski & Frost, 2010). We applied a gamma distribution with ß equal to 0.38 and an offset of 5.30, which placed the 95% highest posterior density (HPD) interval at 5.39–7.42 Ma. We conducted three 25-million generations-long independent runs. We sampled trees and parameters every 1000 generations. We inspected the convergence of sampling parameters using Tracer (Rambaut et al., 2018) and tested the adequacy of a 10% burn-in using the effective sample size of all the parameters (ESS, larger than 200). We combined the outputs from independent runs using LogCombiner. We obtained the maximum clade credibility trees containing the node heights using TreeAnnotator after a burn-in of 10% of the sampled trees (Drummond & Rambaut, 2007). We visualized phylogenetic trees using FigTree v1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/).

Ethical note

The research complied with rules and protocols approved by Instituto para a Biodiversidade e Áreas Protegidas (IBAP, Guinea–Bissau) and adhered to the legal requirements of Guinea–Bissau. We obtained all fecal samples non-invasively from unidentified individuals without manipulation and minimal perturbation of their daily behavior. We obtained six tissue samples opportunistically from dead animals in the hands of local hunters after informing them of the purpose of the study and obtaining the informed consent of carcasses' owners. We kept the identity of hunters and carcass owners secret, and did not report hunting activities to national authorities. We did not pay for these samples, in an attempt to avoid encouraging hunting activities. Instituto para a Biodiversidade e Áreas Protegidas (IBAP — *Institute for*

Biodiversity and Protected Areas), local CITES focal person (Convention on International Trade in Endangered Species of Wild Fauna and Flora) and Instituto para a Conservação da Natureza e Florestas Portugal (ICNF, *Institute for Nature Conservation and Forests*) authorized exportation and importation of fecal and tissue samples from Guinea–Bissau to Portugal (CITES permits N.° 18PTLX005901 and 18PTLX00586).

Data availability DNA sequences produced in this study are deposited in the GenBank database (https://www.ncbi.nlm.nih.gov/genbank/) with accession numbers PP053763–PP053988 and PV329711–PV329797. The geographic location of each sample used for spatially explicit analyses is available as supplementary material (Supplementary Material 2). The R scripts used in this work are deposited in GitHub (https://github.com/Colmonero-CI/Campbelli_sabaeus_GB2024).

Results

DNA extraction, mtDNA amplification and sequencing

Out of 371 fecal samples putatively collected from the species under study, we molecularly assigned 71 fecal samples to *Cercopithecus campbelli* and 76 to *Chlorocebus sabaeus*, using at least one of the mtDNA fragments (*cytb* or HVRI) (Fig. 1). Of the six tissue samples, two were assigned molecularly to *C. campbelli* and four to *Chl. sabaeus*. Using BLAST, *C. campbelli cytb* haplotypes showed 94.49–98.84% identity to NCBI GenBank *C. campbelli* sequences. Our *Chl. sabaeus cytb* haplotypes showed 99.13–100.00% identity to NCBI GenBank *Chl. sabaeus* sequences.

We successfully sequenced 54 samples of *Cercopithecus campbelli* (13 in Bijagós Archipelago, six in Cufada Lagoons Natural Park, ten in Cantanhez Forest National Park, 13 in Dulombi National Park, and 12 in Boé National Park) and 59 samples of *Chlorocebus sabaeus* (17 in Bijagós Archipelago, seven in Cufada Lagoons Natural Park, two in Cantanhez Forest National Park, 14 in Dulombi National Park, and 19 in Boé National Park) for both mtDNA fragments (*cytb* and HVRI) (Fig. 2). After trimming the length to the shortest sequence, the final *cytb* alignment was 345 bp long for both *C. campbelli* and *Chl. sabaeus*,and the final HVRI alignment was 295 bp long for *C. campbelli* and 283 bp for *Chl. sabaeus*.

Species occurrence

During our surveys in southern mainland Guinea–Bissau and the Bijagós Archipelago between 2015 and 2022 we observed seven groups of *Cercopithecus campbelli* and 18 groups of *Chlorocebus sabaeus* (Fig. 1). We observed *C. campbelli* groups in primary and secondary forests at Cufada Lagoons Natural Park (four groups) and Dulombi National Park (three groups). We observed *Chl. sabaeus* groups in mangroves, primary and secondary forests at Cufada Lagoons Natural Park (three

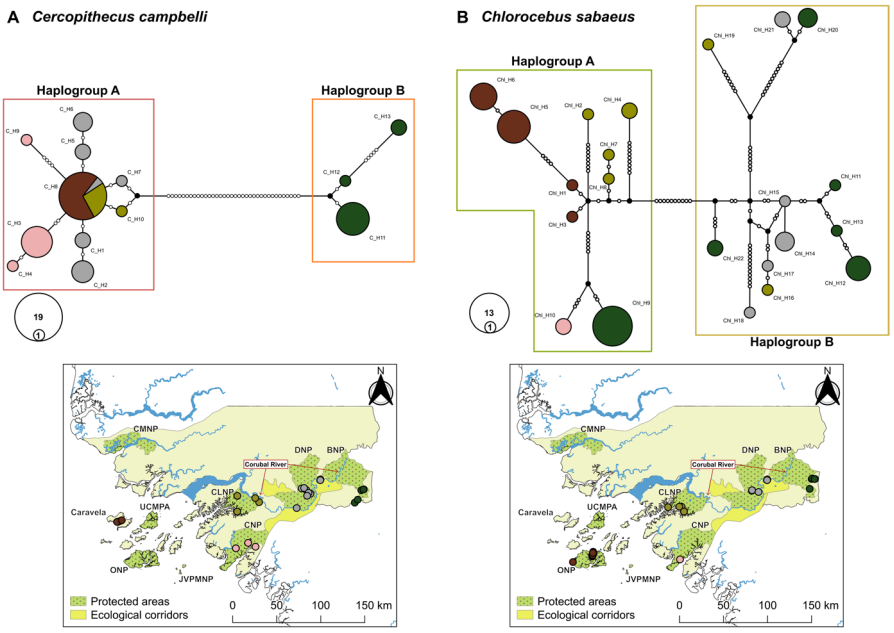

Fig. 2 95% parsimony haplotype network for *Cercopithecus campbelli* (**A**) and *Chlorocebus sabaeus* (**B**) in Guinea–Bissau based on samples collected between 2008 and 2022. *Circles* are colored according to sampling area (indicated on the map) and sizes are proportional to haplotype frequency. The number of nucleotide substitutions between haplotypes are shown as *small white circles* on the branches. Also shown: protected areas and ecological corridors (in *green*) on the mainland: CMNP — Cacheu Mangroves Natural Park, CLNP — Cufada Lagoons Natural Park, CNP — Cantanhez National Park, DNP — Dulombi National Park, BNP — Boé National Park; protected areas in Bijagós Archipelago: UCMPA — Urok Communitarian Marine Protected Area (Formosa, Tchedia, and Nago), ONP — Orango National Park (Orango, Ganogo, Menegue, and Orangozinho), JVPMNP — João Vieira and Poilão Marine National Park (João Vieira, Cavalos, Meio, and Poilão), and Caravela island. We constructed maps in QGIS v3.32.

groups) and Dulombi National Park (three groups), as well as woodland and herbaceous savanna habitats at Boé National Park (12 groups).

We collected the 71 fecal samples molecularly identified as being from *Cercopithecus campbelli* in 24 geographically distinct locations: four in Cufada Lagoons Natural Park, three in Cantanhez Forest National Park, 10 in Dulombi National Park, four in Boé National Park, and three in Caravela island (Fig. 1.). For *Chlorocebus sabaeus*, we collected the 76 molecularly identified fecal samples in 11 geographically distinct locations: two in Cufada Lagoons Natural Park, two in Cantanhez Forest National Park, three in Dulombi National Park, three in Boé National Park, and one in Ganogo island (Fig. 1.)

Mitochondrial genetic diversity and spatial structure

We found 13 unique haplotypes and 53 polymorphic positions in the 640 bp-long concatenated dataset ($N = 54$) for *Cercopithecus campbelli*, and 22 unique haplotypes and 75 polymorphic positions in a 628 bp long mtDNA concatenated dataset ($N = 59$) for *Chlorocebus sabaeus*.

Estimated levels of mitochondrial genetic diversity were high for both species (Table 1). Haplotype richness in *Cercopithecus campbelli* varied between 1 (in the Bijagós Archipelago) and 4.21 (in Dulombi National Park). Haplotype richness in *Chlorocebus sabaeus* was similar between localities but was higher in Cufada Lagoons Natural Park and Dulombi National Park than in the other localities (Table 1). The concatenated haplotype network for *C. campbelli* suggests the existence of two divergent haplogroups distanced by 36 nucleotide substitutions (Fig. 2A). Haplogroup A is composed of ten haplotypes in a star-shaped phylogeny, in which the most frequent haplotype is connected to the surrounding haplotypes by 1–3 nucleotide substitutions. Haplogroup A was present at the Bijagós Archipelago, Cufada Lagoons Natural Park, Cantanhez Forest National Park, and Dulombi National Park. The most frequent haplotype was shared between Cufada Lagoons Natural Park, Dulombi National Park, and the Bijagós Archipelago. Haplogroup B, which we only sampled in Boé National Park, was formed of three haplotypes, separated by a maximum of five nucleotide substitutions. For *Chl. sabaeus*, the concatenated haplotype network also suggests the existence of two divergent haplogroups, distanced by 10 nucleotide substitutions (Fig. 2B). Haplogroup A is formed of ten haplotypes, sampled primarily on the insular and coastal regions (Bijagós Archipelago, Cufada Lagoons Natural Park, and Cantanhez Forest National Park). Haplogroup B is formed of 12 haplotypes, seven sampled in Dulombi National Park and the rest in Boé National Park and Cufada Lagoons Natural Park. We did not find shared haplotypes between sampling locations. In both species, we retrieved haplogroups A and B for both mitochondrial markers independently (*cytb* and HVRI, Supplementary Materials 3, Supplementary Fig. 1 and Fig. 2). Only *C. campbelli* haplogroup A was out of mutation-drift equilibrium (Fu's Fs $= -2.77$, $p < 0.05$; Supplementary Materials 3, Supplementary Table 1). The remaining mutation drift equilibrium test statistics, Tajima's D, Ramos-Onsins and Rozas' R_2 and the raggedness index did not differ significantly from the expectations under a neutral evolution model with constant population size (Supplementary Materials 3, Supplementary Table 1).

Trend–surface analysis revealed a significant signal of geographically induced genetic differentiation, with sampling sites explaining 98.9% of *Cercopithecus campbelli* mitochondrial differentiation (adjusted $R^2 = 0.99$; pseudo-F $= 649.88$, df $= 5$, $p < 0.001$). The fitted site scores from the first significant Canonical Axis explained 99.40% of the constrained variation. After extrapolation, we found a steep gradient of genetic variation from BNP towards the remaining sampling regions (Fig. 3A). When we removed highly divergent haplotypes sampled in Boé National Park for *C. campbelli*, the variance explained by spatial locations of sites was still significant but decreased to 40.06% (adjusted $R^2 = 0.39$; pseudo-F $= 18.56$, df $= 1$, $p < 0.001$). The extrapolated CAP1 fitted site scores (100% of the constrained variation) showed a

Table 1 Mitochondrial genetic diversity of *Cercopithecus campbelli* and *Chlorocebus sabaeus* across insular and Southern mainland Guinea–Bissau based on *cytb* and HVRI mitochondrial DNA fragments

Sampling Area	*cytb*						HVRI						Concatenated[a]					
	N	nH	Hr	S	Hd (SD)	$\pi \times 10^{-2}$ (SD)	*N*	nH	Hr	S	Hd (SD)	$\pi \times 10^{-2}$ (SD)	*N*	nH	Hr	S	Hd (SD)	$\pi \times 10^{-2}$ (SD)
C. campbelli																		
BA	13	1	1.00	0	–	–	13	1	1.00	0	–	–	13	1	1.00	–	–	–
CLNP	7	1	1.00	0	–	–	6	2	2.00	1	0.73 (0.16)	0.30 (0.08)	6	2	2.00	2	0.33 (0.21)	0.10 (0.07)
CNP	16	2	1.44	1	0.13 (0.01)	0.04 (0.03)	10	3	2.20	6	0.38 (0.18)	0.42 (0.27)	10	3	2.20	7	0.38 (0.18)	0.22 (0.14)
DNP	17	2	2.00	1	0.53 (0.05)	0.17 (0.01)	14	5	3.79	4	0.81 (0.07)	0.53 (0.06)	13	6	4.21	5	0.86 (0.06)	0.32 (0.03)
BNP	17	1	1.00	0	–	–	13	3	2.19	7	0.41 (0.15)	0.75 (0.32)	12	3	2.27	7	0.44 (0.25)	0.38 (0.15)
Overall	70	4	–	8	0.54 (0.05)	0.83 (0.10)	56	12	–	45	0.81 (0.04)	4.69 (0.70)	54	13	–	53	0.83 (0.04)	2.48 (0.40)
Chl. sabaeus																		
BA	17	1	1.00	0	–	–	19	5	1.65	8	0.65 (0.09)	0.56 (0.20)	17	4	1.63	7	0.63 (0.08)	0.26 (0.10)
CLNP	9	4	1.99	4	0.58 (0.03)	0.28 (0.12)	7	6	1.95	43	0.95 (0.10)	5.89 (1.18)	7	6	1.95	45	0.95 (0.10)	2.79 (0.56)
CNP	3	1	1.00	0	–	–	2	1	1.00	0	–	–	2	1	1.00	–	–	–
DNP	16	3	1.98	5	0.43 (0.13)	0.52 (0.18)	14	7	1.86	35	0.85 (0.07)	3.68 (0.80)	14	7	1.85	40	0.85 (0.05)	1.87 (0.45)
BNP	26	3	1.96	6	0.61 (0.05)	0.58 (0.12)	21	5	1.55	43	0.55 (0.12)	4.22 (0.87)	19	4	1.52	41	0.52 (0.12)	2.05 (0.48)
Overall	71	6	–	8	0.73 (0.02)	0.49 (0.07)	63	24	–	65	0.91 (0.02)	5.12 (0.31)	59	22	–	75	0.91 (0.02)	2.59 (0.17)

Number of samples (N); number of haplotypes (nH); number of polymorphic positions (S); haplotype diversity (Hd); haplotype richness (Hr) nucleotide diversity (π). Standard deviations are presented in brackets. Sampling areas: BA - Bijagós Archipelago, CLNP - Cufada Lagoons Natural Park, CNP - Cantanhez Forest National Park, DNP - Dulombi National Park, BPN - Boé National Park. a Only samples where both mitochondrial fragments were successfully amplified were included in the concatenated dataset

spatial gradient of genetic variation from Dulombi National Park towards the Cantanhez Forest National Park (Fig. 3A, 3B). Sampling locations explained 38.55% of *Chlorocebus sabaeus* mitochondrial differentiation (adjusted $R^2 = 0.37$; pseudo-F $= 11.29$, df $= 3$, $p < 0.001$). We found a spatial gradient from Dulombi National Park towards the remaining sampling regions (Fig. 3C) based on the extrapolated CAP1 fitted site scores (80.41% of the constrained variation). A significant correlation between genetic and geographic distances was evident in Mantel tests for the overall *C. campbelli* dataset ($r_M = 0.35$, $p < 0.001$) but not for the *C. campbelli* ∅ Boé National Park partial dataset ($r_M = 0.05$, $p = 0.15$). Genetic and geographic distances were also significantly correlated for *Chl. sabaeus* ($r_M = 0.16$, $p < 0.001$).

Phylogenetic reconstruction and lineage divergence time

The tree topology in *Cercopithecus campbelli* is characterized by two divergent haplogroups corresponding to those retrieved in the haplotype network (Supplementary Fig. 3). Monophyly was supported for both haplogroups (PP > 0.99). Haplogroups A and B were estimated to have diverged approximately 1.53 Mya [2.56–0.65 Mya, 95% highest posterior density (HPD)]. We found a similar result for *Chlorocebus*

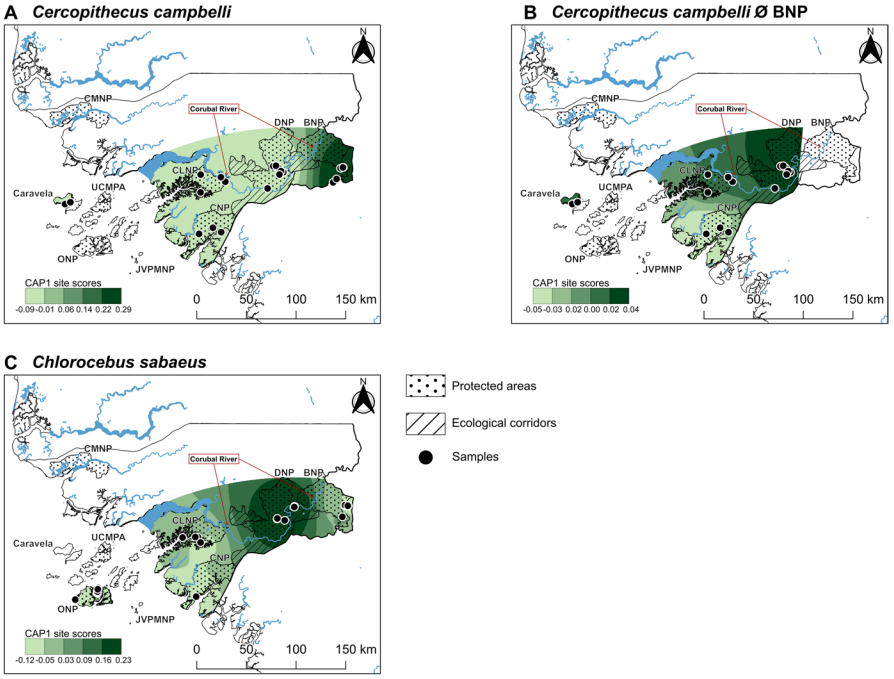

Fig. 3 Spatial patterns of mitochondrial genetic variation of *C. campbelli* (**A**), *C. campbelli* ∅ BNP (**B**) and *Chlorocebus sabaeus* (C) across Guinea–Bissau based on samples collected between 2008 and 2022. Interpolated fitted site scores from the first Canonical Axis (CAP1) of distance-based redundancy analyses are shown in *shades of green*. *Darker shades* represent higher site scores, and *lighter shades* lower site scores. We constructed maps in QGIS v3.32.

sabaeus, for which we identified two divergent haplogroups in the tree (PP > 0.96; Supplementary Fig. 3). Haplogroups A and B were estimated to have diverged approximately 1.16 Mya (1.83–0.67 Mya 95% HPD). The split between *C. campbelli* and *Chl. sabaeus* was estimated as 10.51–3.63 Mya (95% HPD) and the split between the two species and the outgroups at 13.18–7.43 Mya (95% HPD).

Discussion

This study documented a broader distribution of *Cercopithecus campbelli* in Guinea–Bissau (West Africa) than previously reported. For For both *C. campbelli* and *Chlorocebus sabaeus*, our results suggest relatively high mtDNA genetic diversity, with differentiated haplogroups that have diverged more than 1 Mya. Moreover, our findings suggest that mtDNA diversity is spatially structured across the country in both species.

We confirmed the presence of *Cercopithecus campbelli* and *Chlorocebus sabaeus* in areas where they are known to occur (e.g., Gippoliti & Dell'Omo, 2003). We also found molecular and visual evidence for the occurrence of *C. campbelli* in the Boé National Park. This area is outside the distribution range of the most recent IUCN species assessment (Matsuda Goodwin et al., 2020). Our results suggest that *Chl. sabaeus* uses a large diversity of habitats in Guinea–Bissau, namely mangroves, primary forest, and patchy woodland savanna habitats. These findings support those of studies showing that *Chl. sabaeus* in Dulombi National Park uses grassland, woodland, savannah, and cashew orchards (*Anacardium occidentale*) habitats (Bersacola et al., 2018), and do not support the suggestion that *Chl. sabaeus* is predominantly associated with mangrove forests in Guinea–Bissau (Gippoliti & Dell'Omo, 1996, 2003).

We found relatively high mtDNA genetic diversity for *Cercopithecus campbelli* and *Chlorocebus sabaeus* in mainland sampling sites, consistent with patterns found for other primates in the same region (*e.g., Papio papio*: HVRI Hd = 0.81, $\pi = 1.30 \times 10^{-3}$, Ferreira da Silva et al., 2014) and *Pan troglodytes verus*, HVRI Hd = 0.94, $\pi = 3.70 \times 10^{-3}$, Borges, 2017). A significant and negative Fu's Fs found in *C. campbelli* haplogroup A could be interpreted as historical population expansion in the country (Fu, 1997). Although we found similar levels of mtDNA genetic diversity between the regions we sampled in the mainland, insular populations of both species showed the lowest mtDNA diversity. This is particularly evident for the insular *C. campbelli* population in Caravela island, where we found a single haplotype ($N = 13$ samples). Low genetic diversity is frequent in insular or translocated populations and often arises from the founder effect associated with a colonization process mediated by a small number of individuals and subsequent loss of genetic diversity by genetic drift (Allendorf et al., 2013; Eales et al., 2010; Freeland, 2020). However, the specific processes of the colonization of the Bijagós Archipelago by mammals, primates included, are yet to be investigated. Our results suggest close genetic proximity between the insular and mainland coastal populations for the two study species. The single haplotype sampled for *C. campbelli* at Caravela island is indistinguishable from the most frequent haplotype found in Cufada Lagoons Natural

Park, suggesting that the insular populations originate from Cufada or surrounding coastal regions. We sampled multiple closely related and private haplotypes for *Chl. sabaeus* in the Bijagós Archipelago, which suggests a more complex or possibly older colonization process than *C. campbelli* (Hayaishi & Kawamoto, 2006).

Our estimations of mitochondrial spatial structure on the mainland for *Cercopithecus campbelli* and *Chlorocebus sabaeus* are compatible with an isolation-by-distance pattern. In this pattern, gene flow occurs mainly to nearby groups in a stepwise manner (Slatkin, 1993). The location of sampling sites explained approximately 40% of the total genetic differentiation for *C. campbelli* (*C. campbelli* ∅ Boé National Park partial dataset) and for *Chl. sabaeus,* suggesting that variables other than geographic distances contributed to the contemporary and historical mtDNA structure. Considering the ecological features of Guinea–Bissau and the habitat requirements of *Chl. sabaeus* and *C. campbelli,* spatial structuring may have been shaped by a variation of the habitat composition/ecotones (Casado et al., 2010) or by insurmountable barriers, such as permanent water bodies (Telfer et al., 2003).

For *Cercopithecus campbelli*, divergent haplogroups seem to be geographically separated. This is expected in male-biased dispersal species, in which females remain philopatric in natal groups or their movement is very limited in space (Di Fiore et al., 2009). In species with male-biased dispersal, mtDNA variation is expected to be strongly structured compared to nuclear or Y chromosome genetic markers, and demes may show reduced mtDNA haplotype diversity (Di Fiore, 2003; Melnick & Hoelzer, 1992). Our sampling strategy based on non-invasive fecal sample collection from unidentified individuals and mtDNA markers does not allow us to estimate male-specific gene flow. Nonetheless, descriptions of *C. campbelli* in Tai Forest, Côte d'Ivoire suggest female philopatry, where groups are formed by one adult male and multiple females and group males were observed in agonistic interactions with immigrant males (Buzzard & Eckardt, 2007). The spatial distribution of mtDNA variation for *C. campbelli* suggests that both isolation-by-distance and potentially isolation-by-environment may have contributed to shaping historical female gene flow across sampling sites in the mainland. We found that the main axis of genetic variation was from coastal to interior regions of the country. The main axis of genetic variation in *C. campbelli* coincides with an important ecological transition in southern Guinea–Bissau, in which the dominant vegetation changes from Guinea–Congolian sub-humid and dry forest in the coastal areas (Cufada Lagoon Natural Park and Cantanhez Forest National Park) to open forest and savannah woodlands of Sudanese affinity in the western-most regions (Dulombi National Park and Boé National Park) in only 200 km (Catarino et al., 2001).

For *Chlorocebus sabaeus,* the interpolated fitted site scores from distance-based redundancy analyses suggest limited female historical or contemporary gene flow between Dulombi National Park and the remaining sampling areas potentially due to the presence of the Corubal River. Although *Chl. sabaeus* can swim, this behavior seems to be restricted to small distances and shallow waters (Rowe & Myers, 2016) and they may not be able to traverse the Corubal River near Cufada Lagoons Natural Park where it can reach over 1 km in width. The two haplogroups found for *Chl. sabaeus* overlap in Cufada Lagoon Natural Park and Boé National Park (both located in the southern margin of the Corubal River), which suggests a

degree of female historical or contemporary gene flow between these localities (Di Fiore, 2003; e.g., Minhós et al., 2013a; Ferreira da Silva et al., 2014; Kopp et al., 2014). This pattern matches descriptions of females occasionally dispersing in *Chl. sabaeus* (Rowe & Myers, 2016). Our observations at a smaller scale in Guinea–Bissau for *Chl. sabaeus* are similar to the pattern of mtDNA variation in other Cercopithecinae species with female biased dispersal. For example, mtDNA variation of *Papio papio* across its West African range, is characterized by i) haplotype clusters not associated with the geographic origin of samples and which are shared by many distant demes (in some cases, over 500 km), and ii) closely-located demes which harbor very divergent haplotypes (e.g., 16 nucleotide substitutions between haplotypes all sampled in the Republic of Guinea, Kopp et al., 2014). Another female-biased dispersal species in Guinea–Bissau, *Pan troglodytes verus*, shows a similar pattern of mtDNA variation in which Guinea–Bissau populations share haplotypes with populations at Nimba mountains, located over 800 km apart (Sá, 2013).

Our results suggest the presence of two significantly divergent haplogroups for *Cercopithecus campbelli* and *Chlorocebus sabaeus* in Guinea–Bissau, with the estimated lineage divergence within the early Pleistocene (2.58–0.77 Mya; Cohen et al., 2013) and within the timeframe of intraspecific diversification events of many guenon species (1.9–0.1 Mya 95% HPD; Guschanski et al., 2013). The existence of genetically divergent haplogroups in a relatively small area, such as Southern Guinea–Bissau, agrees with the pattern of divergent mitochondrial haplotypes/haplogroups reported for *Papio papio* (Ferreira da Silva et al., 2014 and *Pan troglodytes verus* (Sá, 2013) in the country. Moreover, the spatial distribution of the mtDNA differentiation we found for *C. campbelli* and *Chl. sabaeus* follows the same axis of differentiation between coastal (Cufada Lagoons National Park and Cantanhez Forest National Park) and interior areas (Dulombi National Park and Boé National Park) as in these other primate species. Similar mtDNA diversity levels, spatial structure, and significant divergence between sampled haplogroups across several of the country's primate species could be related to the relatively central position of the country in the distribution of species in West Africa (the center–periphery hypothesis; Pironon et al., 2015). Alternatively, Guinea–Bissau primates may have gone through intra-species vicariant and demographic events thousands of generations in the past (Haus et al., 2013; Zinner et al., 2009). For example, phylogeographic patterns in West African primates (e.g., *Papio* sp., Zinner et al., 2009); *Chlorocebus* sp., Haus et al., 2013; Dolotovskaya et al., 2017), and other mammals (Bertola et al., 2016) have been explained by shifts in the main sub-Saharan biomes due to Pleistocene climatic fluctuations, often accompanied by recurring periods of population range retraction and re-colonization. Guinea–Bissau is at the center of the Fouta Djallon-Casamance differentiation region (Oates et al., 2011). This region likely contained pockets of gallery forest, which could have promoted the differentiation of genetic lineages that later came into contact in Guinea–Bissau after range re-expansion (Oates et al., 2011).

While our sampling scheme was executed at the level of the protected area, the sample distribution encompasses most of the known range of the species (Fig. 1). As such, the patterns of mtDNA variation we observed for *Cercopithecus campbelli* and *Chlorocebus sabaeus* across the sampled protected areas likely represent the general pattern within the country. The lack of geo-referenced data from other West African

populations limits our ability to extrapolate our local mtDNA diversity findings to broader range-wide patterns. Furthermore, our study is limited by the small size of the mitochondrial DNA fragments we amplified (628–640 bp concatenated size), which results in large 95% HPD intervals for the estimated divergence times between the haplogroups sampled. Nevertheless, the estimated 95% HPD intervals across our estimated phylogeny are within the divergence timeframes estimated using complete mitochondrial genomes (Guschanski et al., 2013; Jensen et al., 2023).

Implications for primate conservation and future perspectives

Broad-scale and short-fragment mtDNA databases from non-invasive sampling for multiple populations of co-distributed primates can be a useful tool to obtain updated occurrence data and contribute to improving the effectiveness of conservation actions. Among other factors, conservation areas can be prioritized using levels of inter and intraspecific genetic diversity (Carvalho et al., 2017). Our results suggest that Guinea–Bissau harbors high mitochondrial genetic diversity and multiple mitochondrial lineages for *Cercopithecus campbelli* and *Chlorocebus sabaeu*s These results suggest that Guinea–Bissau could be considered a priority for primate conservation in West Africa since its local populations may contribute significantly to the maintenance of the evolutionary potential of extant primate species (Carvalho et al., 2017). Nevertheless, using short mitochondrial fragments as genetic markers has limitations. The rate of evolution of the mitochondrial D-loop (humans, 2.4×10^{-7} substitutions/site/year; Santos et al., 2005) falls short of what is required for assessing recent and subtle changes in the genetic diversity and fine-scale patterns of population structure (Freeland, 2020), which are expected to be related to the impacts of human activities during the last two to three primate generations. Further studies aiming to improve our understanding of the effects of recent anthropogenic impacts on these primate populations should include genetic markers with higher evolution rates such as autosomal microsatellites ($10x^{-6}$–$10x^{-2}$ mutations/locus/generation), or sequencing of polymorphic positions using next-generation sequencing technologies (Freeland, 2020), which coupled with sequencing of the whole mitochondrial genome would also provide a more accurate estimation of demographic parameters of conservation interest (e.g., Ferreira da Silva et al., 2024b).

In Guinea–Bissau, *Cercopithecus campbelli* and *Chlorocebus sabaeus* were the most hunted and traded primates in urban wild meat markets and dedicated bars (Ferreira da Silva et al., 2021; Minhós et al., 2013b). The impacts of current hunting activities on populations of these two guenons are unknown, but could resemble those proposed for other generalist primates that are hunted in Guinea–Bissau: i) shifts in habitat use and occupancy, ii) increased dispersal distances leading to secondary contact between divergent genetic lineages, iii) preferential movements towards areas where hunting is less frequent causing restriction of gene flow between populations, and iv) low genetic diversity and reduction of effective population sizes (Minhós et al., 2013a; Ferreira da Silva et al., 2014; Minhós et al., 2016; Ferreira da

Silva et al., 2018; Minhós & Ferreira da Silva., 2020; Bersacola et al., 2021; Minhós et al., 2023). Furthermore, the insular populations of *C. campbelli* and *Chl sabaeus* show the lowest levels of mtDNA genetic diversity in the country. The long-term survival of insular primates could be threatened by the ongoing high rate of habitat conversion into agricultural areas and wild meat hunting on the islands (Colmonero-Costeira et al., 2023; Karibuhoye, 2004). Further studies should determine whether recent hunting or deforestation have severely decreased populations in areas previously reported as part of the species' national range but not sampled here, particularly in the northeast (Cacheu Mangroves Natural Park) and northwest parts of the country (Gippoliti & Dell'Omo, 2003), where surveys are urgent.

Inclusion and Diversity statement

This work includes Bissau-Guinean authors who provided invaluable contributions to the sample design and collection.

Abbreviations IUCN: International Union for Conservation of Nature; mtDNA: Mitochondrial DNA; PCR: Polymerase Chain Reaction; Cytb: Cytochrome b gene; HVRI: Hypervariable region I of the mitochondrial D-loop; BLAST: Basic Local Alignment Search Tool; NCBI: National Center for Biotechnology Information database; IBD: Isolation by distance

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Authors contribution MJFS, FG, IAP, MD and NF collected samples and presence data across Guinea-Bissau. IAP, FB, MC, FG and ICC conducted molecular work or contributed to obtaining genetic data. ICC performed data statistical analyses. MJFS and TM designed the study and contributed to funding acquisition. All authors contributed to the writing of the original draft and to review & editing the final manuscript.

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