

Title page

Title: Estimating the effective population size across space and time in the Critically Endangered western chimpanzee in Guinea-Bissau: challenges and implications for conservation management

Running Title:

N_e of western chimpanzees in Guinea-Bissau

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1 **Acknowledgments**

2 We dedicate this chapter to the memory of Michael W Bruford - our mentor,
3 colleague and friend. His enthusiasm, guidance, and support were key to the success of this
4 work and to advance the knowledge on conservation genetics of Guinea-Bissau primates.
5 We would like to acknowledge the Guinea-Bissau governmental agency Instituto de
6 Biodiversidade e Áreas Protegidas (IBAP), namely to the former director Dr. Alfredo Silva
7 and Dr. Justino Biai, and to the directors of protected areas and staff members - Dr. Abilio
8 Said, Dr. Augusto Cá, Dr. Joãozinho Mané and Dr. Sadjo Danfa, for fieldwork and sampling
9 permits and to Abel Vieira, Iaia Cassama, Benjamin Indequé, Braima Bemba Canté for the
10 support in fieldwork logistics. We acknowledge the Direcção Geral de Florestas e Fauna
11 (DGFF) and CITES focal person in GB for samples exportation permits; to the research
12 assistants and guides Sadjo Camará, Mamadu Soares, Mamadu Turé, Idrissa Camará; to the
13 NGO CHIMBO for logistical support for carrying out fieldwork in the Boé region; to I.
14 Espinosa and H. Foito for logistical support in Bissau. We thank Dr. Pedro Melo (vetnatura)
15 and the embassy of the European Union in Bissau for the help in collecting blood samples
16 from chimpanzees. We are grateful to Lara Almeida for facilitating the blood samples of the
17 chimpanzee Simão. This research was funded by Fundação para a Ciência e Tecnologia
18 through the project PRIMATOMICS (PTDC/IVC-ANT/3058/2014), and by funders of the
19 PRIMACTION project (the Born Free Foundation, Chester Zoo Conservation Fund, Primate
20 Conservation Incorporated, Mohamed Bin Zayed (Project 232533027) and by sponsorship by
21 the following Portuguese private companies - CAROSI, Cápsulas do Norte, Camarc, JA-Rolhas
22 e Cápsulas). MJFS worked under an FCT contract
23 (<https://doi.org/10.54499/CEECIND/01937/2017/CP1423/CT0010>). I.C.C., F.B. and I.P. were
24 supported by FCT-doctoral fellowships (ICC:
25 <https://doi.org/10.54499/SFRH/BD/146509/2019>, F.B.: <https://doi.org/10.54499/2020.05839>
26 .BD; I.P.: <https://doi.org/10.54499/SFRH/BD/118444/2016> with
27 <https://doi.org/10.54499/COVID/BD/151758/2021>. M.C. was supported by a postdoctoral
28 research associate contract (BBSRC, BB/R015260/1). R.M.S. was funded under:
29 <https://doi.org/10.54499/DL57/2016/CP1456/CT0002> and UID/00713/2020. C.R.F. thanks
30 the support of CE3C through an assistant researcher contract (FCiência.ID contract #366)
31 and FCT for Portuguese National Funds attributed to CE3C within the projects

32 UIDB/00329/2020, UIDP/00329/2020, and LA/P/0121/2020, and FPUL for a contract of
33 invited assistant professor

34 **Ethics**

35 The manuscript has not been submitted elsewhere. The research complied with
36 ethical guidelines, rules and protocols approved by IBAP and CIBIO-InBIO and adhered to the
37 legal requirements of Guinea-Bissau (GB) and Portugal. All except five samples were
38 obtained non-invasively from unidentified individuals without manipulation or perturbation
39 of their daily behavior. Invasive samples (tissue and blood) were collected opportunistically
40 from animals already deceased (tissue) or collected during health check to individuals living
41 in captivity, by a certified veterinarian (Dr. P. Melo). The blood collection was approved by
42 IBAP. GB CITES focal point (Direção Geral de Florestas e Fauna) authorized collection and
43 exportation of blood and tissue samples. IBAP authorized collection of fecal samples in
44 protected areas and transportation to Portugal. ICNF Portugal (Instituto para a Conservação
45 da Natureza e Florestas) and DGV (Direção Geral de Veterinária) authorized importation of
46 blood and fecal samples (Import Permits Simão 19PTLX00367, Tissue sample run-over
47 individual N.º/ No 18PTLX005871 and Bo, Bella and Emilia blood samples 17-PT-LX00392/I).

48 **Informed Consent**

49 Non-Applicable

50 **Data availability statement**

51
52 The data that supports the findings of this study are available in Dryad Digital Repository.

53

54 **Funding statement**

55 This research was funded by Fundação para a Ciência e Tecnologia (FCT) through the project
56 PRIMATOMICS (PTDC/IVC-ANT/3058/2014), and by funders of the PRIMACTION project (the
57 Born Free Foundation, Chester Zoo Conservation Fund, Primate Conservation Incorporated,
58 Mohamed Bin Zayed (Project 232533027), and by sponsorship by the following Portuguese
59 private - CAROSI, Cápsulas do Norte, Camarc, JA-Rolhas e Cápsulas). MJFS worked under an
60 FCT contract (<https://doi.org/10.54499/CEECIND/01937/2017/CP1423/CT0010>). (ICC:

61 <https://doi.org/10.54499/SFRH/BD/146509/2019>, F.B.:
62 <https://doi.org/10.54499/2020.05839.BD>,
63 I.P. <https://doi.org/10.54499/SFRH/BD/118444/2016> with
64 <https://doi.org/10.54499/COVID/BD/151758/2021>. Rui M. Sá was funded under:
65 <https://doi.org/10.54499/DL57/2016/CP1456/CT0002> and UID/00713/2020. M.C. was
66 supported by a postdoctoral research associate contract (CryoArks project, Biotechnology
67 and Biological Sciences Research Council, BB/R015260/1). C.R.F. was supported by an
68 assistant researcher contract (FCiência.ID contract #366), FCT projects UIDB/00329/2020,
69 and LA/P/0121/2020, and FPUL for a contract of invited assistant professor.

70

71 **Conflict of Interest**

72

73 The authors declare that they have no known competing financial interests or personal
74 relationships that could have appeared to influence the work reported in this paper.

75

76 **Compliance with International Conventions and Regulations on Biological Diversity and** 77 **Endangered Species**

78 We declare that this work complies with the Convention on Biological Diversity and
79 the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CBD
80 and CITES). Within the CBD, we followed the Access to Benefit Sharing (ABS) guidelines. We
81 give credit and equal access to benefits to the countries involved in the study (Guinea-
82 Bissau, Portugal and the UK) and the respective academic institutions and scientists involved
83 in the collection and analysis of data, who are co-authors to this work. We declare that we
84 complied with CITES regulations and obtained export and import permits to move samples
85 from Guinea-Bissau to Portugal for analyses, following CITES guidelines.

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92 **Title:** Estimating the effective population size across space and time in the Critically
93 Endangered western chimpanzee in Guinea-Bissau: challenges and implications for
94 conservation management

95 **Abstract**

96 Effective population size (N_e) is a key concept in evolutionary and conservation
97 biology. The western chimpanzee (*Pan troglodytes verus*) is a Critically Endangered taxon. In
98 Guinea-Bissau, chimpanzees are mainly threatened by habitat loss, hunting and diseases.
99 Guinea-Bissau is considered a key area for its conservation. Genetic tools have not yet been
100 applied to inform management and no estimates of N_e have been obtained. In this study, we
101 use country's range-wide microsatellite data and five whole-genome sequences to estimate
102 several N_e and infer the recent and ancient demographic history of populations using
103 different methods. We also aim to integrate the different N_e estimates to improve our
104 understanding of the evolutionary history and current demography of this great ape and to
105 discuss strengths and limitations of each estimator and their complementarity in informing
106 conservation decisions. Results from the PSMC method suggest a large ancestral N_e , likely
107 due to ancient structure over the whole subspecies distribution until approximately 10-
108 15,000 years ago. After that, a change in connectivity, a real decrease in size or a
109 combination of both occurred, which reduced the then still large ancestral population to a
110 smaller size (MSVAR: ~10,000 decreasing to 1,000-6,000 individuals), possibly indicating a
111 fragmentation into coastal and inner subpopulations. In the most recent past, contemporary
112 N_e is below or close to 500 (GONE: 116-580, NeEstimator: 107-549), suggesting a high risk of
113 extinction. The populations at coastal Parks may have been small or isolated for several
114 generations whereas the Boé Park one exhibit higher long-term N_e estimates and can be
115 considered a stronghold of chimpanzee conservation. Through combining different types of
116 molecular markers and analytical methodologies, we try to overcome the limitations of
117 obtaining high quality DNA sampling from wild threatened populations and estimate N_e at
118 different temporal and spatial scales, which is crucial information to make informed
119 conservation decisions at local and regional scales.

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123 **Keywords:**

124 *Pan troglodytes verus*; Great Ape; Genetic Diversity, Demographic history, Microsatellite
125 loci, Whole-genome sequence, Anthropogenic landscapes

126

127 **1. Introduction**

128 The concept of effective population size (N_e) is central in evolutionary and
129 conservation biology and has important practical applications in conservation management
130 (Frankham et al., 2010, Hoban et al., 2022, Waples, 2022). N_e is considered as probably the
131 most important metric to understand and predict both the populations' short-term risk of
132 extinction by inbreeding depression and their long-term potential to adapt to environmental
133 changes (Hoban et al., 2020, Hoban et al., 2022). N_e is also one of the best-studied metrics
134 for applying minimal viable population thresholds and identifying populations of
135 conservation concern (Frankham, 2005, Jamieson & Allendorf, 2012, Frankham et al., 2014).
136 Moreover, N_e has many practical applications in wildlife management and conservation
137 planning, such as designing human-induced translocations and relocation of populations
138 (Luikart et al., 2010, O'Brien et al., 2022, Waples, 2022, Waples 2024). For instance, N_e has
139 been considered one of the four genetic *Essential Biodiversity Variables* (EBVs, summary
140 measures of biodiversity), which are designed to monitor changes in biodiversity over time
141 and space (Hoban et al., 2022).

142 N_e seems easy to understand and to compute using genetic data (Allendorf et al.,
143 2010). However, it is perhaps one of the most difficult and error-inducing concepts to grasp
144 in population genetics. One reason for this is that N_e is a single number that aims at
145 summarizing a usually highly complex situation, whether we are interested in the
146 demographic history or on the recent dynamics of a species (Chikhi et al., 2010, 2018,
147 Wakeley, 1999, Waples 2022). Furthermore, the estimation and practical integration of the
148 N_e parameter into conservation management and policies is advancing slowly even in
149 regions with high biodiversity and iconic and endangered species (Bertola et al., 2024), such
150 as great apes. This is related to a number of factors concerning feasibility and low financial
151 resources for population genetic studies to estimate N_e and a low reliability of results when

152 the estimation is carried out for non-model species not meeting the assumptions of
153 population genetic models (e.g., Bertola et al., 2024; Waples 2022).

154 The concept of N_e was introduced by Sewall Wright in 1931 (Wright, 1931). N_e
155 quantifies the rate of genetic change (e.g., drift of allele frequencies) of real populations in
156 reference to the Wright-Fisher (WF) idealized population (Wang et al., 2016). WF
157 populations are assumed to have equal sex ratio, constant size and non-overlapping
158 generations, no sexual or natural selection and in which genetic drift is considered the only
159 evolutionary force changing gene frequencies across generations together with mutations
160 (Conner & Hartl, 2004). N_e is thus the size of an idealized WF population with the same
161 properties of genetic drift as the real (more complex) population under consideration (Wang
162 et al., 2016).

163 While the concept seemed straightforward, it was later realized that one could
164 identify different types of N_e depending on the property of interest that N_e was supposed to
165 summarize (e.g., Ryman et al., 2019). For instance, one can define the N_e generating the
166 same rate of inbreeding as the real population of interest (*i.e.*, the probability that a pair of
167 homologous genes in an individual came from the same parent in the previous generation,
168 which was denoted as the inbreeding effective size, N_{ei}) or the N_e generating the same rate
169 of change in variance of gene frequencies (denoted as the variance effective size, N_{eV})
170 (Wang et al., 2016). More recently, the concept of coalescent N_e was also defined to identify
171 the N_e that can explain the patterns of diversity observed in present-day populations under
172 simple demographic models, typically assuming panmixia over long periods of time. This
173 concept has itself been extended by allowing N_e to change through time. Note that under
174 the latter case, there is not one N_e but rather a succession of N_e values, which may thus lead
175 to apparent contradictions between methods estimating one N_e and those estimating a
176 succession of N_e values. Under a standard constant-size WF model, all the different N_e
177 concepts are expected to be the same. However, this is not necessarily the case in real-
178 world situations, where populations are rarely panmictic or at mutation-drift equilibrium.
179 Real populations have likely gone through complex demographic histories involving
180 expansions and contractions, related to environmental changes and fragmentation of
181 habitats (Wang et al., 2016; Ryman et al., 2019). In addition, theoretical work suggests that
182 there may be demographic models for which some N_e cannot be defined (Sjödín et al.,

183 2005). The point we wish to make here is that depending on the research questions asked,
184 one may obtain very different answers. The fact that we obtain different values should be
185 seen as an indication that the species of interest may not be easily summarized by a single
186 N_e number, and that the different estimates obtained might all be useful for devising
187 conservation strategies that account for both the ongoing dynamics of the species but also
188 for its demographic history.

189 The western chimpanzee (*Pan troglodytes verus*, Schwarz, 1934) is one of the four
190 currently recognized subspecies of chimpanzees *P. troglodytes*. Its range extends from
191 Senegal in the west to Ghana in the east (Fig. 1a). The subspecies presently occurs in the
192 following eight West African countries: Côte d'Ivoire, Ghana, Guinea, Guinea-Bissau, Liberia,
193 Mali, Senegal and Sierra Leone, and has most likely disappeared from Benin, Burkina Faso,
194 and Togo (Campbell & Hounbedji, 2015, Ginn et al., 2013, IUCN SSC Primate Specialist
195 Group 2020). This subspecies has been classified as Critically Endangered by the
196 International Union for Conservation of Nature (IUCN) (Humble et al., 2016). The population
197 of *P. t. verus* is estimated to have decreased its abundance by 80% between 1990 and 2014
198 and to have reached a global size between 15,000 and 65,000 individuals (Kühl et al., 2017)
199 or 52,811 individuals (95% CI 17,577–96,564) as more recently estimated by Heinicke et al.
200 (2019a). The subspecies conservation status is expected to deteriorate in the next decades
201 considering that the great majority of the western chimpanzees currently live outside
202 protected areas and within 5 km of an infrastructure (Heinicke et al., 2019a), and it is
203 predicted high rates of deforestation until 2050 for its West African range (Palminteri et al.,
204 2018). Moreover, the western chimpanzee is threatened by hunting to supply the trade of
205 wild meat, live animals and body-parts, and by diseases (Humble et al., 2016; IUCN SSC
206 Primate Specialist Group 2020, Sá et al., 2012). The western chimpanzee has low genetic
207 diversity when compared to the other *P. troglodytes* subspecies, and two recent studies
208 have suggested that N_e could be in the order of 17,378 breeding individuals (de Manuel et
209 al., 2016; Fontseré et al., 2022), even if this number should be interpreted with care.

210 Guinea-Bissau (GB) (area: 36,125 km², population: 2,08 million) is an important
211 biodiversity hotspot holding populations of emblematic and threatened species, such as
212 leopard (*Panthera pardus*, Linnaeus, 1758), lion (*Panthera leo*, Linnaeus, 1758), elephant
213 (*Loxodonta cyclotis*, Matschie, 1900), saltwater hippopotamus (*Hippopotamus amphibius*,

214 Linnaeus, 1758), manatee (*Trichechus manatus*, Lineu, 1758) (Brugiere et al., 2005; Palma et
215 al., 2023), and ten confirmed primate species, including the western chimpanzee, colobus
216 monkeys (*Piliocolobus temminckii*, Kuhl, 1820 and *Colobus polykomos*, Zimmermann, 1780)
217 and the sooty mangabey (*Cercocebus atys*, Audebert, 1797) (Bersacola et al., 2018; Ferreira
218 da Silva et al., 2020, Minhós et al., 2023). Biodiversity conservation has been considered a
219 driver for economic development and consequently, a great effort has been made by
220 national agencies to formally protect a network of areas, which includes six Parks and three
221 ecological corridors in mainland Guinea-Bissau and the Bijagós archipelago (Fig. 1), covering
222 almost 26% of the country size (<https://ibapgbissau.org/areas-protegidas/>). Nevertheless,
223 national conservation management needs some important improvements, such as a
224 corrected list of the species occurring in the country, its present range (e.g., Ferreira da Silva
225 et al., 2020), and baseline estimates of demographic parameters of threatened species, such
226 as population size, to inform prioritization of areas to conserve.

227 The western chimpanzee (*Dari*, in Guinea-Bissau Creole) occurs in the southern part
228 of the country, mainly south of the Corubal River (Bersacola et al., 2018; Carvalho et al.,
229 2013) (Fig. 1b). Chimpanzees were erroneously declared extinct and were rediscovered in
230 the 1990s (Gippoliti & Dell’Omo 1996, 2003). In GB specifically, the main conservation
231 threats faced by the subspecies are habitat loss and fragmentation, hunting to supply the
232 trade of live individuals and retaliatory killing during crop-raiding (Hockings & Sousa, 2013).
233 Please note that the trade of body parts, such as skins and bones, for traditional medicine
234 practices is observed in the capital city markets, although the national origin of these
235 specimens has not been confirmed (Sá et al., 2012), and the trade and consumption of
236 chimpanzee meat does not seem to occur (Ferreira da Silva et al., 2021, Minhós et al., 2013,
237 van Laar 2010), contrary to what happens in other countries (e.g., Côte d’Ivoire; Caspary et
238 al., 2001). Chimpanzee meat in GB is considered non-edible by locals because this primate
239 species is considered to have high resemblance with humans (Amador et al., 2014, Gippoliti
240 & Dell’Omo 2003, Karibuhoye, 2004, Sousa et al., 2017). In contrast, trade of live infant
241 chimpanzees is a very frequent phenomenon (Ferreira da Silva & Regalla, under review,
242 Hockings & Sousa 2013) and may be a significant threat since its capture involves killing the
243 adults (Ferreira da Silva et al., 2021). In addition, chimpanzees appear to be threatened by
244 the propagation of diseases such as leprosy (*Mycobacterium leprae*), which has been

245 detected in several communities of Cantanhez National Park (CNP, Fig. 1) (Hocking et al.,
246 2021). Past studies reporting a high prevalence of parasites shared with humans suggest
247 that habitat disturbance plays a role in the transmission and persistence of pathogens (Sá et
248 al., 2013). GB is an important area in West Africa for the conservation of *P. t. verus*.
249 Specifically, i) the coastal areas of GB together with the ones in Republic of Guinea are
250 considered a priority region (Kormos & Boesch, 2003; IUCN SSC Primate Specialist Group
251 2020), ii) the protected areas of CNP, Dulombi National Park (DNP) and Boé National Park
252 (BNP) (Fig. 1) are considered areas of high value for conservation (Heinicke et al., 2019a),
253 and iii) the Boé region, in the inner part of the country, is one of eight sites across the
254 subspecies distribution that is classified as exceptionally stable or of high-density (Heinicke
255 et al., 2019b).

256 Historically, the overall population in the country has been suggested to be between
257 600 and 1,000 (Gippoliti & Dell’Omo, 2003) and more recently estimated as 1,908
258 individuals (95% confidence interval: 923–6,121 individuals Heinicke et al., 2019a).
259 Improved representative surveys have been recommended for GB given the large
260 confidence intervals of estimates (Heinicke et al., 2019a). The size of local populations has
261 been evaluated for most of the protected areas where chimpanzees occur using various
262 indirect methods (Table 1). Although the estimates from the different studies cannot be
263 compared directly, Cufada Lagoons Natural Park (CLNP) is highlighted as the population with
264 the lowest density, whereas the Boé region stands out as the one displaying the topmost
265 density (reaching > 6 individuals per km², Table 1). Currently, there is no size or density
266 assessment for DNP or for other populations outside areas with formal protection, such as
267 ecological corridors (but see the exception of Gandamael, Table 1, Fig. 1b), although it is
268 estimated that approximately 35% of chimpanzees live outside a park in GB (Heinicke et al.,
269 2019a). Genetic tools have not yet been applied to inform the conservation of the western
270 chimpanzees in GB. Little is known about the genetic diversity or the amount of genetic
271 isolation between populations (but see Borges, 2017 and Gerini, 2018) and no estimates of
272 N_e have been obtained to date.

273 In this study, we use geographically broad genetic data and genomic data from
274 multiple wild-born individuals to estimate the N_e of the western chimpanzee population in
275 GB. We aim to i) estimate N_e and infer the recent and ancient demographic history of

276 populations using different methods as applied to microsatellite loci and whole-genome
277 sequence data (WGS), ii) integrate the different estimates to improve our understanding of
278 both the evolutionary history and current demography of the western chimpanzees, iii)
279 discuss the strengths and limitations of each N_e estimator method and their
280 complementarity in informing conservation decisions for long-lived organisms, and iv)
281 discuss the implications of the results for the conservation management of this emblematic
282 species in GB.

283 **2. Materials and methods**

284 **2.1 Study area**

285 The study area covers a large proportion of the chimpanzee range in GB (Gippoliti &
286 Dell'Omo 2003) (Fig. 1b), encompassing an area of approximately 6,000 km². Sampling of
287 biological material was carried out in four geographically-distinct and formally protected
288 areas – 1. Cantanhez National Park (CNP, 1,067.67 km²), 2. Cufada Lagoons Natural Park
289 (CLNP, 890 km²), 3. Dulombi Natural Park (DNP, 1,600.96 km²) and 4. Boé National Park
290 (BNP, 1,552.95 km²) (<https://ibapgbissau.org/areas-protegidas/>). Chimpanzees were known
291 to be present at these areas prior to our study (Gippoliti & Dell'Omo 2003; Bersacola et al.,
292 2018).

293 **2.2 Microsatellite loci dataset**

294 We generated a dataset of 143 unique genotypes for 10 microsatellite loci derived
295 from non-invasive fecal samples (Borges, 2017, Gerini, 2018) (Fig. 1b). Eighty-five genotypes
296 correspond to samples collected between 2015 and 2017 in CLNP (N=38), BNP (N=34), and
297 DNP (N=13) and the remaining consisted of previously determined genotypes from CNP
298 (N=58) (Sá, 2013) (Fig. 1b). Fecal samples were collected fresh and from unhabituated and
299 unidentified individuals, in sites used by chimpanzee groups for sleeping, foraging and
300 drinking. The techniques and methods to preserve the fecal samples until DNA extraction
301 are described in Ferreira da Silva et al. (2014). DNA extraction was carried out using two
302 methods: i) the QIAamp[®]DNA Stool Mini Kit (QIAGEN[®]) at MWB research group laboratory
303 facilities at School of Biosciences, Cardiff University, UK (Sá, 2013) and ii) the CTAB method
304 (Vallet et al., 2008, adapted by Quéméré et al. 2010) for samples collected between 2015-
305 2017, which were extracted at *Instituto Gulbenkian de Ciência* (IGC, Oeiras, Portugal)

306 laboratory facilities. The procedures to avoid contamination by exogenous DNA are
307 described elsewhere (Ferreira da Silva et al., 2014). DNA samples were identified to the
308 species level using a mitochondrial DNA hypervariable region I fragment (approximately 600
309 base pairs, using primers L15926 and H16555, as described in Sá, 2013). Consensus
310 sequences were derived from forward and reverse sequencing by visual comparison using
311 Geneious Pro v.4.8.5 (Biomatters, Biomatters Ltd, New Zealand). Standard Nucleotide BLAST
312 in NCBI (<http://www.ncbi.nlm.nih.gov/>) was used to identify accessions closely related to
313 the generated sequences and confirm that samples were from *P. troglodytes verus* (i.e.,
314 GenBank Accession code D38113). Allele size standardization between datasets was carried
315 out using re-extraction and re-analyses of DNA extracts of five samples included in Sá (2013)
316 together with the novel samples analyzed in Borges (2017) and Gerini (2018). Allele scoring
317 followed previously described procedures to guarantee minimal impact of allelic dropout
318 and false alleles errors: four replicates were carried out per sample and the rules to reach a
319 consensus genotype were determined per locus (Ferreira da Silva et al., 2014). The
320 consensus genotype was classified according to the Quality Index (QI, Miquel et al., 2006),
321 and genotypes with a mean across loci below 0.55 were excluded from the dataset. The
322 probability of identity (PI) and the probability of identity between siblings (PIsibs) (Waits et
323 al., 2001), estimated using GenAIEx v.6.503 (Peakall & Smouse, 2006), was of 1.5×10^{-11} and
324 8.9×10^{-05} , respectively, which in principle allows to distinguish between unique genotypes
325 using six loci. We could not find genotyping errors (typing errors, large-allele dropout, and
326 locus-specific deficiency in heterozygotes due to null alleles) using MicroChecker v.2.2.3
327 (van Oosterhout et al., 2006) apart from locus D2S1326 for CLNP, which showed excess of
328 homozygotes. We retained the locus in the final dataset as we found no significant
329 departures from Hardy-Weinberg equilibrium per locus using the Bonferroni correction
330 when geographic populations were analyzed separately. The population of chimpanzees in
331 GB does not display significant population structure when assessed using individual-based
332 Bayesian algorithms (e.g., STRUCTURE) (Borges, 2017 estimated $K=1$).

333 **2.3 Genomic data**

334 2.3.1 Sampling

335 Whole-genome sequences were produced from biological material collected from
336 wild born chimpanzees: one road-killed (tissue sample T3-Chimp collected in 2011) and four
337 individuals (blood samples from Bo, Bella, Simão and Emilia chimpanzees, collected between
338 2018 and 2019) confiscated by the Institute for Biodiversity and Protected Areas (IBAP) from
339 private premises. We obtained the information that the individuals were caught by hunters
340 in different sites within GB (*e.g.*, Bo was originally from CNP, Bella was confiscated in Quebo
341 but probably originated from CNP, Emilia was from DNP, and Simão was living in Bafatá but
342 traded in Quebo, Fig. 1). Blood was collected as part of the placement of the individuals in a
343 sanctuary abroad (Sweetwaters Chimpanzee Sanctuary, Ol Pejeta, Kenya, Ferreira da Silva &
344 Regalla, in review³). The blood samples were drawn by a wildlife veterinarian (P. Melo,
345 *vet_natura*, <https://www.vetnatura.pt/>) for health screening and as part of a parasites and
346 virus detection procedure prior to translocation (Melo et al., 2018). Samples were collected
347 in 5 mL collection tubes filled up with the anticoagulant ethylenediamine tetraacetic acid
348 (EDTA) and preserved fresh until DNA extraction. The road-killed individual was found in the
349 road next to CLNP (Fig. 1b) and a sample of muscle tissue was collected and preserved in
350 98% ethanol up to DNA extraction.

351 2.3.2 DNA extraction and data production

352 DNA was extracted from the five samples adapting the method by Vallet et al.,
353 (2008). We used 500 µL of each blood sample and about 10 mg of tissue from the road-
354 killed individual. The details of this two-day DNA extraction protocol can be found in
355 Supplementary material S1. We tested the quality of DNA extractions in 2% agarose gels and
356 quantified DNA concentration using a Nanodrop microvolume spectrophotometer
357 (ThermoFisher Scientific) (Supplementary material S2). Laboratory procedures took place at
358 the IGC, and extractions were carried out in a biological safety cabinet in a Biosafety Level 2
359 dedicated room. Library preparation and sequencing were performed by Macrogen at a
360 coverage of 30-15x using the Illumina Hiseq X and TruSeq platforms.

³ See documentary about rescuing chimpanzees to Ol Pejeta sanctuary, Kenya,
<https://www.youtube.com/watch?v=GxXMk2UPvUM>.

361 2.3.3 Whole-genome sequence (WGS) data assembly, Mapping and Genotype Calling

362 After all samples passed quality control tests, we used the BAM pipeline from
363 PALEOMIX to process the sequences for downstream analysis at the Globe Institute's
364 (University of Copenhagen, Denmark) High-Performance Computing (HPC) cluster. This
365 pipeline trims adapter sequences, filters low quality reads, removes PCR duplicates, and
366 aligns reads and maps them to a reference genome (Schubert et al., 2014). We used a
367 "makefile" (.yaml file), that allows the specification of the tasks to be performed, BWA as
368 the aligner software and the algorithm "mem". The BWA-mem algorithm shows great
369 performance with sequencing errors and is most adequate for short reads, as it is the case
370 of this study (Li, 2013). The "MinQuality" parameter was used to exclude reads with a
371 mapping quality (or Phred score) below zero (Schubert et al., 2014).

372 For the genotype calling, we first selected variants with a minimum Phred quality
373 score of 20, using the HaplotypeCaller algorithm in GATK (version 4.2.0.0; Poplin et al.,
374 2017). HaplotypeCaller uses the input data to calculate the likelihood of each genotype per
375 sample, and then assigns the most likely genotype to that sample. Through the application
376 of the 'SelectVariants', only sites with SNPs were selected. Lastly, *vcftools* (Danecek et al.,
377 2011) was used to remove indels, namely the sites with a missing proportion higher than
378 0.9, a Phred quality score equal to or lower than 30, and genotypes with depth values lower
379 than 5 and higher than 100. A table describing the WGS data summary statistics for each
380 sample, such as coverage, observed number of homozygous genotypes, expected number of
381 homozygous genotypes and inbreeding coefficient (F), can be found in Supplementary
382 material S3.

383 As a quick assessment of the possible presence of genetic structure among the
384 sampled individuals, we performed an analysis with the STRUCTURE software version 2.3.4
385 (Pritchard et al., 2000) using the admixture model and assuming correlated allele
386 frequencies. The parameter set consisted of a burnin period of 50,000 steps, followed by
387 200,000 iterations, and 10 runs for each number of clusters (Van Wyngaarden et al., 2017).
388 The results indicated evidence for a single panmictic cluster ($K=1$) as the best clustering
389 solution to explain the observed genetic variation across individuals, *i.e.* absence of genetic
390 structure (Silva, 2024).

391 **2.4 Effective population size estimation and demographic history**

392 2.4.1 The PSMC (pairwise sequentially Markovian coalescent) and the IICR: principles of 393 demographic inference

394 The PSMC method of Li and Durbin (2011) was applied to the nuclear genomes of
395 the five individuals for which tissue and blood samples were obtained. The PSMC uses the
396 information from the distribution of heterozygous sites along the genome of a single diploid
397 individual (or two haploid genomes) and produces a curve where the x-axis represents time
398 usually represented in a log-scale, and the y-axis is often interpreted as representing the
399 effective population size. Proper scaling of the PSMC in years requires the use of estimates
400 of generation time, mutation, and recombination rates.

401 PSMC version 0.6.5-r67 (Li & Durbin, 2011) (available at
402 <http://github.com/lh3/psmc>) was run on each individual genome using the following
403 settings: `-N25 -t15 -r5 -p "4+25*2+4+6"`. Individual consensus sequences were generated
404 using the *mpileup*, *bcftools* and *vcfutils.pl* (*vcf2fq*) pipeline from SAMTOOLS v. 1.16, with
405 minimum read depth (-d) set to five and maximum read depth (-D) set to 30. The consensus
406 sequence was converted into a fasta-like format using the *fq2psmcfa* program, provided in
407 the PSMC package, with the quality cut off (-q) set to 20. We assumed a mutation rate (μ) of
408 1.2×10^{-8} per base pair per generation and a generation time of 25 years (Venn et al., 2014;
409 Besenbacher et al., 2019; Chintalapati & Moorjani, 2020). To quantify the variance in PSMC
410 curves, we performed 10 bootstraps per individual, following the re-sampling protocol
411 suggested by the authors. The inferred demographic histories for the five analyzed
412 individuals were plotted in a single figure using Ghostscript 9.16 and Gnuplot 5.4.0. The
413 PSMC plots are usually interpreted in terms of N_e changes but can also be interpreted in
414 terms of connectivity changes (see Discussion).

415 2.4.2 MSVAR analysis of microsatellite loci data

416 We also used the Bayesian likelihood-based approach of Storz & Beaumont (2002),
417 as implemented in the MSVAR 1.3 software. This approach assumes a simple model of
418 exponential population size change (allowing for either growth or decline) from an ancient
419 population of size N_1 to a present-day population of size N_0 . In practice, the method uses a
420 Monte Carlo Markov chain algorithm to estimate the posterior probability distribution of N_0

421 and N_1 and of the time at which the population started to increase or decrease (T , in years,
422 assuming that a generation time is given), and the *per* locus mutation rate (μ). We
423 conducted four independent runs with different initial values and varying sets of priors and
424 hyperpriors to reflect assumptions of either constant population size ($N_0 = N_1$), population
425 decline ($N_0 < N_1$), or population growth ($N_0 > N_1$) and therefore control for the impact of the
426 priors on the posterior distributions (Supplementary material, Table S4).

427 Analyses were run using a series of datasets, to discard the possibility that the
428 presence of related individuals, the sampling scheme and genetic structure could impact the
429 inferred demographic histories, and assuming a generational span of 25 years (following
430 Langergraber et al., 2012). We aimed to recover the demographic history of western
431 chimpanzees by analyzing samples from i) GB as one population ($N = 143$ genotypes), ii) by
432 park (CNP $N = 58$, CLNP $N = 38$, DNP $N = 13$ and BNP $N = 34$); iii) a dataset formed by
433 unrelated individuals ($N = 121$, see below how relatedness was estimated); and iv) five
434 “random datasets” obtained by randomly selecting 58 samples, which correspond to the
435 largest dataset from a single area (*i.e.*, CNP). Datasets iii and iv were used to test the
436 influence of the presence of highly related individuals and differences in sample sizes across
437 datasets in demographic estimates, respectively. Analyses were also run for each geographic
438 population since coalescent theory predicts that when populations are structured, samples
439 obtained from one population will tend to exhibit signals of bottlenecks, whereas samples
440 obtained across many demes will tend to have a much weaker bottleneck signal (Beaumont,
441 2004, Wakeley, 1999), as shown on simulated data (Chikhi et al., 2010).

442 To estimate relatedness between individuals, we calculated the correlation
443 coefficient between the observed and simulated values of relatedness (100 pairs) for the
444 Milligan (2003) and Wang (2007) likelihood estimators using the *related* R package (Pew et
445 al., 2015). We also estimated relatedness between pairs of individuals in the overall dataset
446 and per protected area (CNP, CLNP, DNP, and BNP) using the ML-Relate likelihood method
447 (Kalinowski et al., 2006). We performed 100,000 simulations to identify dyads with a likely
448 relationship of Parent-Offspring or Full-Siblings and $r > 0.5$ significantly different ($p < 0.05$)
449 from dyads with a likely relationship of Half-Siblings and Unrelated. For dyads identified

450 using the full dataset and the protected area dataset, one genotype of the dyad, the one
451 which displayed lower QI, was removed from the dataset.

452 The individuals present in the five “random databases” were selected from the
453 dataset i) using *runif* function in R.

454 Each run in MSVAR included 300,000 thinning update steps and 30,000 thinning
455 intervals, totaling 9×10^9 steps. We discarded the first 10% of each simulation to eliminate
456 the influence of initial conditions on parameter estimation (burn-in). We verified
457 convergence between runs both visually and using Brooks, Gelman, and Rubin Convergence
458 Diagnostic test (Gelman & Rubin, 1992; Brooks & Gelman, 1998) conducted in R version
459 2.11.1 (R Development Core Team 2010) using the package BOA version 1.1.7 (Smith, 2007).

460 2.4.3 Linkage disequilibrium-based estimation of current N_e and recent demographic history
461 from genomic data

462 We used GONE (Genetic Optimization for N_e Estimation)
463 (<https://github.com/esrud/GONE>) that implements a genetic algorithm to infer the recent
464 demographic history of a population from single nucleotide polymorphism (SNP) data of one
465 contemporary sample (Santiago et al., 2020). The method can infer the demographic history
466 of a population within the past few hundreds of generations, with the authors stressing that
467 the greatest reliability and resolution is for the last 100 generations (Santiago et al., 2020). It
468 uses the observed spectrum of linkage disequilibrium (LD) between pairs of loci over a wide
469 range of genetic distances (implicit recombination rates) and has been validated by
470 simulation under different demographic scenarios and for small sample sizes (*i.e.*, $n=10$,
471 Santiago et al., 2020). These simulations suggest that even when GONE is not able to
472 accurately infer the recent N_e trajectory, it can estimate the current N_e relatively well. The
473 simulations also suggested that not considering LD data between more distant loci (*e.g.*,
474 with scaled recombination rates > 0.05) in the analyses allows for better estimates of N_e ,
475 particularly when sample sizes are small or when there is population structure and
476 migration rates between subpopulations are low (Santiago et al., 2020). Thus, the authors of
477 the method recommend using a maximum recombination rate of 0.05, this being the default
478 value of this parameter in GONE. Accordingly, we used this value and default settings for all
479 other software parameters, such as no minimum allele frequency cutoff and 40

480 independent replicate runs, with the N_e point estimate for each generation being the
481 geometric mean of the values of the replicates. Analyses were performed using 57,650
482 genome-wide autosomal SNPs with genetic map information. As suggested in Santiago et al.
483 (2020), we obtained an empirical 95% confidence interval by running GONE on 20 random
484 replicates of 57.5K SNPs sampled from the whole-genome sequences. Replicates were
485 generated by variant thinning in PLINK 1.9 (www.cog-genomics.org/plink/1.9/) (Chang et al.,
486 2015).

487 2.4.4 Linkage disequilibrium estimation of contemporary N_e from microsatellite data

488 We used NeEstimator 2.1 (Do et al., 2014) to estimate contemporary N_e from
489 microsatellite loci data using the bias-corrected version of the method based on LD (Hill,
490 1981; Waples, 2006; Waples & Do, 2010), and assuming random mating. The method is
491 robust to equilibrium migration rates up to 10% at lower population sizes (Waples &
492 England, 2011; Gilbert & Whitlock, 2015). We performed analyses both for the whole
493 dataset and separately for each of the four protected areas. The software estimates
494 confidence intervals (CIs), both parametric and based on jackknifing over individuals (Jones
495 et al., 2016), which accounts for the fact that overlapping pairs of loci are being compared
496 and implements a method to correct for possible biases due to missing data (Peel et al.,
497 2013). In any case, for each of the five datasets, we also performed analyses removing loci
498 with more than 10% missing data. When analyzing each dataset, and depending on its
499 sample size, we used a minimum allele frequency (MAF), the Pcrit value, following the
500 recommendations in Waples & Do (2010).

501 **3. Results**

502 **3.1 Effective population size estimation and demographic history**

503 3.1.1 The PSMC (pairwise sequentially Markovian coalescent) and the IICR

504 The PSMC curves exhibited a series of increases and decreases with the last decrease
505 starting around 200 kya, and the previous one starting at around one million years ago
506 (Figure 2a). These curves can be interpreted in terms of changes in N_e , assuming panmixia
507 and no population structure, in terms of changes in connectivity under population structure
508 (Steux et al., 2024) and constant size or as a combination of both types of changes.
509 Altogether they suggest either very large populations in the past that have been significantly

510 reducing for the last 200 ky or are the result of a metapopulation characterized by changes
511 in connectivity with no obvious population decrease during that period. The results for the
512 10 bootstrap replicates for each individual were highly consistent (Supplementary material,
513 Figure S5). One individual (sample T3_chimp) exhibited a PSMC curve that followed the
514 shape of the other curves but was flatter and shifted downward and towards more recent
515 times, a pattern that has been described for individuals having a lower coverage
516 (Nadachowska et al., 2016, see Discussion).

517 3.1.2 MSVAR analysis of microsatellite data

518 The analyses we conducted with the control datasets (unrelated and “random
519 datasets”) did not provide results significantly different from the demographic scenarios
520 obtained for the complete datasets of GB and the parks, hence suggesting that relatedness
521 and sample size did not significantly affect the results.

522 From the whole dataset ($N = 143$ genotypes), MSVAR estimated a contemporary N_e
523 (N_0) between 4,500 and 6,500 individuals, that resulted from a mild bottleneck starting
524 about 44,500-70,000 years ago from a more ancient N_e around 10,000-12,000 (N_1) (Fig. 2b).
525 To account for the possible effects of genetic structure, we also carried out analyses
526 separately for each park (Fig. 2e to 2h). For CNP, the southernmost chimpanzee population
527 of GB, MSVAR identified a stronger signal with estimates of N_0 between approximately 500
528 and 1,125 individuals, whereas N_1 estimates were between 10,000 to 12,000 breeding
529 individuals, with limited overlap between the N_0 and N_1 median posterior distributions and a
530 posterior distribution of N_0/N_1 that was consistently below zero (Fig. 2e, Supplementary
531 material, Table S5). MSVAR estimated that this demographic decrease of the CNP
532 chimpanzee population occurred around 5,000 and 12,500 years ago under the assumption
533 of panmixia (Table 2).

534 We found a similar demographic scenario for the CLNP chimpanzee population,
535 which, like the CNP population, is also located in the coastal area and is geographically the
536 closest to CNP (Fig. 2f). The CLNP chimpanzee population was also found to have undergone
537 a one-order-of-magnitude bottleneck, with very similar values of N_0 and N_1 to the inferred
538 scenario at CNP (Table 2). The putative difference between the demographic histories of the
539 two coastal populations is the fact that the demographic decrease may have occurred later

540 in CLNP than in CNP (CLNP: 3,612 – 7,874 years ago), but the inferred posterior distributions
541 of T for both parks still overlap considerably (Table 2, Fig. 2e and Fig. 2f). The bottleneck
542 signal of both populations is also confirmed by the N_0/N_1 , which in both cases is below 0,
543 across the four simulated scenarios (Fig. 2e and Fig. 2f).

544 As for the chimpanzee populations from the eastern region, at DNP and BNP, we
545 found different demographic histories (Fig. 2g and Fig. 2h). Not only are the current N_e much
546 larger but there is also no clear signal of demographic change for any of these regions. The
547 population from DNP is estimated to have a N_0 of between 2,769 and 7,401 individuals, with
548 a slightly larger estimated N_1 (Table 2). However, the variation in N_0 estimates across the
549 four different simulated scenarios does not allow for a clear signal of population size
550 change. This absence of a clear bottleneck signal is also supported by the variation of the
551 N_0/N_1 estimates and its overlap with zero (meaning no population size change), which is also
552 translated into weak convergence of the T posterior distributions (Fig. 2g). For the
553 easternmost chimpanzee population sampled in BNP, the inferred posterior distributions of
554 N_0 and N_1 are very consistent, which indicates a large and historically stable population in
555 this park (Fig. 2h). The estimated values for the N_0 indicate a population with a current
556 effective population size above 6,500 individuals (Table 2). The estimated posterior
557 distributions of N_1 extensively overlap with the N_0 estimates, as it is also clear from Fig. 2h
558 suggesting no population size changes. The scenario of a large and historically stable
559 population is further supported by the N_0/N_1 estimates consistently overlapping with zero
560 (Fig 2h).

561 3.1.3 Linkage disequilibrium-based estimation of current N_e and recent demographic history
562 from genomic data

563 GONE estimated a contemporary N_e around 300-350 (point estimate of 344 for the
564 previous generation, 95% CI = [116, 580]). Regarding demographic history over the last 100
565 generations, analyses in GONE suggest that after an increase in N_e over 50 generations, the
566 effective population size gradually declined over the last 50 generations up to present
567 times, following an almost symmetrical pattern (Fig. 2c). The N_e decrease was of almost one
568 order of magnitude.

569 3.1.4 Linkage disequilibrium estimation of contemporary N_e from microsatellite data

570 Estimates of contemporary N_e and respective 95% confidence intervals, obtained
571 with NeEstimator from the microsatellite data, both for the global dataset and separately
572 for each of the four parks, are presented in Table 3. The N_e point estimates for the global
573 dataset were around 150-190 individuals, with the 95% CIs spreading around 80-550
574 individuals.

575 Point estimates for CLNP and BNP were of similar magnitude, respectively at about
576 160-230 and 130-150 individuals, but the 95% CIs had infinite upper bounds. This can
577 happen if N_e is large and/or if the data has limited information (e.g., insufficient sample size;
578 reduced number or not very polymorphic loci) (Marandel et al., 2019). The infinite upper
579 bound implies that it is not possible to reject the null hypothesis that LD can be explained
580 entirely by sampling error (Waples & Do, 2010). Still, the finite lower bound provides useful
581 information about the minimum limit of N_e (Waples & Do, 2010). The N_e point estimates for
582 the CNP (54-79 individuals) were substantially lower, but with 95% CIs overlapping with the
583 previous ones. On the other hand, the point estimates for DNP, at 8-12 individuals, were
584 approximately an order of magnitude smaller than for the other datasets and with
585 parametric 95% CIs not overlapping with the other parks; yet, the jackknife CIs had infinite
586 upper bounds. The small sample size from the DNP may contribute to an underestimation of
587 N_e . This underestimation will tend to be smaller if the true N_e is not very large (e.g., ≤ 100)
588 and will be greater the larger the true N_e (Waples & Do, 2010).

589 4. Discussion

590 In this work, we used several methods that aim to estimate either a single effective
591 population size or possible changes in N_e over different temporal scales, using samples
592 obtained over different spatial scales. The genetic diversity of the critically endangered
593 western chimpanzee in GB was estimated using different types of data (microsatellites and
594 WGS SNPs) and the methods applied were thus different. We estimated the demographic
595 trajectories of western chimpanzees' representative of the whole country and, separately,
596 for four geographic populations inhabiting protected areas in the south of the country,
597 which are considered relevant areas given the global conservation of the subspecies.

598 **4.1. Estimation of N_e over different temporal scales**

599 As noted in Luikart et al. (2010) review and in Box 1 in Ryman et al. (2019) one can
600 consider, as a simple approximation, the idea that different N_e estimators should be
601 interpreted by using different time frames (Luikart et al., 2010, Wang, 2005). Some
602 estimates would correspond to the “ancient time” (from hundreds to tens of thousands of
603 generations) whereas others would correspond to the recent or contemporary N_e (from a
604 few to tens or hundreds of generations). Typically, MSVAR (microsatellite data) and PSMC
605 (single genome data) provide information about the former time period whereas
606 NeEstimator (microsatellite data) and GONE (WGS data) provide estimates that are mainly
607 about the recent past. The latter type of methods assume that the long-term N_e can be to
608 some extent neglected regarding some properties of the genetic data such as the LD pattern
609 (at least among some markers) or the variation of allele frequencies in the last few
610 generations. We note that MSVAR also provides an estimate of contemporary N_e but as part
611 of a demographic model of size change, and that GONE also integrates the contemporary N_e
612 in a trajectory of N_e change.

613 The contemporary N_e estimates are considered the most relevant to assess
614 extinction risk because they reflect ongoing or recent demographic or reproductive
615 processes whereas the historical N_e refers to the genetic or demographic processes over
616 much longer periods (Luikart et al., 2010; Santos-del-Blanco et al., 2022). We argue that it is
617 the combination of these estimates that should inform on the best conservation decisions
618 and measures (Fig. 2).

619 Furthermore, although the concept of N_e has been presented as analogous to the
620 census size (N_c), decades of research have shown repeatedly that N_e and N_c are not only
621 distinct but may have nearly opposite trends under some models. We want to stress here
622 that N_e is only informative about some property of the genetic data, and different
623 properties may have different temporal dynamics which are themselves possibly different
624 from the N_c dynamics (see Chikhi et al., 2018, Vishwakarma et al., 2024, Wakeley, 1999). N_c
625 may also be very disconnected from any N_e estimate because N_c informs us about the
626 current individuals living in the environment of interest and are thus directly affecting and
627 affected by major ecological processes, such as predation, competition or density
628 dependence (Waples, 2022). Different N_e values will depend on the probability that

629 individuals have to contribute genes to the next generation but also on how populations
630 may be connected to each other, as this will influence coalescence times (Beaumont, 2004,
631 Chikhi et al., 2010, Wakeley, 1999). Contemporary N_e will be influenced by recent
632 fluctuations in population size, variance in reproductive success among individuals, unequal
633 sex ratio and overlapping generations (Hoban et al., 2020, Waples, 2022). Over longer time
634 periods (“historical N_e ”), population structure and changes in connectivity may generate
635 very contradictory results. For instance, Wakeley (1999) showed that a structured
636 population where all the demes increase in size and between which, gene flow increases at
637 the same time, may exhibit a signal of decrease in N_e in stark contrast with the increase of
638 the actual population size. A similar phenomenon was described by Mazet et al. (2016) on
639 the PSMC method (see also Parreira et al., this Special issue on the effect of population
640 structure on different N_e estimation methods).

641 The PSMC plots that we obtained with the five individuals from GB exhibited a
642 similar trajectory to those obtained by Prado-Martinez et al. (2013) for individuals from the
643 same subspecies (*P. t. verus*) but from other locations. The authors estimated the peak of
644 effective population size at ~150 kyr, which is similar to the time of the highest N_e estimated
645 here (around 200 kyr). The difference in these values could be due to the fact that we used
646 the chimpanzee reference genome, whereas Prado-Martinez et al. (2013) used the human
647 genome as a reference. However, these differences are minimal, probably because the
648 divergence between *Homo sapiens* and *P. troglodytes* is on the order of 1% (see Henrique et
649 al., 2024 and references therein for the effect of divergence of the reference genome).
650 Beyond these important technical issues, the PSMC curves must be interpreted with care as
651 they could indicate changes in N_e through time, changes in connectivity without change in
652 N_e (Steux et al., 2024) or, more likely, a combination of both changes in N_e and connectivity.
653 One particularly striking result was that individual T_3 exhibited a PSMC lower than the
654 other four individuals, and shifted towards the left (more recent times). Several studies have
655 found that this kind of shift can be observed when the coverage is reduced (Nadachowska et
656 al., 2016 on *Ficedula* sp. flycatchers and Henrique et al., 2024 on *Microcebus* sp. mouse
657 lemurs and several other primate species). However, individual T_3 has a higher rather than
658 smaller coverage than the other individuals (T_3 32 X vs. 17 X for Bella, Bo, Emilia and
659 Simão, on average). This result is thus puzzling, and it cannot be explained without further

660 investigation. One possible explanation is that the difference in coverage between samples
661 may have resulted in variable power to call heterozygous sites, which could have resulted in
662 the slightly distinct demographic histories estimated for the five individuals (Frantz et al.,
663 2013).

664 The PSMC method has been used on many endangered species since it only requires
665 one genome sequence and is thus adapted for endangered species for which large genomic
666 data sets are difficult to obtain. This is the case of many endangered primates, including
667 chimpanzees (Prado-Martinez et al., 2013), gibbons (*Hylobatidae sp.*, Carbone et al., 2014),
668 mouse lemurs (Teixeira et al., 2021), and sifakas (*Propithecus sp.*, Guevara et al., 2022).
669 Implicitly, what the PSMC recovers is the distribution of coalescence times along the
670 genome. When two non-recombining fragments are identified by the PSMC and differ by
671 many heterozygous sites, this suggests that their MRCA (most recent common ancestor) is
672 likely very old. The opposite is more likely if there are few or no heterozygous sites. The
673 distribution of these implicit coalescence times can be interpreted as the result of
674 population size changes under the assumption of panmixia and in the absence of significant
675 population structure in the species of interest. The PSMC can thus be interpreted as a series
676 of changes in N_e , and this is the most common interpretation.

677 However, in the last 25 years there has been an increasing recognition that
678 population structure can generate spurious signatures of population size change
679 (Beaumont, 2004, Chikhi et al., 2010, Wakeley, 1999). In the specific case of the PSMC
680 method, Mazet et al. (2016) showed that it is in fact impossible to determine whether a
681 particular PSMC plot is the result of real change in N_e or of a more complex model of
682 population structure with changes in connectivity, without any change in population size. In
683 the latter case, it thus becomes impossible to actually make statements regarding changes
684 in N_e from changes observed in the PSMC curve alone. Mazet et al. (2016) introduced the
685 concept of IICR (inverse instantaneous coalescence rate) and noted that the PSMC method
686 in fact infers the IICR, not N_e . The IICR will be identical to N_e under panmictic models without
687 population structure but very different from any N_e changes as soon as there is population
688 structure. Altogether, this suggests that for species like chimpanzees that are known to be
689 structured (Funfstuck et al., 2015, Lester et al., 2021), signals of population size changes
690 inferred from methods assuming panmixia (PSMC, MSVAR, Bottleneck, StairwayPlot, GONE,

691 etc.) must be interpreted with caution (Steux et al., 2024). However, whether one considers
692 population structure or panmixia, our results suggest that the populations sampled were
693 part of a metapopulation that may have been very large and included all the regions
694 sampled in this paper. We will come back to this later.

695 The analyses using the MSVAR method and microsatellite loci data suggested that
696 chimpanzees from GB have undergone a mild demographic decrease starting around 40,000
697 years ago (when we used the global dataset). However, for the analyses of the different
698 parks, we inferred contrasting histories for inland (eastern, DNP and BNP) and coastal
699 (western, CLNP and CNP) populations with either no changes or recent and minor N_e
700 changes. Also, the estimates for N_1 were very similar for the different parks with ~10,000
701 individuals. This may indicate that the populations at the four parks were connected in the
702 past and part of one metapopulation (itself probably connected to other regions outside
703 GB), in agreement with our interpretation of the PSMC curves.

704 By contrast, the estimates of N_0 were much smaller, indicating either that population
705 connectivity changed towards more recent times, or that these values correspond to the
706 local deme size, as expected under the coalescent theory of structured models. Whichever
707 interpretation one may favor, our results appear to suggest that, whereas the inland eastern
708 populations remained large/connected with other regions (*e.g.*, transnational populations,
709 given the geographic proximity with the Republic of Guinea), the western coastal
710 populations appear more isolated, a process that may have started thousands of years ago
711 at the scale of the country. We have to be cautious with these figures as the timing of size
712 change inferred by MSVAR may not correspond to any particular timing of change in gene
713 flow, since models without changes in connectivity would also generate signals of
714 bottleneck. Despite these cautionary remarks, we believe that the CNP and CLNP may have
715 become separated from the metapopulation, but could have remained connected to each
716 other, forming a smaller sub-population not exceeding 1,200 breeding individuals. This
717 result also suggests that chimpanzees may have still been able to disperse between these
718 two parks.

719 In a recent study, Fontsero et al. (2022) analyzed chromosome 21 genome-wide data
720 across the species and subspecies distribution and inferred a large exchange of migrants
721 during the last ~800 years (range 117 to 2,200 years) for the populations located in the

722 northern range of the distribution, which includes Senegal, Mali, northern Guinea and GB
723 (using samples from BNP only). Thus, no signs of long-term isolation were detected for GB.
724 In another study, Heinicke et al. (2019a) investigated the existence of subpopulations across
725 the *P. t. verus* distribution based on field survey data and spatial modeling tools. According
726 to these authors, one large subpopulation (> 33,000 individuals, approximately 50% of the
727 total population size) was predicted at the northern range of the subspecies, in areas
728 characterized by savanna-mosaic habitats and extending across the Fouta Djallon highland
729 region and the neighboring areas of Senegal and GB (including the four parks of our study),
730 up to Mali and Sierra Leone. Thus, these studies could explain why we inferred large N_1
731 estimates of ~10,000 reproductive individuals (compared to N_0). These estimates could
732 correspond to the whole GB population, possibly reflecting an historical connection of the
733 GB population to a large metapopulation centered in the Fouta Djallon highland region. In a
734 more recent study, Steux et al. (2024) suggested that patterns of genomic variation as
735 observed in the PSMC curves could be modeled as part of a metapopulation of small demes
736 characterized by periods of changing connectivity. They estimated that, in comparison to
737 other *P. troglodytes* subspecies, *P. t. verus* population was characterized by smaller demes,
738 which could explain the lower nucleotide diversity observed in western chimpanzees. Their
739 study however focused on the rather ancient past (older than ca. 50 kyr) due to the
740 uncertainties on the PSMC curves they analyzed in the recent past. Other genetic studies
741 based on microsatellite loci and a fragment of the mitochondrial DNA D-loop region, carried
742 out in GB, do not indicate a strong population structure (Borges, 2017; Sá, 2013), which
743 suggest that the chimpanzees are able to disperse between parks. The change in
744 connectivity between inland areas (DNP-BNP) and coastal (CNP-CLNP) within GB cannot be
745 directly inferred from our analyses. We must thus be careful in result interpretations.
746 However, our analyses do identify a large ancestral population that might have been
747 fragmented as a consequence of environmental changes that occurred around 10,000 years
748 ago, including climate instability in West Africa and the more recent increased human
749 impact resulting from the development of agriculture. The Younger-Dryas Holocene
750 transition marks a very abrupt transition between the African Humid Period (14,500 - 6,000
751 years ago, characterized by the expansion of forests and lakes across the Sahara region),
752 and a sequence of time periods characterized by arid conditions towards the late Holocene

753 (Gasse & Van Campo, 1994). This climate instability, with a succession of warm and cooling
754 events in West Africa, impacted the extension of forest cover (deMenocal et al., 2000) and,
755 most likely, the size and connectivity of the populations of forest-dwelling fauna, as is the
756 case of the western chimpanzee.

757 The GONE analyses suggested a surprising growth of N_e between 2,500 and 1,250
758 years ago (*i.e.*, in generations 100-50) followed by a short stationary period and a more
759 recent symmetrical decrease. This pattern may be an artifact of the method, due to the
760 small number of individuals analyzed (five chimpanzees). For example, Beichman et al.
761 (2018) illustrated the general difficulties of demographic history methods in inferring
762 demographic events over the past hundred generations using whole-genome data for fewer
763 than 10 individuals. Reid & Pinsky (2022) also emphasized the importance of large sample
764 sizes for different demographic history methods regarding power and precision to detect
765 and quantify population declines in the last 100 generations, with GONE being no exception
766 to a sharp deterioration in performance when sample sizes are small. Similar and even
767 larger humps have also been described by Santiago et al. (2020) when they simulated data
768 from a simple structured model (panel F of their Figure 2), but at this stage one should be
769 cautious to interpret these results until larger sample sizes are analyzed.

770 The result that however appears to be consistent with the other methods used in
771 this study, is that a gradual decline of N_e took place during the last 1,250 years (*i.e.*, the last
772 50 chimpanzee generations) towards the present, with the whole population reaching a
773 contemporary N_e around 300-350 breeding individuals, estimates that are qualitatively in
774 agreement with those of NeEstimator and microsatellite data (N_e of 107-549 or 138-294,
775 respectively).

776 **4.2 Implications for conservation management of the western chimpanzee in Guinea-** 777 **Bissau**

778 N_e should be seen as a key parameter in conservation management and genetic
779 biodiversity monitoring because it is supposed to provide information on population
780 viability, on inbreeding depression risk, on population isolation, and on effectiveness of
781 selective processes and adaptation in relation to drift (Charlesworth, 2009, Hoban et al.,
782 2022). In populations with small N_e , genetic diversity is lost at a faster rate over time, and
783 the random fluctuations in allele frequency by drift can neutralize the effect of natural

784 selection and increase the probability of fixation of deleterious mutations, potentially
785 leading the population to inbreeding depression (Charlesworth 2009, Hoban et al., 2022,
786 Newman & Pilson, 1997).

787 Franklin (1980) proposed the thresholds of 50/500 for a minimum effective size
788 required for a viable population in the short and long term, respectively. This
789 recommendation became an established rule of thumb in conservation biology and has
790 been proposed as a genetic indicator to assess progress towards global conservation targets
791 (Frankham et al., 2014, Hoban et al., 2020). The 50 short-term rule refers to an effective size
792 quantifying the rate of inbreeding (N_{ei}). The minimum N_{ei} of 50 individuals is thought to be
793 enough to prevent the rapid inbreeding of the population (*i.e.*, 1% per generation), which
794 could lead to excessive homozygosity for deleterious recessive alleles and reduced fitness by
795 inbreeding depression. The 500 long-term rule refers to the effective size related to the loss
796 of additive genetic variation (N_{eAV}). This threshold defines the N_e above which a population
797 should retain enough evolutionary potential to adapt to new selective forces (*i.e.*, future
798 environmental conditions, Jamieson & Allendorf, 2012). More recently, these numbers have
799 been doubled, with 100 individuals being presented as more adequate to prevent
800 inbreeding depression over five generations for wild populations (*i.e.*, limiting to 10% the
801 loss in total fitness) and 1,000 individuals as necessary to protect evolutionary potential in
802 the long-term (Frankham et al., 2014), particularly when the species' reproductive rates are
803 low (Perez-Pereira et al., 2022). When a population is detected to have a small or declining
804 N_e , managers and conservationists should be called to investigate the most likely causes and
805 to reverse the demographic trajectory (Wang et al., 2016). This is why estimating N_e is
806 increasingly recognized as central to conservation programs.

807 In this work, we used different approaches that estimated historical and
808 contemporary N_e . While the estimates by the different methods could differ, results were
809 consistent in suggesting that current N_e is below 500 breeding individuals for the GB
810 chimpanzee's population. For instance, GONE and NeEstimator suggested values that were
811 typically between 100 and 500, even if some estimates could have larger upper bounds,
812 most likely due to small sample sizes (see Results section). These results were qualitatively
813 in agreement with the N_o estimates obtained with MSVAR even if the latter were higher
814 (between 500 and 1,000). While no N_e estimate should be taken at face value, the fact that

815 these estimates were qualitatively similar when using different methods making different
816 assumptions and using different types of genetic markers, suggests that the contemporary
817 demographic dynamics of chimpanzees in GB is driven by small and isolated populations
818 that derive from, what used to be, a very large metapopulation. Furthermore, these results
819 confirm the selection of the coastal areas of GB and Republic of Guinea by Kormos & Boesch
820 (2003) as priority regions for conservation of the subspecies and are aligned with estimates
821 of density that points to a small population in CLNP (Carvalho et al., 2013), highlighting the
822 critical conservation situation of these populations.

823 Small populations (*i.e.*, < 500 breeding individuals) may go extinct through a
824 phenomenon referred as extinction vortex (Gilpin & Soule, 1986), in which genetic and
825 demographic issues interact synergistically to decrease genetic diversity and cause
826 population growth rate to drop due to the reduction of the mean fitness. This results in
827 further decreases in genetic diversity and promotes the subsequent processes to happen in
828 a cascade, until the extinction of the population. In the case of the western chimpanzees in
829 GB, the main conservation threats have been identified and characterized to some extent.
830 Natural habitats have generally been converted into subsistence crop plantations, such as
831 rice (*Oryza spp.*) or cassava (*Manihot esculenta*), and cashew (*Anacardium occidentale*)
832 monoculture agroforests at least since the last two decades (Hockings & Sousa, 2013,
833 Temudo & Abrantes, 2014). Additionally, the construction of roads and infrastructures
834 increased the accessibility to remote areas and promoted more encounters with humans,
835 which may have increased chimpanzees' mortality. Although it was found that chimpanzees
836 can cross and use human-altered habitats to some degree, namely sharing the use of
837 forested and village areas with local communities (*e.g.*, in CNP, Bersacola et al., 2021),
838 extensive habitat loss and conversion into crop fields and villages are expected to reduce
839 connectivity between populations and diminish population size rapidly (Torres et al., 2010).
840 In CNP for instance, it was estimated a loss of 11% of suitable habitat and the death of
841 between 157 and 1,103 individuals in the population for the period between 1986 and 2003
842 (Torres et al., 2010), which corresponds to less than one chimpanzee generation. Moreover,
843 as reported by Stiles (2023) and as illustrated here (with the four blood samples of
844 confiscated individuals), hunting for live individuals to supply the national and international
845 illegal pet trade occurs in the country (Ferreira da Silva & Regalla, in review, Stiles 2023).

846 Quantitative data on the number of traded chimpanzees originating from the GB in
847 international trade routes is missing (Clough & Channing, 2018; Stiles et al., 2013; Stiles,
848 2023). However, given the ease of detecting chimpanzees in private houses and hotels (*e.g.*,
849 18 individuals between 2006 and 2022 found by chance, in Ferreira da Silva & Regalla in
850 review) and considering that five to 10 adults can be killed to harvest only one infant
851 chimpanzee (Teleki, 1980), it can be suggested that hunting to supply the trade of live
852 individuals may have contributed to a reduction of the population size and consequently of
853 the N_e . Furthermore, as conservation threats tend to act synergistically at the local and
854 regional scale - habitat fragmentation increases accessibility to natural habitats by local
855 communities, which in turn, increase poaching, negative interactions with farmers and
856 diseases transmission (Humble et al., 2016), the negative impacts of human-derived activities
857 on the population effective size may be larger than each threat is individually.

858 Specifically, chimpanzees inhabiting CNP and CLNP - the two populations identified
859 by this study at a high risk of extinction - are currently negatively impacted by habitat loss
860 and fragmentation, and by retaliatory killing by farmers during crop-raiding (Hockings &
861 Sousa, 2013). Chimpanzees in CNP may also be subjected to higher reproductive isolation
862 since the Park is in a peninsula surrounded by two permanent water bodies which are
863 insurmountable by chimpanzees, and suitable habitat for the subspecies was considerably
864 lost in northwestern areas, where the isthmus connects the peninsula to the mainland (*i.e.*,
865 Bantael Sila, Cumbijã and Guiledge villages, Torres et al., 2010). These coastal areas have
866 been considered important to maintain gene flow with GB mainland for another primate
867 species (*e.g.*, Guinea baboons, *Papio papio*, Ferreira da Silva et al., 2014, Ferreira da Silva et
868 al., in press). Other forest-dwelling and hunted primate species at CNP may have gone
869 through a decline in N_e of a similar magnitude. Minhós et al. (2016) found a pattern of N_e
870 decrease for co-distributed populations of colobus monkeys inhabiting CNP (*Ptilocolobis*
871 *badius temminckii* and *Colobus polykomos*) at a similar time period as estimated here for
872 CNP chimpanzees (*i.e.*, ca. 10,000 to 3,000 years). The N_e for colobus monkeys was
873 estimated using alike models and genetic markers (*i.e.*, MSVAR runs and microsatellite loci).
874 The fact that a similar demographic event was observed for other co-distributed species in
875 CNP despite different socio-ecology features, strengthens our interpretation that CNP
876 chimpanzees experienced low N_e in recent times. Moreover, several chimpanzee

877 communities across CNP show symptoms of leprosy (caused by *Mycobacterium leprae*,
878 Hockings et al., 2021), which is likely to have negative consequences for the longevity and
879 reproductive success of individuals, but so far unknown. On the other hand, In the southern
880 region of CLNP, the construction of a large road and a thermoelectric plant with respective
881 electricity transmission lines, led to the loss of one of the best-preserved forest patches of
882 the park (Catarino, 2019) and increased the accessibility to areas used by chimpanzees for
883 nesting (Carvalho et al., 2013). Cases of live chimpanzee captures have been recorded in
884 both parks, which most likely implies adult mortality (Ferreira da Silva & Regalla in review).
885 Furthermore, CLNP is bordered in the east by the main road connecting the south of the
886 country to the capital city - Bissau and, as demonstrated in this study, wildlife-vehicle
887 collisions do happen. Our results suggest that CLNP and CNP are at high risk of extinction
888 and the impact of human-derived activities potentially threatening the two chimpanzee
889 populations should be investigated.

890 Our study provides the first estimates of N_e for DNP. We estimated an historical N_e of
891 2,769 - 7,401 breeding individuals using MSVAR but could not detect a strong departure
892 from mutation-drift equilibrium. This result was at odds with the very low contemporary N_e
893 of 6-30 obtained using NeEstimator. DNP is located at the northern margin of the Corubal
894 River and is currently at one edge of the subspecies distribution (see Fig. 1b). Our estimates
895 of a stable demographic trajectory and a large historical N_e suggest that DNP was at some
896 point in time connected to other large chimpanzee populations. Connectivity to populations
897 located in the south of the Corubal River (*e.g.*, BNP) should be more reduced at present
898 times because the current width of the water body, which can surpass one kilometer in
899 some sites, may be a significant barrier to primate's gene-flow (*e.g.*, the green monkey,
900 *Chlorocebus sabaues*, Colmonero-Costeira et al., in review, the Guinea baboon, *Papio papio*,
901 Ferreira da Silva et al., in press). Nevertheless, the configuration and discharge of the
902 Corubal River may have been different in the past (*e.g.*, the mouth may have been in the
903 southwest part of the country, located by the Rio Grande de Buba channel area, Alves
904 2007), which could have allowed chimpanzees to cross to what is the south margin. The
905 small contemporary N_e may be explained by the fact that present environmental conditions
906 do not support a large population of chimpanzees. DNP is located at the edge of the
907 distribution of the subspecies and has low density of villages and other human

908 infrastructures. This area is mostly dominated by woodlands and savannah woodland
909 formations (Catarino et al., 2008), and found to be of low habitat suitability for chimpanzees
910 by modeling exercises (<100, range 0-1,000, in Figure S2.2. Carvalho et al., 2021), which
911 could be either related to environmental conditions or a small sample size (J. Carvalho,
912 personal communication). During field work, chimpanzees were mostly detected (and fecal
913 samples collected) in greater proximity to gallery forests along smaller streams or next to
914 the Corubal River (Fig. 1b), and we observed that the subspecies was not widely distributed
915 in the park area, as in CNP, for instance (Sá, 2013). Chimpanzees at Fongoli in Senegal,
916 inhabiting a similar open, savanna-woodland environment, do not suffer from nutritional
917 stress but display physiological stress from dehydration and heat, which does not seem to
918 be behaviorally compensated (from sitting for longer periods in the shade or using pools or
919 caves for instance, Wessling et al., 2018). Such adverse environmental conditions may be
920 determinant for constraining the distribution at the biogeographical range limits of the
921 subspecies (Wessling et al., 2018) and similarly, limiting the size of the population at DNP.

922 By contrast, our estimates of historical N_e of the population of chimpanzees
923 inhabiting the BNP (MSVAR N_D : 6,716 - 24,642 breeding individuals) are large and confirm
924 the classification of the area as stable or of high-density (Heinicke et al., 2019b). Boé
925 population has been included in a previous population genomic study, using samples
926 collected across the subspecies range (Fontesere et al., 2022), which estimated high and
927 recent connectivity (for the last ~780 years, range 117-2,200 years) between communities at
928 the northern range (localities in the Republic of Guinea and south of Senegal, Fig. 3 in
929 Fontesere et al., 2022). Boé was found to be genetically closer to samples collected in
930 southern Senegal (Fontesere et al., 2022), possibly due to long-term connectivity between
931 the two neighboring populations. Present-day high density of chimpanzees in the Boé region
932 has been justified by i) remoteness of the area and difficult access, ii) rare hunting of
933 chimpanzee to comply with religious taboos, iii) high habitat suitability for chimpanzees, and
934 iv) slow habitat loss and conversion, and in a large area, habitats are undisturbed (Binczik et
935 al., 2019; Carvalho et al., 2021, van Laar 2010). Although DNP and BNP are closely located
936 and share similar environmental conditions, within the Boé region there is a wide network
937 of rivers and waterbodies surrounded by relatively well-preserved gallery forests, which are
938 used by chimpanzees to nest and feed (Binczik et al., 2019). Our results suggest that Boé is a

939 stronghold for the chimpanzee population in GB. The effective protection and restoration of
940 the natural habitats in ecological corridors connecting BNP and the remaining parks located
941 south of the Corubal River (Fig. 1b) could be beneficial to promote dispersal, potentially
942 increasing gene flow and improving the probability for long-term persistence of
943 chimpanzees in coastal areas of GB.

944 **4.3 Implications for the estimation of N_e of wild populations of primates**

945 The most common approaches to estimate the effective size of real populations are
946 based on its genetic properties (Luikart et al., 2010). However, obtaining genetic
947 information of threatened species can be challenging. The main issue is related to attaining
948 high quality DNA, which is usually extracted from fresh blood and tissue samples. Species of
949 conservation concern are frequently found in low densities in the wild and commonly live in
950 inaccessible areas and in habitats of low visibility. Hence, it is difficult to trap or handle
951 individuals (Beja-Pereira et al., 2009). Moreover, invasive methods are considered unethical
952 as the contact with humans to retrieve blood or tissue samples increases the risk for disease
953 transmission (Beja-Pereira et al., 2009). Thus, invasive sampling of wild-born individuals for
954 threatened species is typically opportunistic and carried out for a few individuals, for
955 instance during veterinarian interventions or post-mortem (*e.g.*, Prado-Martinez et al.,
956 2013). Such procedures can take several years to complete (Xue et al., 2015), and sampling
957 is usually geographically restricted, and not representative, of the species distribution and
958 variability. In primates, genomic data used to infer parameters of interest for conservation,
959 has been obtained from high quality DNA collected from individuals in zoos or rescuing
960 centers (*e.g.*, Rogers et al., 2019 but see Fontseré et al., 2022 who obtained genomic
961 diversity estimates of wild chimpanzee populations from 828 non-invasively fecal samples).
962 Yet, specific environmental conditions or breeding practices that inadvertently reduce
963 natural selection pressures or increase inbreeding (Christie et al., 2012) can lead to wrong or
964 limited inferences of demographic parameters. Also, small and spatially restricted sampling
965 can introduce bias in contemporary N_e estimates (*e.g.*, Santos-del-Blanco et al., 2021) and
966 geographic-broad genetic data, such as the ones obtained using non-invasive fecal samples,
967 are preferable.

968 Here, we show that the estimated values of N_e using genomic data and more classic
969 genetic markers, like microsatellite loci obtained using non-invasive fecal samples, are

970 largely concordant, although we found that median N_e estimates produced by SNP data
971 were higher than estimates generated using microsatellite data. This pattern was also
972 reported by Clarke et al. (2024) meta-analysis. Our study reinforces that datasets generated
973 with traditional genetic markers, such as legacy or baseline microsatellite loci datasets for
974 local populations, are of great value; these can be used to estimate parameters relevant to
975 inform conservation management in species for which obtaining genomic data is not
976 straightforward, or in studies carried out in countries with limited access to sequencing
977 units, funding and trained researchers in genomic data (Bertola et al., 2024). Moreover, our
978 study shows that the combination of different molecular markers and analytical methods
979 can be a useful strategy to overcome the limitations of obtaining high quality DNA from wild
980 threatened populations, to investigate species evolutionary history in time and space, and
981 to integrate genetic information in conservation management decisions at local and regional
982 scales.
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986 **Data Archiving Statement**

987 Data for this study are available at Dryad Digital Repository: *to be completed after*
988 *manuscript is accepted for publication.*

989

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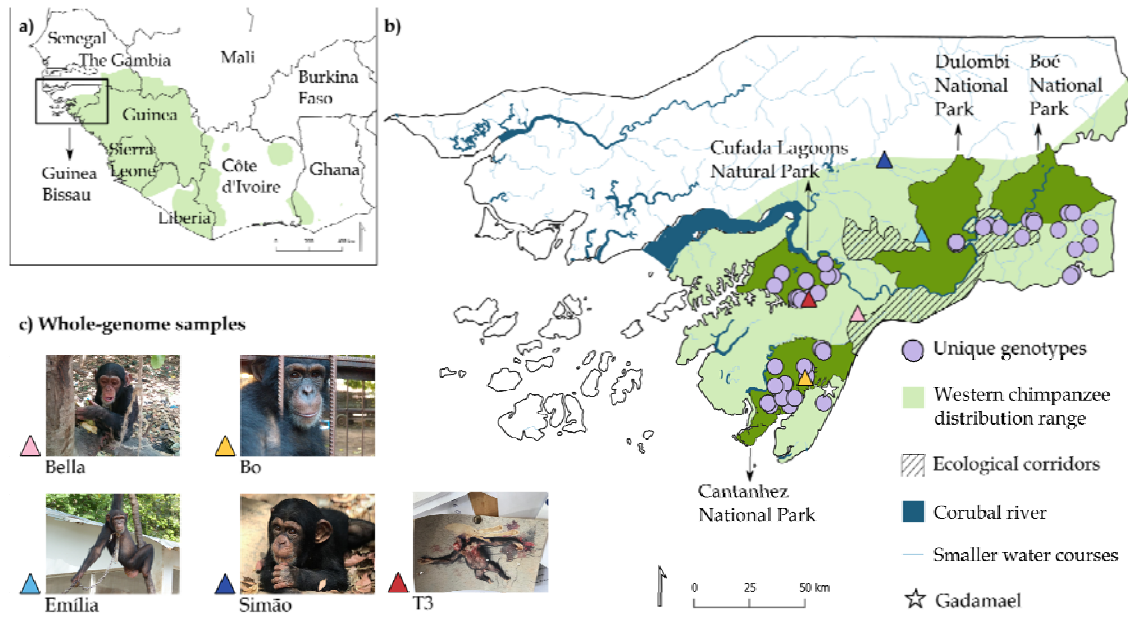
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1474 **Tables and Figures**
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Figure 1 a) Distribution of the western chimpanzee (*Pan troglodytes verus*) in West Africa and b) the location of the study area in Guinea-Bissau. Unique genotypes for 10 microsatellite loci (represented by purple circles, N=143) were obtained from fecal samples collected non-invasively in four parks located in southern Guinea-Bissau and encompassing the national range of chimpanzees – Cufada Lagoons Natural Park (CLNP), Cantanhez National Park (CNP), Dulombi National Park (DNP) and Boé National Park (BNP). The location of ecological corridors is also indicated. Gadamael area (referred to in table 1) is also mapped and represented as a white star. c) Pictures show the four confiscated chimpanzees and one road-killed individual that were sampled to generate whole-genome sequencing data. Blood samples were drawn from the confiscated individuals during placement in a sanctuary abroad. One tissue sample was obtained from one road killed minutes after fatality. The location of confiscated individuals in the map in b) (represented by triangles) is estimated and reflects information on the individual's origin obtained from national authorities (e.g., Bo from CNP) or where individuals were found in private premises. Photo credits by MJFS, H. Foito (European Union Embassy Bissau), C. Casanova, L. Almeida and I. Camará.

Table 1. Compilation of results from past studies estimating the density of local populations of chimpanzees in Guinea-Bissau

Site	Reference	Method	Estimated density (ind./km ²)	Estimated number of individuals
Cantanhez National Park	Sousa J. 2009	nest count method for density estimation	1.937 and 2.340	*2,070 and 2,454
	Torres et al., 2010	modeling	MI	376 and 2632
	Hockings et al., 2013	MI	3.00	MI
Cufada Lagoons Natural Park	Carvalho et al., 2013	nest count method for density estimation	0.22	137 (95% C.I. 51–390)
	Sousa et al., 2013	marked nest count method	0.79 (CI 95%: 0.61–1.04)	300 (CI 95%: 230–390)
Gadamael	Sousa F. (2009)	nest count method	0.897	33

		for density estimation		
Boé National Park	Schwarz et al., 2007	Interviews	MI	710
	Dias et al., 2018++	nest count method for density estimation	0.38	18
	Wenceslau, 2014	nest count method for density estimation	1.8	MI
	Binczik et al., 2017	standing crop nest count method	0.77 (95% CI 0.45–1.34)	1,465 to 4,415
	Heinicke et al., 2019a	modeling	6.76	MI
Overall population	Heinicke et al., 2019a	modeling		1,908 (923–6121 95% CI)

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The study areas of the majority of studies are National Parks, except for Gadamael (see Fig. 1). The geographic location of sites within the country is indicated in Fig. 1. Details on the methodology can be found in the respective publications. We show the 95% confidence intervals (CI) when reported by the original study. *indicates that the estimated number of individuals was calculated here by multiplying the density reported in the study by the area of the respective protected area in <https://ibapgbissau.org/areas-protegidas/>. ++Please note that the study by Dias et al., 2018 only considers 47km² of study area. MI – indicates missing information in the original study

Table 2 Estimation of long-term N_e for the whole dataset and per park by employing the Bayesian likelihood-based approach implemented in MSVAR 1.3 (Storz & Beaumont 2002) and using microsatellite loci data.

Site	N	N_0 (Historical)	N_1 (ancestral)	Time ago of demographic change (years)	Estimated demographic trajectory
Global dataset	143	4,500 - 6,500	10,705 – 11,910	44,596 – 69,518	Evidence of mild demographic bottleneck
Cantanez National Park	58	500 - 1,125	10,615 – 11,644	5,288 – 12,477	One order of magnitude demographic bottleneck
Cufada Lagoons Natural Park	38	534 - 1,225	10,397 – 11,416	3,612 – 7,874	One order of magnitude demographic bottleneck
Dulombi National Park	13	2,769 - 7,401	9,356 – 10,651	13,110 – 32,085	Stable
Boé National Park	34	6,716 - 24,642	7,320 – 8,555	6,753 – 85,645	Stable

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In the table is the number of genotypes (N), the posterior distributions of present-day population size N_0 , the ancient population size N_1 (reproductive individuals), the time at which the population started to change (T , in years, assuming a 25 years generation time) for four independent runs for each dataset considered and the estimated demographic trajectory. The median and 90% highest posterior density intervals (HPD90%) are indicated for N_0 , N_1 and T .