

<https://doi.org/10.1038/s42003-024-06901-3>

Whole genomes of Amazonian uakari monkeys reveal complex connectivity and fast differentiation driven by high environmental dynamism



Núria Hermosilla-Albala ^{1,17}✉, Felipe Ennes Silva^{2,3,17}, Sebastián Cuadros-Espinoza¹, Claudia Fontserè ^{1,4}, Alejandro Valenzuela-Seba ¹, Harvinder Pawar¹, Marta Gut⁵, Joanna L. Kelley ⁶, Sandra Ruibal-Puertas¹, Pol Alentorn-Moron ¹, Armida Faella¹, Esther Lizano ^{1,7}, Izeni Farias ⁸, Tomas Hrbek^{8,9}, Joao Valsecchi ^{10,11,12}, Ivo G. Gut⁵, Jeffrey Rogers ¹³, Kyle Kai-How Farh¹⁴, Lukas F. K. Kuderna ^{1,14}, Tomas Marques-Bonet ^{1,5,7,15,18} & Jean P. Boubli^{16,18}

Despite showing the greatest primate diversity on the planet, genomic studies on Amazonian primates show very little representation in the literature. With 48 geolocalized high coverage whole genomes from wild uakari monkeys, we present the first population-level study on platyrrhines using whole genome data. In a very restricted range of the Amazon rainforest, eight uakari species (*Cacajao* genus) have been described and categorized into the bald and black uakari groups, based on phenotypic and ecological differences. Despite a slight habitat overlap, we show that posterior to their split 0.92 Mya, bald and black uakaris have remained independent, without gene flow. Nowadays, these two groups present distinct genetic diversity and group-specific variation linked to pathogens. We propose differing hydrology patterns and effectiveness of geographic barriers have modulated the intra-group connectivity and structure of bald and black uakari populations. With this work we have explored the effects of the Amazon rainforest's dynamism on wild primates' genetics and increased the representation of platyrrhine genomes, thus opening the door to future research on the complexity and diversity of primate genomics.

The current global framework poses new challenges to the scientific community, these being related to the salvage of biodiversity in the face of global climate change and other anthropogenic threats to wild species^{1,2}. It is in this context that there has been an increased effort to describe wild mammalian populations³ from an evolutionary and conservation perspective^{4–8}. This has been particularly noteworthy in the highly endangered Primate order^{9,10}, for which the Amazon has the greatest diversity on the planet. Surprisingly, ~20% of Amazonian primate species have been described since 1990¹¹, evidencing the scarcity of knowledge on this group. For the first time, whole genomes have been generated for many long-neglected and not yet investigated primate species^{10,12–15}. In particular, platyrrhine genomes (those of primates found in Central and Southern America) remain largely unexplored compared to other primate groups, likely due to their phylogenetic distance to the human species and the difficulty of obtaining biomaterials^{16,17}. In the face of the above-mentioned dangers wild

biodiversity is and will be exposed to, the framework of conservation genomics is becoming more and more relevant: describing the detailed population structure and evolutionary history of wild lineages can provide key insights to the design and implementation of conservation strategies (<https://doi.org/10.1111/mec.15720>)^{8,17,18}.

In this study, we focus on *Cacajao* or uakari monkeys of the Pitheciidae¹⁹, which remains one of the least known primate genera in the Americas. Endemic to the Amazon, uakaris are part of the rich yet poorly known local biodiversity found in it. Their geographic distribution is the most restricted of the three Pitheciinae genera (*Cacajao*, *Chiropotes*, and *Pithecia*), occurring between the Negro–Branco and Ucayali–Solimões–Juruá Rivers²⁰. Eight species have been described in the *Cacajao* genus, which in turn are clustered into two groups, the bald and the black uakaris¹⁹. They show contrasting phenotypic (pelage coloration^{19,21,22} and skin pigmentation²³), ecological (habitat preference²¹, geographic

A full list of affiliations appears at the end of the paper. ✉e-mail: nuria.hermosilla@upf.edu

distribution^{21,24}), and genomic traits^{21,22}. The bald uakaris comprise five species (*C. calvus*, *C. amuna*, *C. novaesi*, *C. rubicundus*, *C. ucayalii*)²⁴ and black uakaris, three (*C. ayresi*, *C. hosomi*, *C. melanocephalus*)²⁵. Black uakaris are, as their name suggests, black-haired and partially brown in some cases¹⁹. They are mostly found in the forests of Solimões-Japurá and Negro-Branco river systems, and inhabit extensive areas of *terra firme* forest during some months, including high altitude areas²⁰. On the other hand, bald uakaris are mainly flooded-forest specialists found around white water rivers (*várzea*) and present a red bald face with pelage that ranges from very light-greyish white (white bald uakaris) to red-chestnut (red bald uakaris)^{23,24}. The populations of the five species of bald uakaris are found in the Ucayali, Solimões, and Juruá river systems, nearly overlapping but in general south of the black uakaris' distribution range²⁶ (Fig. 1A).

Complex demographic histories (recent divergence times, incomplete lineage sorting, complex gene flow patterns), combined with limited resolution of previous studies^{21, 24,27–29} have resulted in an incomplete understanding of the evolutionary history of many platyrrhine lineages. This includes the uakaris^{14,21,24,30}. Since these show shallow divergence times and rapid radiation^{21,24,27} high phenotypic variability and a very limited distribution range, their study provides a rich experiment setting to better understand the impact on the genome of such habitat heterogeneity both through time and space in larger systems.

Multiple studies have addressed the evolutionary history of *Cacajao* employing mitochondrial and double digest restriction-site (ddRAD) associated sequences, whose resolution was insufficient to fully resolve their evolutionary history^{21,24,27}. In accordance with the described phenotypes, genetic data in these studies has suggested that bald and black uakaris form two differentiated phylogenetic clades²¹, which in turn can be split into red and white bald uakaris, and north and south bank of the Negro River populations for black uakari species^{21,24,27}. Nonetheless, neither the detailed population structure nor gene flow patterns among the populations of wild uakaris have been described, the population-level phylogenetic resolution has not been reached, and the reported divergence time estimates inside the genus have not been consistent among studies^{21,24,27}. Here we present the first population-level study on whole genome data of a platyrrhine genus. Our dataset consists of 48 geolocalized uakari whole genomes at ~30× coverage, with which we describe the detailed dynamics, population structure, and broad functional heterogeneity of wild *Cacajao* populations with increased statistical power^{16,17}. This is also key to refining previous demographic estimates^{8,16,17,31}; we obtained the first whole genome phylogeny for uakaris with population-level resolution in most of the cases, narrower or new confidence intervals for divergence times and effective population size (*N_e*) estimates in the genus. Our work provides insights for future efforts to protect *Cacajao* wild populations, whose habitat integrity is becoming increasingly vulnerable in the face of global climate change and anthropogenic threats³².

Results

Here, we generated 48 whole genomes at a mean coverage of 31× ranging from 14× to 43× (Supplementary Data 1), in which we discovered a total of 191,4 million single-nucleotide polymorphisms (SNPs). Twelve populations from all described *Cacajao* species were analyzed here, which were defined based on taxonomy and sampling localities (Supplementary Table S1).

Population structure and phylogenetics follow pelage coloration and agree with intra-group geographic distribution patterns

To determine the structure of current wild *Cacajao* populations (Fig. 1A), we ran a principal component analysis (PCA) (Fig. 1B–D), ADMIXTURE (Fig. S4–6) and phylogenetic analyses (Fig. 2). PCA and ADMIXTURE analyses consistently show that population structure in the genus follows patterns described by pelage coloration: the largest genetic dissimilarity being found between bald and black uakari populations (variance explained by PC1: 6.93%; Fig. 1C, S4A), followed by differences within each group which were addressed independently (bald: red bald uakaris—white bald uakaris with a variance explained of 4.02% by PC1; black: ML (*C. melanocephalus*)—AY (*C. ayresi*) + HS (*C. hosomi*) with PC1 accounting for 3.62% of the

variance) (Figs. 1B, D, S5A, S6A) overall in the absence of admixed individuals (Figs. S4A, S5A, S6A). Strong intra-species population structure was identified in white CV (*C. calvus*) and red RB (*C. rubicundus*) bald uakari populations (Fig. 1D), as well as for ML in black uakaris (Fig. 1B). In the latter group, AY and HS consistently cluster together (Fig. 1B, C). To further investigate the identified genomic clusters, *F_{ST}* was estimated as a proxy for genetic differentiation between populations. As expected, the highest differences in the genus were between bald and a black populations: the red bald RB-JutaíSolimões population and both black HS-Salmeri and AY (*F_{ST}* = 0.546) (Fig. S11). Then, AY and ML-JapuráR were the most differentiated intra-group pair with an *F_{ST}* of 0.247, close to that between CV-Jutaí and RB-JutaíSolimões (*F_{ST}* = 0.233) (Fig. S11). Overall, we observed that the geographic distribution of the samples aligned with the intra-group genomic clusters, and even at the species level, where subpopulations could be identified both geographically and at the genomic level in some cases (Fig. 1).

Moreover, the phylogenetic signal in the *Cacajao* lineage was explored by generating the first set of whole autosomal genome maximum clade-credibility phylogenies. Two independent phylogenies were generated to assess the influence of variable window sizes on the final support of the respective topologies. We built a first phylogeny based on 2144 1 Mb maximum likelihood (ML) trees covering ~80% of the genome (Fig. 2), and a second phylogeny based on 9313 250 kb ML trees covering ~85% of the genome (Fig. S8A, B). The node support in the phylogeny based on 1 Mb windows appeared overall higher (Fig. 2, S9A) when compared to the second phylogeny based on 250 kb windows (Fig. S8). Furthermore, the phylogenetic signal retrieved was observed to be homogeneously distributed through the genome rather than driven by specific regions (Fig. S9B, C). The topologies described here were in accordance with previous phylogenies built from restricted autosomal regions (ddRAD sequences)²⁴, although employing the whole autosome reaching population-level resolution.

Focusing on the highly supported phylogenetic tree (1 Mb windows) and in accordance with PCA, ADMIXTURE analyses and previous studies, black and bald uakaris split following pelage coloration patterns as well as based on geographic distribution. This was with the exception of a very low-supported deviation (Phylogenetic_support = 0.08) for one sample from HS-LowV (PD_0914), which clusters with AY. This was not surprising, as these populations showed very little genomic differentiation (*F_{ST}* (HS-LowV,AY) = 0.04, Figs. 1B, C). On the other hand, in accordance with previous studies²⁴, the phylogeny built from the respective whole mitochondrial genomes (Fig. S10) showed lower resolution: it recovered species-level classification for black uakaris, and bald uakaris were correctly classified into red and white except for one sample from CV-MSDR (PD_0434), which clustered into red uakaris.

Lastly, a major difference in the topology of red bald uakaris arose when the two phylogenies based on the whole autosome were compared. The 250 kb windows-based tree showed RB to diverge earlier from the ancestor of UC (*C. ucayalii*) and NV (*C. novaesi*) (Phylogenetic_support = 0.67, Fig. S8). This was in disagreement with the 1 Mb windows-based tree (Phylogenetic_support = 0.91, Fig. 2) and previous studies based on ddRAD²⁴, both of which describe the split between RB and UC happened after the split of their common ancestor from NV. This divergence has been estimated to be quite recent (0.26 Mya)²⁴, which may explain why the complete resolution of the phylogenetic relationship inside red bald uakaris appears harder to reach.

Recent divergence times in *Cacajao* have resulted in different genetic diversity ranges

In order to uncover the past demographic history of wild uakaris, constant effective population sizes (*N_e*) and divergence times were estimated employing an ML approach with fastsimcoal2 and three different model topologies (Fig. 3A, Supplementary Methods 1.3.1, Supplementary Table S4). Among these, split times were estimated providing a refined and narrower confidence interval in three of the cases when compared to previous ddRAD estimates (Bald-black: 0.92 Mya [0.89–0.98], bald_red-bald_white: 0.66 Mya [0.64–0.68], CV-AM: 0.16 Mya [0.15–0.18])²⁴, a

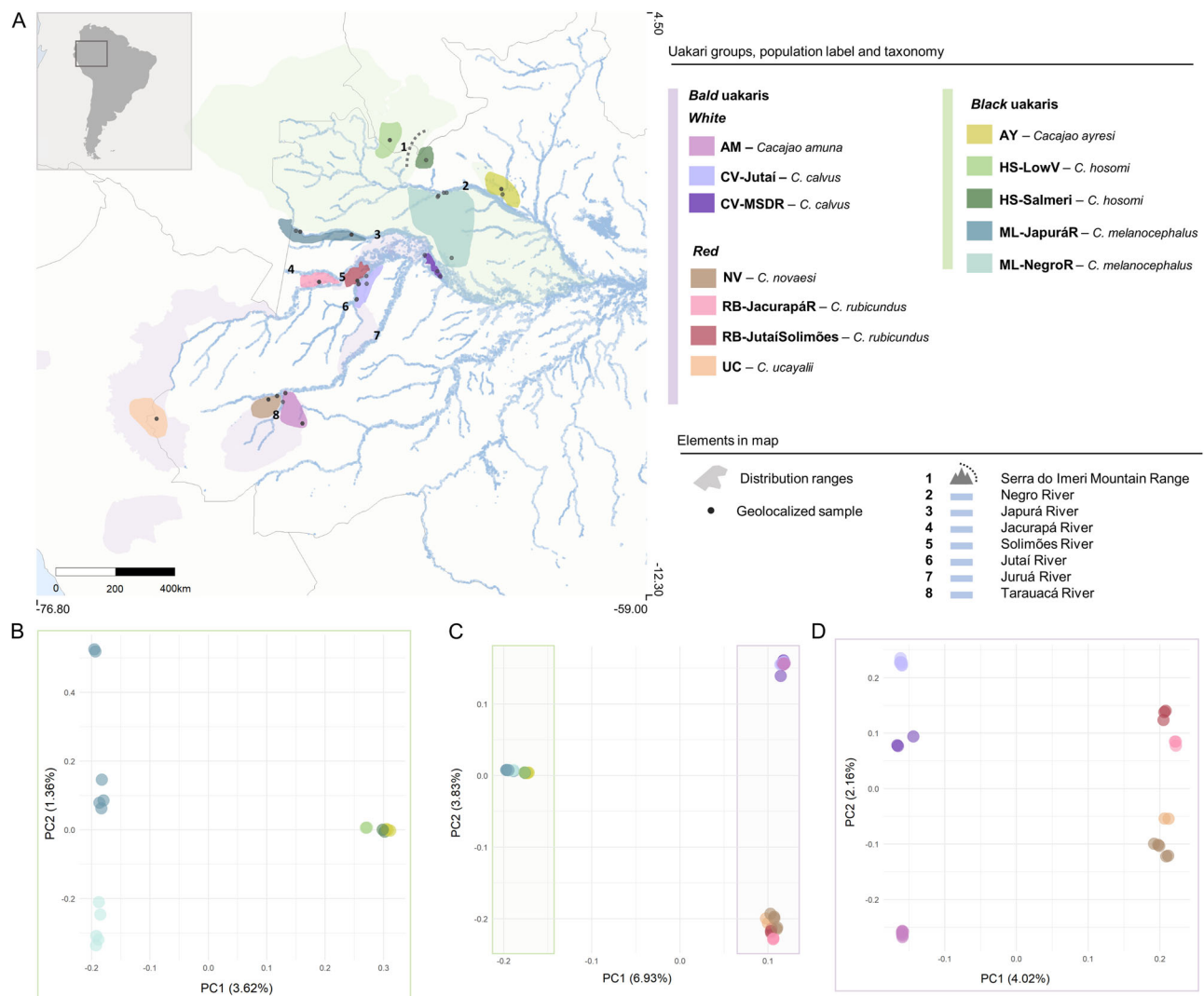


Fig. 1 | Sampled populations' distribution and structure. **A** Sampled localities and population geographic distributions. **B** Principal component analysis on the black uakari dataset ($n = 2.24$ M SNPs). **C** Principal component analysis on the global

dataset ($n = 10.46$ M SNPs). **D** Principal component analysis on the bald uakari dataset ($n = 6.10$ M SNPs).

similar estimate for the divergence time inside black uakaris to previous studies (0.66 Mya [0.27–0.82])²⁴ and lastly, a new estimate for the divergence between the two ML populations (0.03 Mya [0.02–0.15]).

Focusing on the N_e estimates for extant populations, a trend can be observed where bald uakaris tend to have an overall larger N_e and mean genome-wide heterozygosity estimates when compared to black populations—with the exception of CV-Jutai, which shows lower N_e than the ML-NegroR population; and both RB populations, whose mean heterozygosity falls in the black range. The black AY population presented the lowest genetic diversity among *Cacajao* populations (Fig. 3B). Nonetheless, inbreeding was not widespread in the genus and remarkably non-existent in the sampled black uakari populations (Supplementary Data 1). Overall, *Cacajao* showed a lower genetic diversity than other genera in the Pitheciidae in accordance with Kuderna et al.¹⁰ (Fig. 3B). This ranged between 0.0014 and 0.0025 heterozygotes per base pair (Fig. 3B) with variable density distribution in each population (Fig. S3).

Geographic barriers of distinct effectiveness drive independent population dynamics in bald and black uakaris

The dynamics of wild uakari populations were investigated here as a potential underlying force driving the observed genomic clustering in our dataset. No significant signals of gene flow were detected between bald and

black uakaris (Fig. 4). Population dynamics in black uakaris were shaped by stable allopatric (geographic) barriers (Negro River and Serra do Imeri mountain range, Fig. 1A), which determined a discrete genomic structure in the group (Fig. 1B, S6B) while not preventing gene flow in some regions (Fig. 4). On the other hand, while the genomic structure in bald uakari populations was identifiable and populations were delimited by rivers too, we hypothesized variations in the hydrology in the floodplains of the Amazon basin and related river rearrangements have had an impact on these populations' dynamics²², given their proposed variable effect as effective barriers to gene flow^{33–36}.

Bald uakaris

Consistently elevated f -branch values (Fig. 4B) indicated widespread gene flow between the ancestors of white bald uakaris and the red bald RB. Focusing on CV-Jutai, this approximated Isolation by Distance (IBD) with RB populations. This was geographically plausible particularly in the case of the RB-Jutai-Solimões, where the dynamics of the Juruá, Solimões, and Jutai rivers would have affected the connectivity between the two groups^{34–36} found at opposite banks of it (Fig. 1A), allowing the approximation to a population following an IBD model (Fig. 4).

On the other hand, after the split of white bald uakaris, deviations from IBD appeared: (i) Positive effective migration rates were observed between

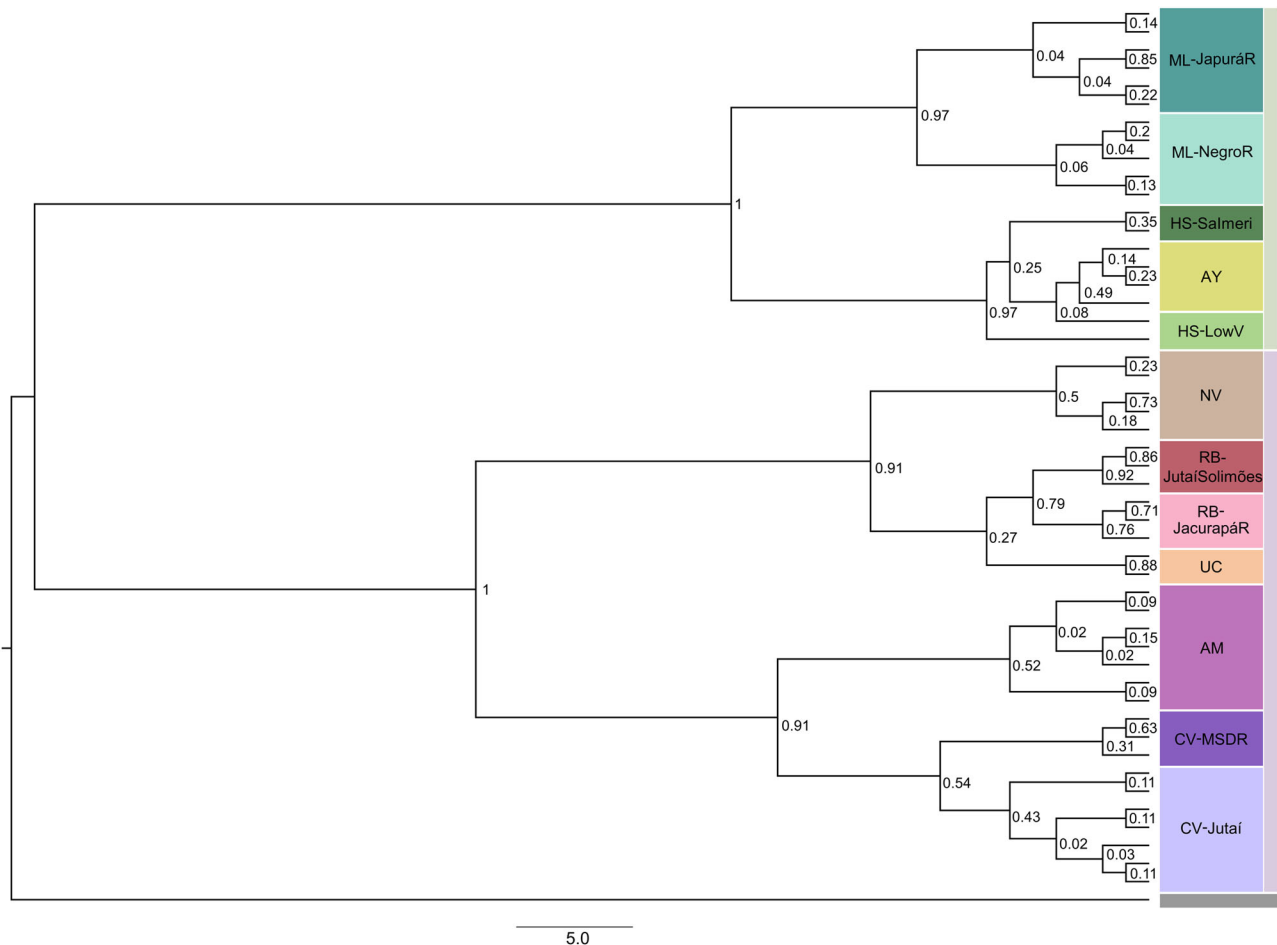


Fig. 2 | Whole genome phylogeny phylogram. Whole genome maximum clade-credibility phylogeny from 2144 maximum likelihood trees based on 1 Mb non-overlapping sliding windows. Phylogenetic support is indicated in each split. Phylogram where branch lengths are proportional to the observed phylogenetic distance

in terms of substitutions. Colors depict population labeling system, outer bar indicates bald (plum) or black (green) uakari group and bottom gray bar indicates outgroup node (*Pithecia pithecia*).

the two CV populations ($F_{ST} = 0.067$) as shown in Fig. 4A, although apart from recurrent interbreeding, strong shared ancestry could intensify such a signal. (ii) CV-MSDR and AM (*C. amuna*) ($F_{ST} = 0.065$) did show connectivity (Fig. 4A), and although ADMIXTURE analyses (Fig. S5B) showed the first population would share a small ancestry component with AM, we suggest that CV-MSDR represents a unique ancestral component on its own, as this signal was not captured by any other method. (iii) Connectivity was limited between CV-Jutaí and AM ($F_{ST} (AM, CV-Jutaí) = 0.092$) (Fig. 4B).

Gene flow signals were detected between AM and NV (Fig. 4B), species currently found on opposite banks of the Tarauacá River (Fig. 1A), where no migration was previously detected (Fig. 4A).

Black uakaris

Negro River—at its widest—together with Serra do Imeri appeared to fragment black uakari populations (Figs. 1A and 4A). In agreement with field observations which propose that ML distribution expands beyond Negro River's headwaters towards Colombia, results suggest that at the north-west of their distribution range, ML had historically interbred with HS from lowland Venezuela (HS-LowV) (Fig. 4B) ($F_{ST} (HS-LowV, ML) = 0.019$, $F_{ST} (HS-Salmeri, ML) = 0.021$). On the other hand, we observed high connectivity between ML populations potentially related to river channels formed in the past between Japurá and Negro River³⁷ (Figs. 1A and 4A), although this signal is most likely influenced by their very shallow divergence time (0.03 Mya [0.02–0.15]). Finally, at the northern bank of Negro River, connectivity was found between AY and HS (Fig. 4A) ($F_{ST} (HS-$

SaImeri, AY) = 0.041, $F_{ST} (HS-LowV, AY) = 0.046$) although it had been historically more intense between the first and the population of HS-SaImeri (Fig. 4B). These results explain the persistent clustering between these species in structure analyses (Figs. 1B, S6B, S11). The latter observations were also anticipated by field observations given the absence of clear barriers between HS-SaImeri and AY versus the allopatric barriers between them and the rest of the black populations.

In spite of the high connectivity among HS populations (Fig. 4A), these showed very distinct gene flow patterns (Fig. 4B). The split between AY and HS was estimated to be as recent as 0.2 Mya²⁴, therefore all the events these are involved in—population split in HS, interspecies gene flow—must have been even more recent. Furthermore, the effective migration rates among HS populations were likely overestimated because of their strong shared ancestry. All this would be consistent with the independent gene flow signals shown by these two populations with AY and ML respectively.

Functional genetic differentiation between groups is led by Malaria and Integrin-related pathways

Given the consistent genomic separation observed between bald and black uakaris (Figs. 1 and 2, S11), their distinct phenotype and independent population dynamics (Fig. 4), we aimed to explore the trace of their genome-wide differentiation at the functional level. For that, we identified those coding regions appearing most divergent in all intergroup comparisons: differentially fixed non-synonymous (NS) variants among bald and black uakaris were filtered by alignability of the given amino acid and conservation levels of these across the primate lineage (See Methods—Genetic

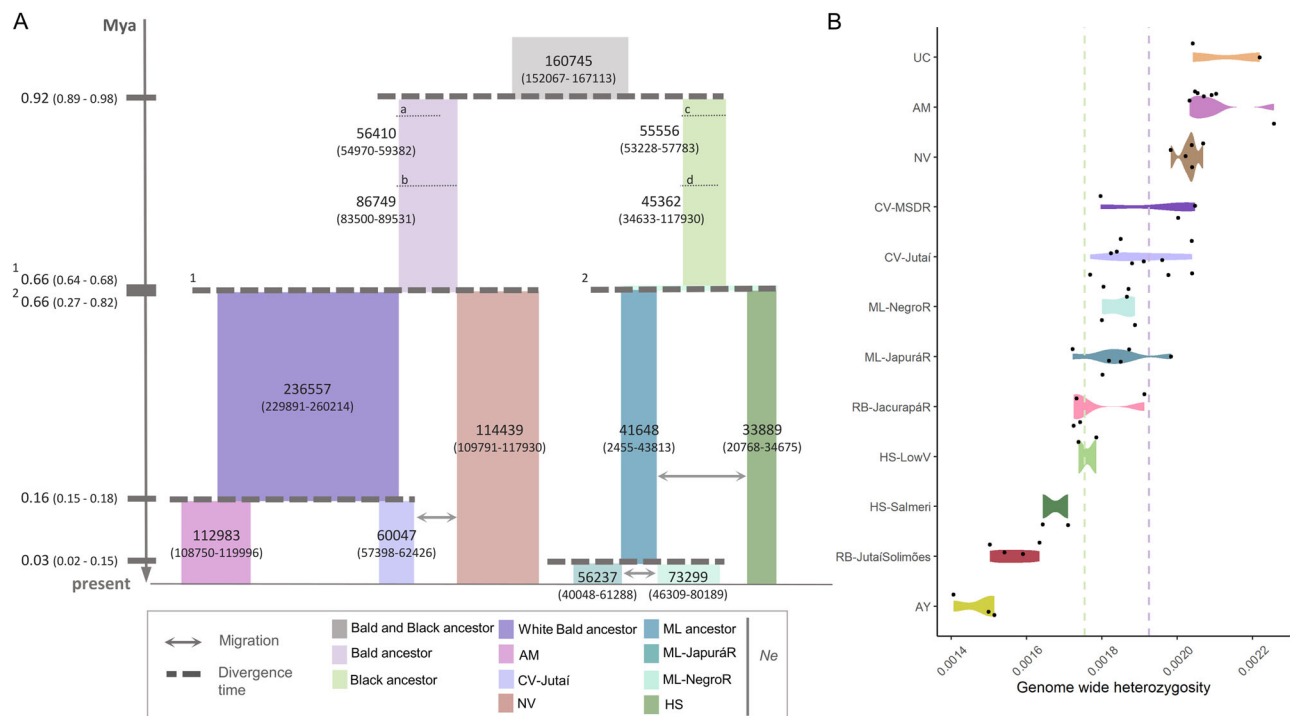


Fig. 3 | Demographic history of Cacajao and genetic diversity in extant populations. **A** Maximum likelihood model topologies and estimates for divergence times (depicted by dashed lines) and effective population sizes (depicted by color bars width, ML estimate indicated above confidence interval). Three models summarized in Fig. 3A: (i) bald and black uakaris general model, (ii) bald uakaris model and (iii)

black uakaris model. Bald and black ancestor Ne estimates were calculated twice: in the general model (a, c) and in the bald (b) and black (d) models respectively. **B** Genome-wide heterozygosity per population, points depicting individual observations and colored shape its distribution. D, dashed lines indicate mean values per group (bald: plum, black: green).

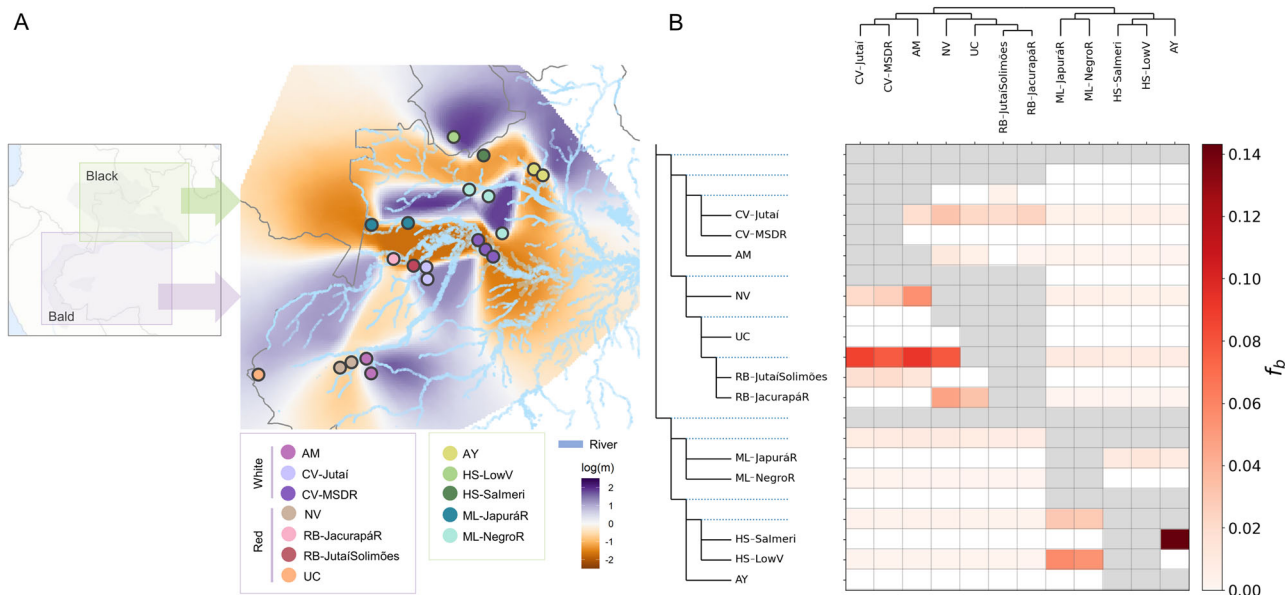


Fig. 4 | Migration patterns and gene flow in the Cacajao genus. **A** Estimated effective migration surfaces (EEMS) on black and bald uakaris complete dataset. Color palette depicting effective migration rate values ($\log(m)$), where white reflects

isolation by distance and color, deviations from it. **B** F-branch statistic on full dataset, color intensity reflecting intensity of event; significant values considered (f -branch ≥ 0.05).

differentiation using *Fst*) obtaining 1126 NS variants. The identified NS mutations clustered into 956 genes (Supplementary Data 2), specifically, 336 were detected only in bald uakaris, 406 only in black, and 98 had different mutations in both groups.

Focusing on genes with NS variation in bald uakaris, the dopachrome tautomerase gene (*DCT*) involved in melanin biosynthesis downstream to *MC1R*, showed a mutation in the position tarseq_64:7868470 (T > C) that

causes a NS change ($Q > E$). This gene has been linked to hair and skin color variation in animals^{38–40}.

Further, through a functional over-representation analysis (ORA), categories of OMIM, KEGG and PANTHER pathway were found to be significantly enriched in the above described sets of genes, as well as GO-Biological process categories for bald and black uakaris and HPO in the case of genes affected in both groups (Table 1). The strongest enrichment in the

Table 1 | Significant functional differences between bald and black uakaris

ORA significant results (FDR<0.05)	
<p><u>336</u> genes show fixed mutations only in bald uakaris</p> <p>Malaria ECM-receptor interaction Hematopoietic cell lineage Cytokine-cytokine receptor interaction KEGG pathway</p>	<p>Myocardial infarction Malaria, susceptibility to Malaria, resistance to Lung cancer alveolar cell carcinoma OMIM</p>
<p><u>406</u> genes show fixed mutations only in black uakaris</p> <p>Integrin activation Regulation of cell cycle phase transition GO – Biological process</p> <p>Integrin signalling pathway PANTHER pathway</p>	<p>Hypercholesterolemia familial Schizophrenia Tracheoesophageal fistula Colorectal cancer OMIM</p>
<p><u>98</u> genes show different fixed mutations for the two groups</p> <p>Lipoprotein localization Regulation of innate immune response Positive regulation of defense response Immune response-regulating signaling pathway GO - Biological Process</p> <p>Tracheoesophageal fistula Breast cancer Prostate cancer OMIM</p>	<p>Posterior pharyngeal cleft Recurrent bronchitis IgG deficiency Emphysema Leukopenia Lymphadenopathy Abnormality of the lymph nodes Hematological neoplasm Elevated hepatic transaminase Abnormal leukocyte count Human Phenotype Ontology</p>

Significant over-representation analysis (ORA) results (FDR ≤ 0.05) on genes with non-synonymous mutations in conserved sites across the primate lineage, which were differentially fixed between groups.

common list of genes (different mutation in each group) was of the *Lipo-protein localization* category from KEGG pathway, nonetheless, there was an overall enrichment for immune-related categories in the three databases (Table 1, Fig. S14). On the other hand, *Malaria*-related categories were detected both from OMIM and KEGG pathway databases for bald uakaris (Table 1, Fig. S15), although in the latter database the most enriched category was *Myocardial infarction*. Lastly, for genes with fixed NS variation in black uakaris, *Integrin*-related categories were the most significantly enriched in GO-Biological process and KEGG pathway databases, further, *Hypercholesterolemia familial* was the strongest enriched category from the OMIM database (Table 1, Fig. S16).

Discussion

We have generated the first population-level study based on whole genome data of a platyrrhine genus: the *Cacajao*, endemic to the Amazon and divided into bald and black uakaris¹⁹. By the analysis of 48 geolocalized whole genomes of wild uakari populations, we provide a detailed description of population structure and dynamics as well as refine the pre-existing demographic estimates^{14, 21,24,30}. Using population-level data, we built a phylogeny based on 1Mb-windows across the whole genome of uakaris. Moreover, narrower confidence intervals have been obtained for divergence times and ancestral or current *Ne* estimates, while migration rates were estimated for the first time to the best of our knowledge. Through this, we have begun to detangle the complex relationship between a highly dynamic environment such as the Amazon rainforest and primate whole genomes. We believe our dataset provides an exceptional representation of all wild uakari species considering the difficulty in sampling wild mammalian populations. Nonetheless, we are aware our study might be constrained by

the lack of evenness in-population sampling and sometimes limited sample sizes.

Bald and black uakaris

Bald and black uakaris present strong differences both phenotypically and ecologically^{19,21,23,24}, which hint at differences at the genomic level that have been described in this study. Since their split, the two groups have taken divergent phylogenetic trajectories and come to represent (i) two distinguished genetic clusters of populations (Figs. 1B–D and 2) with (ii) different genetic diversity ranges (Fig. 3B) and (iii) effective population size (*Ne*) estimates (Fig. 3A) that display (iv) independent population dynamics (Fig. 4).

Population structure and phylogenetics

By considering the whole autosomal genome, the samples in the dataset clustered into north and south bank of the Negro River for black uakaris and into red and white for bald, overall showing strong population structure sometimes even past the species level. In the phylogenetic inference, we were able to go beyond previous ddRAD-based analysis²⁴ by classifying all the samples into their designated populations (Fig. 2) in accordance with population structure analyses (Figs. 1B–D and S4–6). The only exception to this fine clustering was a sample of HS_LowV, which appeared closer to AY with very low probability (0.08). The overall high level of resolution was reached with high support homogeneously distributed along the genome, which means the signal was not driven by specific regions in the genome when 1 Mb windows were employed (Figs. 2 and S9B, C). Nonetheless, slight discrepancies between the latter and the results obtained from employing 250 kb windows were observed specifically regarding the red

bald uakaris clade (Fig. S8). This could be reflecting incomplete lineage sorting due to shallow divergence times in this clade: such signal would be better captured when smaller portions of the genome are employed to build independent window trees, leading to a more topologically divergent set of trees to build the consensus from.

On the other hand, the phylogeny built from the whole mitochondrial genomes (Fig. S10) only recovered the main splits ((white bald, red bald), (ML, (HS, AY))), being unable to get to the species level as reflected in all preceding publications^{14,21,24,30}. The signal retrieved from mitochondrial genomes is limiting since it reflects a partial evolutionary history, that of the female lineage⁴¹. It has been proposed that given the high levels of male-male affiliation observed, dispersal in *Cacajao* could be female-biased⁴². If this was the case, we could hypothesize that prevalent female dispersal inside the resolved clades could be the cause of the observed topology: the phylogenetic trajectory described by a limited number of female lineages does not necessarily have to coincide with the trajectory of all inspected lineages in the genus. Besides, in comparison to the autosomal range, analyses based on mitochondrial genomes rely on a much lower number of markers proportionally, which can hinder the resolution of the most terminal nodes of a tree because of decreased statistical power^{16,17}.

Demographic history and current genetic diversity

The first split identified by all phylogenies, which depicts the divergence of black and bald uakaris, was estimated to be 0.91 Mya [0.89–0.98], falling in the Pleistocene as previously suggested^{21,24} (Figs. 3A and S13). This estimate falls inside the confidence interval of ddRAD estimates (1.13 Mya [0.67–1.72]²⁴), but far outside that of estimates based on cytochrome B data (5 Mya²¹ and 2.38 Mya [1.3–3.58]²⁴). Further, the first intra-group divergences were estimated to happen simultaneously in both groups at around 0.66 Mya (0.66 Mya [0.64–0.68] in bald, 0.66 [0.27–0.82] in black) (Figs. 3A and S13). While we remain confident 0.66 Mya is an accurate estimate for the first divergence in bald uakaris, given the wide confidence interval in the estimate for black uakaris we do not rule out the exact match might be artefactual. Nonetheless, in accordance with previous studies based on ddRAD sequences (0.46 Mya [0.29–0.7] in bald, 0.48 Mya [0.27–0.78] in black)²⁴, we believe the divergence time between ML and (HS, AY) does fall inside the estimated confidence interval, still being a close time frame to the first split in bald uakaris and thus a relevant point worth exploring further. In this case, estimates based on mitochondrial sequences are again far from those obtained with whole genomes²⁴. Overall, these comparisons indicate that despite being a partial representation of the whole genome, the level of resolution obtained through the employment of ddRAD sequences is much closer to that obtained via whole genomes than through mitochondrial gene sequences, yet not complete.

On the other hand, we tested three demographic models considering constant N_e and estimated values for ancestral (*Cacajao*, bald uakaris, white bald uakaris, black uakaris, ML) and extant populations of AM, CV-Jutai, NV, HS, and ML (Fig. 3A). Given that our sample size was not even among populations and this was limited in some cases, we estimated constant N_e s to reduce the complexity of the models, hence acknowledging the constraints of our estimates. Although confidence intervals in the black model were overall wider than those in bald, current estimates reflected that bald N_e s were in general bigger than those of black in those populations included (Figs. 3A and S13). In accordance, we saw that genetic diversity in bald populations was higher than black's, being highest in UC and lowest in AY (Fig. 3B), likely reflecting the known largest distribution range in bald uakaris in the case of UC²⁶ and the smallest in black AY²⁵. In this context, both RB populations were the only exception to bald's higher genome-wide heterozygosity when compared to black's: RB populations show a very disjunct and small distribution range²⁶, which together with their diversity falling below bald's range, could point towards a past bottleneck⁸, although this would need to be explicitly tested. Regarding the estimates of N_e , again bald's higher sizes were with the exception of ML-NegroR ($N_e = 73499$ (43309–80189)), which showed a slightly higher point estimate than CV-Jutai ($N_e = 60047$ (57298–62436))—yet displaying a much wider confidence

interval. In context with genome-wide heterozygosity, we find that their closeness in N_e is not unreasonable given that ML-NegroR was the black population with the highest mean genetic diversity in the group and CV-Jutai that with the lowest in bald (Fig. 3B), with overlapping ranges. We are aware these are constant estimates of effective population size and hence may fail to reflect the true demographic history of the inspected lineages throughout time; nonetheless, the presented estimates are overall coherent with found heterozygosity ranges and thus useful in their interpretation.

Independent population dynamics

Despite the disparities in habitat preference and mostly disjunct distribution of bald and black uakaris, their range limits become very close in the area between the right bank of the Negro River and the Solimões River at opposite banks of the Japurá River (Fig. 1A). In this region, a new and isolated population of the white bald-headed uakari *C. calvus* was found on an oxbow island of flooded forest that moved from the right bank of the Japurá River to the left; therefore in the same bank of the black uakari *C. melanocephalus*²⁶. Yet, no significant gene flow signal was detected between groups (Fig. 4B). We are unable to describe the origin of the observed reproductive isolation, but given the proximity of these two distribution ranges at this point and the fact that other populations in this study showed connectivity despite the presence of the Japurá River (Fig. 4A), we hypothesize that the isolation between bald and black uakaris is currently maintained by overall independent population dynamics driven by differential habitat preference, dissimilar phenotypes and relevantly by reproductive incompatibilities. Chromosomal rearrangements have been reported to alter recombination rates in the best cases leading to infertile descendants between differing karyotype-species⁴³. Two distinguishing chromosomal rearrangements have been identified in the karyotypes of *C. rubicundus* and *C. melanocephalus*⁴⁴, which are probably related to the observed reproductive isolation of bald and black uakaris. On the other hand, divergent habitat specializations have been proposed to affect patterns of gene flow³⁶—despite populations of bald and black uakaris can be found both in flooded and upland forests, these do not share overall habitat preferences: flooded areas around white or black water rivers and general migratory patterns between habitat types^{21,45}. Furthermore, differing phenotypic features—in this case, notably pelage coloration¹⁹ and bald's characteristic red face²³—have been described as drivers to reproductive isolation in primates^{39,46} and could be playing a major role in mate choice of the same *Cacajao* group. Likewise, the highly vasculated red face of bald uakaris, not present in black, has been suggested to represent a group-specific communication signal of health status to potential sexual mates²³.

On the other hand, past admixture is detected in wild populations in this study (Fig. 4), although no admixed individuals are found (Fig. S4–6) and relevantly, population structure is maintained.

The genomic structure of black uakari populations is driven by Negro River²⁵ and Serra do Imeri (Figs. 1A and 4A). River width has been suggested to be determinant of population connectivity hindering gene flow in the wider sections of its course^{35,47,48}. Along its course, the Negro River poses a barrier of variable intensity to black uakari populations: at its lower course, the river gets wider⁴⁷ and this could explain the absence of gene flow signals between AY and ML populations (Fig. 4B). Nonetheless, connectivity has been enabled between ML and HS-Salmeri populations in the north-west distribution of black uakaris, beyond the highwaters of the Negro River (Fig. 4A)⁴⁷. Moreover, the populations of HS show differential gene flow patterns by interbreeding with different populations (Fig. 4B), which could have been influenced by the presence of the mountain range acting as a barrier: HS-LowV would have interbred with ML and on the right side of the mountain range at a higher altitude, and HS-Salmeri with AY (Fig. 4B), the latter relevantly being the strongest detected gene flow signal. The two ML populations, found in the upper Japurá River (ML-JapuráR) and in the right bank of Negro River up to the Solimões-Japurá confluence (ML-NegroR) respectively, displayed disjunct genomic clustering (Fig. 1B) despite the fact that these appeared connected and shared gene flow patterns (Fig. 4). The time split between these two populations was estimated at 0.03 Mya

[0.02–0.15] (Fig. 3A). Field observations have suggested these populations interbreed irrespective of Japurá River, moreover, the existence of a paleo-channel connecting Negro and Japurá River has been proposed based on digital elevation model analyses³⁷. However, considering all our results we are not able to discern between high connectivity between these populations or strong ancestry sharing caused by their very recent split time.

In contrast to black populations, whose structure is mainly driven by two prominent barriers, bald uakaris are mostly found in flooded forests of western Amazonia²⁶, a seasonally⁴⁹ and historically dynamic environment^{24,36,37}. This dynamism could hypothetically explain why a priori, current disjunct geographic distribution alone does not hint at the genomic pattern drawn by white and red bald uakari populations. These groups were estimated to diverge 0.66 million years ago [0.64–0.68] (Fig. 3A). This is a more recent estimate than those previously introduced based on cytochrome B (0.91 Mya [0.5–1.42]²⁴), but falling in the estimated interval of those based on ddRAD sequences (0.46 Mya [0.29–0.7]²⁴), now with higher confidence. In the first place, seasonal variations in hydrology, which are particularly common in the floodplains of the Amazon basin linked to rain patterns⁵⁰, have been proposed to affect gene flow by intensifying and diminishing the effect of rivers as effective barriers^{22,33–36}. River dynamics have been proposed to particularly affect population connectivity in systems found in the western part of the Amazon^{14,22,36}, where lowlands are of a much younger age than those in the east, thus shaping a less stable geological topography^{33,35}. In this framework, intermittency of rivers as effective barriers could hypothetically contribute to explain the observed interspecies connectivity in bald uakaris in spite of apparent geographic barriers—in the case of RB populations and CV-Jutai separated by the Jutai River, and AM and NV far in the south delimited by the Tarauacá River (Figs. 1A and 4) as previously suggested by Silva et al.²². Secondly, it is widely understood that current geographic arrangement represents a very precise moment in time, hence evolutionarily relevant geographic barriers for a given lineage might not be present anymore^{25,34}. In this sense, geography does not need to agree with the observed genomic landscape, which on the contrary summarizes the evolutionary history of a given lineage. Related to this, major geographic rearrangements such as the formation of riverine channels⁵⁰, specifically between Juruá and Jutai Rivers^{22,26,37}, have been reported in the Amazon basin throughout the recent geological past (100 kya). This river rearrangement could have led to the isolation of white bald uakari populations from red bald populations in opposite banks (Fig. 1A) by altering their patterns of geographic distribution²².

Genetic differentiation

In order to explore what was the functional impact of the observed independent genomic clustering of bald and black populations, we further inspected which were consistently, the most divergent coding genomic regions between all intergroup pairs of populations (Table 1). Stringent filtering was applied to these to account for the limitations of the annotation being used—which despite being crucial to our study as others alike are to explore non-model organism genomics, was known to be constrained. By focusing specifically on NS variants found in codons that showed diversity across the primate phylogeny, we first detected 98 genes with differentially fixed SNPs in both groups (Supplementary Data 2). These were mostly enriched for functions involved in the regulation and performance of the immune system (Table 1, Fig. S14), which was not striking in accordance with the extensive number of studies done on genes under selection in primates⁵¹. Additionally, 336 (Supplementary Data 2) and 406 genes (Supplementary Data 2) were detected to harbor differentially fixed NS SNPs only in bald or black uakaris respectively. In the set of genes of bald uakaris, we show a NS mutation in the *DCT*. Involved in melanin biosynthesis, this gene has been found to be under positive selection in East Asian human populations related to light skin pigmentation³⁹, and to dilute coat color phenotypes when knockout in mice due to reduced melanin content in hair³⁸. Here, from a maximum parsimony perspective, one could assume the ancestral phenotype for *Cacajao* is dark for coat coloration, given that its closest phylogenetic genus, *Chiropotes*¹⁰, shares this with black

uakaris²⁷. Considering this, our results would match the expectation that bald uakaris should be the group to have diverged from black uakaris, which on the contrary share the ancestral state with the *Pithecia pithecia* reference genome. We are aware that our results do not imply causality for the phenotype of bald uakaris and that further targeted functional analyses should be addressed in order to confirm the link between this mutation and the observed variation in coat coloration. Nevertheless, the genetic landscape of non-human primates has been inspected before with the aim of identifying precise genetic patterns to explain light-dark phenotypic transitions in the phylogeny without concluding results^{39,40,52}, hence we consider this to be a relevant advance and starting point for potential further research. On the other hand, regarding the functional categories enriched in these sets of genes, we found these were less specific than in the common set. Nonetheless, Malaria-related categories for bald (Table 1, Fig. S15) and integrin pathway-related categories for black uakaris (Table 1, Fig. S16) arised more than once in independent databases. Although the underlying mechanisms leading to the enrichment of integrin pathway-related categories only in black uakaris remain unresolved in this study, differential *Platyrrhini*-specific variation inside the primate lineage has been identified in the integrin transmembrane receptor family of proteins, which beyond being linked to immune function, have been recognized specifically as receptors to viruses in primates⁵³. On the other hand, a specific species of *Plasmodium* that is regarded as zoonotic Malaria in South American primates (*Plasmodium brasilianum*) is currently known and was first ever reported on the blood of a bald uakari in 1908⁵⁴. Regarding this, black uakari species have been hypothesized to be at lower risk of infection⁵⁵ given that the density of Malaria-vector mosquitoes is low⁵⁶ in their usual habitat close to black water rivers. On the contrary, bald uakaris mostly inhabit areas nearby white water rivers—with the only exception of the *Jutai River*³⁵—, specifically flooded areas which are considered Malaria hotspots⁵⁷. We hypothesize higher exposure to the parasite throughout time in bald uakaris would explain the observed enrichment for Malaria-related categories in this group in contrast to black. Overall, we consider these are preliminary results on the functional differentiation between bald and black uakaris and encourage future studies to further explore this topic.

Conservation perspective

Connectivity is broadly widespread between populations of the same *Cacajao* group (Fig. 4), which is particularly relevant for bald uakaris which had been regarded as mostly isolated populations²⁶. This indicates connectivity is a relevant factor in these genus' population dynamics and to maintaining the health of *Cacajao* populations in the wild⁸. Climate change effects combined with anthropogenic threats have led to population decline in the past in lemur⁵⁸, langur⁵⁹ and tamarin⁶⁰ lineages via habitat fragmentation and changes in connectivity⁹. In the case of *Cacajao* populations, black uakaris have been categorized by the IUCN red list of endangered species as low concern (ML⁶¹, AY⁶²) and vulnerable (HS⁶³), and display a wider and rather continuous distribution range in contrast to bald uakaris²⁵, being reported to migrate between different habitat types²¹. Despite these populations show higher habitat versatility and are found in the northern distribution in the Amazon Rainforest—further from the deforestation focus⁶⁴—their conservation status is still of concern, in particular, because of the high hunting pressure they are exposed to⁶³.

Bald uakaris are largely specialists of flooded forests, which makes them highly dependent on the wellbeing of this very particular habitat^{21,24}. The distribution of bald uakaris is disjunct (Fig. 1A), with the most extreme example being the sampled population of CV-MSDR, which is known to be found in a flooded-forest area limited by the Solimões and Japurá Rivers isolated from the remaining bald populations²⁴, and effectively isolated from a genomics perspective as observed in this study too. The *Cacajao* genus has been under thorough taxonomic review in the recent past^{19,24}, probably the reason why all bald uakaris are still assessed as subspecies of *C. calvus*, following the taxonomic classification of Hershkovitz (1987). Accordingly, two taxa were categorized as Vulnerable—*ucayaliti*⁶⁵ and *novaesi*⁶⁶ and two more—*calvus*⁶⁷ and *rubicundus*⁶⁸—as Least Concern, likely in relation to

their northern distributions⁶⁴. Also, *C. amuna* is the only taxon not assessed yet as it was recently described²⁴.

Although habitat loss is currently not an immediate threat for all these populations, particularly for those of northern distributions (e.g., *C. calvus* and *C. rubicundus*)⁶⁴, it is relevant to acknowledge their vulnerability linked to habitat specificity²⁴. There is yet a relevant exception in populations of *C. ucayalii* (UC was sampled in this study), which are the only bald uakaris to be reported at high altitude non-flooded forests and show the largest distribution range^{26,69}. In accordance with field observations²⁶, the UC population sampled in this study showed no significant signals of interbreeding with any other bald population, which significantly may suggest a crucial interplay between their habitat versatility and such high genetic diversity levels, highest in the *Cacajao* genus, despite being reproductively isolated from the rest of the populations in this study. Contrary to this, CV and RB populations are the most flooded-forest specialists in the genus and present the most restricted distribution²⁶, accordingly, in this study the sampled populations from these species show the lowest genetic diversity and estimated *N_e* (CV) in bald uakaris.

Cacajao is however one more case in the global panorama, where we find more than half of the extant primate lineages are under extinction threat^{9,10}. With the aim of starting to fill the notable gap of knowledge in non-Catarrhini (mostly non-great ape) wild populations' genomics^{10,12–14}, we hope the understanding of uakari wild population dynamics and their respective degree of genetic diversity may contribute to the implementation of effective conservation actions to face increasing rates of wild biodiversity loss in the near future^{4,8,9,70}.

Methods

Sample processing and data generation

We used samples from 48 *Cacajao* individuals (*N_{bald}* = 30, *N_{black}* = 18) and 1 *Pithecia* from wild populations on the Brazilian Amazon with known geographical coordinates. The latter was added as an outgroup in certain analyses. All samples in this study were muscle tissue samples from wild-caught uakaris preserved in 70–90% ethanol obtained from Brazilian zoological collections in Instituto Nacional de Pesquisas da Amazônia (INPA), Universidade Federal do Amazonas (UFAM) and Instituto de Desenvolvimento Sustentável Mamirauá (IDSM). The individuals analyzed were either sampled during multiple commissioned large field surveys of the biodiversity of the Amazon rainforest by the Brazilian government (2000–2017) or as part of monitoring programs, from hunted individuals by local communities. Collection permits were obtained from the Biodiversity Authorization and Information System (SISBIO; permit nos. 55777, 42111, 32095–1, 7795–1) and exported under CITES permits (19BR033597/DF and 15BR019039/DF), always in compliance with all relevant ethical regulations for animal use. We extracted the DNA with the MagAttract HMW DNA extraction kit and prepared short-inserted paired-end libraries for the whole genome with the KAPA HyperPrep kit (Roche) PCR-free protocol. From these, we sequenced paired-end reads with a $2 \times 151 + 18 + 8$ bp length on NovaSeq6000 (Illumina) to reach at $\sim 30\times$ coverage per sample. For more details on the data generation steps see Supplementary Methods in Kuderna et al.¹⁰.

Data processing

We interleaved the reads with seqtk mergepe (v1.3)⁷¹ and subsequently trimmed the adapters with cutadapt (v3.4, `-interleaved`)⁷². We then mapped the trimmed reads using bwa-mem (v0.7.17, default settings)⁷³ to the reference assembly of the *Pithecia pithecia* species at the scaffold level presented in Shao et al.¹⁵. We used a reference genome from a genus outside *Cacajao* for the following reasons, (i) given that *Cacajao* is a non-model organism *Pithecia pithecia* was the closest species with an available reference genome and (ii) we considered using a sister but external taxon as reference to our analyses was a necessary direction in order to minimize biases in our work related to reference bias and ancestry sharing. The reference genome had a length of 2.72 Gbp and a 10,87 Mbp scaffold N50. We proceeded to mark duplicates and add read groups employing bbmarkduplicates (default

settings) from biobambam (v2.0.182)⁷⁴ and the AddOrReplaceReadgroups (default settings) from PicardTools (v2.8.2)⁷⁵ respectively. The mode of the depth was calculated for all samples independently using MOSDEPTH (v0.3.3)⁷⁶.

We employed the resulting cram files for the calling of variants in a three-step manner using the GATK (v4.1.7.0)⁷⁷ toolkit. We generated eighty-nine equal-size windows from the reference genome to parallelize the process for the sake of computation time. At first, we generated a GVCF file using the HaplotypeCaller algorithm (`-ERC BP_RESOLUTION`) per each of the samples and chunks respectively. Secondly, we combined all variants called in all samples for a given chunk in the reference genome using CombineGVCFs (default settings) to be finally jointly genotyped employing GenotypeGVCFs (default settings).

Hard filtering was applied to the resulting SNPs in each chunk by removing those variants that were not biallelic (vcflib vcfliballelic (v10.5)⁷⁸), those with depth values outside the range between 70 (double the median maximum coverage across samples) and 5 (one third of the median minimum coverage across samples), and missingness higher than 60% using vcftools (v0.1.12)^{78,79}. Furthermore, following GATK Best Practices Protocol, we excluded those SNPs not complying with the following expression: `"QD < 2 | FS > 60 | MQ < 40 | SOR > 3 | ReadPosRankSum < -8.0 | MQRankSum < -12.5"` employing GATK VariantFiltration. We merged the remaining SNPs in each chunk-VCF into a single dataset, which we then filtered for allele imbalance by keeping variants with frequencies within the range of 0.25–0.75 (Supplementary Methods (Section 1.2), Fig. S1). Lastly, we removed SNPs in scaffolds belonging to sex chromosomes (identified in Kuderna et al.¹⁰) and those in scaffolds shorter than 0.5 Mb from the dataset, thus keeping 367 scaffolds.

Data analysis

Genome-wide heterozygosity. We calculated genome-wide heterozygosity in windows of 100kbp. For each sample independently, we extracted the number of callable base pairs in the given window region together with the number of heterozygous positions. These values were used (i) to calculate the mean heterozygosity per individual in the dataset as well as (ii) to plot the heterozygosity distribution across the genome. Plots were generated using the ggplot2⁸⁰ package in R (v4.2.2)⁸¹.

Relatedness and inbreeding. We used NGSRelate2 (v2.0)⁸² to calculate relatedness and inbreeding in the dataset, which employs the Jacquard coefficients to estimate the kinship and inbreeding coefficients respectively. We assessed relatedness based on the *theta* parameter (Fig. S2). We then split the full dataset into sub-datasets based on the taxonomy to calculate relatedness and inbreeding so these were not overestimated due to population structure. Plots were generated using the ggplot2 package in RStudio.

At this point, we independently filtered the full dataset and the subset of bald individuals using PLINK (v2.0, `--maf 0.019 --geno 0.4`)⁸³. Furthermore, we only filtered the dataset including the black species by `--maf 0.05 --geno 0.4`, employing a different minimum allele frequency filter due to the difference in number of individuals. These datasets were used to run EEMS and SMC++ (See below). Then, in all three datasets, we obtained independent sites by pruning the datasets of linkage disequilibrium using the default settings (`--indep-pairwise 50 5 0.5`). These datasets were employed to run PCA and ADMIXTURE (See below).

Population structure

PCA. We generated three PCA plots using smartPCA from EIGENSOFT (v7.2.1)⁸⁴. First, we calculated the eigenvalues and eigenvectors on the full dataset (bald and black samples), on the bald species subset, and on the black species subset. Plots were generated using the ggplot2 package in R (v4.2.2). When compared, Fig. 1C and Supplementary Fig. 7A depict differences before and after applying the above-mentioned LD pruning filter. Supplementary Figs. were generated with PLINK (v2.0) `--pca` and plotted in the same way as the main figures.

ADMIXTURE. ADMIXTURE⁸⁵ software was run on the full dataset (bald and black), and on both partial datasets independently. We generated fifty different random seeds for the runs on the different datasets so as to compare the results of independent runs and assess the model convergence in each of the three cases. The tested number of ancestral populations (K) ranged from 2–13 in the full dataset and from 2 to 8 in the partial datasets. Plots were generated with the ggplot2 package in R (v4.2.2) using the data in those runs with the lowest cross-validation error.

Connectivity and gene flow

Testing deviations from isolation by distance. Using the Effective Migration Surfaces (EEMS)⁸⁶ software, we estimated migration and diversity rates in our dataset by looking for deviations from an isolation by distance model. We run this three times, one on the full dataset (bald and black), and the other two on the partial datasets. We generated dissimilarity matrices with EEMS' bed2diffs_v1 program. We defined a geographic range in each of the cases based on geographic distribution knowledge obtained from the IUCN website. The software was run for 9 M iterations in each of the cases with a number of thinning iterations of 9999. We assessed the convergence of the MCMC and completeness in each of the times by placing parallel and independent runs. Plots were generated in R (v4.2.2) using the reemplots2 package⁸⁷, including Fig. 4A when combined with individual sampling locations.

Gene flow quantification using D and f statistics. To calculate D and f statistics we ran the DSUITE software⁸⁸ on 1914 M bi- and multi-allelic SNPs. We also calculated the f -branch metric⁸⁰ as a simplification of f statistics in large phylogenies with a high proportion of correlated values. We used the sequenced *Pithecia pithecia* sample (different from the reference) as an out-group. To obtain f -branch values depicting gene flow evidence in the dataset⁸⁰, the *Dtrios*, *Fbranch* and *dtools.py* tools from DSUITE were employed.

Phylogenetics

Whole genome phylogeny. From each of the samples and the one *Pithecia pithecia* outgroup sample above-mentioned, we generated 2144 1 Mb-long and 9313 250kb-long windows based on the autosomal regions in the *Pithecia pithecia* reference genome using bedtools makewindows⁸⁹. We wanted to use more than one window size to see its effect on the results. These covered 78.78% and 85.55% of the reference genome respectively. We obtained consensus sequences using ANGSD (v0.931, -doFasta 1)⁹⁰ multiple sequence alignment (MSA), which was trimmed with trimAL (v1.4.1)⁹¹. We then independently inputted these to iqTree2 (v2.1.2, -B 1000)⁹² to generate a ML tree per window after 1000 bootstrap replicates and automatic model selection. Two consensus trees were built by merging trees obtained from windows of a given length using BEAST's TreeAnnotator⁹³ following Pajmians et al.⁹⁴.

Topological distance between 1 Mb window trees vs. consensus tree. To assess the distribution of branch support across the genome, we calculated the topological distances from each 1 Mb window tree to the respective consensus tree. Two metrics were applied using ape.dist.topo function from ape R package (v5.6–2)⁹⁵: PH85 and score. The latter metric takes into account branch lengths.

Mitochondrial phylogeny. To run MitoFinder⁹⁶, we used a subset of the trimmed raw reads representing 4% of the total set per sample. Inside MitoFinder, we employed MetaSPADES to assemble into and Arwen⁹⁷ to annotate the mitochondrial genomes of each of the samples. We reordered all the contigs with Fasta-tools SHIT⁹⁸ option so all start at the CYTB gene. MAFFT⁹⁶ was then used to generate a MSA of the mitochondrial contigs, which was in turn trimmed using trimAL to generate a phylogenetic tree using iqTree2 (v2.1.2, -B 1000).

All figures of phylogenetic trees were built using FigTree (v1.4.4, <http://tree.bio.ed.ac.uk/software/figtree>).

Genetic differentiation using Fst

Average differentiation genome wide. We generated PLINK (v2.0) binary files with a 0.6 allowed missingness which was fed to the KRIS R package (<https://github.com/kridsadakorn/kris>). The fst.hudson function was used to calculate the average pairwise Hudson F_{ST} ⁹⁹ along the genome among all the identified populations in the dataset combining taxonomic data and observed population structure.

Differentiation in coding regions, analysis of NS variants. To calculate Hudson F_{ST} values per SNP in each pairwise comparison between the studied populations we used PLINK (v2.0). After this, we kept variants in the 99%-upper quantile of the F_{ST} distribution detected in all intergroup comparisons. As the employed VCF here was biallelic, these corresponded to differentially fixed variants in each of the two groups. Out of these, we investigated those variants in coding sequence (CDS) regions. These were identified using the annotation of the respective assembly based on human orthologs (Valenzuela-Seba 2024¹⁰⁰). We retrieved the sequence of those codons containing the kept variants both (i) in the REF state from the original reference genome employing Samtools faidx¹⁰¹ and (ii) in the ALT state from the filtered VCF employing Bcftools consensus¹⁰¹. We then proceeded to translate these codons and kept only those that contained a variant causing a NS mutation (6442 SNPs). These were then filtered based on their alignability and on their conservation in the primate order. First, given that the quality of the genomic annotation employed was low, after filtering we only kept codons that i) were confidentially aligned throughout the primate lineage and ii) those falling in genes without frameshifts (99.99% of the total) (3992 SNPs). The MSA including most primate lineages was obtained from Valenzuela-Seba 2024¹⁰⁰. We then grouped the filtered codons by gene, getting a total of 3143. Second, by assessing the amino acidic conservation across the primate lineage based on the Shannon entropy coefficient calculated in Valenzuela-Seba 2024¹⁰⁰, we kept only those positions in our dataset with lower levels of conservation across the whole primate phylogeny (positions in the top 75% quantile of the Shannon entropy distribution) in order to filter out positions so highly conserved at the order level that would show no signal at the genus level. By doing this, the list was reduced to 1126 positions, which fell into 956 genes. We divided this list of genes into three groups: genes, which showed NS mutations only in bald uakaris (336) only in black (406) and finally those affected in both groups—although by different variants (98) (Supplementary Data 2). We then employed the ORA option from the WebGestalt tool¹⁰² with default parameters to inspect functional enrichment in each of these gene lists independently. Here, we used the initial set of ortholog genes as background—excluding the genes with frameshifts. The following databases were tested for significant over-representation: GO (Biological Process non-redundant), KEGG, PANTHER pathway, OMIM and HPO. We report significant results ($FDR \leq 0.05$).

Modeling demographic history

ML modeling with fastsimcoal2. We generated a VCF including monomorphic positions by adding --select-variant-type NO_VARIATION with the GATK (v4.1.7.0) VariantFiltration option including those autosomal scaffolds longer than 0.5 Mb (367). No missingness was allowed across all samples and only biallelic sites were included. In order to minimize intra-population structure, widespread gene flow and coherent sample sizes between populations, we only included a subset of the samples in the general dataset (Supplementary Table S3). Coding regions predicted on the *Pithecia pithecia* reference genome were removed in order to keep putatively neutral positions based on the annotation from Valenzuela-Seba 2024¹⁰⁰. Folded multiSFS to be used as input for fastsimcoal2¹⁰³ were generated with the associated vcf2sfs.py and foldSFS.py scripts based on 162,75 M SNPs and 1.953 M monomorphic positions.

Models details. We generated three models, a general one including bald and black samples, and two more for each of the groups alone. We included seven samples from the population of *C. calvus* from Jutai River (CV-Jutai)

and six from *C. melanocephalus* from Japurá River (ML-JapuráR) in the bald and black model. Then, we sampled seven individuals from *C. calvus* from Jutai River (CV-Jutai) and *C. amuna* (AM) representing white bald uakaris and six from *C. novaesi* (NV) for red bald uakaris for the bald model. Finally, six samples from *C. melanocephalus* from Japurá (ML-JapuráR), five from Negro River's (ML-NegroR) and four samples from both *C. hosomi* populations—given their very low genetic differentiation—as HS, were included in the black model. For more details about the models see Supplementary Methods (Section 1.3.1).

The mutation rates used in these models were calculated in Kuderna et al.¹⁰: the mean between *C. calvus* and *C. melanocephalus*' mutation rates was used for the bald and black model, *C. calvus*' mutation rate was used in the bald model and the mean between *C. hosomi*'s and *C. melanocephalus*' in the black one.

SMC++ demographic inference. To compensate for the fact that N_e is assumed constant in the previous models, we used the SMC++ software to investigate the fluctuations of N_e through time. Making use of the four populations defined and respective sample sets used for the group-specific ML models (White bald uakaris, red bald uakaris, south bank Negro River black uakaris and north bank Negro River black uakaris), we created a .smc file employing the vcf2smc program from the SMC++ (v. 1.15.2)¹⁰⁴ software per group and each of the 367 remaining scaffolds after filtering in previous steps. After that, using the associated estimate program (--spline cubic --timepoints 0 100,000), we generated a .model file per group applying an averaged mutation rate per generation across all *Cacajao* estimates in Kuderna et al.¹⁰. We built one single plot with all group estimates together using the plot script from SMC++ and indicating a generation time of 10 years based on Kuderna et al.¹⁰.

Statistics and reproducibility

Statistical tests, samples used, filters applied and file formats used in each analysis can be found in each Methods' subsection. By following these guidelines and employing the available trimmed genetic sequences all analyses are to be fully reproducible.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data and materials availability

All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Information as well as all the necessary parameters to run the used programs. This is with the exception of data used in some filtering steps of the Genetic differentiation using Fst Methods section—presented in the Valenzuela-Seba 2024¹⁰⁰ PhD thesis—which can be provided upon request. All main plots in manuscript figures can be reproduced following the Methods section from the trimmed fastq files. Furthermore, the Supplementary Data 2 file provides the list of genes with NS mutations by uakari group to run the Overrepresentation Analysis depicted in Table 1 and the Supplementary Data 3 file encloses the input file to BEAST's TreeAnnotator to reproduce the phylogenetic tree in Fig. 2. Reference genome assembly for *Pithecia pithecia* is available in GenBank with ID GCA_023779675.1. Trimmed fastq files for the *Cacajao* samples analyzed and the *Pithecia* outgroup sample can be found on the ENA Portal under the identifiers PRJEB49549 and PRJEB77610. Unique sample IDs in each repository can be linked to sample metadata through Supplementary Data 1. For further doubts or inquiries, contact nuria.hermosilla@upf.edu.

Received: 2 October 2023; Accepted: 16 September 2024;

Published online: 08 October 2024

References

1. Ortiz, A. M. D., Outhwaite, C. L., Dalin, C. & Newbold, T. A review of the interactions between biodiversity, agriculture, climate change,

and international trade: research and policy priorities. *One Earth* **4**, 88–101 (2021).

2. Habibullah, M. S., Din, B. H., Tan, S.-H. & Zahid, H. Impact of climate change on biodiversity loss: global evidence. *Environ. Sci. Pollut. Res.* **29**, 1073–1086 (2021).
3. Wilder, A. P. et al. The contribution of historical processes to contemporary extinction risk in placental mammals. *Science* **380**, eabn5856 (2023).
4. Xue, Y. et al. Mountain gorilla genomes reveal the impact of long-term population decline and inbreeding. *Science* **348**, 242–245 (2015).
5. de Manuel, M. et al. Chimpanzee genomic diversity reveals ancient admixture with bonobos. *Science* **354**, 477–481 (2016).
6. de Manuel, M. et al. The evolutionary history of extinct and living lions. *Proc. Natl. Acad. Sci. USA* **117**, 10927–10934 (2020).
7. Coimbra, R. T. F. et al. Whole-genome analysis of giraffe supports four distinct species. *Curr. Biol.* **31**, 2929–2938.e5 (2021).
8. Fontseré, C. et al. Population dynamics and genetic connectivity in recent chimpanzee history. *Cell Genom.* **2** (2022).
9. Estrada, A. et al. Impending extinction crisis of the world's primates: why primates matter. *Sci. Adv.* **3**, e1600946 (2017).
10. Kuderna, L. F. K. et al. A global catalog of whole-genome diversity from 233 primate species. *Science* **380**, 906–913 (2023).
11. Russell, A. & Mittermeier, A. B. R. New primates described from 1 January 1990 to 1 May 2023. *Primate Specialist Group IUCN/SSC* http://www.primatesg.org/new_species/ (2023).
12. Rogers, J. et al. The comparative genomics and complex population history of baboons. *Sci. Adv.* **5**, eaau6947 (2019).
13. Ancient and modern genomes unravel the evolutionary history of the rhinoceros family. *Cell* **184**, 4874–4885.e16 (2021).
14. Janiak, M. C. et al. Two hundred and five newly assembled mitogenomes provide mixed evidence for rivers as drivers of speciation for Amazonian primates. *Mol. Ecol.* **31**, 3739–3970 (2022).
15. Shao, Y. et al. Phylogenomic analyses provide insights into primate evolution. *Science* **380**, 913–924 (2023).
16. Walters, A. D. & Schwartz, M. K. Population genomics for the management of wild vertebrate populations. In: *Population Genomics* 419–436 (Springer International Publishing, Cham, 2020).
17. Angela, P. & Fuentes-Pardo, D. E. R. Whole-genome sequencing approaches for conservation biology: advantages, limitations and practical recommendations. *Mol. Ecol.* **26**, 5359–5868 (2017).
18. Supple, M. A. & Shapiro, B. Conservation of biodiversity in the genomics era. *Genome Biol.* **19**, 1–12 (2018).
19. Hershkovitz, P. Uacaries, new world monkeys of the genus *Cacajao* (Cebidae, Platyrrhini): a preliminary taxonomic review with the description of a new subspecies. *Am. J. Primatol.* **12**, 1–53 (1987).
20. Veiga, L. M., Barnett, A. A., Ferrari, S. F. & Norconk, M. A. Evolutionary biology and conservation of Titis, Sakis and Uacaris. (Cambridge University Press, 2013).
21. Boubli, J. P. & Ditchfield, A. D. The time of divergence between the two species of uakari monkeys: *Cacajao calvus* and *Cacajao melanocephalus*. *Folia Primatol.* **71**, 387–391 (2000).
22. Silva, F. E. et al. Impact of Quaternary Amazonian river dynamics on the diversification of uakari monkeys (genus *Cacajao*). *Journal of Biogeography*, **51**, 1505–1517 (2024).
23. Mayor, P. et al. Proximate causes of the red face of the bald uakari monkey (*Cacajao calvus*). *R. Soc. Open Sci.* **2**, 150145 (2015).
24. Silva, F. E. et al. Molecular phylogeny and systematics of bald uakaris, genus *Cacajao* (Primates: Pitheciidae), with the description of a new species. *Mol. Phylogenet. Evol.* **173**, 107509 (2022).
25. Boubli, J. P. et al. A taxonomic reassessment of *Cacajao melanocephalus* Humboldt (1811), with the description of two new species. *Int. J. Primatol.* **29**, 723–741 (2008).

26. Silva, F. E. et al. On the geographic distribution of the Bald Uakaris (*Cacajao calvus* spp.) in Brazilian Amazonia. *Primate Conserv.* **35**, 69–86 (2021).
27. Boubli, J. P. et al. Spatial and temporal patterns of diversification on the Amazon: a test of the riverine hypothesis for all diurnal primates of Rio Negro and Rio Branco in Brazil. *Mol. Phylogenet. Evol.* **82**, 400–412 (2015).
28. Collins, A. C. & Dubach, J. M. Phylogenetic relationships of spider monkeys (*Ateles*) based on mitochondrial DNA variation. *Int. J. Primatol.* **21**, 381–420 (2000).
29. Casado, F. et al. Mitochondrial divergence between 2 populations of the hooded Capuchin, *Cebus (Sapajus)* cay (Platyrrhini, Primates). *J. Hered.* **101**, 261–269 (2010).
30. Valencia, L. M., Martins, A., Ortiz, E. M. & Di Fiore, A. A RAD-sequencing approach to genome-wide marker discovery, genotyping, and phylogenetic inference in a diverse radiation of primates. *PLoS One* **13**, e0201254 (2018).
31. Hohenlohe, P. A., Funk, W. C. & Rajora, O. P. Population genomics for wildlife conservation and management. *Mol. Ecol.* <https://doi.org/10.1111/mec.15720> (2020).
32. Almond, R. E. A., M., G. & Petersen, T. Living planet report 2020: bending the curve of biodiversity loss. <https://icriforum.org/living-planet-report-2020-bending-the-curve-of-biodiversity-loss/> (2020).
33. Bowler, M., Murrieta, J. N., Recharte, M., Puertas, P. & Bodmer, R. Peruvian Red Uakari Monkeys (*Cacajao calvus ucayalii*) in the Pacaya-Samiria national reserve — a range extension across a major river barrier. *Neotrop. Primates* **16**, 34–37 (2009).
34. Sawakuchi, A. O. et al. Rainfall and sea level drove the expansion of seasonally flooded habitats and associated bird populations across Amazonia. *Nat. Commun.* **13**, 4945 (2022).
35. Fordham, G., Shanee, S. & Peck, M. Effect of river size on Amazonian primate community structure: a biogeographic analysis using updated taxonomic assessments. *Am. J. Primatol.* **82**, e23136 (2020).
36. Luna, L. W. et al. Late Pleistocene landscape changes and habitat specialization as promoters of population genomic divergence in Amazonian floodplain birds. *Mol. Ecol.* **32**, 214–228 (2023).
37. Ruokolainen, K., Moulatlet, G. M., Zuquim, G., Hoorn, C. & Tuomisto, H. Geologically recent rearrangements in central Amazonian river network and their importance for the riverine barrier hypothesis. *Front. Biogeogr.* **11**, <https://doi.org/10.21425/F5FBG45046> (2019).
38. Guyonneau, L., Murisier, F., Rossier, A., Moulin, A. & Beermann, F. Melanocytes and pigmentation are affected in dopachrome tautomerase knockout mice. *Mol. Cell. Biol.* **24**, 3396–3403 (2004).
39. Bradley, B. J. & Mundy, N. I. The primate palette: the evolution of primate coloration. *Evolut. Anthropol.* **11**, 97–111 (2008).
40. Munds, R. A., Titus, C. L., Moreira, L. A. A., Eggert, L. S. & Blomquist, G. E. Examining the molecular basis of coat color in a nocturnal primate family (*Lorisidae*). *Ecol. Evol.* **11**, 4442–4459 (2021).
41. Chen, X. J. & Butow, R. A. The organization and inheritance of the mitochondrial genome. *Nat. Rev. Genet.* **6**, 815–825 (2005).
42. Gregory, T. & Bowler, M. Male-male affiliation and cooperation characterize the social behavior of the large-bodied pitheciids, *Chiropotes* and *Cacajao*: a review. *Am. J. Primatol.* **78**, 550–560 (2016).
43. Faria, R. & Navarro, A. Chromosomal speciation revisited: rearranging theory with pieces of evidence. *Trends Ecol. Evol.* **25**, 660–669 (2010).
44. Péquignot, V., Koifmann, C. P. & Dutrillaux, B. Chromosomal phylogeny of *Lagothrix*, *Brachyteles*, and *Cacajao*. *Cytogenet. Genome Res.* **39**, 99–104 (2008).
45. Barnett, A. A., Castilho, C. V., de, Shapley, R. L. & Anicácio, A. Diet, habitat selection and natural history of *Cacajao melanocephalus ouakary* in Jaú National Park, Brazil. *Int. J. Primatol.* **26**, 949–969 (2005).
46. Ferrari, S. F., Guedes, P. G., Figueiredo-Ready, W. M. B. & Barnett, A. A. Reconsidering the taxonomy of the black-faced Uacaris, *Cacajao melanocephalus* group (Mammalia: Pitheciidae), from the northern Amazon Basin. *Zootaxa* **3866**, 353–370 (2014).
47. Defler, T. The genesis of the Modern Amazon river basin and Andean uplift and their roles in mammalian diversification. *Hist. Terrestrial Mamm. South Am.* **235**, 257 (2019).
48. Figueiredo-Vázquez, C., Lourenço, A. & Velo-Antón, G. Riverine barriers to gene flow in a salamander with both aquatic and terrestrial reproduction. *Evol. Ecol.* **35**, 483–511 (2021).
49. Michael, B. et al. Seasonal fluctuations in the mass of the Amazon River system and Earth's elastic response. *Geophys. Res. Lett.* <https://doi.org/10.1029/2005GL023491> (2005).
50. do Prado, A. H., de Almeida, R. P., Galeazzi, C. P., Sacek, V. & Schlunegger, F. Climate changes and the formation of fluvial terraces in central Amazonia inferred from landscape evolution modeling. *Earth Surf. Dyn.* **10**, 457–471 (2022).
51. Nielsen, R. et al. A scan for positively selected genes in the genomes of humans and chimpanzees. *PLoS Biol.* **3**, e170 (2005).
52. Bradley, B. J., Gerald, M. S., Widdig, A. & Mundy, N. I. Coat color variation and pigmentation gene expression in rhesus macaques (*Macaca mulatta*). *J. Mamm. Evol.* **20**, 263–270 (2012).
53. Darc, M. et al. Polymorphisms in the $\alpha 4$ integrin of neotropical primates: insights for binding of natural ligands and HIV-1 gp120 to the human $\alpha 4 \beta 7$. *PLoS One* **6**, e24461 (2011).
54. Fuehrer, H.-P., Campino, S. & Sutherland, C. J. The primate malaria parasites *Plasmodium malariae*, *Plasmodium brasilianum* and *Plasmodium ovale* spp.: genomic insights into distribution, dispersal and host transitions. *Malar. J.* **21**, 138 (2022).
55. Barnett, A. A., Shaw, P., Spironello, W. R., MacLarnon, A. & Ross, C. Sleeping site selection by golden-backed uacaris, *Cacajao melanocephalus ouakary* (Pitheciidae), in Amazonian flooded forests. *Primates* **53**, 273–285 (2012).
56. Goulding, M., Carvalho, M. L. & Ferreira, E. J. G. Rio Negro, rich life in poor water: amazonian diversity and foodchain ecology as seen through fish communities. (Balogh Scientific Books, 1988).
57. Tackling malaria hotspots in the Amazon. *WHO* <https://www.who.int/news-room/feature-stories/tackling-malaria-hotspots-in-the-amazon> (2019).
58. Salmona, J., Heller, R., Quéméré, E. & Chikhi, L. Climate change and human colonization triggered habitat loss and fragmentation in Madagascar. *Mol. Ecol.* **26**, 5203–5222 (2017).
59. Zhao, X. et al. Climate change, grazing, and collecting accelerate habitat contraction in an endangered primate. *Biol. Conserv.* **231**, 88–97 (2019).
60. Farias, I. P., Santos, W. G., Gordo, M. & Hrbek, T. Effects of forest fragmentation on genetic diversity of the critically endangered primate, the pied tamarin (*Saguinus bicolor*): implications for conservation. *J. Hered.* **106**, 512–521 (2015).
61. Russell A. Mittermeier (Global Wildlife Conservation), Bezerra, B. M., Boubli, J. P., Urbani, B. & Erwin Palacios (Conservación Internacional Colombia, Bogotá, Colombia). IUCN Red List of Threatened Species: *Cacajao melanocephalus*. IUCN Red List of Threatened Species (2020).
62. Russell A. Mittermeier (Global Wildlife Conservation), Bezerra, B. M., Boubli, J. P. & Urbani, B. IUCN Red List of Threatened Species: *Cacajao ayresi*. IUCN Red List of Threatened Species (2015).
63. Mittermeier, R.A., Boubli, J.P., Bezerra, B.M. & Urbani, B. *Cacajao hosomi* (amended version of 2020 assessment). The IUCN Red List of Threatened Species 2021: e.T136640A191697668. <https://doi.org/10.2305/IUCN.UK.2021-1.RLTS.T136640A191697668.en> (2021).
64. Silva, R. M., da, Lopes, A. G. & Santos, C. A. G. Deforestation and fires in the Brazilian Amazon from 2001 to 2020: impacts on rainfall

- variability and land surface temperature. *J. Environ. Manag.* **326**, 116664 (2023).
65. Boubli, J. P. et al. *Cacajao calvus* ssp. *ucayalii*. The IUCN Red List of Threatened Species 2021: e.T3420A206546797. <https://doi.org/10.2305/IUCN.UK.2021-3.RLTS.T3420A206546797.en> (2021).
66. Silva, F. E. & Boubli, J. *Cacajao calvus* ssp. *novaesi*. The IUCN Red List of Threatened Species 2020: e.T3421A17992061. <https://doi.org/10.2305/IUCN.UK.2020-2.RLTS.T3421A17992061.en> (2020).
67. Silva, F. E. & B. *Cacajao calvus* ssp. *calvus*. The IUCN Red List of Threatened Species 2020: e.T3419A17992129. <https://doi.org/10.2305/IUCN.UK.2020-2.RLTS.T3419A17992129.en> (2020).
68. Silva, F. E., de Azevedo, R. B. & Boubli, J. P. *Cacajao calvus* ssp. *rubicundus*. The IUCN red list of threatened species 2021: e.T3422A206546924. <https://doi.org/10.2305/IUCN.UK.2021-3.RLTS.T3422A206546924.en> (2021).
69. Heymann, E. W. & Aquino, R. Peruvian red Uakaris (*Cacajao calvus ucayalii*) are not flooded-forest specialists. *Int. J. Primatol.* **31**, 751–758 (2010).
70. Orkin, J. D., Kuderna, L. F. K. & Marques-Bonet, T. The diversity of primates: from biomedicine to conservation genomics. *Annu. Rev. Anim. Biosci.* **9**, 103–124 (2021).
71. GitHub—lh3/seqtk: toolkit for processing sequences in FASTA/Q formats. [GitHub https://github.com/lh3/seqtk](https://github.com/lh3/seqtk).
72. Martin, M. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet. J.* **17**, 10–12 (2011).
73. Li, H. & Durbin, R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* **25**, 1754–1760 (2009).
74. Tischler, G. & Leonard, S. biobambam: tools for read pair collation based algorithms on BAM files. *Source Code Biol. Med.* **9**, 1–18 (2014).
75. GitHub—broadinstitute/picard: a set of command line tools (in Java) for manipulating high-throughput sequencing (HTS) data and formats such as SAM/BAM/CRAM and VCF. [GitHub https://github.com/broadinstitute/picard](https://github.com/broadinstitute/picard).
76. Pedersen, B. S. & Quinlan, A. R. Mosdepth: quick coverage calculation for genomes and exomes. *Bioinformatics* **34**, 867–868 (2017).
77. McKenna, A. et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res.* **20**, 1297–1303 (2010).
78. Garrison, E., Kronenberg, Z. N., Dawson, E. T., Pedersen, B. S. & Prins, P. A spectrum of free software tools for processing the VCF variant call format: vcflib, bio-vcf, cyvcf2, hts-nim and slivar. *PLoS Comput. Biol.* **18**, e1009123 (2022).
79. Danecek, P. et al. The variant call format and VCFtools. *Bioinformatics* **27**, 2156–2158 (2011).
80. Wickham, H. *ggplot2: elegant graphics for data analysis*. 2nd edn (Springer, New York, 2009).
81. The R project for statistical computing. <https://www.r-project.org/>.
82. Hanghøj, K., Moltke, I., Andersen, P. A., Manica, A. & Korneliussen, T. S. Fast and accurate relatedness estimation from high-throughput sequencing data in the presence of inbreeding. *Gigascience* **8**, giz034 (2019).
83. Chang, C. C. et al. Second-generation PLINK: rising to the challenge of larger and richer datasets. *Gigascience* **4**, s13742–015–0047–8 (2015).
84. Price, A. L. et al. Principal components analysis corrects for stratification in genome-wide association studies. *Nat. Genet.* **38**, 904–909 (2006).
85. Alexander, D. H., Novembre, J. & Lange, K. Fast model-based estimation of ancestry in unrelated individuals. *Genome Res.* **19**, 1655–1664 (2009).
86. Petkova, D., Novembre, J. & Stephens, M. Visualizing spatial population structure with estimated effective migration surfaces. *Nat. Genet.* **48**, 94–100 (2015).
87. GitHub—dipetkov/reemplots2. [GitHub https://github.com/dipetkov/reemplots2](https://github.com/dipetkov/reemplots2).
88. Malinsky, M., Matschiner, M. & Svardal, H. Dsuite—fast D-statistics and related admixture evidence from VCF files. *Mol. Ecol. Resour.* **21**, 584–595 (2021).
89. Quinlan, A. R. & Hall, I. M. BEDTools: a flexible suite of utilities for comparing genomic features. *Bioinformatics* **26**, 841–842 (2010).
90. Korneliussen, T. S., Albrechtsen, A. & Nielsen, R. ANGSD: analysis of next generation sequencing data. *BMC Bioinform.* **15**, 356 (2014).
91. Capella-Gutiérrez, S., Silla-Martínez, J. M. & Gabaldón, T. trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics* **25**, 1972–1973 (2009).
92. Minh, B. Q. et al. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Mol. Biol. Evol.* **37**, 1530–1534 (2020).
93. Drummond, A. J. & Bouckaert, R. R. Bayesian evolutionary analysis with BEAST. (Cambridge University Press, 2015).
94. Pajmans, J. L. A. et al. African and Asian leopards are highly differentiated at the genomic level. *Curr. Biol.* **31**, 1872–1882.e5 (2021).
95. Paradis, E. & Schliep, K. ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics* **35**, 526–528 (2019).
96. Allio, R. et al. MitoFinder: efficient automated large-scale extraction of mitogenomic data in target enrichment phylogenomics. *Mol. Ecol. Resour.* **20**, 892–905 (2020).
97. Laslett, D. & Canbäck, B. ARWEN: a program to detect tRNA genes in metazoan mitochondrial nucleotide sequences. *Bioinformatics* **24**, 172–175 (2008).
98. GitHub—CFSAN-Biostatistics/fastatools: tools for working with fasta files. [GitHub https://github.com/CFSAN-Biostatistics/fastatools](https://github.com/CFSAN-Biostatistics/fastatools).
99. Hudson, R. R., Slatkin, M. & Maddison, W. P. Estimation of levels of gene flow from DNA sequence data. *Genetics* **132**, 583–589 (1992).
100. Valenzuela Seba, A. Mapping protein-coding genomes to phenomes: a phylogenetic perspective. (Universitat Pompeu Fabra, 2024).
101. Danecek, P. et al. Twelve years of SAMtools and BCFtools. *Gigascience* **10**, giab008 (2021).
102. Liao, Y., Wang, J., Jaehnig, E. J., Shi, Z. & Zhang, B. WebGestalt 2019: gene set analysis toolkit with revamped UIs and APIs. *Nucleic Acids Res.* **47**, W199–W205 (2019).
103. Excoffier, L. et al. fastsimcoal2: demographic inference under complex evolutionary scenarios. *Bioinformatics* **37**, 4882–4885 (2021).
104. Terhorst, J., Kamm, J. A. & Song, Y. S. Robust and scalable inference of population history from hundreds of unphased whole genomes. *Nat. Genet.* **49**, 303–309 (2017).

Acknowledgements

T.M.B. gratefully acknowledges the financial support from the European Research Council (ERC) under the European Union’s Horizon 2020 research and innovation program (grant agreement no. 864203), (PID2021-126004NB-I00) (MICIIN/FEDER, UE) and from the Secretaria d’Universitats i Recerca and CERCA Program del Departament d’Economia i Coneixement de la Generalitat de Catalunya (GRC 2021 SGR 00177). J.P.B. gratefully acknowledges the financial support from the Natural Environment Research Council (NERC) (NE/T000341/1). F.E.S. gratefully acknowledges the financial support from the European Union’s Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement

(801505), the Fonds National de la Recherche Scientifique (F.R.S.-FNRS, Belgium; grant 40017464) Brazilian National Council for Scientific and Technological Development (CNPq) (Processes 303286/2014-8, 303579/2014-5, 200502/2015-8, 302140/2020-4, 300365/2021-7, 301407/2021-5, #301925/2021-6), the International Primatological Society (Conservation grant). The Rufford Foundation (14861-1, 23117-2, 38786-B), the Margot Marsh Biodiversity Foundation (SMA-CCO-G0023, SMA-CCOG0037), the Primate Conservation Inc. (1713 and 1689) and the Gordon and Betty Moore Foundation (Grant 5344) (Mamirauá Institute for Sustainable Development). N.H.-A. gratefully acknowledges the financial support from the Government of Catalonia | Agència de Gestió d'Ajuts Universitaris i de Recerca (Agency for Management of University and Research Grants) (FI_00040).

Competing interests

Employees of Illumina, Inc. are indicated in the list of author affiliations. The other authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s42003-024-06901-3>.

Correspondence and requests for materials should be addressed to Núria Hermosilla-Albala.

Peer review information *Communications Biology* thanks Amely Martins and the other, anonymous, reviewer(s) for their contribution to the peer

review of this work. Primary Handling Editors: Luke Grinham and Laura Rodríguez Pérez.

Reprints and permissions information is available at <http://www.nature.com/reprints>

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>.

© The Author(s) 2024

¹IBE, Institute of Evolutionary Biology (UPF-CSIC), Department of Medicine and Life Sciences, Universitat Pompeu Fabra. PRBB, C. Doctor Aiguader N88, 08003 Barcelona, Spain. ²Research Unit of Evolutionary Biology and Ecology, Département de Biologie des Organismes, Université libre de Bruxelles (ULB), Brussels, Belgium. ³Research Group on Primate Biology and Conservation, Mamirauá Institute for Sustainable Development, Tefé, Amazonas, Brazil. ⁴Center for Evolutionary Hologonomics, The Globe Institute, University of Copenhagen, Øster Farimagsgade 5A, 1352 Copenhagen, Denmark. ⁵Centro Nacional de Análisis Genómico (CNAG), C/ Baldori Reixac 4, 08028 Barcelona, Spain. ⁶Department of Ecology and Evolutionary Biology, University of California Santa Cruz, 130 McAllister Way, Santa Cruz, CA 95060, USA. ⁷Institut Català de Paleontologia Miquel Crusafont, Universitat Autònoma de Barcelona, Edifici ICTA-ICP, Cerdanyola del Vallès, 08193 Barcelona, Spain. ⁸Universidade Federal do Amazonas, Departamento de Genética, Laboratório de Evolução e Genética Animal (LEGAL), Manaus, Amazonas 69080-900, Brazil. ⁹Department of Biology, Trinity University, San Antonio, TX 78212, USA. ¹⁰Research Group on Terrestrial Vertebrate Ecology, Mamirauá Institute for Sustainable Development, Tefé, Amazonas, Brazil. ¹¹Rede de Pesquisa para Estudos sobre Diversidade, Conservação e Uso da Fauna na Amazônia—RedeFauna, Manaus, Amazonas, Brazil. ¹²Comunidad de Manejo de Fauna Silvestre en la Amazonía y en Latinoamérica—ComFauna, Iquitos, Loreto, Peru. ¹³Human Genome Sequencing Center and Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX 77030, USA. ¹⁴Illumina Artificial Intelligence Laboratory, Illumina Inc., San Diego, CA 94404, USA. ¹⁵Institució Catalana de Recerca i Estudis Avançats (ICREA) and Universitat Pompeu Fabra. Pg. Lluís Companys 23, 08010 Barcelona, Spain. ¹⁶School of Science, Engineering & Environment, University of Salford, Salford M5 4WT, UK. ¹⁷These authors contributed equally: Núria Hermosilla-Albala, Felipe Ennes Silva. ²³These authors jointly supervised this work: Tomas Marques-Bonet, Jean P. Boubli. ✉e-mail: nuria.hermosilla@upf.edu