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Running head: Diversity and distribution of African rope squirrels

REASSESSING THE DIVERSITY AND DISTRIBUTION OF AFRICAN ROPE SQUIRRELS (*FUNISCIURUS* TROUESSART, 1880)

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Teaser text

We studied the diversity and distribution of rope squirrels, genus *Funisciurus* Trouessart 1880, in the Congo Basin. By gathering the most comprehensive genetic and morphometric data to date on a significant part of the geographical and taxonomic range of these squirrels (specimens from seven provinces of the Democratic Republic of Congo), we identified six main taxa: one previously undescribed species, three allopatric taxa, and two genetically distinct sympatric taxa. We then mapped their distribution,

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showing that the Congo and Kasai rivers represent significant biogeographical barriers limiting the distribution of *Funisciurus* species. These results support research into the role of rope squirrel species in the transmission of pathogens of public health importance.

Image for the online table of contents: African rope squirrel

Photo credit: Bushnell photo trap set by Pascal Baelo

ABSTRACT

The diversity, taxonomy, distribution and ecology of sub-Saharan tree squirrels remain under-researched. This study aims to elucidate the diversity and distribution of rope squirrels, genus *Funisciurus* Trouessart 1880, in the Congo Basin. We assembled the most comprehensive genetic and morphometric data set to date, on a significant portion of the geographical and taxonomic range of these squirrels (470 specimens from seven provinces of the Democratic Republic of Congo). We identified six main taxa: a previously undescribed *Funisciurus* species (here, Fx), three allopatric taxa of *Funisciurus anerythrus* (Fc, FaRB and FaLB), and two sympatric genetically distinct taxa (FbLB1, FbLB2), potentially belonging to a *Funisciurus bayonii* species complex. We then mapped their distribution, showing that the Congo and Kasaï Rivers represent significant biogeographical barriers limiting the distribution of *Funisciurus* species. In addition to providing a baseline for research on the impact of climate change and habitat degradation on the abundance and distribution of rope squirrels, our findings support research on the role of rope squirrel species in the transmission of pathogens of public health importance.

Keywords: Biodiversity, Congo Basin, DNA-barcoding, Sciuridae, rope squirrels

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INTRODUCTION

The Sciuridae family, which includes approximately 50 genera and 302 species (**Zachos et al., 2021, Steiner & Huettmann, 2023**), is distributed globally, with the exception of Antarctica. While some squirrel species are valued as a food source in certain regions (**Fa et al., 2009; Baelo et al., 2018**), they are increasingly recognized as important reservoirs and incidental hosts for zoonotic pathogens (**Han et al., 2015; e.g., Sklenovska et al., 2018; Schulze et al., 2020**). However, the phylogenetic, ecological, and life history characteristics of many squirrels, particularly those in sub-Saharan Africa, remain poorly understood. In Central Africa, the Sciuridae family is represented by eight genera, including the African pygmy squirrel (*Myosciurus pumilio*) and several species within the rope (*Funisciurus*), sun (*Heliosciurus*), ground (*Xerus*), bush (*Paraxerus*), palm (*Epixerus*), and giant (*Protoxerus*) genera (**Kingdon & Hoffmann, 2013; Thorington et al., 2012**). Four of these genera—*Funisciurus*, *Paraxerus*, *Heliosciurus*, and *Protoxerus*—are common across diverse habitats in the Democratic Republic of Congo (DRC), spanning dense rainforest to savannah ecosystems.

Unlike many large mammals that are highly susceptible to habitat disturbances, a few Sciuridae species have shown a remarkable capacity to adapt to human-altered environments. For instance, *Funisciurus anerythrus* has been observed at high population densities in palm oil plantations, reaching up to 500 individuals per square kilometer—far exceeding densities in primary lowland rainforests (**Tiee et al., 2018**). This adaptability heightens the frequency of human-squirrel interactions and raises concerns about potential zoonotic spillover risks, particularly in agricultural and peri-domestic settings (**Rimoin et al., 2017; Kumakamba et al., 2021**).

Recent studies underscore the role of squirrels in zoonotic disease transmission, for example bornavirus (**Hoffmann et al., 2015; Schulze et al., 2020**). Sub-Saharan

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99 squirrels, particularly those within the *Funisciurus* and *Heliosciurus* genera, have also
100 been associated with the mpox virus (MPXV), of which a variant led to a global
101 outbreak infecting over 80,000 people in 2022, emphasizing the importance of
102 understanding the status of these squirrels as zoonotic reservoirs (**Chen et al., 2012;**
103 **Curaudeau et al., 2023; Kinganda-Lusamaki et al., 2025**).

104 Studying the distribution and phylogenetic relationships of *Funisciurus* in sub-
105 Saharan Africa could illuminate patterns of pathogen-host co-evolution within this
106 genus. MPXV, for instance, comprises two main clades: the more virulent clade I and
107 the less virulent clade II (**Nakazawa et al., 2015; Kinganda-Lusamaki et al., 2025**).
108 Clade I MPXV occurs in the Congo Basin, and clade II in West Africa - though both
109 clades have been exported via human infections to other regions in Africa and the rest of
110 the world (**Otieno et al., 2025**). The distinct clades found to be infecting humans could
111 be a reflection of genetically and geographically distinct natural reservoir(s). These
112 geographically distinct clades may correlate with specific squirrel populations,
113 suggesting possible co-evolutionary and biogeographical relationships affecting disease
114 transmission dynamics.

115 Geographical barriers, particularly rivers, can influence the distribution of rodents
116 and the spread of pathogens, limiting the ranges of arboreal mammals in West and
117 Central Africa. While natural barriers such as the Volta, Sanaga and Congo rivers are
118 known to limit the ranges of several small mammal species (**Kennis et al., 2011**), the
119 influence of the Congo River on the distribution and evolutionary history of
120 Sciuromorph rodents remains underexplored, leaving significant gaps in our
121 understanding of their evolutionary history across the Congo Basin.

122 This study addresses these gaps by clarifying the diversity of *Funisciurus* taxa within the
123 Congo Basin and assessing whether the Congo River serves as a significant geographic

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124 barrier affecting their distribution. Along with advancing biodiversity, evolutionary and
125 biogeographic insights, our research provides critical data on the distribution of
126 *Funisciurus* taxa across the Congo Basin, to serve as a baseline for comparison with the
127 phylogeographies of zoonotic pathogens relevant for public health.

128 2. MATERIALS AND METHODS

129 2.1. SAMPLE COLLECTION

130 ***2.1.1. SQUIRREL CAPTURE AND SAMPLING***; we trapped and sampled squirrels
131 from 2014 to 2022 in 17 localities across four provinces (Tshopo, Bas-Uele, Ituri and
132 Tshuapa) in the Democratic Republic of Congo (DRC). Further, we used specimens
133 from three other localities in three provinces (Kwilu, Kinshasa and Kongo Central),
134 where squirrels were sampled in 1995 and preserved at the Royal Museum for Central
135 Africa-Tervuren (Belgium) (**Supplementary Figure S1**).

136 We captured squirrels using traditional traps (**Supplementary Figure S2**), baited
137 with a ripe palm nut and placed in different habitats (primary forests, secondary forests
138 and fallow land), at heights ranging from 0.00 m (on the ground) to 7.37 m (tree
139 branches) (average height =1.58m±1.29m). The various standard traps and techniques
140 used to capture rodents (pitfall traps, Havahart traps, flap traps, Sherman traps) proved
141 unsuitable or ineffective when applied to squirrels. Consequently, traditional traps were
142 used, given their effectiveness in capturing tree squirrels.

143 The traditional traps were constructed from stems, flexible vines and rattan, which
144 were wrapped around a branch to facilitate the animals' movement towards the bait
145 (**Supplementary Figure S2**). A wooden wedge was then attached to the wire to act as a
146 trigger for the bait. The trap was baited to attract the animal and catch it by the neck.
147 Traditional traps had one or two sliding knots, allowing squirrels to be caught in both

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directions of approach. Other traps were constructed from flexible rods and wires with a radius of 0.5 mm and rattan. These traps had a single noose and functioned in the same way as traps with two nooses.

We euthanized live captured squirrels with an overdose of isoflurane, adhering to the guidelines of the American Society of Mammalogists for euthanasia (**Sikes et al., 2016**), before collecting blood samples on Whatman filter paper and collecting tissue samples (tongue, kidney, liver, lung, spleen, and colon) and feces. These tissues were preserved either in 70-94% ethanol or in a DNA/RNA preservation solution (e.g. RNAlater or DNA/RNA Shield, Zymo Research). The preserved samples were sent to the Royal Belgian Institute of Natural Sciences (RBINS) and the University of Antwerp (Belgium) for genetic analysis. The carcasses were preserved in a 10% formalin solution and stored partly at the Centre de Surveillance de la Biodiversité in Kisangani (DRC) and partly at the University of Antwerp (Belgium). A list of all specimens is provided in Supplementary Table S1.

Field research permits were granted by the Centre de Surveillance de la Biodiversité in Kisangani of University of Kisangani at the University of Kisangani, with provincial and local authorities approving all mission orders. Samples exported to the Royal Belgian Institute of Natural Sciences and the University of Antwerp (Belgium) prior to the implementation of the Nagoya Protocol were covered by Centre de Surveillance de la Biodiversité material transfer agreements, while those exported after 2018 were authorized by a Nagoya export permit issued by the Congolese Ministry of the Environment and Sustainable Development (MEDD) (Permit numbers 003/ANCCB-RDC/SG-EDD/BTB/08/2019, 018/ANCCB-RDC/SG-EDD/BTB/07/2022, 021/SG-EDD/BTB/ANCCB-RDC/08/2023, 011/ANCCB-RDC/SG-EDD/BTB/2023). No CITES permits were required. Permission to import the samples into Belgium was

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173 granted by the Federal Agency for the Safety of the Food Chain (FASFC), permit
174 numbers 2022DBP504, 2023DBP370, 2023DBP371, 2024DBP424.

175 **2.1.2. *EXTERNAL MORPHOMETRY AND CRANIOMETRY;*** Squirrels were first
176 identified *in situ* to genus or species level using **Kingdon & Hoffmann (2013**). We
177 examined captured squirrels on the basis of external morphological criteria, *i.e.* coat,
178 body length, tail length, hind foot length, and ear length. Sex and reproductive stages
179 were determined by external observation, including the presence or absence of nipples
180 (small or swollen) and the appearance of the vagina (closed or open) in females or the
181 presence of testes (abdominal or scrotal) in males. Length was measured to the nearest
182 millimeter on fresh specimens using a Mitutoyo caliper to an accuracy of 0.05 mm.
183 Weight was measured using a spring balance (Pesola) and expressed in grams.

184 Only the skulls of adult squirrels were extracted and measured (**Supplementary**
185 **Table S1**). A total of 23 measurements were taken on each skull (**Verheyen & Bracke,**
186 **1966; Van Der Straeten & Dieterlen, 1987; Verheyen et al., 1996**). The used
187 measurements are listed with its corresponding number, abbreviation, and
188 morphological character (**Supplementary Table S2).**
189 We also measured 29 skulls from the collections of the Royal Museum for Central
190 Africa-Tervuren to link a craniometrically defined Operational Taxonomic Unit (OTU)
191 to cytochrome b sequences previously published in GenBank (**Falendysz at al., 2017**).

192 **2.1.3. *ANALYSIS OF MORPHOMETRIC AND CRANIOMETRIC DATA;*** The
193 approach consisted of several steps. First, OTUs were designated based on preliminary
194 Molecular Taxonomic Units (MOTUs) identified using cytochrome b data. Next, we
195 tested the hypothesis that variation in craniometrics and external morphological data

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196 aligns with the MOTUs through multivariate analysis using R version 4.2.3 and
197 Statistica version 12 (StatSoft Inc).

198 To evaluate sexual dimorphism within OTUs, all measurements were analyzed.
199 However, as no significant sexual dimorphism was detected (**Supplementary Tables S3**
200 **and S4)**, subsequent analyses were conducted using specimens of both sexes. Basic
201 statistics, including the number of observations, mean, minimum, maximum, standard
202 deviation, and coefficient of variation, were calculated for all OTUs (**Supplementary**
203 **Tables S5 and S6**)
204 A forward discriminant analysis was then performed using all 23 variables from
205 the complete craniometric dataset, clearly highlighting the sequenced specimens. A
206 second discriminant analysis focused on the OTUs *Funisciurus anerythrus* Left Bank
207 (FaLB), *Funisciurus anerythrus* Right Bank (FaRB), and *Funisciurus cf congicus* (Fc)
208 near Kikwit, using a partial craniometric dataset. For the Kikwit specimens, where some
209 measurements were unavailable due to skull damage, a mean substitution method was
210 applied to estimate missing values (Statistica) (**Supplementary Figure S4).** Finally, a
211 third discriminant analysis incorporated all available external measurements for all
212 OTUs, again clearly highlighting the sequenced specimens.
213 Not all specimens were intact, the tail and/or ear having been cut off and the skull
214 bones fractured, the skull not extracted or not recovered, which explains the variations in
215 the number of individuals (N) used in each type of analysis. The subset of tissues
216 bearing a DNA barcode was selected at random on the basis of the geographical origin
217 of the capture and the availability of morphometric measurements. We also measured 41
218 squirrel skulls collected in Kikwit, Kinshasa and Kongo Central (DRC) and stored at the
219 Royal Museum for Central Africa, and analyzed their tissue samples stored at the Royal
220 Belgian Institute of Natural Sciences.

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221 **2.2. MOLECULAR DATA ACQUISITION**

222 We extracted DNA of squirrel tissue samples (tongue or liver or kidney or lung) using
223 the Nucleospin Tissue mini kit according to the manufacturer's instructions (Machery-
224 Nagel, Düren, Germany), with an overnight pre-lysis incubation instead of 1-3h, and a
225 one-minute elution at 70°C instead of RT. We then amplified a mitochondrial gene
226 fragment using universal cytochrome b primers L14723 and H15915 (**Montgelard et**
227 **al., 2002**) with Promega MasterMix PCR following the manufacturer's instructions
228 (Promega, Madison, USA). The initial denaturation was at 94°C for 3 min, followed by
229 35 cycles at 94°C for 1 min, 52°C for 1 min and 72°C for 1 min. Final extension was
230 carried out at 72°C for 7 minutes.

231 We then purified the amplified products using ThermoFisher Exo-SAP IT PCR
232 product clean-up reagent (ThermoFisher Scientific, Santa Clara, USA) and sent them to
233 Macrogen (Amsterdam, The Netherlands) for sequencing. Finally, we manually cleaned
234 the sequences based on the chromatograms and aligned them using the MAFFT
235 algorithm within Geneious 2019.2.3. This alignment included in-house generated
236 *Funisciurus* sequences, as well as two *Paraxerus* sequences serving as an outgroup. This
237 alignment also included *Funisciurus sp* sequences downloaded from GenBank, on the
238 condition that they did not contain stop-codons and clustered with our *Funisciurus*
239 sequences rather than with the outgroup (accession numbers: MF694315, MF694313,
240 MF694317, MF694320, MN597594 and MN597668), yielding a total of 143 sequences.

241 **2.3. SPECIES DELIMITATION USING GENETIC DATA**

242 We first inferred an approximately-maximum-likelihood phylogenetic tree using
243 MAFFT-aligned mitochondrial sequences and the FastTree plugin in Geneious 2019.2.3,
244 using default settings and rooting on the *Paraxerus* outgroup (**Price et al., 2009**).

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245 Briefly, the FastTree plugin worked as follows: a rough tree topology was first obtained
246 through a neighbour-joining approach; tree length was then reduced using nearest-
247 neighbour interchanges and subtree-prune-regraft-moves. Tree topology and branch
248 length were improved using maximum-likelihood rearrangements, where each site was
249 assigned to one of 20 geometrically spaced categories (0.05-20) using a Bayesian
250 approach (gamma prior), representing each site's most likely evolutionary category.
251 Finally, to estimate the reliability of each split in the tree, the Shimodaira-Hasegawa test
252 was used on three alternate topologies (NNIs) around the focal split. The resampling size
253 was 1,000 (**Price et al., 2009**).

254 To further explore *Funisciurus* taxon delineation within our dataset, we used
255 species delimitation algorithms that generate species partitions based either on distance
256 (ASAP, Assemble Species by Autonomous Partitioning) or phylograms (bPTP,
257 Bayesian Poisson Tree Processes), to offer complementary perspectives (**DeSalle, 2024**).
258 First, we employed the ASAP method on our MAFFT alignment, with the following
259 adjustments to meet ASAP requirements: five GenBank sequences (MF694315,
260 MF694313, MF694317, MF694318, and MF694320) were removed to ensure overlap
261 across sequences. ASAP is a frugal, computationally efficient method, which is not
262 based on the reconstruction of phylogenies (**Puillandre et al., 2021**). Rather, this
263 distance-based approach identifies species partitions by detecting barcode gaps—the
264 difference between small intraspecific and large interspecific sequence distances
265 (**Puillandre et al., 2021**).

266 ASAP uses an ascending hierarchical clustering algorithm, which sequentially
267 merges sequences, based on pairwise distances, until all sequences are grouped within
268 the same partition (**Additional file 1**). For each partition, a *p*-value (likelihood of

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269 panmixia, *i.e.*, a low *p*-value signifies that merging the proposed subsets would lead to
270 intra-subset genetic diversity that is higher than expected within a single panmictic
271 species), and W-score (barcode gap width metric, calculated using the threshold
272 distance, *i.e.*, the midpoint between the distance that triggered the merging of subsets
273 and the distance before merging) are computed (**Additional file 1**). A larger W-score
274 reflects a larger barcoding gap, *i.e.*, species are well separated. After clustering, the *p*-
275 value and W-score of each partition are sorted by ascending and descending order,
276 respectively, to compute the ASAP-score (averaged ranks of *p*-value and W-score for
277 each partition). Consequently, smaller ASAP-scores reflect better partitions. Its main
278 advantage over other automated barcode gap discovery approaches is that the maximal
279 intraspecific genetic distance does not need to be specified by the user *a priori*, which
280 makes it suitable for under-researched taxa such as African rope squirrels. We used the
281 most recent ASAP tool, (**Puillandre et al., 2021**), and compared simple p-distances,
282 Jukes-Cantor (JC69) and Kimura 2-parameter (K80) substitution models to investigate
283 whether substitution models impacted the proposed species delimitation. Only results
284 from JC69 are presented here.

285 For additional robustness, we applied the bPTP (**Zhang et al., 2013**) method. This
286 method uses phylogenetic tree branching patterns to propose species partitions (*i.e.*,
287 evaluating whether the number of substitutions follow a Poisson distribution within
288 *versus* between species) and provides Bayesian support values for these partitions.To
289 meet bPTP requirements (**Zhang et al., 2013**), we ran an IQ-TREE analysis (using
290 default settings from the IQ-TREE tool, (**Nguyen et al., 2015; Hoang, et al., 2017;**
291 **Kalyaanamoorthy et al., 2017; Minh et al., 2020**) on the MAFFT alignment used in
292 the ASAP method, with the following adjustments: only *Funisciurus* sequences, non-
293 redundant haplotypes, and fully overlapping sequences were included, for a total of 47

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294 sequences. The resulting phylogenetic tree was used as an input file for the bPTP
295 analysis (**Additional file 2**).
296 Finally, we compared the species partitions proposed by ASAP and bPTP with the
297 topology of the FastTree and the results of discriminant analyses based on morphometric
298 data.

299 **3. RESULTS**

300 **3.1. TRAPPING EFFORT**

301 During a total of 99,035 trapping nights, the trapping effort resulted in the capture of
302 429 squirrels belonging to the genus *Funisciurus*, (success rate of 0.053%). Of these
303 squirrels, 301 were caught on the left bank of the Congo River and 128 on the right
304 bank. No reliable classification could be established based on external morphometric
305 characteristics alone.

306 **3.2. SPECIES PARTITIONING**

307 Overall, approaches based on mitochondrial sequences yielded between four and six
308 MOTUs, when excluding singletons and outgroups. The following MOTUs could be
309 identified based on ASAP and bPTP results: FaLB, FaRB, Fc, FbLB and Fx. Based on
310 ASAP results only, FbLB was further split into FbLB1 and FbLB2. The general species
311 partitioning obtained using mitochondrial data was supported by craniometric data, with
312 the exception of Fx, for which an insufficient number of cranial measures could be
313 taken.

314 **3.2.1.** ***SPECIES PARTITIONING USING ASAP;*** Using the best available species
315 partition, with the lowest ASAP score (ASAP score = 1.50, **Table 1**, hereafter ASAP1),

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316 seven MOTUs of *Funisciurus* were distinguished, in addition to two outgroup MOTUs.
317 However, one MOTU is represented by a single sequence (YK156), giving a total of six
318 well-supported MOTUs consisting of two or more specimens each (**Figure 1,**
319 **Additional file 1**). Using the second-best species partition, with the second-lowest
320 ASAP score, (ASAP score = 3, **Table 1**), eight MOTUs of *Funisciurus* were
321 distinguished, in addition to two outgroup MOTUs. However, two MOTUs are
322 represented by a single sequence each (YK156 and NDU803), resulting in the same six
323 well-supported MOTUs identified in the first partition (**Additional file 1**). Using the
324 smallest *p*-value to determine the best available species partition (ASAP score = 5.50, p
325 = 0.00022, **Table 1,** hereafter ASAP2), five *Funisciurus* MOTUs can be distinguished,
326 in addition to two outgroup taxa. These include, again, a MOTU represented by a single
327 sequence (YK156), yielding a total of four well supported MOTUs consisting of two or
328 more specimens each. In contrast to previous partitions, FaLB, FaRB and Fc are merged
329 within a single MOTU (**Figure 1, Additional file 1**).

330 **3.2.2. *SPECIES PARTITIONING USING bPTP;*** Using the bPTP method applied to
331 our IQ-TREE, six MOTUs of *Funisciurus* were identified with support values ranging
332 from 0.697-0.984. However, one of them consists of a single sequence (YK110, support
333 value: 1.000). Thus, bPTP identifies five well supported MOTUs represented by two or
334 more specimens: Fx (0.984), Fc (0.900), FaLB (0.820), FaRB (0.817), and FbLB (0.697)
335 (**Figure 1, Additional file 2**).

336 **3.2.3. *SPECIES PARTITIONING BASED ON EXTERNAL MORPHOMETRIC***
337 ***CHARACTERISTICS AND SEXUAL DIMORPHISM;*** Comparing Student's t-tests
338 with the craniometric (**Supplementary Table S3**) and external (**Supplementary Table**

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339 **S4**) measurements of males and females of each species did not reveal any sexual
340 dimorphism. In all subsequent analyses, sexes were therefore lumped.

341 The discriminant analysis carried out on the external measurements (Weight,
342 Length of hind foot, Length of ear, Length of tail, Length of body) revealed low
343 discriminating power (Wilk's Lambda = 0.229) (**Supplementary Figure S3**). There is
344 no distinction between *F. anerythrus* from the right bank and from the left bank. *F.cf*
345 *bayonii* (Fb) is clearly separated from *F. anerythrus* along root 1. No external
346 measurements are available for *F.cf congicus*.

347 **3.2.4. *SPECIES PARTITIONING BASED ON CRANIAL MORPHOMETRIC***
348 ***CHARACTERISTICS;*** Unlike external measurements, craniometric measurements of
349 adult rodents offer greater stability and are among the most effective methods for
350 identifying rodent species. For this reason, 220 adult squirrel skulls were measured and
351 23 measurements were taken on each skull (**Supplementary Table S2**).

352 A forward discriminant analysis, (**Supplementary Figure S5**) omitting measures
353 M8 (width of the interorbital narrowing), M9 (width of the zygomatic arch) and M19
354 (maximum width of the cranial cavity) to maximize the number of observations for *F. cf*
355 *congicus* (Fc), measures (M1: maximum length of the skull; M2: condilo-basal length;
356 M3: henselion-basion length; M4: henselion-palation length; M5: length of the cleft
357 palate; M6: length of the diastema; M7: distance between the anterior edge of the
358 alveolus of M1 and the cutting edge of the upper incisor; M10: minimum width of the
359 palate; M11: length of the upper molar row; M12 : external width of upper molar row at
360 M1; M13: maximum molar width; M14: minimum width of zygomatic plate; M15:
361 maximum width of nasals; M16: maximum length of nasals; M17: length of lower molar
362 row; M18: length of the tympanic bulla; M20: depth of the upper incisors; M21: height

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363 of the rostrum at the anterior edge of the alveolus of M1; M22: maximum width of the
364 rostrum; M23: distance between the extreme points of the coronoid and angular
365 processes) reveals a good discrimination. *F. anerythrus* (FaRB and FaLB) and *F cf*
366 *bayonii* (FbLb) are clearly separated along root 1.

367 *F.cf congicus* (only six sequenced specimens with mesured skulls, see **figure 1**) is
368 clearly different from both other species. *F. anerythrus* Right Bank (FaRB) and Left
369 Bank (FaLB) are well differentiated along root 2. The number of sequenced specimens
370 of the two genotypes of *F.cf bayonii* (FbLB1 and FbLB2) are too small to separate them
371 in this analysis (**Supplementary Figure S5**).

372 A discriminant analysis, performed on the OTUs of *F. anerythrus* from the right
373 bank and left bank, as well as FbLB1 and FbLB2 using a craniometric dataset limited to
374 the skulls of specimens with matching genetic sequences, while omitting the often
375 missing M6, M7 and M20 measurements (**Supplementary Figure S3**), reveals subtle
376 differences between *F. cf bayonii* sp1 (FbLB1) and *F. cf bayonii* sp2 (FbLB2). However,
377 the limited availability of specimens with both sequence data and craniometric
378 measurements has hampered the differentiation of these potentially distinct OTUs. The
379 same figure also reveals clear differences between *F. anerythrus* from the two banks of
380 the Congo River (FaRB and FaLB) and no difference between *F. anerythrus* from the
381 right bank (FaRB and Fx in **Supplementary Figure S6**).

382 **3.2.5 *OVERALL SPECIES PARTITIONING;*** Our most conservative results, found
383 using a barcoding gap analysis with partition scoring where we retained the partition
384 with the lowest *p*-value (ASAP2), suggest the presence of four MOTUs consisting of at
385 least two specimens each. Here, *F. anerythrus* and *F. cf congicus* form one MOTU,
386 whereas *F. cf bayonii*, and a previously undescribed *Funisciurus* species (here, Fx), each
387 form another MOTU.

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388 Our second most conservative result, using a tree-based algorithm inferring
389 species delimitation based on the distribution of substitutions between and within
390 species (bPTP), suggests the presence of five MOTUs. These are *F. cf bayonii* (FbLB),
391 the undescribed *Funisciurus* species (Fx), *F. anerythrus* individuals found on the left
392 bank of the Congo River (FaLB), *F. anerythrus* individuals found on the right bank of
393 the Congo River (FaRB), and *F. congicus* (Fc).

394 The discriminant analysis conducted on craniometric data suggests the presence of
395 five OTUs, however differently distributed: *F.cf bayonii* individuals form two weakly
396 supported OTUs (FbLB1 and FbLB2), while Fc, FaLB and FaRB each form a well-
397 supported OTU. While Fx does not appear to be different from FaRB, the limited
398 number of measurable skulls for Fx prevents any conclusions about craniometric
399 differences between Fx and FaRB.

400 Last, our least conservative results, based on a barcoding gap analysis with
401 partition scoring where we retained the partition with the lowest ASAP score (ASAP1),
402 suggest the presence of six well-supported MOTUs of *Funisciurus* represented by at
403 least two individuals each. These MOTUs are Fc, FaLB, FaRB, Fx, FbLB1, and FbLB2,
404 and are in line with the FastTree topology (**Figure 1**).

405 **3.3. DISTRIBUTION AND HYDROLOGICAL BARRIERS**

406 The six main well-supported taxa occupy distribution areas separated by hydrological
407 barriers (**Figure 2**). The previously undescribed *Funisciurus* species (Fx) originates
408 exclusively from the right bank of the Congo River (Figure 2a) and the *F. cf bayonii*
409 species (FbLB1 and FbLB2) (Figure 2a) are sympatric and distributed exclusively on the
410 left bank of the Congo River. In addition, FaRB is distributed exclusively on the right
411 bank of the Congo River, and FaLB is distributed exclusively on the left bank of the

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Congo River. *F. cf congicus* (Fc) is distributed exclusively on the left bank of the Kasaï River (Figure 2b).

4. DISCUSSION

This study had two main objectives: first, to ascertain the number of *Funisciurus* OTUs present in the Congo Basin; and second, to investigate the role of the Congo River as a geographical barrier between *Funisciurus* taxa. For this purpose, we integrated morphometric, molecular and zoogeographic data from the most extensive *Funisciurus* species dataset to date. We first identified a discrepancy between the previously described species of *Funisciurus* in our study area, and the individuals present in our dataset (**Table 2, Figure 1**). We then found that both the Congo River and the Kasaï River delineate the distribution of *Funisciurus* OTUs (**Figure 2**). This highlights the importance of hydrological barriers in the speciation processes of small arboreal mammals in the Congo Basin and calls for an update in the taxonomy and IUCN distribution maps of rope squirrels.

Previously, five rope squirrel species had been described in our study area: *F. anerythrus* (Thomas, 1890), *F. bayonii* (Bocage, 1890), *F. congicus* (Kuhl, 1820), *F. pyrropus* (F. Cuvier, 1833), and the mountain-dwelling *F. carruthersi* Thomas, 1906 (**Kingdon & Hoffmann, 2013; Thorington et al., 2012; Gerrie et al., 2016**).

To minimise the risk of OTU over-segregation or fusion in our analysis (**Sloan et al., 2017; Nesi et al., 2011**), our final OTU partitioning is based on both craniometric and molecular OTU delineation, as well as a zoogeographic analysis of their distribution. To do so, we have integrated multiple OTU delineation methods (**Demos et al., 2013**), *i.e.,* a discriminant analysis of craniometric data, two barcoding gap analyses with partition scoring (ASAP1 and ASAP2), and a tree-based algorithm inferring species

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436 delimitation based on the distribution of substitutions between and within species
437 (bPTP), with OTU distribution maps, where well-supported OTUs distributed in the
438 same hydrological basin indicate the presence of non-interbreeding, sympatric species;
439 while allopatric OTUs could theoretically interbreed, but are prevented to do so by
440 hydrological barriers. Thus, using our most conservative results (ASAP2) and our
441 updated OTU distribution, we suggest that there exist four *Funisciurus* species in the
442 Congo Basin, namely *F. anerythrus* (composed of three allopatric subspecies: Fc, FaLB,
443 and FaRB); two sympatric, but genetically and craniometrically different, thus non-
444 interbreeding *F. cf bayonii* species (FbLB1, FbLB2); and the previously undescribed
445 *Funisciurus* species (Fx).

446 Several key points regarding the taxonomy of *F. anerythrus*, *F. bayonii*, and an
447 undescribed *Funisciurus* species warrant further discussion. According to previous
448 studies, *F. anerythrus*—originally described from Buguera, Uganda—has a distribution
449 spanning from Uganda to Angola and Togo (**Thorington & Hoffmann, 2005**), covering
450 all central Congolese forest blocks (**Colin, 1991**). **Kingdon & Hoffmann (2013)**
451 identified four subspecies based on geographical and morphological variations. The
452 southernmost, *F. a. anerythrus*, occurs from western Uganda to southern DRC, reaching
453 Mount Kabobo and northern Angola. It has a reddish-brown dorsal coat and a yellow to
454 red ventral coat. The northernmost, *F. a. bandarum*, ranges from the Central African
455 Republic to southern Chad, featuring a buff-tinged dorsal coat and a pale grey to orange
456 ventral side. Either of these may correspond to our OTU FaRB, but limited skull and
457 DNA data prevent verification. *F. a. mystax*, found in southern Cameroon and Gabon,
458 has a reddish-brown dorsal and orange ventral coat, while *F. a. raptorum*, endemic to
459 Benin and Nigeria, displays a grey dorsal and whitish ventral surface. Due to

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460 morphological similarities and limited overlap in our dataset, these findings reinforce the
461 need for a thorough taxonomic reassessment of the *F. anerythrus* complex.

462 Conversely, previous studies indicate that *F. bayonii* is found mostly in western
463 DRC, with no described subspecies (**Thorington et al., 2012; Kingdon & Hoffmann,**
464 **2013; Gerrie et al., 2016**). This species has only been documented outside the Central
465 African forest block and was previously considered a species of wooded savannah
466 mosaics (**Thorington et al. 2012**). However, our results show that two species
467 morphologically similar to *F. bayonii* are present in Yoko and Yasikia, located near the
468 left bank of the Congo River, in evergreen tropical forests. This result corroborates the
469 need for further taxonomic revision to clarify the status of these species, including their
470 ecological niches.

471 Last, we report the existence of a species that does not cluster with either *F.*
472 *anerythrus* nor *F.* cf *bayonii*, and which is distributed north of the Congo River (e.g.,
473 UMA30). Its range does not overlap with the known range of the mountain-restricted *F.*
474 *carruthersi* Thomas, 1906, and it does not display the typical red-leggedness of *F.*
475 *pyrropus* (F. Cuvier, 1833). Although its appearance resembles that of individuals of *F.*
476 *anerythrus* found on the right bank of the Congo River (FaRB), all OTU delineation
477 techniques based on genetic data suggest that it is a yet undescribed species of rope
478 squirrel.

479 Beyond taxonomic considerations, we highlight that the distribution ranges of
480 these squirrels are different, and much smaller than previously thought, rendering their
481 populations more vulnerable to environmental change and habitat degradation (**Steiner**
482 **M. & Huettmann F., 2023**). Current IUCN distribution maps indicate that *F. congicus*'
483 northeastern expansion is limited by the Congo River, while *F. bayonii* is confined
484 southwest of the Kasaï River. (data retrieved from IUCN Red List

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485 (https://www.iucnredlist.org)**; Gerrie et al., 2016**). However, our findings contradict
486 this. We observed that *F. congicus* (Fc) is restricted southwest of the Kasaï River, with
487 no specimens found between the Kasaï and Congo Rivers. Additionally, unlike the
488 IUCN map for *F. bayonii*, we found that the sympatric FbLB1 and FbLB2 OTUs are
489 exclusively limited to this interfluvial region, where the Kasaï River restricts
490 southwestern expansion, and the Congo River blocks northeastern expansion. Similarly,
491 contrary to IUCN data for *F. anerythrus*, the Congo River separates FaLB (southwest)
492 and FaRB (northeast). The undescribed Fx is found only northeast of the Congo River
493 (**Figure 2**). These findings highlight the role of hydrological barriers in driving
494 allopatric speciation in arboreal Sciuridae, consistent with research on primates
495 (**Anthony et al., 2007; Harcourt & Wood, 2012; Fonteyn et al., 2023**) and small
496 mammals rodentia (**Katuala et al., 2008; Kennis et al., 2011**). They further highlight
497 that the distribution range of each *Funisciurus* OTU is smaller than previously thought
498 (**Supplementary Figure S7)**. Consequently, habitat degradation or environmental
499 change may have unexpectedly severe impacts on *Funisciuru*s populations
500 (**Supplementary Figure S8)**, of which regular monitoring is necessary. We therefore
501 advocate for updated IUCN distribution maps and regular monitoring of the *Funisciurus*
502 OTUs identified above, to ensure that conservation measures can be taken where
503 appropriate.Regular monitoring will also support research on the potential
504 epidemiological importance of *Funisciurus* species.

505 **CONCLUSION**

506 Our morphometric and molecular analyses enabled us to identify six main rope squirrel
507 OTUs in the Congo Basin, namely *F. cf congicus*, *F. anerythrus*_LB, *F. anerythrus*_RB,
508 *Funisciurus sp*, *F. cf bayonii*_LB1, and *F. cf bayonii*_LB2. Further integration of these
509 approaches with zoogeographic analyses suggests that Fc (*F. cf congicus* (Kuhl, 1820)), *F.*

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510 *anerythrus*_LB and *F. anerythrus*_RB are subspecies of *F. anerythrus* (Thomas, 1890),
511 while the two sympatric and non-interbreeding *F. cf bayonii*_LB1, and *F. cf bayonii*_LB2
512 each are a separate species, possibly forming a complex with *F. bayonii* (Bocage, 1890).
513 Last, we report on a previously undescribed species of *Funisciurus* (Fx). We further
514 highlight the role of the Congo and Kasaï as hydrological barriers, delineating the
515 distribution of our OTUs, with *F. cf congicus* found exclusively southwest of the Kasaï, *F.*
516 *anerythrus*_LB, *F. cf bayonii*_LB1 and *F. cf bayonii*_LB2 found between the Kasaï and
517 Congo, and *F. anerythrus*_RB and Fx found northeast of the Congo River. These results
518 call for an update of IUCN maps, and for regular monitoring of *Funisciurus* populations,
519 which may be more vulnerable to environmental change and habitat degradation than
520 previously thought. Last, we highlight that further taxonomic and ecological studies are
521 needed to describe each OTU and ascertain its ecological niche, which will support
522 research on zoonotic pathogen spillover risks associated with each OTU. In this
523 perspective, we also recommend a complete review of the taxonomic status of other tree
524 squirrels, in particular the genera *Paraxerus, Heliosciurus* and *Protoxerus.*
525

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REFERENCES

Anthony NM, Johnson-Bawe M, Jeffery K, Clifford SL, Abernethy KA, Tutin CE, Lahm SA, White LJT, Utley JF, Wickings EJ, et al. 2007. The role of Pleistocene refugia and rivers in shaping gorilla genetic diversity in central Africa. Proceedings of the National Academy of Sciences, 104(51), 2043220436. https://doi.org/10.1073/pnas.0704816105

Baelo P, Asimonyio J, Amundala N, Akaibe D, Gambalemoke S, Erik V, Anne L, Bongo G, Koto-te-Nyiwa Ngbolua K. 2018. Preliminary Inventory of Squirrels (Sciuridae, Rodentia) of Kisangani Region in the Democratic Republic of the Congo. American Journal of Zoology . Vol. 1, No. 1, pp. 15-19. https://doi.org/10.11648/j.ajz.20180101.13

Chen NLiG, Liszewski MK, Atkinson JP, Jahrling PB, Feng Z. 2012.Virulence differences between monkeypox virus isolates from West Africa and the Congo basin. Virology Journal. 340:46–63. https://doi.org/10.1016/j.virol.2005.05.030

Colyn M. 1991. L'importance zoogéographique du bassin du fleuve Zaïre pour la spéciation: le cas des primates simiens. Annales-Musée Royal de l'Afrique Centrale-Sciences Zoologiques (Belgium). Annalen-Koninklijk Museum voor Midden-Afrika-Zoologische Wetenschappen. no. 264. http://agris.fao.org

Curaudeau M, Besombes C, Nakouné E, Fontanet A, Gessain A, and Hassanin A. 2023. Identifying the most probable mammal reservoir hosts for monkeypox virus based on ecologicalniche comparisons. Viruses, 15(3), 727. https://doi.org/10.3390/v15030727

Baelo et al, submitted

559 Demos TC, Agwanda B, Hickerson MJ. 2013. Integrative taxonomy within the
560 *Hylomyscus denniae* complex (Rodentia: Muridae) and a new species from Kenya.
561 Journal of Mammalogy 2013, 95(1):E1-E15. https://doi.org/10.1644/13-MAMM-
562 A-268
563 DeSalle R. 2024. DNA Barcoding: Methods and Protocols ISBN978-1-0716-3580-
564 3ISBN978-1-0716-3581-0(eBook) https://doi.org/10.1007/978-1-0716-3581-0
565 Fa J E, Peres CA, & Meeuwig J. 2002. Bush meat exploitation in tropical forests: an
566 intercontinental comparison. Conservation biology, 16(1), 232-237.
567 https://doi.org/10.1046/j.1523-1739.2002.00275.x
568 Falendysz E A, Lopera JG, Doty JB, Nakazawa Y, Crill C, Lorenzsonn F, Kalemba, F.,
569 Ronderos, L.N, Mejia, M.D., Malekani, J.M, Karem K., Carroll D.S., Osorio J.O.,
570 and Rocke, T. E. (2017). Characterization of Monkeypox virus infection in
571 African rope squirrels (*Funisciurus sp*.). PLoS neglected tropical diseases, 11(8),
572 e0005809. https://doi.org/10.1371/journal.pntd.0005809
573 Fonteyn D, Vermeulen C, Gorel AP, Silva de Miranda PL, Lhoest S, and Fayolle A.
574 2023. Biogeography of central African forests: Determinants, ongoing threats and
575 conservation priorities of mammal assemblages. Diversity and
576 Distributions, 29(6), 698-712. DOI: 10.1111/ddi.13677
577 Gerrie R, Kennerley and Koprowski RJ. 2016. *Funisciurus bayonii*. The IUCN Red List
578 of Threatened Species; http://dx.doi.org/10.2305/IUCN.UK.2016-
579 3.RLTS.T8757A22270331.
580 Han BA, Schmidt JP, Bowden SE et Drake JM. 2015. Réservoirs de rongeurs de futures
581 zoonoses. Actes de l'Académie nationale des sciences, 112(22), 7039-7044.
582 https://doi.org/10.1073/pnas.1501598112

Baelo et al., submitted

583 Harcourt AH & Wood MA. 2012. Rivers as Barriers to Primate Distributions in
584 Africa. International Jornal of Primatolology **33**, 168–183
585 https://doi.org/10.1007/s10764-011-9558-z
586 Hoang DT, Chernomor O, Haeseler A, Minh BQ, and Vinh LS. 2017. UFBoot2:
587 Improving the ultrafast bootstrapapproximation. Molecular Biology and Evolution,
588 in press. https://doi.org/10.1093/molbev/msx281
589 Bernd Hoffmann DVM., Dennis Tappe MD, Dirk Höper MSc, Christiane Herden DVM,
590 Annemarie Boldt MD, Christian Mawrin MD, Olaf Niederstraßer MD, Tobias
591 Müller MD, Maria Jenckel MSc, Elisabeth van der Grinten DVM, et al. 2015. A
592 variegated squirrel bornavirus associated with fatal human encephalitis. New
593 England Journal of Medicine, 373(2), 154-162.
594 https://doi.org/10.1056/NEJMoa1415627
595 Kalyaanamoorthy S, Minh BQ, Wong TKF, Haeseler A, and Jermiin LS. 2017.
596 ModelFinder: Fast model selection foraccurate phylogenetic estimates. Nature
597 Methods, 14:587–589.https://doi.org/10.1038/nmeth.4285
598 Katuala P, Kennis J, Nicolas V, Wendelen W, Hulselmans J, Verheyen E, Van Houtte
599 N, Dierckx T, Dudu A andLeirs H. 2008. The presence of *Praomys*, *Lophuromys*,
600 and *Deomys* species (Muridae, Mammalia) in the forest blocks separated by the
601 Congo River and its tributaries (Kisangani region, Democratic Republic of
602 Congo). Mammalia, 72(3), 223-228. https://doi.org/10.1515/MAMM.2008.044
603 Kennis J, Nicolas V, Hulsemans J, Katuala GBP, Wendlen W, Verheyen E, Dudu A,
604 Leirs H, 2011. The impact of the Congo River and its tributaries on the rodent
605 genus Praomys: speciation origin or range expansion limit?, Zoological Journal of
606 the Linnean Society, Volume 163, Issue 3, November 2011, Page 983-1002,
607 https://doi.org/10.1111/j.1096-3642.2011.00733.x

Baelo et al., submitted

608 Kinganda LE, Amuri AA, Nuñez FN, Makangara JC, Pratt C, Vakaniaki EH, Hoff NA,
609 Luakanda GN, Sabiti SN, Mulopo NM et al. 2024. Clade I mpox virus genomic
610 diversity in the Democratic Republic of the Congo, 2018–2024: predominance of
611 zoonotic transmission. Cell journal. DOI: 10.1016/j.cell.2024.10.017
612 Kingdon J & Hoffmann M. 2013. 'Mammals of Africa. Volume V: Carnivores,
613 Pangolins, Equids and Rhinoceroses' 2nd ed. Ed J Kingdon. (Bloomsbury
614 Publishing Plc: London) https://doi.org/10.5860/CHOICE.50-4188
615 Kumakamba C, Niama FR, Muyembe F, Mombouli JV, Kingebeni PM, Nina RA, Ngay
616 LI, Bounga G, N'Kawa F, Goma NC et al. 2021. Coronavirus surveillance in
617 wildlife from two Congo basin countries detects RNA of multiple species
618 circulating in bats and rodents. PLoS One, 16(6), e0236971.
619 https://doi.org/10.1371/journal.pone.0236971
620 Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, Haeseler A,
621 Lanfear R. 2020. IQ-TREE 2: New Models and Efficient Methods for
622 Phylogenetic Inference in the Genomic Era, Molecular Biology and Evolution,
623 Volume 37, Issue 5, May 2020, Pages 1530–
624 1534, https://doi.org/10.1093/molbev/msaa015

Baelo et al., submitted

625 Montgelard C, Sophie B, Claire T, Olivier V, François M. 2002. Systématique
626 moléculaire de Sciurognathi (Rodentia) : les gènes mitochondriaux du
627 cytochrome *b* et de l'ARNr 12S soutiennent les Anomaluroidea (Pedetidae et
628 Anomaluridae) Volume 22, Numéro 2, février 2002, pages 220-233
629 https://doi.org/10.1006/mpev.2001.1056
630 Nakazawa Y, Mauldin MR, Emerson GL, Reynolds MG, Lash RR, Gao J, Zhao H, Yu
631 Li, Muyembe JJ et al. 2015. A phylogeographic investigation of African
632 monkeypox. *Viruses*, *7*(4), 2168-2184. https://doi.org/10.3390/v7042168
633 Nesi N, Nakouné E, Cruaud C, and Hassanin A. 2011. DNA barcoding of African fruit
634 bats (Mammalia, Pteropodidae). The mitochondrial genome does not provide a
635 reliable discrimination between *Epomophorus gambianus* and *Micropteropus*
636 *pusillus*. Comptes Rendus. Biologies, Volume 3334 (2011) no. 7, pp. 544-
637 554.https://comptes-rendus.academie
638 sciences.fr/biologies/articles/10.1016/j.crvi.2011.05.003/
639 Nguyen LT, Schmidt HA, Haeseler A, and Minh BQ. 2015. IQ-TREE: A fast and effective
640 stochastic algorithm for estimating maximum likelihood phylogenies. Molecular
641 Biology and Evolution, 32:268-274. https://doi.org/10.1093/molbev/msu300

642 Otieno JR, Ruis C, Onoja AB, Kuppalli K, Hoxha A, Nitsche A, Brinkmann A, Michel
643 J, Kingebeni PM, Bamuleka DM, et al. 2025. Global genomic surveillance of
644 monkeypox virus. Natural Medicines **31**, 342–350 (2025).
645 https://doi.org/10.1038/s41591-024-03370-3

646 Price MN., Dehal PS, & Arkin AP. 2009. FastTree: computing large minimum evolution
647 trees with profiles instead of a distance matrix. Molecular biology and
648 evolution, 26(7), 1641-1650. https://doi.org/10.1093/molbev/msp077

Raelo et al., submitted

649 Puillandre N, Brouillet S, Achez G. 2021. ASAP: assemble species by automatic
650 partitioning. Molecular Ecology Resources 21:609−620.
651 https://doi.org/10.1111/1755-0998.13281
652 Rimoin AW, Alfonso VH, Hoff NA, Doshi RH, Mulembakani P, Kisalu NK, Wright
653 LL. 2017. Human exposure to wild animals in the Sankuru Province of the
654 Democratic Republic of the Congo. Ecohealth, 14, 552-
655 563.https://doi.org/10.1007/s10393-017-1262-9
656 Schulze V, Lurz PW, Ferrari N, Romeo C, Steele MA, Marino S, Vittoria MM,
657 Calvignac SS, Schlottau K, Beer M, Ulrich RG, and Ehlers B. 2020. Search for
658 polyoma-, herpes-, and bornaviruses in squirrels of the family Sciuridae. Virology
659 journal, 17, 1-21. https://doi.org/10.1186/s12985-020-01310-4
660 Sikes RS, & Animal Care and Use Committee of the American Society of
661 Mammalogists. 2016. 2016 Guidelines of the American Society of Mammalogists
662 for the use of wild mammals in research and education. Journal of
663 mammalogy, 97(3), 663-688. https://doi.org/10.1093/jmammal/gyw078.
664 Sklenovská N, & Van Ranst M. 2018. Emergence of monkeypox as the most important
665 orthopoxvirus infection in humans. *Frontiers in public health*, *6*, 383729.
666 https://doi.org/10.3389/fpubh.2018.00241
667 Sloan DB, Havird JC, & Sharbrough J. 2017. The on-again, off-again relationship
668 between mitochondrial genomes and species boundaries. Molecular
669 ecology, 26(8), 2212-2236. https://doi.org/10.1111/mec.13959
670 StatSoft, Inc. 2014. STATISTICA (data analysis software system), version 12. Available
671 at: https://www.statsoft.com.

Baelo et al., submitted

Steiner M. & Huettmann F. 2023. Chapter 1. Taxonomy for the Squirrels of the World: Hairy Classifications and Conservation Category Games. in: Sustainable Squirrel Conservation, https://doi.org/10.1007/978-3-031-23547-4_1

Thorington JRW, Koprowski JL, Steele MA, & Whatton JF. 2012. Squirrels of the world. JHU Press.

Thorington RW & Hoffmann RS. Family Sciuridae. Pp 754-818 in Wilson D.E. & Reeder, D.M. (Eds), (2005). Mammals species of the World: A taxonomic and Geographic reference. The Johns Hopkins University Press, Baltimore; Vol. 2, 21-42 pp.

Tiee MS, Harrigan RJ, Thomassen HA, & Smith TB. 2018. Ghosts of infections past: using archival samples to understand a century of monkeypox virus prevalence among host communities across space and time. Royal Society Open Science, 5(1), 171089.

Van der Straeten E, & Dieterlen F. 1987. *Praomys misonnei*, a new species of Muridae from Eastern Zaïre (Mammalia). *Stuttg. Beitr. Naturk.*, 402: 1-11.

Verheyen W, & Bracke E. 1966. The influence of aging on the craniometrical characters of *Praomys jacksoni* (De Winton 1897). Annales du Musée Royal d'Afrique Centrale de Tervuren, 144, 91-101.

Verheyen WN, Colyn M, & Hulselmans J. 1996. Re-evaluation of the *Lophuromys nudicaudus* HELLER, 1911 species-complex with a description of a new species from Zaire (Muridae-Rodentia). Bulletin de l'Institut Royal des Sciences Naturelles de Belgique Biologie, 66, 241-273.

Zachos FE, Burgin CJ, Wilson DE, Mittermeier RA, Rylands AB, Lacher TE, Sechrest W. 2021. Illustrated Checklist of the Mammals of the World. Boxed set of two

Baelo et al., submitted

696 volumes, with a foreword by Razan Al Mubarak. Mammalian Biology 101, 125–
697 126 (2021). https://doi.org/10.1007/s42991-020-00088-w
698 Zhang J, Kapli P, Pavlidis P, Stamatakis P. 2013. A general species delimitation method
699 With applications to phylogenetic placements. Bioinformatics 29:2869–2876.
700 https://doi.org/10.1093/bioinformatics/btt499
701

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Fig. 1. Proposed rope squirrel Operational Taxonomic Units using a combination of methods. Above the branches, methods used to propose species partitions.

Fig. 2. Maps showing the distribution of the species and subspecies identified in relation to the Congo River and its tributaries.

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List of table

Table 1. Species partitions ranked in ascending order of ASAP scores. The *p*-value reflects the likelihood that Partition is different from $Partition_{n-1}$ and is ranked in ascending order. The threshold distance is used to compute the W-score and represents the mid-point between the distance that triggered the merging of subsets into Partition and the previous distance in the list. The W-score is the relative barcode gap width associated with $Partition_{n-1}$ and is ranked in descending order. The ASAP-score is the average of the ranks of the *p*-value and of the W-score. A smaller ASAP-score thus signifies a better partition (**Puillandre et al., 2021**). Read: Considering an ASAP-score of 2.00 and a p-value of 0.0177, 9 subsets are identifiable in the dataset, including two outgroups.

Number of subsets	ASAP-score	P-val (rank)	W (rank)	Threshold dist.
*9	2.00	1.77e-02 (3)	7.83e-04 (1)	0.020430
5	3.50	1.96e-01	2.03e-04 (3)	0.118381
*8	4.50	3.46e-03 (02)	4.68e-05 (7)	0.029208
6	5.00	3.80e-04 (1)	2.56e-05 (9)	0.075144
*12	7.00	5.45e-01 (8)	4.72e-05 (6)	0.0008821
8	11.50	6.87e-01 (15)	3.11e-05 (8)	0.052757
*10	12.00	8.40e-01 (22)	5.20e-04 (2)	0.015671
59	14.00	5.73e-01 (10)	6.22e-06 (18)	0.000899
60	15.50	6.79e-01 (14)	6.22e-06 (17)	0.000450
2	15.50	8.14e-01 (20)	1.15e-05 (11)	0.180112

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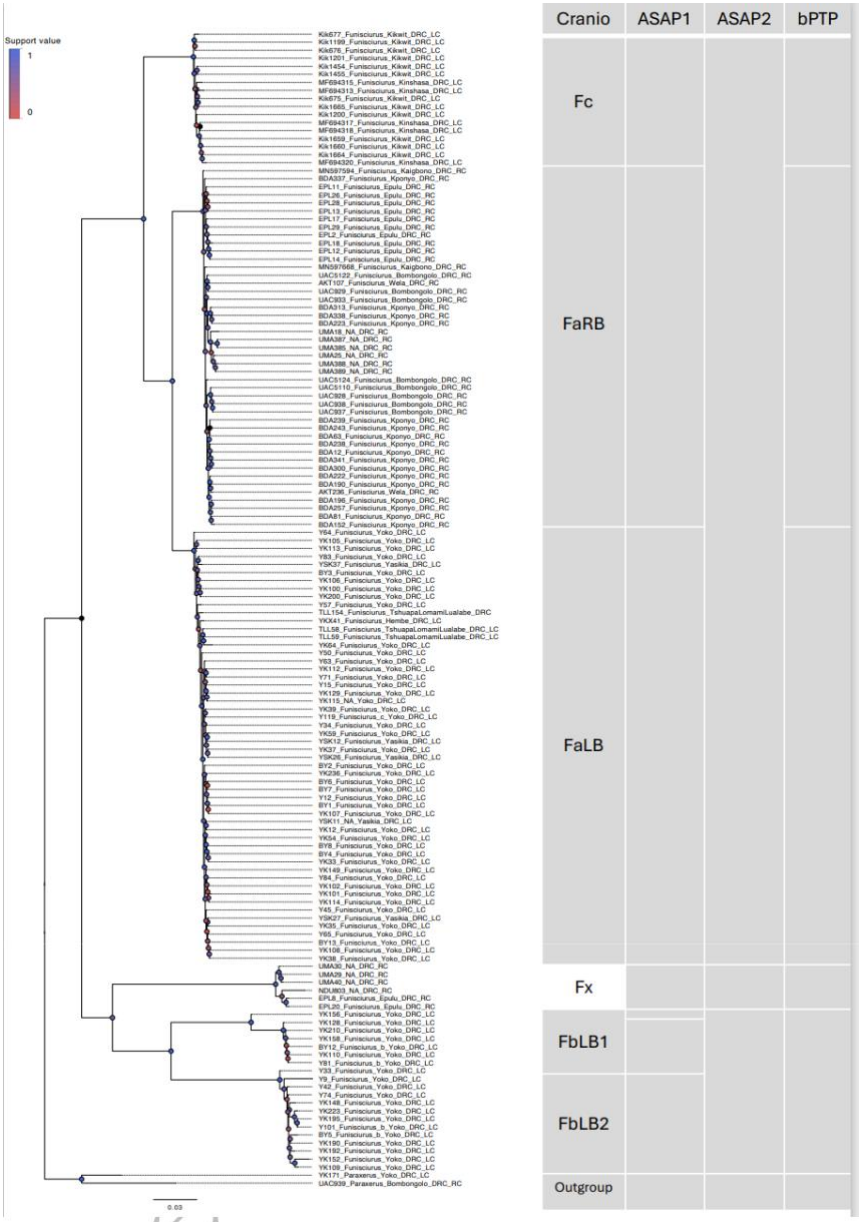
722 **Figures**



723

724 **Figure 1.** Proposed rope squirrel Operational Taxonomic Units using a combination of

725 methods. Above the branches, methods used to propose species partitions. Cranio:

726 Craniometry; ASAP1: Assemble Species by Autonomous Partitioning (ASAP), lowest

727 ASAP score; ASAP2: *idem*, lowest p-value; bPTP: bayesian Poisson Tree Processes;

728 RB: right bank of the Congo River; LB: left bank of the Congo River. Fx: previously

729 undescribed *Funisciurus* species; Fb: *Funisciurus cf bayonii*; Fc: *Funisciurus cf*

Bae
nitted

730 *congicus*; Fa: *Funisciurus anerythrus*. The tree was made using FastTree on MAFFT-
731 assembled cytochrome b sequences.

732

733

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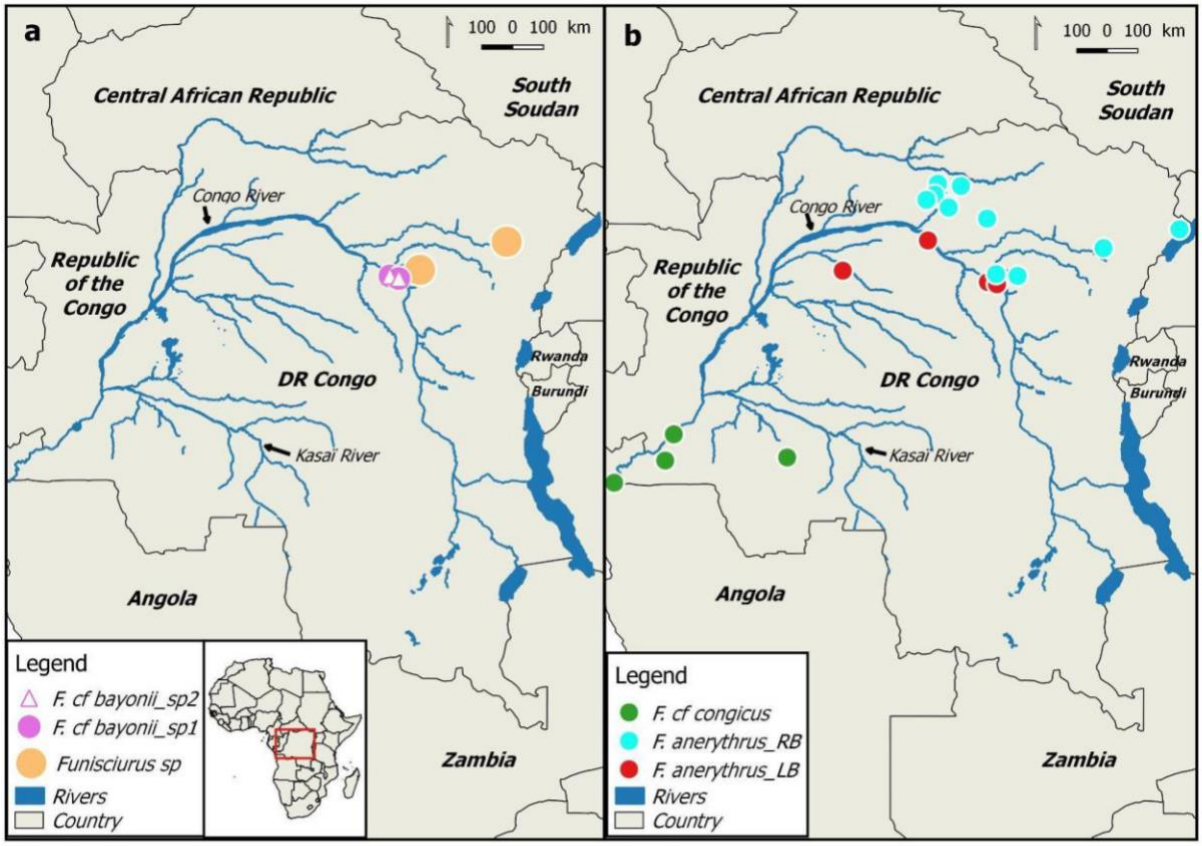

Figure 2. Maps showing the distribution of the species and subspecies identified in relation to the Congo River and its tributaries, (**a**) *Funisciurus sp* from the right bank of the Congo River and the sympatric *F. cf bayonii sp1* and *F. cf bayonii sp2* on the left bank of the Congo River; (**b**) *F. anerythrus_RB* on the right bank, *F. anerythrus_LB* on the left bank of the Congo River, and *F. cf congicus* on the left bank of the Kasaï River.

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Contributions of the authors

PB, EV and JH conceptualized the study and designed the methodology. PB collected the data and identified the species in the field. LF, PB, GTS and NL generated molecular data. PB, JH, and EV analyzed the morphometric data. PB produced the map. LF, SG and EV analysed the molecular data. PB, LF, JH and EV wrote the original version. PB, LF, CM, AL, NA, NL, GTS, JN, JM, SG, GG, JH and EV reviewed and edited the document. All authors approved the final manuscript.

Data availability

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765 The generated and/or analyzed datasets can be made available for research purposes upon
766 request to the corresponding authors. The sequences from this study will be submitted to
767 GenBank upon acceptance.
768
769 **Conflict of interest declaration.**
770 The authors declare no conflict of interest.
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