# A multidisciplinary approach to identify the animal reservoir(s) of the Monkeypox virus in Africa

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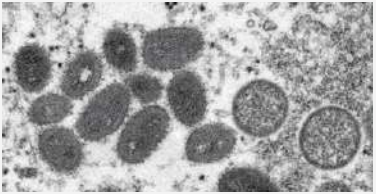
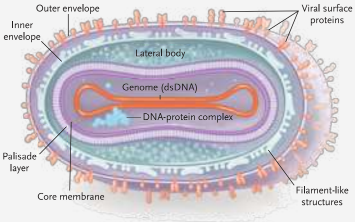
Manon Curaudeau
October 30th, 2024

PhD funded by Fondation SCOR
Part of the ANR Afripox Project

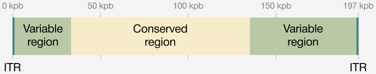
Supervised by:
Antoine GESSAIN – Institut Pasteur – Epidémiologie et Physiopathologie des Virus Oncogènes (UMR 3569)
Alexandre HASSANIN – Muséum national d'Histoire naturelle & Sorbonne Université – Institut de Systématique, Évolution, Biodiversité (UMR 7205)

# Monkeypox virus





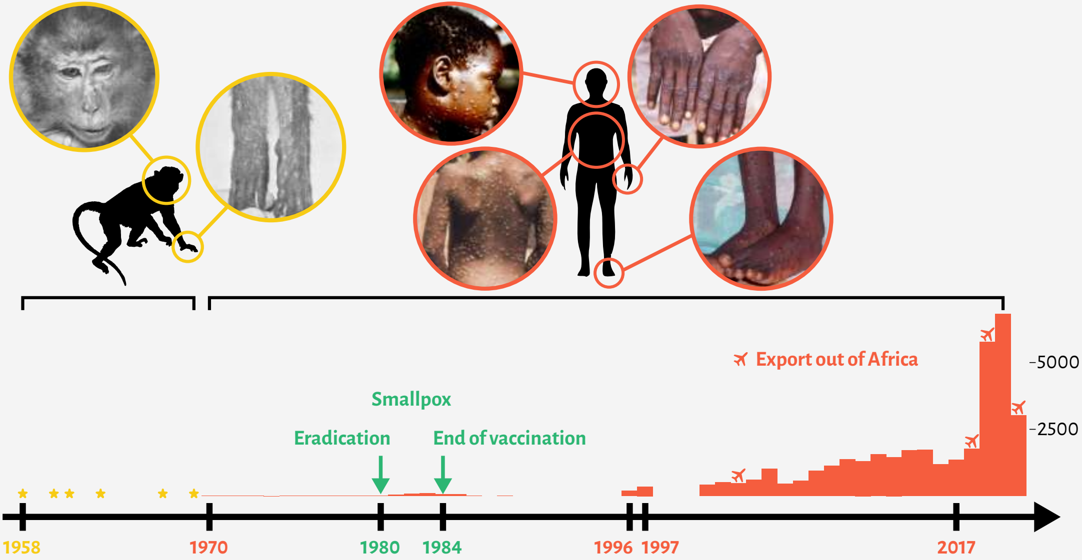
Gessain *et al.*, 2022



Mpox is caused by a virus: Monkeypox virus (MPXV)

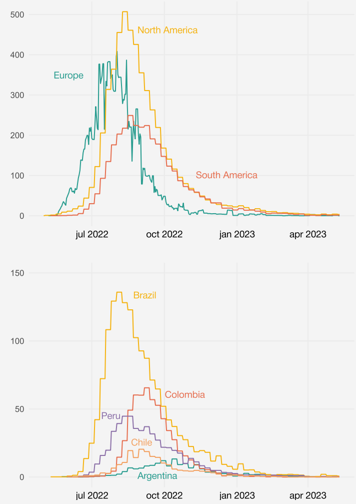
- Poxviridae, like smallpox (Fenner and Nakano, 1988)
- Specifically *Orthopoxvirus* (Fenner and Nakano, 1988)
- Double-stranded DNA genome
- 197kb genome and 186 ORFs

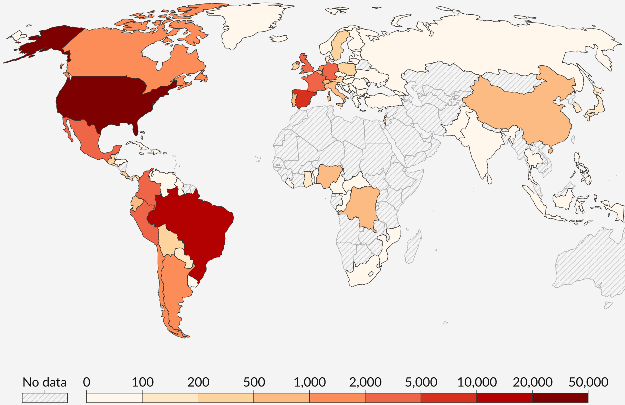
# A brief history of Mpox



Sklenovská and Van Ranst, 2018

# Mpox pandemic started in May 2022

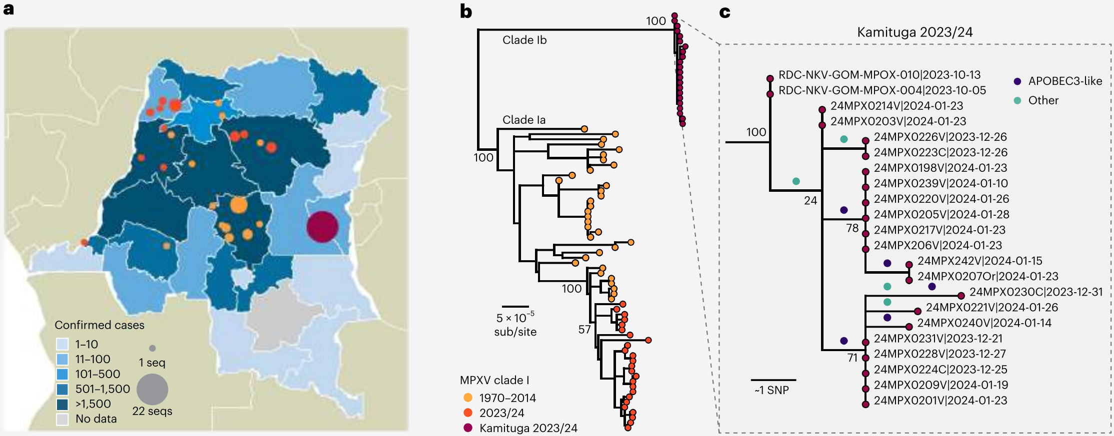




Our World in Data

World Health Organization, 2024

# A new major outbreak in 2024

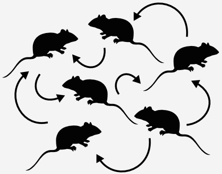


Vakaniaki *et al.*, 2024

## Mpox is a zoonotic disease but the reservoir is still unknown

# A multidisciplinary approach to identify the animal reservoir(s) of the Monkeypox virus in Africa

# Reservoir of a zoonotic virus



Reservoir species

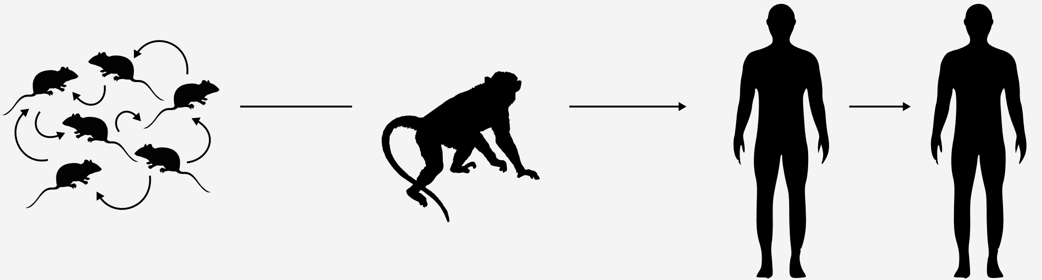
# Reservoir of a zoonotic virus

Reservoir species

Index case

Secondary case

# Reservoir of a zoonotic virus



Reservoir species

Intermediary host

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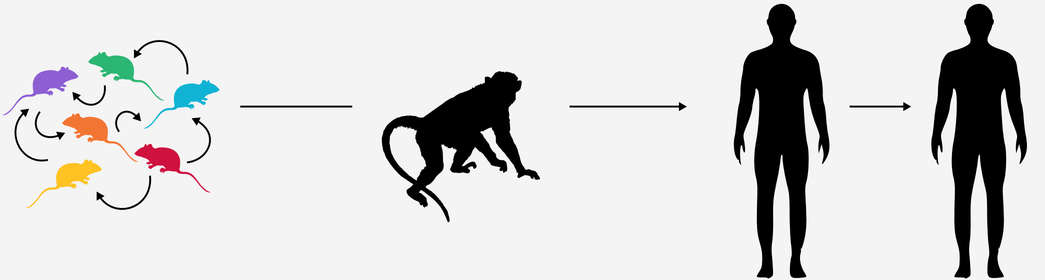
# Reservoir of a zoonotic virus



Reservoir species

Intermediary host

Index case

Secondary case

**Objective 1:** Enhance knowledge on the biodiversity of the most probable reservoirs for MPXV

**Objective 2:** Supply public health authorities with key data on these reservoirs to aid mpox prevention

**Goal:** Support prevention efforts by reducing the risk of spillover from reservoirs to humans, lowering the chances of mpox outbreaks

# Bibliographic study to identify potential reservoir or secondary hosts of MPXV

1. MPXV belongs to *Orthopoxvirus*, a genus exclusive to mammals

   → MPXV reservoir is a **mammal**: 5973 species

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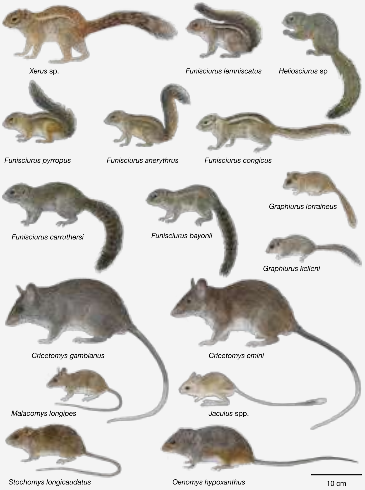
4. 19 genera of mammals with at least one evidence:

   - Virus isolation
   - MPXV fragment amplified by PCR
   - Anti-OPXV antibodies

   → MPXV reservoir belongs to one of this genera: **213 species**



Lacher *et al.*, 2016
Wilson *et al.*, 2017

# Strategy to identify the reservoir(s) of Monkeypox virus

1. Ecological niche overlap analyses between MPXV and African mammals

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2. Comparisons of phylogeographic patterns between MPXV and its reservoir

3. Perspectives: Detection of MPXV in museum specimens of MPXV reservoirs

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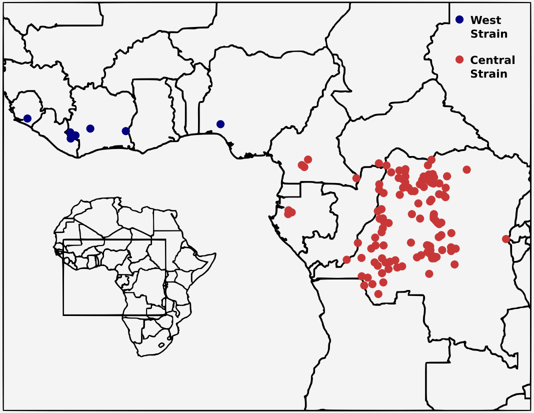
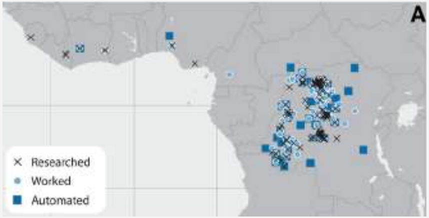
# Previous Monkeypox niche (ENM)



Levine *et al.*, 2007: 156 occurrences, **all unavailable**

Monkeypox ENM has been done but mixes together:

- Index and secondary cases
- Confirmed and suspect cases
- GPS of villages, hospitals, and health centers

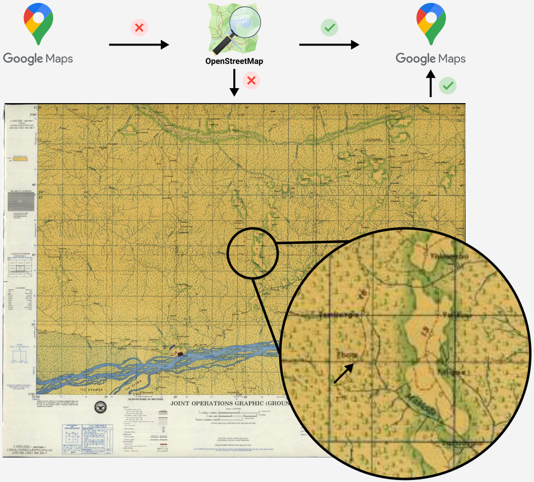


Lash *et al.*, 2012: 69-116 occurrences, **all unavailable**

→ Updated niche with only confirmed index cases

# Monkeypox virus occurrences

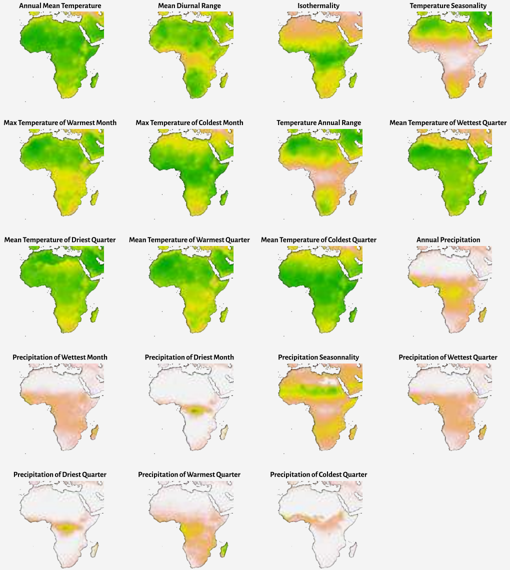
From index cases in the literature and maps



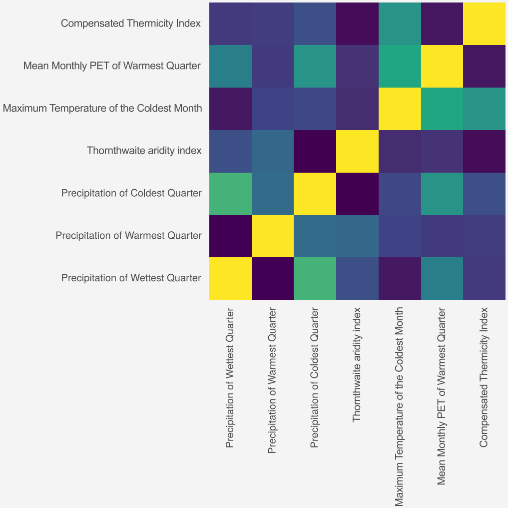
n = 96

109 reliable GPS points (96 filtered)

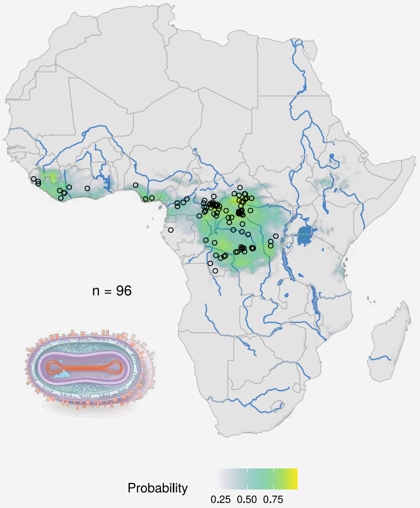
# Bioclimatic and topological variables

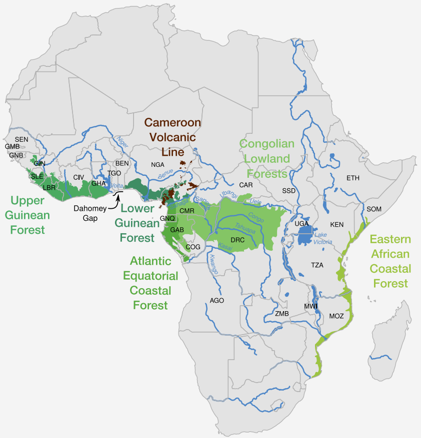


→ Least correlated variables ( $|r| < 0.7$ )

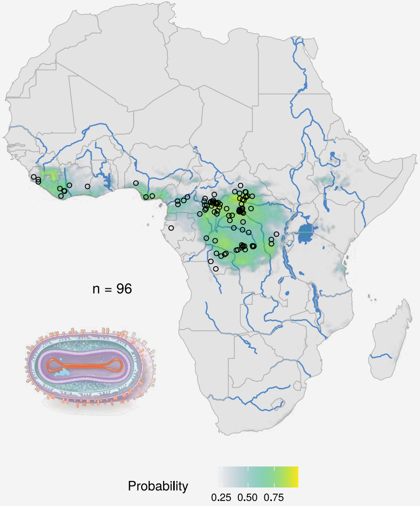


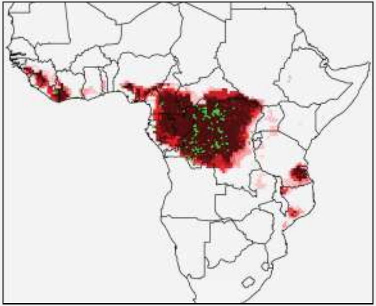
# Monkeypox virus niche compared to African forests



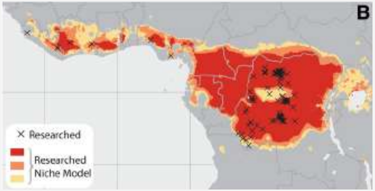


# Monkeypox virus niche compared to previously published niches





Levine *et al.*, 2007



Lash *et al.*, 2012

# Getting occurrence data points for the 213 mammal species

GBIF

iDigBio
Integrated Digitized Biocollections

VertNet

Specimen databases

iNaturalist

Observation database

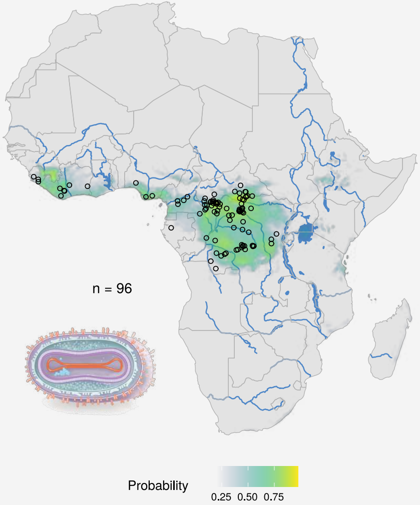
IUCN RED LIST©

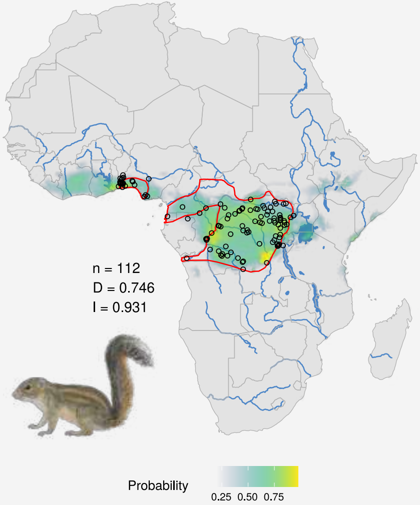
Distribution database

99 species with sufficient occurrence data points (> 16)

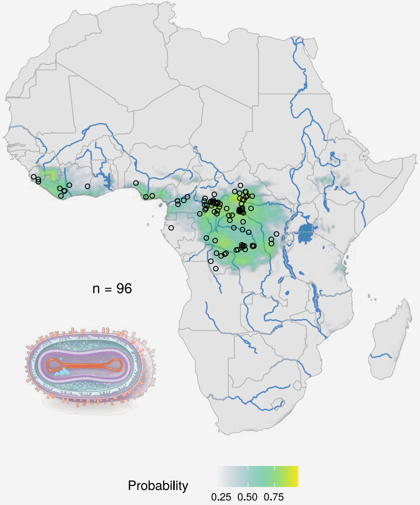
→ Other species are too rare to be the reservoir

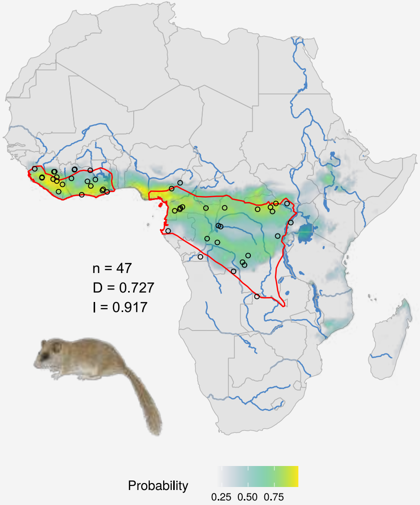
# MPXV niche *versus Funisciurus anerythrus* niche



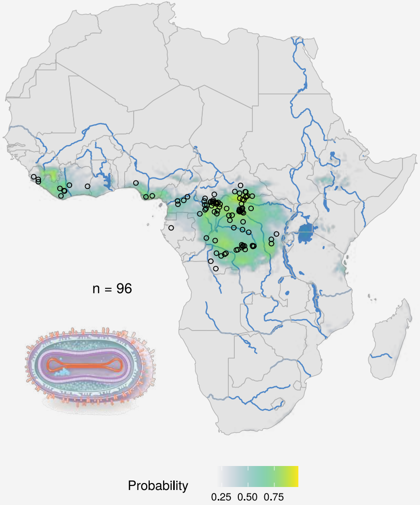


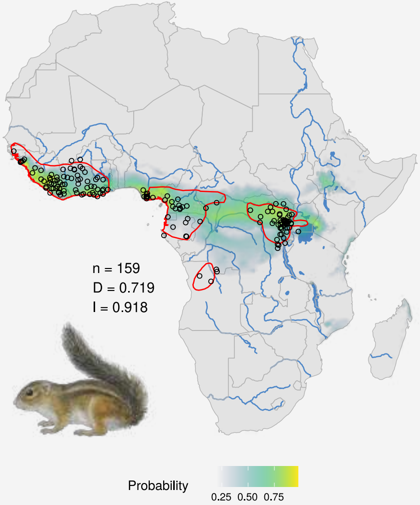
# MPXV niche *versus Graphiurus lorraineus* niche



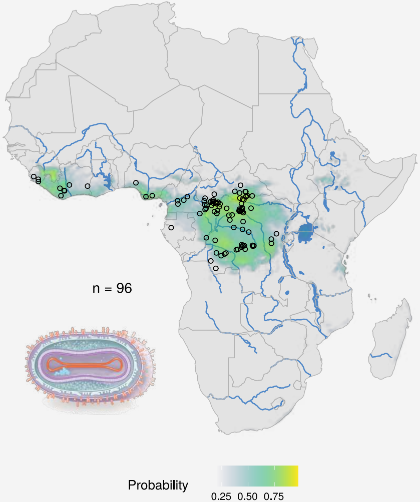


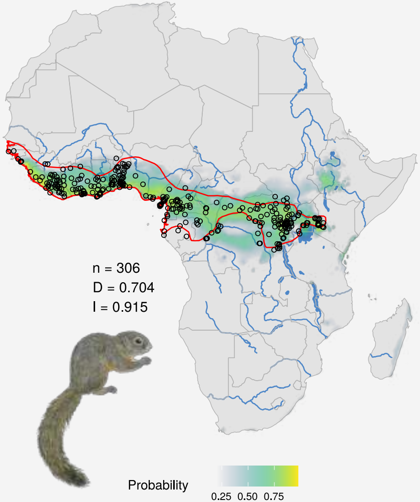
# MPXV niche *versus Funisciurus pyrropus* niche





# MPXV niche *versus Heliosciurus rufobrachium* niche





# Article published in *Viruses*

viruses

MDPI

*Article*

## Identifying the Most Probable Mammal Reservoir Hosts for Monkeypox Virus Based on Ecological Niche Comparisons

**Manon Curaudeau [1,2], Camille Besombes [3], Emmanuel Nakouné [4], Arnaud Fontanet [3,5], Antoine Gessain [2] and Alexandre Hassanin [1,*]**

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[4] Department of Arboviruses, Emerging Viruses and Zoonosis, Institut Pasteur, Bangui BP 923, Central African Republic; emmanuel.nakoune@pasteur-bangui.cf

[5] Conservatoire National des Arts et Métiers, Unité PACRI, 75003 Paris, France

* Correspondence: alexandre.hassanin@mnhn.fr

**Abstract:** Previous human cases or epidemics have suggested that Monkeypox virus (MPXV) can be transmitted through contact with animals of African rainforests. Although MPXV has been identified in many mammal species, most are likely secondary hosts, and the reservoir host has yet to be discovered. In this study, we provide the full list of African mammal genera (and species) in which MPXV was previously detected, and predict the geographic distributions of all species of these genera based on museum specimens and an ecological niche modelling (ENM) method. Then, we reconstruct the ecological niche of MPXV using georeferenced data on animal MPXV sequences and human index cases, and conduct overlap analyses with the ecological niches inferred for 99 mammal species, in order to identify the most probable animal reservoir. Our results show that the MPXV niche covers three African rainforests: the Congo Basin, and Upper and Lower Guinean forests. The four mammal species showing the best niche overlap with MPXV are all arboreal rodents, including three squirrels: *Funisciurus anerythrus*, *Funisciurus pyrropus*, *Heliosciurus rufobrachium*, and *Graphiurus lorraineus*. We conclude that the most probable MPXV reservoir is *F. anerythrus* based on two niche overlap metrics, the areas of higher probabilities of occurrence, and available data on MPXV detection.

**Keywords:** Monkeypox; animal reservoir; ecological niche model; tropical Africa; evergreen forests; Sciuridae

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Academic Editor: Benjamin Liu

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Revised: 7 March 2023
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### 1. Introduction

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Taxonomically, MPXV belongs to Poxviridae, a family of large double-stranded DNA viruses (130–375 kbp) represented by 22 genera and 83 species. The family is divided into two subfamilies: Entomopoxvirinae, in which hosts are insects; and Chordopoxvirinae, in which hosts are vertebrates (birds, crocodiles, mammals, and teleost fishes) [4–7]. Within Chordopoxvirinae, all viruses of the genus *Orthopoxvirus* (OPXV) are related to mammalian hosts, and phylogenetic analyses have supported the existence of an Old World group composed of MPXV and eight other species, such as variola virus (the agent of smallpox), vaccinia virus (the source of modern smallpox vaccines), and cowpox [8]. Mpox was first described in 1958 in Asian macaques used for polio vaccine production and research at the

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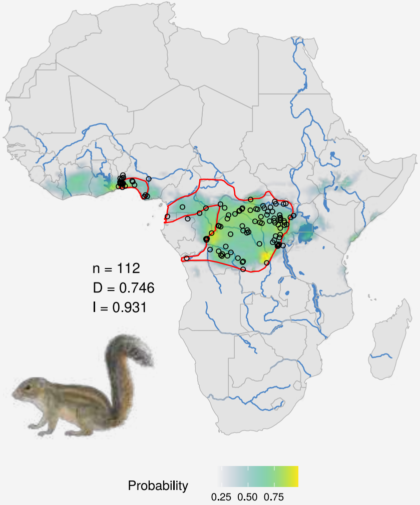

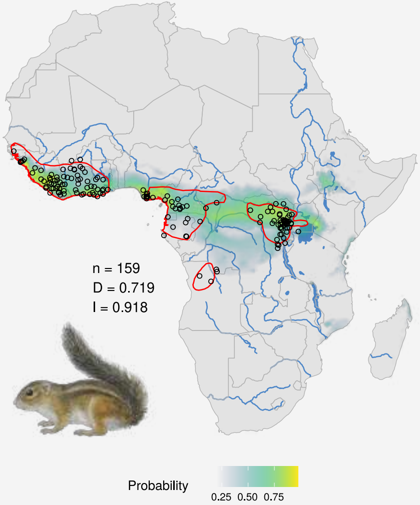

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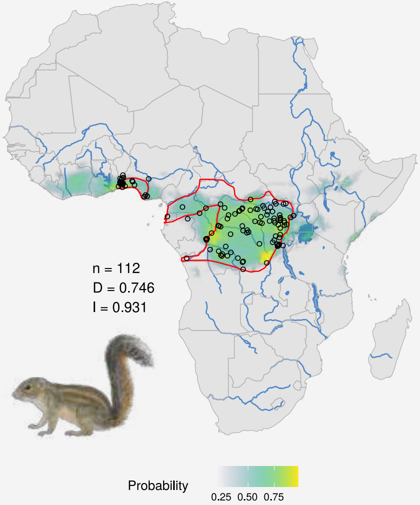

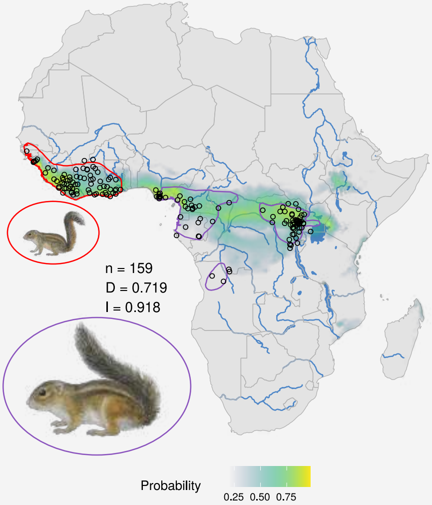

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4. Are some *F. pyrropus* actually *F. anerythrus*?

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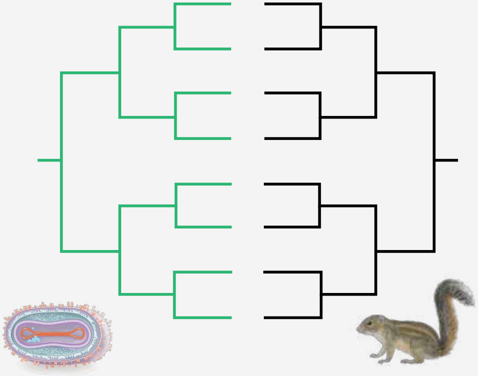
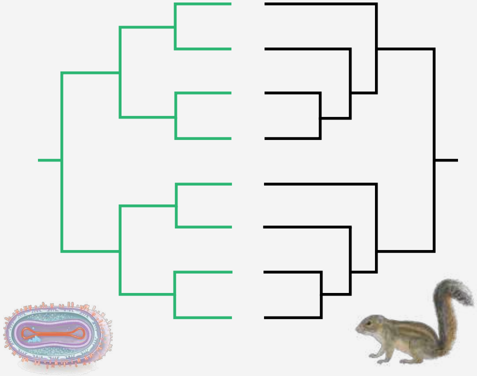
# Comparative phylogeography between MPXV and *Funisciurus anerythrus*

Perfect coevolution

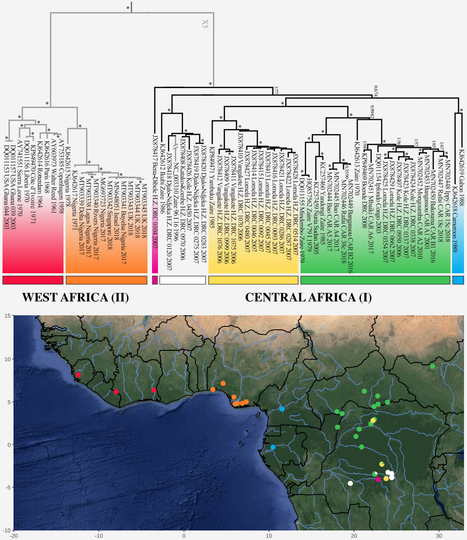


No coevolution



Comparisons of phylogeographic patterns + datations

# Phylogeography of MPXV



Two main phylogenetic groups of MPXV

- West Africa
- Central Africa/Congo Basin
- Smaller groups within the two clades

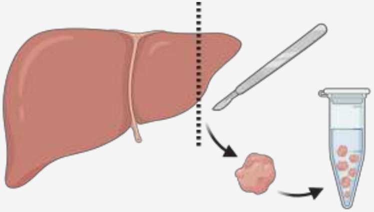
Is this deep divergence linked to the reservoir isolation?

- Co-evolution between MPXV and its reservoirs
- Same groups bewteen the virus and the reservoir

modified from Berthet, Descorps-Declère, Besombes, Curaudeau *et al.*, 2021

# Specimens collected for the *museomics* study

*"Fresh"* specimens: 140 including 46 *Funisciurus*



MUSÉUM NAL HIST NATURELLE

CBGP Centre de Biologie pour la Gestion des Populations

INSTITUTE OF VERTEBRATE BIOLOGY CZECH ACADEMY OF SCIENCES

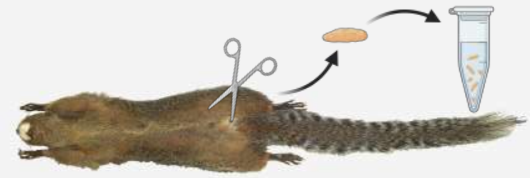
FIELD MUSEUM

AMERICAN MUSEUM OF NATURAL HISTORY

FORSCHUNGS museum KOENIG

MVZ

*"Historic"* specimens: 303 including 232 *Funisciurus*



MUSÉUM NAL HIST NATURELLE

naturhistorisches museum Basel archive des lebens

AFRICA museum

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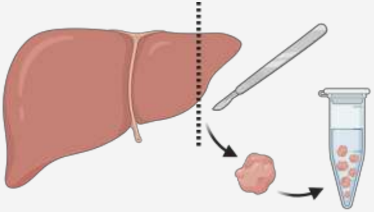

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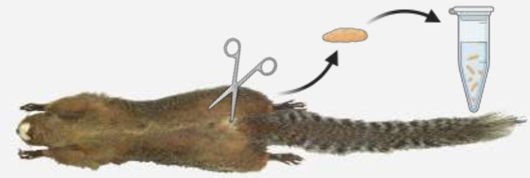
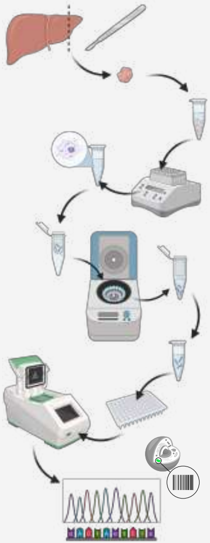
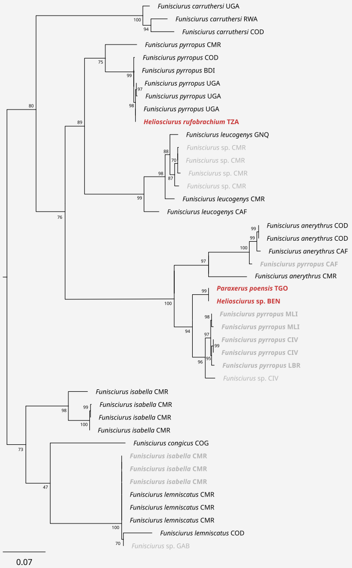


# Phylogeny of *Funisciurus* based on the CO1 (705 bp) with Maximum Likelihood (ML)





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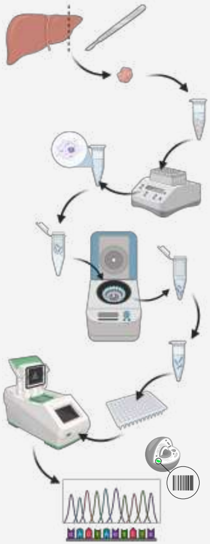

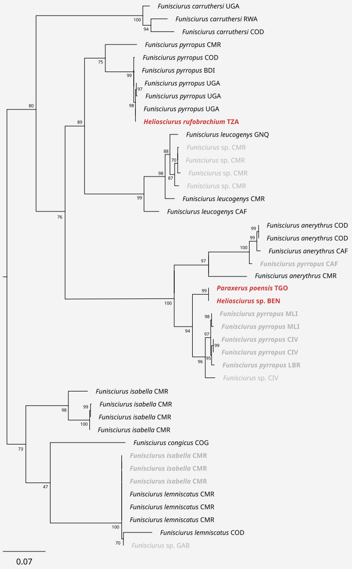

Many identification errors

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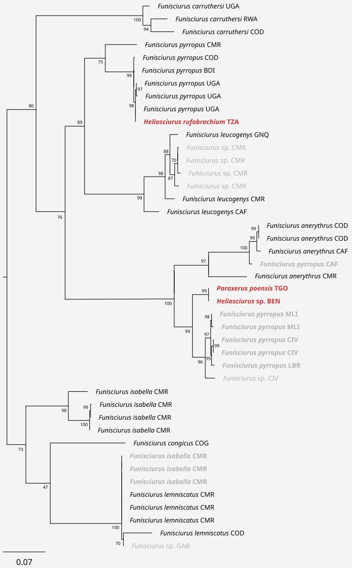

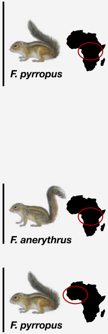

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Niche result confirmed

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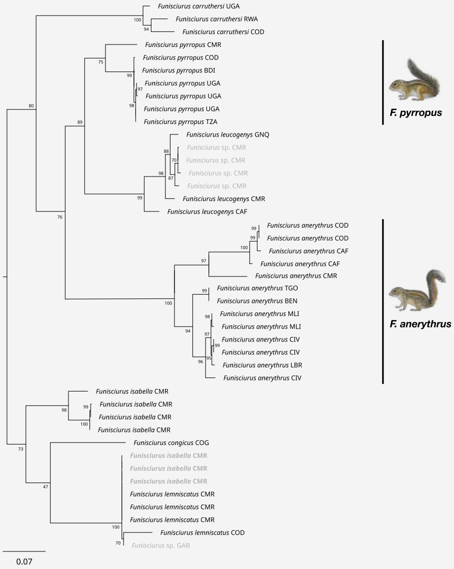

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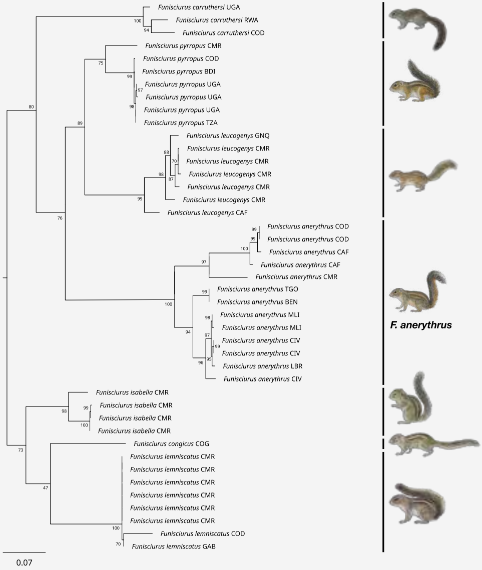

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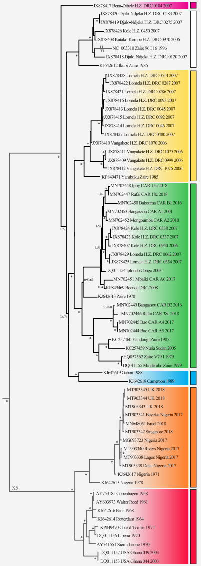

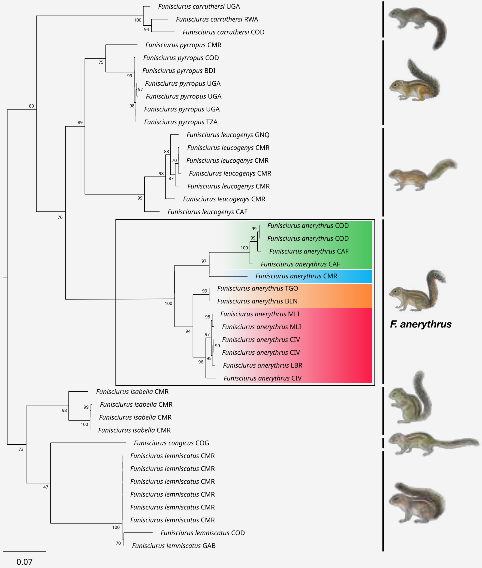

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Phylogeny similar to MPXV

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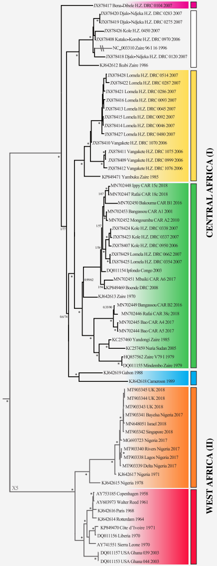

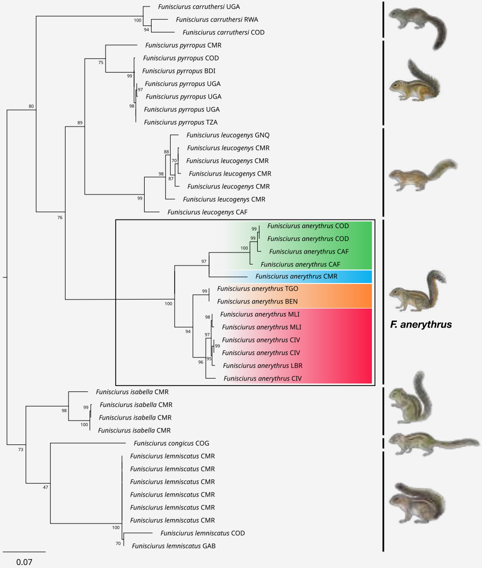

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Niche result confirmed

Phylogeny similar to MPXV

⚠ Small fragment

⚠ Maternal lineage only

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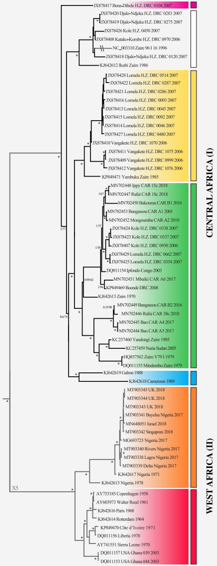
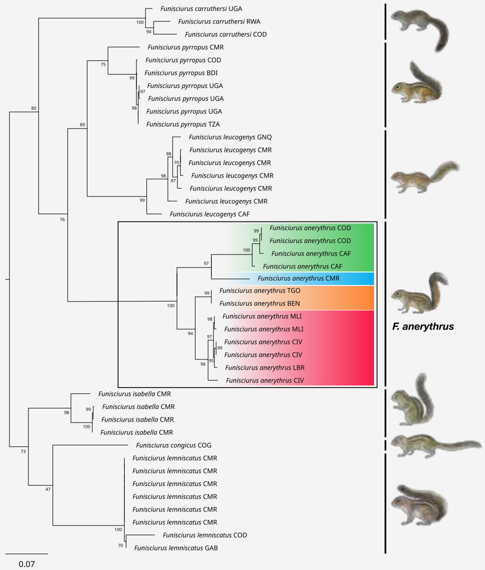




Many identification errors

Niche result confirmed

Phylogeny similar to MPXV

⚠ Small fragment

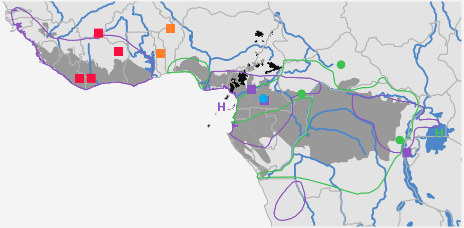
⚠ Maternal lineage only

→ Genome sequencing

# Whole genome sequencing of 32 Protoxerini squirrels (3 Gbp)

Illumina sequencing of ten *F. anerythrus*

- Six in West Africa
- Three in Central Africa/Congo Basin
- One in Cameroon

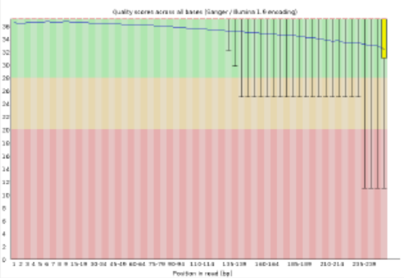


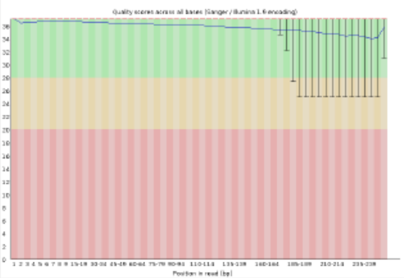
Illumina sequencing of other Protoxerini squirrels

- Twelve *Funisciurus* including 3 *F. pyrropus*
- One *Protoxerus*
- Five *Paraxerus*
- Four *Heliosciurus*

+ One Sciuridae genome per genus on genbank

# Pre-processing of genomic data



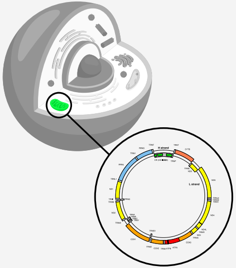


Multiple steps before genomic analyses

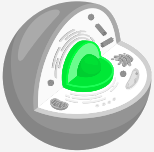
1. Estimate data quality with FastQC
2. Trim data with Trimmomatic
3. Estimate data quality with FastQC
4. Use of Kraken2 to see if contaminants

    → One *Funisciurus* from CAR removed

# Selection of genomic markers used for phylogeny



1. Mitochondrial dataset: Complete mitochondrial genomes
    - 16000 – 17000 bp
    - Two strategies to assemble the genome
        - ***De novo***: GetOrganelle
        - **With annotations**: MitoZ



with OrthoMaM

2. Nuclear dataset: Exons of genes present in only one copy
    - 1017 exons
        - Exons present in both squirrels and humans
        - GC content between 40 and 60 %
        - Exons > 1000 bp in *Marmota marmota*
    - Mapping with NextGenMap
    - *De novo* assembly using Spades

# Selection of genomic markers used for phylogeny

| Specimen | Number of exons |
|---|---|
| MAM20130014 | 503 |
| C07XAR49 | 63 |
| JCK11056 | 43 |
| 149422 | 29 |
| MLIPIA90 | 20 |
| M4356 | 16 |
| M6070 | 13 |
| R12021 | 9 |
| NC0402 | 8 |
| R24205 | 1 |

Exons present in both squirrels and humans

GC content between 40 and 60 %

Exons > 1000 bp in *Marmota marmota*

→ 1017 exons

Mapping of the 10 *F. anerythrus sensu lato* on the 1017 exons

→ 705 exons in at least 1 *F. anerythrus sensu lato*

→ Chimera reference

Only ones with no missing data in *F. anerythrus sensu lato*

→ 167 exons

# Exons repartition on chromosomes

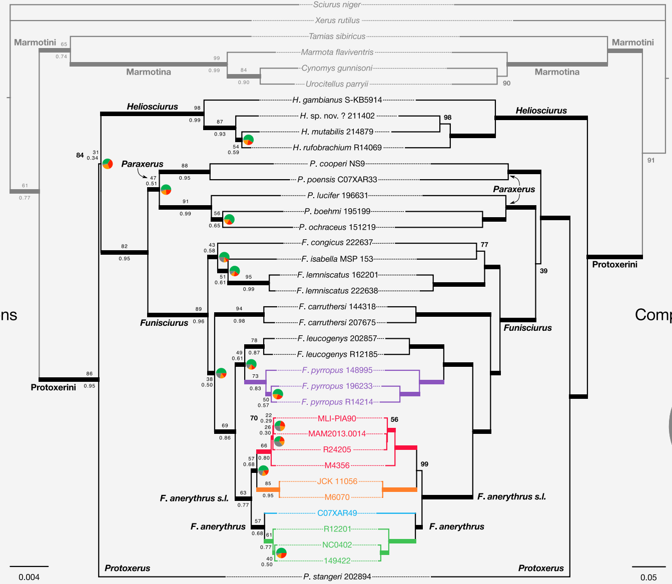
| Chromosome | 1017 exons | 705 exons | 167 exons |
|---:|:---:|:---:|:---:|
| 1 | 78 | 64 | 16 |
| 2 | 119 | 83 | 23 |
| 3 | 89 | 66 | 16 |
| 4 | 58 | 46 | 8 |
| 5 | 48 | 36 | 10 |
| 6 | 54 | 34 | 10 |
| 7 | 76 | 51 | 8 |
| 8 | 50 | 35 | 11 |
| 9 | 58 | 35 | 11 |
| 10 | 39 | 16 | 1 |
| 11 | 57 | 41 | 10 |
| 12 | 39 | 27 | 5 |
| 13 | 25 | 18 | 7 |
| 14 | 38 | 22 | 10 |
| 15 | 23 | 16 | 5 |
| 16 | 39 | 24 | 2 |
| 17 | 33 | 20 | 8 |
| 18 | 26 | 17 | 5 |
| 19 | 5 | 2 | 0 |
| X | 58 | 50 | 1 |
| Y | 0 | 0 | 0 |
| unplaced | 5 | 2 | 0 |

Based on *Sciurus carolinensis*' chromosomes

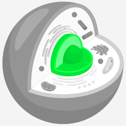
Obtained with BLAST using *Marmota marmota*'s sequences

→ Almost all chromosomes represented in the dataset

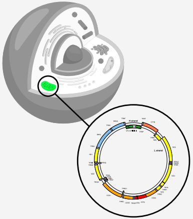
# Maximum likelihood trees with 1000 bootstraps in IQ-TREE2



Concatenation of 167 exons
255 958 bp



Complete mitochondrial genome
16 326 bp

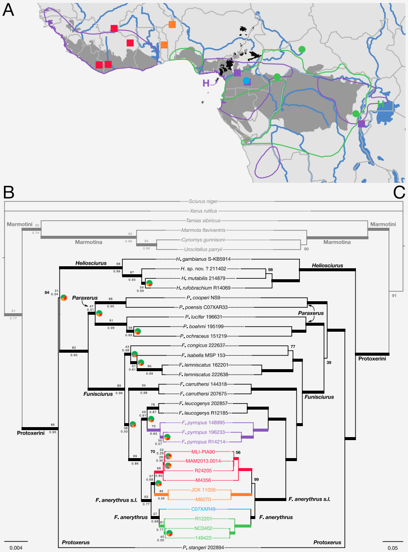


# Datations

| Clade | tMRCA | 95% HPDL | 95% HPDH |
|---|---:|---:|---:|
| *F. anerythrus* CAR + DRC | 0.32 | 0.28 | 0.36 |
| *F. anerythrus* | 1.17 | 1.08 | 1.23 |
| *F. anerythrus* Togo + Benin | 0.06 | 0.05 | 0.08 |
| *F. anerythrus* Liberia + Côte d'Ivoire + Mali | 0.42 | 0.36 | 0.46 |
| *F. anerythrus* West Africa | 1.96 | 1.87 | 2.24 |
| *F. anerythrus* | 2.46 | 2.37 | 2.70 |
| *F. pyrropus* | 1.05 | 0.99 | 1.14 |
| *F. leucogenys* | 0.97 | 0.90 | 1.05 |
| *F. pyrropus* + *F. leucogenys* | 2.97 | 2.83 | 3.21 |
| *F. pyrropus* + *F. leucogenys* + *F. anerythrus* | 3.93 | 3.79 | 4.25 |
| *F. carruthersi* | 1.13 | 1.05 | 1.24 |
| *F. pyrropus* + *F. leucogenys* + *F. anerythrus* + *F. carruthersi* | 5.92 | 5.75 | 6.57 |
| *F. lemniscatus* | 0.78 | 0.68 | 0.84 |
| *F. lemniscatus* + *F. isabella* | 4.70 | 4.58 | 5.10 |
| *F. lemniscatus* + *F. isabella* + *F. congicus* | 5.73 | 5.58 | 6.22 |
| *Funisciurus* | 6.58 | 6.40 | 7.33 |
| *P. cooperi* + *P. poensis* | 6.53 | 6.29 | 6.76 |
| *P. lucifer* + *P. boehmi* + *P. ochraceus* | 6.65 | 6.27 | 6.86 |
| *Paraxerus* | 10.98 | 10.74 | 11.18 |
| *Funisciurus* + *Paraxerus* | 11.92 | 11.73 | 12.15 |
| *Heliosciurus* | 5.25 | 5.04 | 5.56 |
| Protoxerini | 15.45 | 15.16 | 15.65 |
| Marmotina | 5.43 | 5.22 | 5.57 |
| Marmotini | 18.35 | 17.76 | 18.65 |
| Marmotini + Protoxerini | 21.13 | 20.74 | 21.46 |
| Xerinae | 23.56 | 22.96 | 23.96 |

| Clade | tMRCA | 95% HPDL | 95% HPDH |
|---|---:|---:|---:|
| Clade Ia I | 90.2 | 65.2 | 109.2 |
| Clade Ia II | 90.3 | 62.3 | 102.9 |
| Clade Ia III | 53 | 37.5 | 74.7 |
| Clade Ia IV | 83.6 | 59.6 | 96.8 |
| Clade Ia III and VI | 104.8 | 60.9 | 117 |
| Clade Ia II, III and VI | 111.7 | 68.8 | 121.9 |
| Clade Ia I, II, III and VI | 118.2 | 74 | 128.5 |
| Clade Ia I, II, III, IV and VI | 121.3 | 82.1 | 133.8 |
| Clade Ia | 134 | 90.3 | 152.5 |
| Clade Ib | 2.3 | 1 | 4 |
| Clade I | 186.6 | 124.4 | 220 |
| Clade IIa | 212.4 | 144 | 233.6 |
| Clade IIb | 266.2 | 176.6 | 300 |
| Clade II | 408.9 | 286.8 | 455 |
| MPXV | 1059.3 | 998.6 | 1169.4 |

# Article *in prep.*



Conclusions of the article:

1. *F. anerythrus* is present in both Central and West Africa
2. Mitochondrial and nuclear trees are similar
   → We can use only the mitogenome for further analyses
3. *F. anerythrus* has the same phylogeny as MPXV
   - Same dichotomy between West and Central Africa
   - Two clades in West Africa separated by the Volta river
   - Several clades in Central Africa
4. When using conventional susbstitution rates, divergence dates of MPXV and *F. anerythrus* do not match
   → More investigation needed on MPXV substitution rate

# Strategy to identify the reservoir(s) of Monkeypox virus

1. Ecological niche overlap analyses between MPXV and African mammals

   → Curaudeau, M. *et al.*, 2023. **Identifying the Most Probable Mammal Reservoir Hosts for Monkeypox Virus Based on Ecological Niche Comparisons.** *Viruses*, 15(3): 727

2. Comparisons of phylogeographic patterns between MPXV and *F. anerythrus*

3. Perspectives: Detection of MPXV in museum specimens of *F. anerythrus*

# *Museomics*: detection of MPXV in squirrel museum specimens

Monkeypox virus has a double-stranded DNA genome

- DNA molecules more stable than RNA molecules
- MPXV could be preserved in museum specimens

→ **Is there MPXV DNA in squirrel museum specimens?**

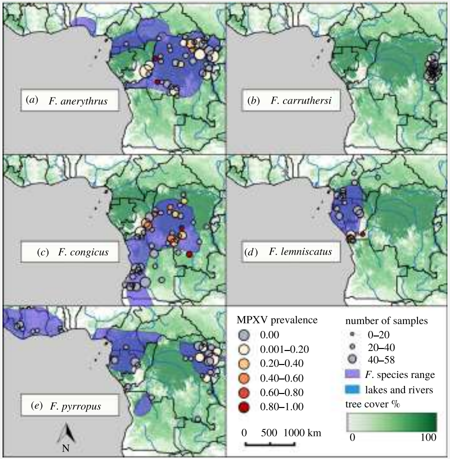
# *Museomics*: detection of MPXV in squirrel museum specimens from the RMCA and AMNH

Tiee *et al.* (2018) found MPXV DNA in *Funisciurus* specimens:

- Small fragments (< 123 bp)
- Specimens from the 19th and 20th century
- 9.0 % (93 of 1038) specimens
- Five species including 2 new species

However, very low variability in the fragment:

- No phylogenetic signal
- Only positive specimens from Central Africa



Tiee *et al.*, 2018

# *Museomics*: detection of MPXV in squirrel museum specimens from the MNHN and RMCA

To study if MPXV DNA fragments are preserved in skin of squirrel specimens

1. We extracted DNA from 303 specimens of the late 19th and 20th century
2. We amplified the cytb to check if samples have DNA: 131 sequences
   - → All *F. pyrropus* in West Africa are *F. anerythrus*
3. We want to amplify short (around 100 bp) variable fragments of MPXV
   - → Phylogenetic signal + cover the whole MPXV genome
   - → MPXV is a MOT → We could not do it before studying the squirrels

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If MPXV DNA in collection specimens

1. We will sequence the mitogenome of the squirrel
2. We will sequence the MPXV genome

→ **Molecular datations**: Better estimation of MPXV substitution rate

- → Correlation with squirrel divergence dates?

## Conclusion

# Both the niche analyses and the phylogenetic patterns suggest *Funisciurus anerythrus* is the main reservoir of MPXV

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# African tree squirrels: the Protoxerini tribe

31 species but only 5 CO1 sequences *a.k.a* the barcode of life