Infection, Genetics and Evolution 98 (2022) 105204

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Research paper

Detection and genetic diversity of Mopeia virus in *Mastomys natalensis* from different habitats in the Limpopo National Park, Mozambique

Lourenço Mapaco [a,b,c], Laurent Crespin [d,e], Dércio Rodrigues [a,1], Joelle Gouy de Bellocq [f], Josef Bryja [f], Mathieu Bourgarel [b], Dorothée Missé [g], Alexandre Caron [b,h], Jose Fafetine [h], Julien Cappelle [b,c,*,2], Florian Liégeois [g,i,**,2]

[a] Agrarian Research Institute of Mozambique, Directorate of Animal Sciences, IIAM, P. O. Box 1922, Maputo, Mozambique
[b] ASTRE, Univ Montpellier, CIRAD, INRA, Montpellier, France
[c] CIRAD, UMR ASTRE, Montpellier, France
[d] Université Clermont Auvergne, INRAE, VetAgro Sup, UMR EPIA, 63122 Saint-Genès-Champanelle, France
[e] Université Lyon, INRAE, VetAgro Sup, UMR EPIA, 69280 Marcy-L'Etoile, France
[f] Institute of Vertebrate Biology, Czech Academy of Sciences, Květná 8, 603 65 Brno, Czech Republic
[g] MIVEGEC, University of Montpellier, IRD, CNRS, 34394 Montpellier, France
[h] Veterinary Faculty, UEM, P. O. Box 257, Maputo, Mozambique
[i] Faculty of Veterinary Science, University of Zimbabwe, P.O. Box MP167, Harare, Zimbabwe

ARTICLE INFO

Keywords:
Mammarenavirus
Phylogeny
Mastomys natalensis
Mozambique

ABSTRACT

Mammarenaviruses have been a growing concern for public health in Africa since the 1970s when *Lassa virus* cases in humans were first described in west Africa. In southern Africa, a single outbreak of *Lujo virus* was reported to date in South Africa in 2008 with a case fatality rate of 80%. The natural reservoir of *Lassa virus* is *Mastomys natalensis* while for the *Lujo virus* the natural host has yet to be identified. *Mopeia virus* was described for the first time in *M. natalensis* in the central Mozambique in 1977 but few studies have been conducted in the region. In this study, rodents were trapped between March and November 2019in villages, croplands fields and mopane woodland forest. The aim was to assess the potential circulation and to evaluate the genetic diversity of mammarenaviruses in *M. natalensis* trapped in the Limpopo National Park and its buffer zone in Massingir district, Mozambique. A total of 534 *M. natalensis* were screened by RT-PCR and the overall proportion of positive individuals was 16.9%. No significant differences were detected between the sampled habitats ($\chi^2 = 0.018$; DF = 1; $p = 0.893$). The *Mopeia virus* (bootstrap value 91%) was the *Mammarenavirus* circulating in the study area sites, forming a specific sub-clade with eight different sub-clusters. We concluded that *Mopeia virus* circulates in all habitats investigated and it forms a different sub-clade to the one reported in central Mozambique in 1977.

1. Introduction

Mammarenaviruses are enveloped, ambisense or negative single-strand RNA microorganisms and their genome (≈10.5 kb) consists in two or three RNA segments named large (L), medium (M) and small (S). Currently, there are four genera in the *Arenaviridae* family (*Mammarenavirus, Antennavirus*, *Hartmanivirus* and *Reptarenavirus*). The genus *Mammarenavirus* infects mainly mammals including rodents (Radoshitzky et al., 2019). In Africa the viruses of the *Mammarenavirus* genus have been a growing concern for public health since the 1970s when the first cases of human disease associated with haemorrhagic fevers caused by the *Lassa virus* were described in west Africa (Buckley et al., 1970; Frame et al., 1970).Based on geographical occurrence, genetic and serological features, mammarenaviruses are divided into old world group (OWG) and new world group (NWG) (Bowen et al., 1997; Albariño et al., 1998). The OWG is restricted to the Eastern Hemisphere while the NWG occur in the Western Hemisphere. The *Lymphocytic choriomeningitis virus* an OWG *Mammarenavirus* has a worldwide

* Correspondence to: J. Cappelle, CIRAD, UMR ASTRE, TA A 117/E, Campus International de Baillarguet, 34398 Montpellier Cedex 5, France.
** Correspondence to: F. Liégeois, IRD-UMR 224 MIVEGEC, 911 Avenue Agropolis, 34394 Montpelier, France.
E-mail addresses: Julien.cappelle@cirad.fr (J. Cappelle), florian.liegeois@ird.fr (F. Liégeois).
[1] Dércio Rodrigues was the expert on rodent identification and he was murdered early 2020.
[2] J. Cappelle and F. Liegeois were equally involved in the study.

https://doi.org/10.1016/j.meegid.2022.105204
Received 20 September 2021; Received in revised form 15 December 2021; Accepted 2 January 2022
Available online 5 January 2022
1567-1348/© 2022 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

distribution (Gratz, 2006; Fornůsková et al., 2021). To date, 16 species of *Mammarenavirus* have been isolated from African mainland and all belong to the OWG (Burrell et al., 2017; Radoshitzky et al., 2019). Two of them, *Lassa virus* and *Lujo virus*, are associated with human lethal diseases. *Lassa virus* is responsible for 300,000 to 500,000 infection cases resulting in about 5000 deaths each year in west Africa (Ogbu et al., 2007) while a single outbreak of *Lujo virus* has been reported to date in South Africa in 2008 where four out of the five infected patients died (Paweska et al., 2009). The natural reservoir of *Lassa virus* is the rodent *M. natalensis* (Monath et al., 1974) while for the *Lujo virus* the natural host is yet to be identified (Simulundu et al., 2016). *M. natalensis* is largely distributed in sub-Saharan African (IUCN, 2016a), with a wide ecological range and can be found in savannas, grasslands, agricultural fields and houses (Coetzee, 1975; Monadjem et al., 2015). It hosts a number of non-pathogenic mammarenaviruses for humans such as *Mopeia virus, Luna virus*, *Lunk virus, Morogoro virus*, *Gairo virus* (Wulff et al., 1977; Taylor et al., 1981; Günther et al., 2009; Ishii et al., 2011; Gryseels et al., 2015; Cuypers et al., 2020).

The *Mopeia virus,* identified for the very first time in Mopeia district, Zambézia province (Fig. 1A), central Mozambique (Wulff et al., 1977), was the second African *Mammarenavirus* described in *M. natalensis* after the *Lassa virus.* No human cases of *Mopeia virus* diseases have been reported so far. However, this virus has been foreseen as an useful model to understand *Lassa virus* transmission ecology and immune responses (Borremans et al., 2011; Russier et al., 2012; Schaeffer et al., 2019). Grobbelaar et al. (2021), highlighted the importance of information on the occurrence and diversity of mammarenaviruses in Africa to understand possible risks to human health.A new *Mammarenavirus* non-pathogenic for humans (*Dhati Welel virus*) has been recently described in *M. natalensis* in eastern Africa (Gouy de Bellocq et al., 2020); which suggests that with further studies new mammarenaviruses could be identified in Africa and that a systematic mapping of *Mastomys* and *Mammarenavirus* deserves more attention. Indeed, apart from an early study from the 1970s (Wulff et al., 1977), there is a lack of data about *Mammarenavirus* circulation in Mozambique. Thus, this study was set out to investigate the diversity of mammarenaviruses circulating in *M. natalensis* trapped in villages, cropland fields and mopane woodland forest in the Limpopo National Park (LNP) and its buffer zone.

2. Materials and methods

2.1. Study sites

The study was carried out in the Massingir district counterpart of the LNP in Gaza province, south Mozambique (Fig. 1A). The rodents were trapped between March and November 2019 in three different habitats (villages, croplands fields and mopane woodland forest) from localities inside the LNP (Bingo, Macavene and Mavoze) and its buffer zone (Chibotane, Machavule and Madingane). (Fig. 1B). The Massingir district has 5893 km^2 of surface, 37,664 inhabitants, is dominated by dry semi-arid type zones with average annual temperatures of 30 °C and rainfall 600 mm. The study area falls within the *Great Limpopo Transfrontier Conservation Area* adjacent to the South African Kruger National Park (KNP) and the Zimbabwe's Gonarezhou National Park. The area supports subsistence farming dominated by rain-fed cropping in elevated land and with small irrigation schemes close to rivers and to Massingir dam. There are an extensive livestock production. Four localities are located inside the LNP with no physical barrier to separate wildlife and human. The wildlife mammal community includes large, medium and small sized terrestrial animals, such as elephants, buffaloes, lions, wild dogs, wild pigs and zebras.

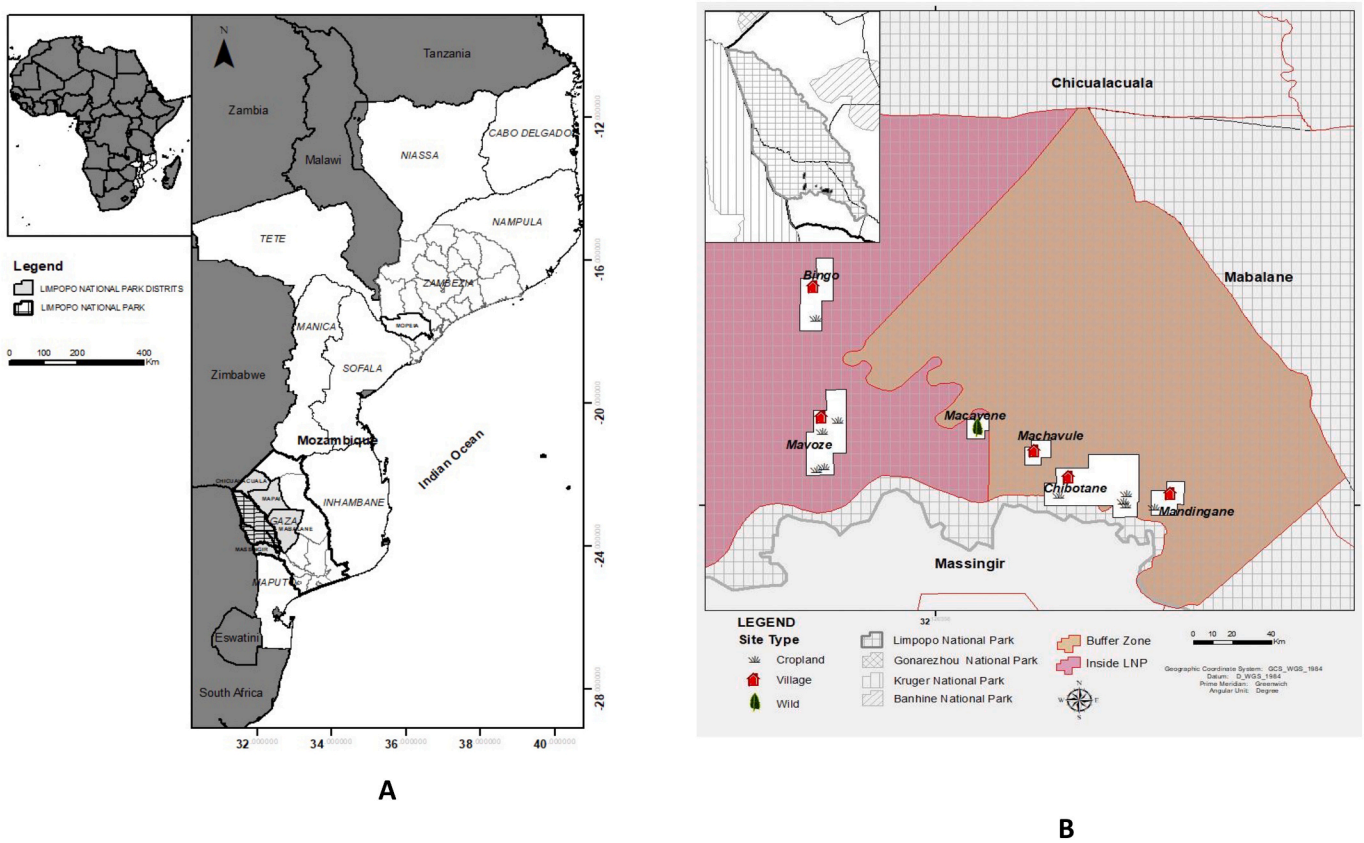

Fig. 1. Study area. A. Map of Africa in the upper left corner showing the location of Mozambique. On the right side the map of Mozambique indicating the location of the LNP and the districts covered by the park. The map of Mozambique also illustrates the location of Zambézia province where *Mopeia virus* was first described for the first time. B. The LNP map showing the sampled sites inside the park and in the buffer zone.

2.2. Rodent trapping and sampling

The following experimental protocol was approved by the Ethical Committee of VetAgro-Sup in Lyon, France (*Comité d'Ethique* de VetAgro Sup n°18, Avis 1905) and the credential for rodent capture in the LNP was obtained from the Mozambican National Administration for Conservation Areas (Credential Nr. 1/02/2021).

Two types of sites were included during this study: capture mark and recapture (CMR) sites and removal sites. In CMR sites trapped rodents were marked and released for potential recapture for rodent ecology studies (results not shown here). Only rodents found dead in the traps in the CMR sites were sampled for *Mammarenavirus* screening. By contrast in the removal sites, all *M. natalensis* rodents captured were sacrificed and the samples were screened for *Mammarenavirus* RNA detection. In villages, rodents were trapped inside houses and granaries. The captures in cropland fields included irrigated and rain-fed sites. The sites were selected based on the information collected from the local population about the presence of rodents and we also checked for signs of rodent's presence before setting the traps. During the sampling, crops (mainly bean and maize) were abundant in irrigated cropland fields while in the rain-fed cropland field, the sampling occurred after peanut harvesting.

In houses and granaries, the rodents were trapped using mesh traps baited with chorizo and Sherman (small and medium sizes) baited with toasted coconuts, peanut butter and oats. In cropland fields and mopane woodland only Sherman traps were set up. The number of traps deployed in each site was adjusted according to the house, granary and field size. In each house or granary, the number of traps varied between two and five while in cropland fields and mopane woodland the minimum number was 24 and the maximum 144 traps. Due to logistic and safety reasons (i.e., presence of dangerous wildlife) a single mopane woodland was sampled four times inside the LNP. The traps were armed at the end of the day and inspected the following morning. Traps were set for one to five consecutive nights, depending on the trapping success. Captured rodents were transported to the laboratory located in the LNP headquarters where the team was based during the trapping sessions. Rodents were euthanised with isoflurane, dissected and tissues (lung, heart, liver, spleen, kidney, cerebral, tail and ear samples) as well as blood (Dried Blood Spot, DBS) were collected. All tissue samples were conserved in home-made RNAlater solution (http://www.protocol-online.org/prot/Protocols/RNAlater-3999.html) and tail and ear for rodent species genetic identification were conserved in 70% ethanol (Herbreteau et al., 2011).

2.3. Rodent species genetic identification

Initially, morphological characteristics were used for rodent species in-field identification (Herbreteau et al., 2011). Because species in some African rodent genera are difficult to recognize and the taxonomy of many groups is still not well resolved (Monadjem et al., 2015), at least 10% of rodents morphologically identified as *M. natalensis* were selected for genetic identification at each capture site. We applied the DNA-barcoding approach to amplify and sequence (by the Sanger method) the complete mitochondrial cytochrome *b* gene (*CYTB*; 1140 bp) of selected individuals. We followed the protocols described by Bryja (2014) for DNA extraction, polymerase chain reaction (PCR) and sequencing. Species and intraspecific lineages were identified by a maximum likelihood (ML) analysis in RAxML 8.2.8 (Stamatakis, 2014), using GTRCAT substitution model recommended by the author. The robustness of the nodes was evaluated by the default bootstrap procedure with 1000 replications and new sequences were considered as part of previously defined taxa/lineages if the bootstrap support was >95%. For this study, we specifically compared the new sequences with those from the phylogenetic studies of the genus *Mastomys* (Colangelo et al., 2013; Martynov et al., 2020).

2.4. Mammarenavirus detection and phylogeny

Only *M. natalensis* individuals were considered for *Mammarenavirus* screening. RNA was extracted from lung tissues using Qiagen RNeasy Mini kit (Hilden, Germany). Briefly, 30 mg of lung tissue samples were disrupted for RNA release by adding 20 μL of proteinase K, one 2 mm inox bead and 500 μL of lysis buffer in a 2 mL tube and vortexed using a Qiagen TissueLyser LT system for 5 min at 50 Hz. The homogeneous lysate in the 2 mL tube from the above step was centrifuged at 11,000 ×*g* for 3 min and then the supernatant was collected and used for viral RNA extraction following the manufacturer's instructions.

Two pairs of primers P1-LSF/R and P2-LSF/R (Li et al., 2015) targeted to conserved regions of the L segment of mammarenaviruses were used to amplify a 611 bp fragment. The total volume of the reaction mix for the first-strand cDNA synthesis was 20 μL containing 5 μL of template RNA, 20 pmol of *Mammarenavirus* genus-specific primer (P1-LSF), 0.5 mM of each dNTP, 4 μL of 5× RT Buffer, 20 units of RiboLock RNase Inhibitor and 100 units of Maxima H Minus Reverse Transcriptase. The thermo profile conditions were those described in the manufacture protocol (Maxima H minus Reverse Transcriptase, Thermo Scientific). The pair P1-LSF/R was used on the first amplification and the P2-LSF/R on the second PCR. The final volume of PCR mix for the two reactions was 50 μL and the volume or concentration of mix components were: 5 μL of cDNA, 1.25 units of Dream Taq Hot Start DNA Polymerase (Thermo Scientific, city and country), 1× Dream Taq Buffer (Thermo Scientific) with 2 mM $MgCl_2$, 0.2 mM of each dNTP (Thermo Scientific) and 3 pmol of each primer. The same thermal profile was used for each primer pair: initial denaturation at 95 °C for 3 min, followed by 40 cycles of denaturation at 95 °C for 30 s, annealing at 50 °C for 30 s and extension at 72 °C for 1 min. The final extension was at 72 °C during 5 min. The expected RT-PCR products were visualized on 1% agarose gel.

Positive RT-PCR amplicons including the positive control were purified using a Wizard SV Gel and PCR Clean-up system (Promega, Wisconsin, USA). Only purified amplicons with sufficient concentration were sent to LGC Genomics (Berlin, Germany) using the primer pair P2-LSF/R.

The final ML analysis of *Mammarenavirus* included 67 sequences of which 50 generated in this study, one *Mopeia virus* used in this study as positive control, 15 *Mammarenavirus* partial L gene fragment of representatives of the OWG and one representative of the NWG as outgroup from GenBank. *Mammarenavirus* nucleic acid sequences were aligned using MEGA 7 (Kumar et al., 2016), with minor manual adjustments. Sites that could not be unambiguously aligned were excluded and divergent regions were excluded from subsequent analysis. Thus, the final size of our alignment used for the phylogenetic analysis was of 468 bp. Phylogenies were inferred using ML method implemented in PhyML (Guindon et al., 2010). The reliability of branching orders was tested using the bootstrap approach (1000 replicates). The suited evolution model (GTR + I + G) was selected by Akaike's Information criterion (AIC) using Topali software (Milne et al., 2009). *Mopeia virus* nucleic acid sequences generated in this study were deposited in GenBank under accession numbers MZ512094 to MZ512143.

2.5. Statistical analysis

The proportion of *Mopeia virus* RNA positive individuals were calculated by habitat and locality with 95% confidence intervals (CI) using Wilson score test (Agresti and Coull, 1998). The χ^2 test of independence was performed to evaluate differences in proportions of positive individuals among the habitat types as well as among the localities.

3. Results

3.1. Rodent trapping and sampling

During a total of 7290 trap nights; 1235 rodents were captured and

82.5% ($n = 1019$) were morphologically identified as *M. natalensis*. In CMR sites we caught 541 *M. natalensis* individuals out of which 56 were found died in the traps and in removal sites we trapped 478 individuals. A total of 534 lung tissue samples were obtained from all *M. natalensis* trapped and sacrificed in removal sites and from all individuals found died in the traps deployed in CMR sites h. *M. natalensis* were captured in all the three types of habitats and across all the localities trapped. The highest number of *M. natalensis* was trapped from cropland fields and the lowest number of individuals was caught in the mopane woodland (Table 1 and Table S1).

3.2. Rodent species genetic identification

Mitochondrial CYTB genotyping of selected 101 out of 534 specimens (Table S2) confirmed all of them to be *M. natalensis* and all belonging to phylogroup B-VI (Colangelo et al., 2013).

3.3. Mammarenavirus molecular detection

The overall proportion of positive individuals for *Mammarenavirus* RNA detection was 16.9% [95% CI: 13.9–20.3]. Neither croplands nor villages were sampled in Macavene locality because the entire population that lived there was resettled outside the park. *Mammarenavirus* RNA was detected in 16.9% [95% IC: 13.8–20.5] and 16.3% [95% IC: 8.1–30.0] of specimens, respectively from cropland fields and villages. A single mopane woodland forest was sampled in the Macavene locality where 16.7% [95% CI: 3.0–56.4] of individuals tested positive. Nevertheless, the proportions of *Mopeia virus* positive rodents did not differ significantly between the habitats ($\chi^2 = 0.018$; DF = 1; $p = 0.893$). Across the localities, Machavule (only two individuals were caught) had the highest proportion 100% [$n = 2$; 95% CI: 34.2–100] of positive individuals followed by Bingo with 22.7% [95% CI: 15.2–32.5] and the lowest proportion 2.9% [95% CI: 0.8–9.8] was found in Madingane locality (Table 1).

Concerning the differences among habitats (or localities), five samples that had doubtful RT-PCR results were excluded from the chi-square test. Besides the mopane woodland forest habitat and two localities (Macavene and Machavule) were excluded from the analysis because of low expected values (McHugh, 2013). The proportion of positive individuals differed significantly among the localities ($\chi^2 = 12.512$; DF = 3; $p = 0.0058$).

3.4. Phylogenetic analysis

A total of 50 out of 90 purified amplicons had sufficient concentration for sequencing, thus the phylogenetic analysis was performed for all the positive samples from villages ($n = 7$) and mopane woodland forest ($n = 1$) and for 51% (42/82) of the positive samples from the cropland fields.

All our *Mammarenavirus* sequences clustered together with *Mopeia virus* (bootstrap value 91%) (Fig. 2). However, all the *Mopeia virus* identified in this study formed a specific *Mopeia virus* sub-clade with eight different sub-clusters. The sub-clusters I, III, IV, VI, VII and VIII were well sustained with bootstrap value >70%, whereas for sub-clusters II and IV the tree topology seemed unresolved with bootstrap values of 48% and 56%, respectively. The nodes of divergence between the sub-clusters I and II; and the sub-clusters IV, V, VI, VII were also not well supported with bootstrap value <70% (Fig. 2).

4. Discussion

In the present study, we screened *M. natalensis* by RT-PCR and we detected *Mopeia virus* in all habitats and localities across the study area at the interface between LNP and communal land in its buffer zone. We reported the presence of *Mopeia virus* in 16.9% (90/534) of tested *M. natalensis* with no significant differences between habitats. Phylogenetic analysis indicated that the viruses detected formed a specific *Mopeia virus* sub-clade with eight sub-clusters.

Several villages and cropland sites were surveyed inside the LNP and its buffer zone while a single mopane woodland was sampled four times inside the LNP. *M. natalensis* has a very similar external morphology with *Mastomys coucha* which is largely endemic in southern Africa (IUCN, 2016b). Sympatric occurrence of *M. natalensis* and *M. coucha* was reported in some regions of South Africa including the KNP (Kneidinger et al., 2014) which is adjacent to the LNP in our study area. The mitochondrial phylogenetic analysis split *M. natalensis* into six matrilineage phylogroups: A-I, A-II, A-III, B-IV, B—V, B-VI (Colangelo et al., 2013) and all were identified as natural reservoirs of mammarenaviruses (Gouy de Bellocq et al., 2020). The CYTB mitochondrial gene sequence phylogenetic analysis indicated that all captured *Mastomys* in our study area were *M. natalensis* belonging to the B-VI lineage. Previous studies reported that two lineages of *M. natalensis* (B—V and B-VI) occur in parapatry in Mozambique with the former being geographically restricted to the northernmost part of the country (Colangelo et al., 2013; Gouy de Bellocq et al., 2020).

Mammarenavirus detection in Mozambique dates back to 1977 when the *Mopeia virus* was first identified in *M. natalensis* population from Mopeia district in central Mozambique (Wulff et al., 1977). Studies have been carried out in southern Africa and indeed detected non-pathogenic *Mammarenavirus* such as *Mopeia virus, Morogoro virus, Gairo virus and Luna virus* in *M. natalensis* (Wulff et al., 1977; Günther et al., 2009; Ishii et al., 2011; Gryseels et al., 2015). Here, we detected the *Mopeia virus* in 16.9% (90/534) of tested *M. natalensis* samples by the viral RNA molecular detection system (RT-PCR). The proportion of positives is below the ones detected by previous serological studies: 21% in Mozambique (Wulff et al., 1977), 20% in Zimbabwe (Johnson et al., 1981) and the highest of 31.8% from retrospective samples collected South Africa and Zimbabwe (Grobbelaar et al., 2021). In Tanzania Borremans et al.

Table 1
Number of *M. natalensis* tested (n) and number of positives samples in different habitats and localities of the LNP and its buffer zone.

Locality	Habitat						Total	
	Cropland fields		Villages		Mopane woodland			
	Nr. of positives (n)	Proportion [CI, 95%]	Nr. of positives (n)	Proportion [CI, 95%]	Nr. of positives (n)	Proportion [CI, 95%]	Nr. of positives (n)	Proportion [CI, 95%]
Bingo	17 (78)	21.8 [14.1–32.2]	3 (10)	30.0 [10.8–60.3]	/	/	20 (88)	22.7 [15.2–32.5]
Chibotane	28 (144)	19.4 [13.8–26.7]	0 (19)	0.0 [0.0–16.8]	/	/	28 (163)	17.2 [12.2–23.7]
Macavene	/	/		/	1 (6)	16.7 [3.0–56.4]	1 (6)	16.7 [3.0–56.4]
Machavule	/	/	2 (2)	100.0 [34.2–100.0]		/	2 (2)	100.0 [34.2–100.0]
Madingane	1 (62)	1.6 [0.3–8.6]	1 (8)	12.5 [2.2–47.1]		/	2 (70)	2.9 [0.8–9.8]
Mavoze	36 (201)	17.9 [13.2–23.8]	1 (4)	25.0 [4.6–69.9]		/	37 (205)	18.0 [13.4–23.9]
Grand Total	82 (485)	16.9 [13.8–20.5]	7 (43)	16.3 [8.1–30.0]	1 (6)	16.7 [3.0–56.4]	90 (534)	16.9 [13.9–20.3]

Five individuals had dubious RT-PCR results in Bingo ($n = 1$), Chibotane ($n = 2$) and Mavoze ($n = 2$).

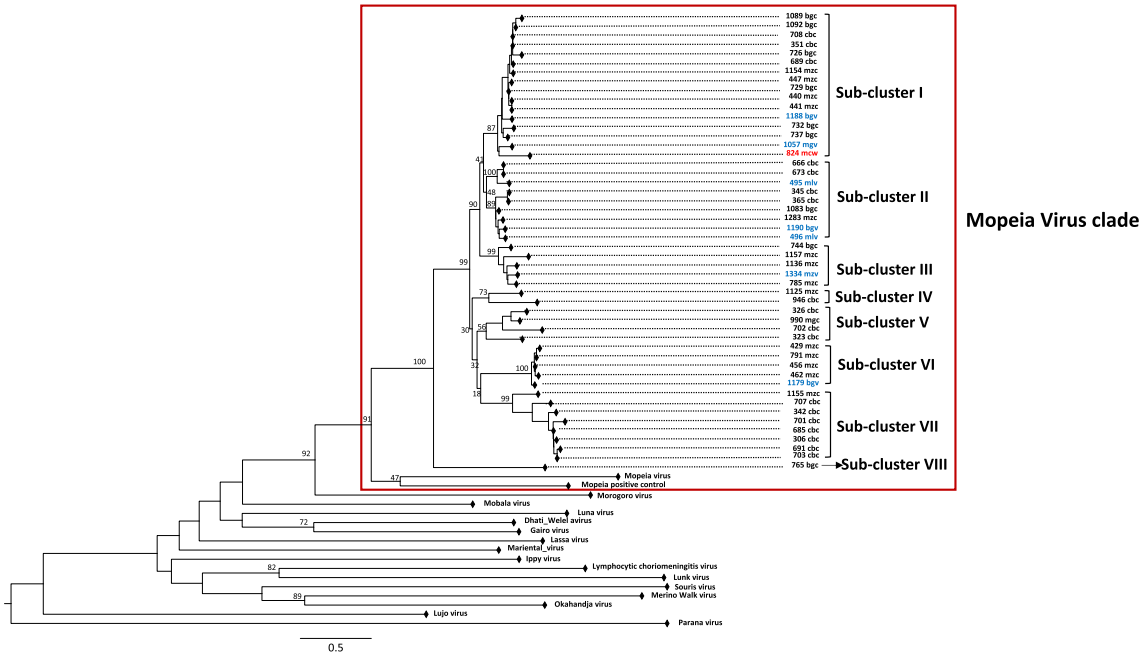

Fig. 2. Maximum Likelihood analysis, implemented in PhyML, of a partial nucleotide sequence of *Mammarenavirus* L gene fragment from the samples, our positive control genotyped, OWG *Mammarenavirus* reference sequences previously identified in Africa and available in the GenBank (Table S4). One *Mammar*enavirus sequence of the NWG (Table S4) were used as outgroup. The asterisks at nodes represent the bootstrap values ≥70% calculated from 1000 bootstrap replicates. Scale bars indicate the number of base substitutions per site. The sample reference number is followed by three letters indicating the collection site as following: bgc = Bingo cropland; bgv = Bingo village; cbc = Chibotane cropland; mcw = Macavene mopan woodland; mzc = Mavoze cropland; mzv = Mavoze village; mlv = Machavule village; mgc = Madingane cropland and mgv = Madingane village. Village. The colors differentiate the types of habitats: black = cropland fields, blue = villages and red = mopane woodland. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(2011) found 12.1% [95% CI: 9.5–15.3] by serological testing and 8.4% [95% CI: 6.2–11.2] by the viral RNA molecular detection system. Due to the difference in the diagnostic tests used in these four studies, the comparison of prevalence is difficult. Comparing serological and molecular studies would require more studies in which both techniques are implemented on the same individuals (i.e., the presence of viral antibodies is supposed to be much long-lasting than viral RNA in individuals). At the molecular level (RT-PCR testing), the estimate of prevalence in our study was about twice the one reported in Tanzania, indicating a higher proportion of positive cases at the time of sampling in our study area. However, as these studies were carried out in different contexts, factors such as age of tested animals, habitat types and season may contribute to the difference in the proportion of positive individuals observed among them. Indeed, variation of *Mammarenavirus* prevalence estimators have been reported between geographic regions in Tanzania (Cuypers et al., 2020).

We sampled three different habitats (only two individuals were caught in the mopane woodland forest) and no significant differences were observed on the proportion of individuals positive for *Mopeia virus* RNA across the habitats. This observation suggests that the level of *Mopeia virus* circulation is the same among *M. natalensis* populations living in these three habitats. The possible explanation for this homogeneity could be that the studied habitats offer similar environmental conditions for viral viability and transmission. Borremans et al. (2011), also reported a great homogeneity in *Mopeia virus* prevalence across different habitat types in Tanzania and suggested that the abiotic environment may not be an important determinant of virus prevalence.

The proportion of positive individuals differed significantly among the localities; however, the 100% [95% CI: 34.2–100.0] found in Machavule is less informative because of the very small number of rodents ($N = 2$) captured in this locality. Thus, the 22.7% [95% CI: 15.2–32.5] detected in Bingo is referenced as the highest proportion of positive rodents across the localities in our study area. On the other hand, our statistical inference within the localities was influenced by the lowest proportion of *Mopeia virus* positive rodents detected in Madingane 2.9% [95% CI: 0.8–9.8]. The differences observed within localities suggests that in some localities humans are relatively more exposed to potentially zoonotic Mammarenaviruses. Nonetheless, the localities were sampled in different periods and this could possibly explain the differences of proportion of positive samples detected in Madingane locality. Rodent-host population dynamics is an important factor of the epidemiology of mammarenaviruses, whereby seasonal peaks of virus circulation may be associated with increased rodent populations and could explain prevalence differences between different months (Jay et al., 2005; Akhmetzhanov et al., 2019). The level of Mammarenavirus circulation in rodent population depend on the interaction between the virus, rodent-hosts and ecological variables (Childs and Peters, 1993). Therefore, a study incorporating an analysis of epidemiological and ecological parameters in our study area is needed to understand the homogeneity of virus circulation in habitats, the heterogenicity of prevalence in the localities and possibly during the different sampling months.

The mammarenaviruses detected in three different habitats (villages, croplands and mopane woodland forest) clustered with *Mopeia virus,* the unique *Mammarenavirus* species described so far in Mozambique (Wulff et al., 1977). The mammarenaviruses from the study area formed a specific sub-clade that did not include the reference *Mopeia virus* isolate from Mozambique (GenBank accessory Nr. AY772169.1) as well as the *Mopeia virus* positive control isolate ("Mopeia virus, strain UVE/MOPV/UNK/MZ/Mozambique 20410 | EVAg"). The mammarenaviruses detected in the study area formed eight sub-clusters and the sub-cluster I is the one most widespread with viruses from the three habitats and from five out of six localities included in the study. However, the sub-clusters II and V were not well sustained. Likewise, the bootstrap values at the roots of sub-clusters I and II as well as for sub-clusters IV to VII were low suggesting an unresolved tree topology. Consequently, it is probable that

the viruses could change the place between the sub-clusters I and II as well as among the sub-clusters IV to VII. This is likely due to the small size (468 bp) of the fragment analyzed. Therefore, we think that the analysis of the whole L segment would give better insight of the genetic diversity of the mammareviruses in our study area. Besides, the basal position of sample 765 from Bingo cropland field may suggest that in fact there is some degree of genetic variability of *Mopeia virus* in the study area. The detection of these probable different sub-clusters in the different habitats may indicate wide circulation of viruses of different sub-clusters within the study area. Evidence of *Mammarenavirus* circulation in other species than *M. natalensis* had been reported. *Mopeia virus* antibodies were detected in *Aethomys chrysophilus* in Zimbabwe (Johnson et al., 1981), *Mus minutoides* carries *Lunk virus* in Zambia and *Kodoko virus* in West Africa (Lecompte et al., 2007; Ishii et al., 2012). Recently, two novel mammarenaviruses were detected in Angola: the first one, *Bitu virus*, related to *Okahandja virus,* was found in *Micaelamys namaquensis*; the second one, *Kwanza virus,* related to *Mobala virus, was* detected in *Mus triton* (Těšíková et al., 2021). Results obtained in samples from South Africa and Zimbabwe indicated presence of mammarenavirues in 14 rodent species that included *Aethomys. chrysophilus*, *Mastomys. coucha*, *Rattus norvegicus*, *Rattus rattus*, *Saccostomus campestris* and *Gerbilliscus leucogaster* (Grobbelaar et al., 2021). The assessment of the potential circulation of mammarenaviruses in other rodent species from our study area is therefore important.

Mammarenaviruses transmission from rodent to humans occur through contaminated rodent excreta and secretions and crop harvesting or hunting activities, poor hygiene in households and rodent consumption increase the risk of transmission (Paweska, 2014; Akhmetzhanov et al., 2019) The human population in our study area practices agriculture, hunting (although prohibited by law), some consume rodents and like in other parts of the country the environment sanitation is deficient. Here we reported that *Mopeia virus* circulates in *M. natalensis* rodents, thus we hypothesize that humans in the study area may be exposed to mammareviruses. Unpublished data cited by Grobbelaar et al. (2021), indicated that antibodies to *Mopeia virus* were detected in humans' sera but with no association with clinical disease resembling mammarenaviruses. Serological and molecular investigation of *Mopeia virus* in humans will inform about viral exposure and the viral genetic profile in the study area.

5. Conclusions

Mopeia virus was detected in *M. natalensis* trapped in villages, cropland fields and mopane woodland from all the six surveyed localities. The *Mopeia virus* we detected formed a specific *Mopeia virus* sub-clade than the previously described in Mozambique. Habitat type does not have influence on the proportion of positive individuals in the study area. The genus *Mammarenavirus* contain zoonotic species and possibly others potentially zoonotic could emerge, thus, our results suggest that humans could be exposed to mammarenaviruses in all habitats, so awareness campaigns could be designed to sensitize local citizens about the potential risks of zoonotic disease spill over from rodents as well as further risk-based studies to explore which group of stakeholders are more exposed than others. We have expanded and updated the data on *Mopeia virus* in Mozambique and made more sequences available for this virus.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2022.105204.

Data availability

The authors declare that all the data analyzed here will be available without any restrictions.

Funding

This work was publicly funded through ANR (the French National Research Agency) under the "*Investissements d'avenir*" program with the reference ANR-16-IDEX-0006.

Competing interests

The authors have no competing interests to declare.

Acknowledgments

The authors gratefully acknowledge and thank the International Atomic Energy Agency for making available the sequencing services used for genotyping the virus. Extensive thanks to Albert Nicolau, Amélia Zandamela, Lucas Mandlate, Remigio Mungoi and Emuna Frechaut for valuable assistance during the field and laboratory works. The authors also acknowledge Dr. Laurent Granjon and Dr. Philippe Gauthier for the kind help with rodent molecular identification.

References

Agresti, A., Coull, B.A., 1998. Approximate is better than "exact" for interval estimation of binomial proportions. Am. Stat. 52, 119–126. https://doi.org/10.2307/2685469.

Akhmetzhanov, A.R., Asai, Y., Nishiura, H., 2019. Quantifying the seasonal drivers of transmission for Lassa fever in Nigeria. Philos. Trans. R. Soc. B Biol. Sci. 374, 20180268. https://doi.org/10.1098/rstb.2018.0268.

Albariño, C.G., Posik, D.M., Ghiringhelli, P.D., Lozano, M.E., Romanowski, V., 1998. Arenavirus phylogeny: a new insight. Virus Genes 16, 39–46.

Borremans, B., Leirs, H., Gryseels, S., Günther, S., Makundi, R., de Bellocq, J.G., 2011. Presence of Mopeia virus, an African arenavirus, related to biotope and individual rodent host characteristics: implications for virus transmission. Vector-Borne Zoonotic Dis. 11, 1125–1131. https://doi.org/10.1089/vbz.2010.0010.

Bowen, M.D., Peters, C.J., Nichol, S.T., 1997. Phylogenetic analysis of the Arenaviridae: patterns of virus evolution and evidence for cospeciation between arenaviruses and their rodent hosts. Mol. Phylogenet. Evol. 8, 301–316. https://doi.org/10.1006/mpev.1997.0436.

Bryja, J., et al., 2014. Pan-African phylogeny of Mus (subgenus Nannomys) reveals one of the most successful mammal radiations in Africa. BMC Evol. Biol. 14 (1), 256. https://doi.org/10.1186/s12862-014-0256-2.

Buckley, S.M., Casals, J., Downs, W.G., 1970. Isolation and antigenic characterization of Lassa virus. Nature 227, 174. https://doi.org/10.1038/227174a0.

Burrell, C.J., Howard, C.R., Murphy, F.A., 2017. Arenaviruses. In: Fenner and White's Medical Virology. Elsevier, pp. 425–436. https://doi.org/10.1016/B978-0-12-375156-0.00030-8.

Childs, J.E., Peters, C.J., 1993. Ecology and epidemiology of arenaviruses and their hosts. In: Salvato, M.S. (Ed.), The Arenaviridae, The Viruses. Springer US, Boston, MA, pp. 331–384. https://doi.org/10.1007/978-1-4615-3028-2_19.

Coetzee, C.G., 1975. The Biology, Behaviour, and Ecology of *Mastomys natalensis* in Southern Africa, Vol. 8.

Colangelo, P., Verheyen, E., Leirs, H., Tatard, C., Denys, C., Dobigny, G., Duplantier, J.-M., Brouat, C., Granjon, L., Lecompte, E., 2013. A mitochondrial phylogeographic scenario for the most widespread African rodent, *Mastomys natalensis*: Phylogeography of *Mastomys natalensis*. Biol. J. Linn. Soc. 108, 901–916. https://doi.org/10.1111/bij.12013.

Cuypers, L.N., Baird, S.J.E., Hánová, A., Locus, T., Katakweba, A.S., Gryseels, S., Bryja, J., Leirs, H., Goüy de Bellocq, J., 2020. Three arenaviruses in three subspecific natal multimammate mouse taxa in Tanzania: same host specificity, but different spatial genetic structure? Virus Evol. 6, veaa039. https://doi.org/10.1093/ve/veaa039.

Fornůsková, A., Hiadlovská, Z., Macholán, M., Piálek, J., de Bellocq, J.G., 2021. New perspective on the geographic distribution and evolution of lymphocytic Choriomeningitis virus, Central Europe. Emerg. Infect. Dis. 27, 2638–2647. https://doi.org/10.3201/eid2710.210224.

Frame, J.D., Baldwin, J.M., Gocke, D.J., Troup, J.M., 1970. Lassa fever, a new virus disease of man from West Africa. I. Clinical description and pathological findings. Am. J. Trop. Med. Hyg. 19, 670–676. https://doi.org/10.4269/ajtmh.1970.19.670.

Gouy de Bellocq, J., Bryjová, A., Martynov, A., Lavrenchenko, L., 2020. Dhati Welel virus, the missing mammarenavirus of the widespread Mastomys natalensis. J. Vertebr. Biol. 69, 20018. https://doi.org/10.25225/jvb.20018.

Gratz, N., 2006. Vector- and Rodent-Borne Diseases in Europe and North America: Distribution, Public Health Burden, and Control. Cambridge University Press.

Grobbelaar, A.A., Jardine, J., Burt, F.J., Shepherd, A.J., Shepherd, S.P., Leman, P.A., Kemp, A., Braack, L.E.O., Weyer, J., Paweska, J.T., Swanepoel, R., 2021. Mammarenaviruses of Rodents, South Africa and Zimbabwe. Emerg. Infect. Dis. 27, 3092–3102. https://doi.org/10.3201/eid2712.211088.

Gryseels, S., Rieger, T., Oestereich, L., Cuypers, B., Borremans, B., Makundi, R., Leirs, H., Günther, S., Goüy de Bellocq, J., 2015. Gairo virus, a novel arenavirus of the widespread Mastomys natalensis: genetically divergent, but ecologically similar to

Lassa and Morogoro viruses. Virology 476, 249–256. https://doi.org/10.1016/j.virol.2014.12.011.

Guindon, S., Dufayard, J.F., Lefort, V., Anisimova, M., Hordijk, W., Gascuel, O., 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst. Biol. 59, 307–321. https://doi.org/10.1093/sysbio/syq010.

Günther, S., Hoofd, G., Charrel, R., Röser, C., Becker-Ziaja, B., Lloyd, G., Sabuni, C., Verhagen, R., van der Groen, G., Kennis, J., Katakweba, A., Makundi, R., Leirs, H., 2009. Mopeia virus–related arenavirus in Natal Multimammate mice, Morogoro, Tanzania. Emerg. Infect. Dis. 15, 2008–2010. https://doi.org/10.3201/eid1512.090864.

Herbreteau, V., Jittapalapong, S., Rerkamnuaychoke, W., Chaval, Y., Cosson, J.-F., Morand, S., 2011. Protocols for Field and Laboratory Rodent Studies. Kasetsart University.

Ishii, A., Thomas, Y., Moonga, L., Nakamura, I., Ohnuma, A., Hang'ombe, B., Takada, A., Mweene, A., Sawa, H., 2011. Novel arenavirus, Zambia. Emerg. Infect. Dis. 17, 1921–1924. https://doi.org/10.3201/eid1710.10452.

Ishii, A., Thomas, Y., Moonga, L., Nakamura, I., Ohnuma, A., Hang'ombe, B.M., Takada, A., Mweene, A.S., Sawa, H., 2012. Molecular surveillance and phylogenetic analysis of Old World arenaviruses in Zambia. J. Gen. Virol. 93, 2247–2251. https://doi.org/10.1099/vir.0.044099-0.

IUCN, 2016a. *Mastomys natalensis*: Granjon, L.: The IUCN Red List of Threatened Species 2016. e.T12868A115107375. https://doi.org/10.2305/IUCN.UK.2016-3.RLTS.T12868A22425266.en.

IUCN, 2016b. *Mastomys coucha*: Cassola, F.: The IUCN Red List of Threatened Species 2016. e.T12865A22425161. https://doi.org/10.2305/IUCN.UK.2016-2.RLTS.T12865A22425161.en.

Jay, M.T., Glaser, C., Fulhorst, C.F., 2005. The arenaviruses. J. Am. Vet. Med. Assoc. 227, 904–915. https://doi.org/10.2460/javma.2005.227.904.

Johnson, K.M., Taylor, P., Elliott, L.H., Tomori, O., 1981. Recovery of a Lassa-related arenavirus in Zimbabwe. Am. J. Trop. Med. Hyg. 30, 1291–1293. https://doi.org/10.4269/ajtmh.1981.30.1291.

Kneidinger, C.M., van Heerden, H., MacFadyen, D., van der Merwe, M., Avenant, N.L., van der Ban, H., 2014. Species identification, habitat preferences and population genetics of Mastomys natalensis (A. Smith, 1834) and M. coucha (A. Smith, 1836) in an enclosed area. In: , , 3rd20. Kruger National Park, South Africa, Navors. nas. Mus., Bloemfontein, pp. 31–45.

Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33, 1870–1874. https://doi.org/10.1093/molbev/msw054.

Lecompte, E., ter Meulen, J., Emonet, S., Daffis, S., Charrel, R.N., 2007. Genetic identification of Kodoko virus, a novel arenavirus of the African pigmy mouse (Mus Nannomys minutoides) in West Africa. Virology 364, 178–183. https://doi.org/10.1016/j.virol.2007.02.008.

Li, K., Lin, X.-D., Wang, W., Shi, M., Guo, W.-P., Zhang, X.-H., Xing, J.-G., He, J.-R., Wang, K., Li, M.-H., Cao, J.-H., Jiang, M.-L., Holmes, E.C., Zhang, Y.-Z., 2015. Isolation and characterization of a novel arenavirus harbored by Rodents and Shrews in Zhejiang province, China. Virology 476, 37–42. https://doi.org/10.1016/j.virol.2014.11.026.

Martynov, A.A., Bryja, J., Meheretu, Y., Lavrenchenko, L.A., 2020. Multimammate mice of the genus Mastomys (Rodentia: Muridae) in Ethiopia – diversity and distribution assessed by genetic approaches and environmental niche modelling. J. Vertebr. Biol. 69, 20006.1. https://doi.org/10.25225/jvb.20006.

McHugh, M.L., 2013. The Chi-square test of independence. Biochem. Medica 23, 143–149. https://doi.org/10.11613/BM.2013.018.

Milne, I., Lindner, D., Bayer, M., Husmeier, D., Mcguire, G., Marshall, D.F., Wright, F., 2009. TOPALi v2: a rich graphical interface for evolutionary analyses of multiple alignments on HPC clusters and multi-core desktops. Bioinformatics 25, 126–127. https://doi.org/10.1093/bioinformatics/btn575.

Monadjem, A., Taylor, P., Denys, C., Cotterill, F., 2015. Rodents of Sub-Saharan Africa: A Biogeographic and Taxonomic Synthesis. https://doi.org/10.1515/9783110301915.

Monath, T.P., Newhouse, V.F., Kemp, G.E., Setzer, H.W., Cacciapuoti, A., 1974. Lassa Virus Isolation from Mastomys natalensis Rodents during an Epidemic in Sierra Leone. Science 185, 263–265. https://doi.org/10.1126/science.185.4147.263.

Mopeia virus, strain UVE/MOPV/UNK/MZ/Mozambique 20410 | EVAg [WWW Document], n.d. URL https://www.european-virus-archive.com/virus/mopeia-virus-strain-uvemopvunkmzmozambique-20410 (accessed 6.15.21).

Ogbu, O., Ajuluchukwu, E., Uneke, C.J., 2007. Lassa fever in West African sub-region: an overview. J. Vector Borne Dis. 44, 1–11.

Paweska, J.T., 2014. Chapter 7 - Lujo virus hemorrhagic fever. In: Ergönül, Ö., Can, F., Madoff, L., Akova, M. (Eds.), Emerging Infectious Diseases. Academic Press, Amsterdam, pp. 95–110. https://doi.org/10.1016/B978-0-12-416975-3.00007-8.

Paweska, J.T., Sewlall, N.H., Ksiazek, T.G., Blumberg, L.H., Hale, M.J., Lipkin, W.I., Weyer, J., Nichol, S.T., Rollin, P.E., McMullan, L.K., Paddock, C.D., Briese, T., Mnyaluza, J., Dinh, T.-H., Mukonka, V., Ching, P., Duse, A., Richards, G., de Jong, G., Cohen, C., Ikalafeng, B., Mugero, C., Asomugha, C., Malotle, M.M., Nteo, D. M., Misiani, E., Swanepoel, R., Zaki, S.R., Teams, I., 2009. Nosocomial outbreak of novel arenavirus infection, southern Africa. Emerg. Infect. Dis. 15, 1598–1602. https://doi.org/10.3201/eid1510.090211.

Radoshitzky, S.R., Buchmeier, M.J., Charrel, R.N., Clegg, J.C.S., Gonzalez, J.-P.J., Günther, S., Hepojoki, J., Kuhn, J.H., Lukashevich, I.S., Romanowski, V., Salvato, M. S., Sironi, M., Stenglein, M.D., de la Torre, J.C., ICTV Report Consortium, 2019. ICTV virus taxonomy profile: Arenaviridae. J. Gen. Virol. 100, 1200–1201. https://doi.org/10.1099/jgv.0.001280.

Russier, M., Reynard, S., Tordo, N., Baize, S., 2012. NK cells are strongly activated by Lassa and Mopeia virus-infected human macrophages in vitro but do not mediate virus suppression. Eur. J. Immunol. 42, 1822–1832. https://doi.org/10.1002/eji.201142099.

Schaeffer, J., Reynard, S., Carnec, X., Pietrosemoli, N., Dillies, M.-A., Baize, S., 2019. Non-Pathogenic Mopeia Virus Induces More Robust Activation of Plasmacytoid Dendritic Cells than Lassa Virus. Viruses 11, 287. https://doi.org/10.3390/v11030287.

Simulundu, E., Mweene, A.S., Changula, K., Monze, M., Chizema, E., Mwaba, P., Takada, A., Ippolito, G., Kasolo, F., Zumla, A., Bates, M., 2016. Lujo viral hemorrhagic fever: considering diagnostic capacity and preparedness in the wake of recent Ebola and Zika virus outbreaks. Rev. Med. Virol. 26, 446–454. https://doi.org/10.1002/rmv.1903.

Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinforma. Oxf. Engl. 30, 1312–1313. https://doi.org/10.1093/bioinformatics/btu033.

Taylor, P., Johnson, K.M., Tomori, O., Elliott, L.H., 1981. Recovery of a Lassa-related arenavirus in Zimbabwe. Am. J. Trop. Med. Hyg. 30, 1291–1293. https://doi.org/10.4269/ajtmh.1981.30.1291.

Těšíková, J., Krásová, J., Goüy de Bellocq, J., 2021. Multiple mammarenaviruses circulating in angolan rodents. Viruses 13, 982. https://doi.org/10.3390/v13060982.

Wulff, H., McIntosh, B.M., Hamner, D.B., Johnson, K.M., 1977. Isolation of an arenavirus closely related to Lassa virus from Mastomys natalensis in south-East Africa. Bull. World Health Organ. 55, 441–444.