VIRUS EVOLUTION

Virus Evolution, 2025, **11(1)**, veaf041
DOI: https://doi.org/10.1093/ve/veaf041
Advance Access Publication 22 May 2025
Research Article

Paramyxo- and coronavirus diversity and host associations in non-volant small mammals: evidence of viral sharing

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Abstract

Rodents and other non-volant small mammals (like shrews) maintain major ecological and epidemiological roles as reservoirs of zoonotic pathogens. Their presence within human-modified landscapes and interfaces with people, wildlife, and livestock create frequent opportunities for viral spillover. Despite this, the pathogen diversity and true risk of viral transmission are poorly understood by these hosts in Africa. Here, we explored the diversity and host association of paramyxoviruses and coronaviruses in non-volant small mammals from South Africa through longitudinal and opportunistic sample collection and molecular detection of viral RNA and host genetic barcoding. A high diversity of viruses was identified, with prevalences of 11.9% and 1.79% for paramyxoviruses and coronaviruses, respectively. Five instances of coinfections involving multiple paramyxoviruses and a coronavirus were detected, as well as nine Bayesian-supported paramyxovirus host genus, subfamily, and family switching, signifying frequent unrestrained viral sharing. Though the zoonotic potential of these identified viruses is unknown, the frequency of host switching suggests that these viruses may be more prone to adaptation to new host species or utilize highly conserved entry mechanisms. This highlights the risks for potential cross-species transmission events to livestock, domestic animals, and people, warranting continued surveillance.

Keywords: rodent; shrew; sengi; paramyxovirus; coronavirus; biosurveillance; viral sharing; cross-species transmission

Introduction

The consideration of wildlife species as hosts to an array of diverse viruses has gained traction over the past few decades with the emergence of wildlife-borne zoonoses such as Marburg virus (*Filoviridae*), Hendra virus (*Paramyxoviridae*), Lassa virus (*Arenavirus*), and Hantaan virus (*Hantavirus*), severe acute respiratory syndrome coronavirus (SARS-CoV) 1 and 2 as well as Middle East respiratory coronavirus (*Coronaviridae*). Bats and rodents, the most specious orders of mammals, have been among the wildlife hosts and reservoir species for these viruses (Mollentze and Streicker 2020). Biosurveillance efforts for RNA viruses in the *Paramyxoviridae* and *Coronaviridae* families in bat species currently far outweigh that of rodents and shrews (termed non-volant small mammals)—highlighting the need to improve our understanding of the viral diversity, prevalence, and maintenance in these hosts (Drexler *et al.* 2012, Geldenhuys *et al.* 2021, Mortlock *et al.* 2021).

The *Paramyxoviridae* family is known for its diverse host range across the 4 described subfamilies and 16 encompassed genera (Kuhn *et al.* 2023). Rodent-borne paramyxoviruses have been officially classified as species within the *Orthoparamyxovirinae* subfamily to the *Narmo-*, *Jeilong-*, *Henipa-*, and *Respirovirus* genera, with the number of fully characterized viruses rapidly increasing. The rodent-associated Mojiang virus from the *Henipavirus* genus has been speculated to be the causative agent of fatal pneumonia in three mine workers from China (Wu *et al.* 2014). However, no direct evidence has been provided to support this. More recently, a shrew-borne Henipavirus-related paramyxovirus, Langya virus, was identified following sentinel surveillance of febrile patients in China, with a documented history of recent animal exposure (Zhang *et al.* 2022). Contrary to other zoonotic henipaviruses, this virus has not resulted in any human fatalities to date, and human

infection is believed to be sporadic through independent spillovers as opposed to human-to-human transmissions.

Coronavirus diversity from rodents sampled in Asia and Europe includes betacoronaviruses from the *Embecovirus* subgenus and novel alphacoronaviruses. In both cases, evolutionary histories inferred from phylogenetic reconstruction suggest that rodents and coronaviruses have shared a long-term association (Lau *et al.* 2015, Tsoleridis *et al.* 2016). Coronavirus surveillance among small, non-volant mammals in Africa has been limited (Geldenhuys *et al.* 2021). However, this has been improving with the inclusion of rodent and shrew species in more surveillance studies. From the current literature, the prevalence of coronaviruses among small non-volant mammals has been reported to be low, accounting for less than 1% of sampled animals (Geldenhuys *et al.* 2021, Kumakamba *et al.* 2021, Ntumvi *et al.* 2022) or absent (Onyuok *et al.* 2019, Maganga *et al.* 2020, Suu-Ire *et al.* 2022, Wang *et al.* 2024).

Non-volant small mammals are of particular importance as potential virus reservoirs given their interaction with the human population across various contexts spanning from domestic cohabitation to agriculture, urban living, and wild environments, i.e. as household and agricultural pests, domesticated pets, or even as a food source (Pauciullo *et al.* 2024). These interactions have profound implications for both public health and wildlife conservation. From a zoonotic disease perspective, our understanding will require acquiring viral surveillance data to determine viral diversity and identify key reservoir species.

In this study, we investigated the presence and genetic diversity of paramyxo- and coronaviruses among non-volant small mammals in South Africa from opportunistic and dedicated longitudinal sampling, identified key natural host species, and evaluated viral sharing among different species.

Materials and methods

Study sites

Sample collection for this research included longitudinal seasonal surveillance at one site (Meletse, Limpopo), semi-longitudinal at another (Secunda, Mpumalanga), and opportunistic sample collection at four locations among three provinces in South Africa (Free State, Eastern Cape, and Gauteng; Fig. 1). Seasonal surveillance (2014–17) was conducted in the Rooiberg region surrounding the Madimatle cave (24.5914° S, 27.6258° E) in Meletse, Limpopo province. Sampling was conducted near Secunda town (26.4773° S, 29.19463° E) in the Mpumalanga province for five non-consecutive months (2015–16). The study was a collaboration between the Biosurveillance and Ecology of Emerging Zoonoses research group, the Mammal Research Institute at the University of Pretoria, and the Ditsong National Museum of Natural History (DNMNH). Species of non-volant mammals included in the study represent rodents, sengis, and shrews.

Approval for non-volant mammal research (Section 20) was obtained from the Department of Agriculture, Land Reform and Rural Development (12/11/1/1/8). A sampling permit for non-volant small mammal research was obtained from the Department of Economic Development, Environment, and Tourism of the Limpopo Province (ZA/LP/73972; CPM402-00007; ZA/LP/83642) and from the Free State Department of Economic Development, Tourism, and Environmental Affairs (01/21659; 01/26238).

Ethics statement

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the University of Pretoria's Animal Ethics Committee under H003-16, H004-16, and EC071-15.

Sample collection and processing

Personalized protective equipment was worn when capturing and sampling non-volant small mammals, including double-layer nitrile examination gloves, thick leather gloves for animal handling, disposable surgical gowns (Foliodress Hartmann, Johannesburg, South Africa), coveralls (Tyvek, DuPont, Johannesburg, South Africa), and gumboots. Powered air-purifying respirators (3M, Johannesburg, South Africa) were worn for respiratory protection during sample collection. All sampling and protective equipment, as well as sample containers, were decontaminated after use with a 10% liquid bleach solution (5500 ppm hypochlorite solution), and disposable PPE was discarded as biohazardous waste.

Seasonal sampling was performed between November 2014 and November 2017 at the Meletse site in Rooiberg, and from June to August 2015 ($n = 449$) and January/April 2016 at Secunda ($n = 100$; Supplementary Table S1). A small number of samples submitted to our research group (between 2004-2016), were opportunistically tested ($n = 14$). Active sample collection was performed as described in Geldenhuys *et al.* (2023). Briefly, non-volant small mammals were trapped using Sherman traps (H.B Sherman Traps, Inc., Tallahassee, FL, USA) and snap traps baited with a mixture of peanut butter and rolled oats. Each trapping session was started 2 h before sunset, and traps were inspected at sunrise. Captured animals were held in cotton bags until processing. Morphological and morphometric information of each mammal was recorded, and animals not collected as voucher specimens were released at the capture site.

Several sample types were collected as part of a larger biosurveillance study, including fur, excreta (urine and faeces), ectoparasites, oral swabs, and blood (Geldenhuys *et al.* 2023). Samples were stored in 1× DNA/RNA Shield (ZymoResearch, Irvine, CA, USA) inactivation and preservation media, and immediately frozen in a dryshipper (MVE Vapor Shippers, Ball Ground, GA, USA). Selected animals taken as voucher specimens received an isoflurane (Safeline Pharmaceuticals) overdose for euthanasia. During necropsy, using sterile scissors and forceps, soft organ tissues were collected in 1× DNA/RNA Shield (ZymoResearch, Irvine, CA, USA). Voucher carcasses were inactivated in 10% formalin for 24 h and transferred to ethanol, after which they were submitted to the DNMNH's Small Mammal Collection and the Natural History Collection of Public Health and Economics. For viral surveillance, sample types (faecal, rectum, intestine, and kidney) associated with coronaviruses and paramyxoviruses were tested. Samples were extracted in the BSL3 facility at the Department of Medical Virology, Centre of Viral Zoonoses of the University of Pretoria using the Zymogen Quick-RNA MiniPrep Plus kit (Zymo Research) according to the manufacturer's specifications.

Molecular detection of coronaviruses and paramyxoviruses and statistical analyses

Sample RNA was subjected to randomly primer cDNA synthesis using SuperScript reverse transcriptase IV (ThermoScientific, South Africa) as previously described (Mortlock *et al.* 2021). Paramyxo- and coronaviral testing was done using two assays targeting the *Orthoparamyxovirinae* subfamily and *Coronaviridae* family, respectively, using previously published methodologies (Geldenhuys *et al.* 2018, Mortlock *et al.* 2021). Amplicons of the correct size observed during 1.5% agarose gel analysis (Lonza, Johannesburg, South Africa) were excised, purified, and

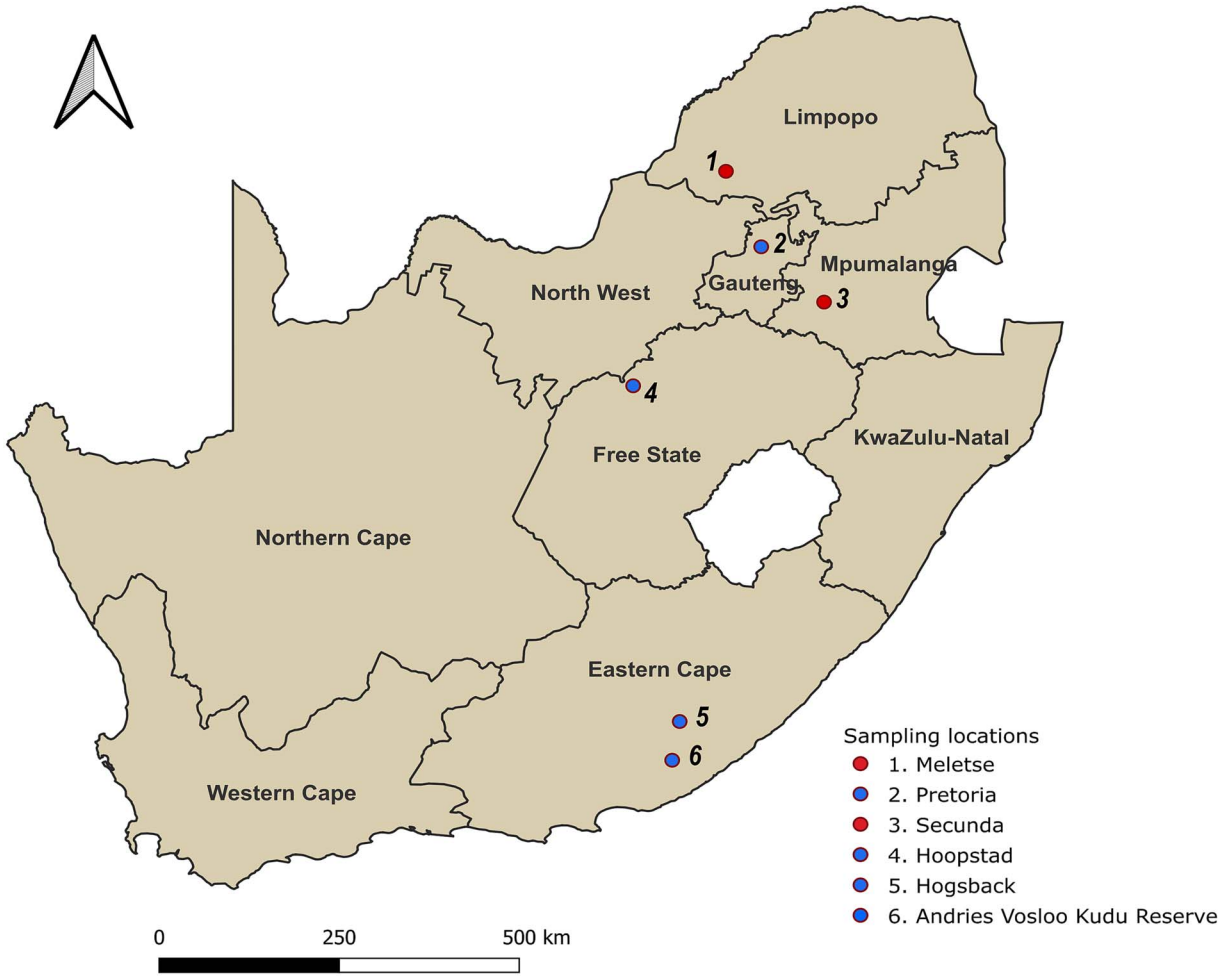

Figure 1. Locations in South Africa from which non-volant small mammal samples originated, with sites 1 and 3 representing where semi-/longitudinal sampling was conducted, and the remaining sites show where opportunistic sampling was done, or samples were obtained through submissions (hand-ins).

sequenced as previously described (Geldenhuys *et al.* 2023). Sequencing was performed on the ABI 3500xl at the DNA sequencing facility of the University of Pretoria.

For the longitudinal paramyxovirus surveillance conducted at the Matlapitsi site, sufficient data were available, and statistical analyses (Chi-Square) were conducted to assess the independence between the sex, month of sampling and host genus categorical variables and the test outcome. All analyses were performed in R version 4.3.0 (R_Core_Team 2022).

Molecular host identification

Host species identification was performed in the field using published keys (Happold and Happold 2013, Kingdon *et al.* 2013, Monadjem *et al.* 2015), while additional morphological identification was carried out at the DNMNH using extracted skulls of voucher specimens—which received museum voucher numbers (Supplementary Table S1). Genetic barcoding was performed on select specimens found to contain surveyed viral RNA for accurate association between morphological and molecular taxonomic identification and accurate host identification and implication. Partial Cytochrome B sequences were produced as described previously (Greenberg *et al.* 2012). Amplicons of correct size based on 1.5% agarose gel analyses were sequenced as described above and compared to morphological assignments and available reference sequences on the National Center for Biotechnology Information (NCBI).

Bioinformatic analyses

All nucleotide and amino acid sequence alignments were performed with CLUSTALW in the BioEdit sequence alignment editor (v.7.2.5; Hall 1999). Reference genomes from respective viral genera were obtained from GenBank (NCBI) for comparative analysis. Estimations of pairwise similarities were performed with p-distance analyses in MEGA v.7 (Kumar *et al.* 2016). Coronavirus phylogenies were constructed with a general time reversible (GTR) model and paramyxoviruses with the Transition Model 3, both with gamma distribution and invariant sites. Bayesian Markov Chain Monte Carlo (MCMC) chains were set to 20 000 000 states, sampling every 2000 steps, and convergence was confirmed *via* an effective sample size (ESS) of >200. Final Bayesian trees were calculated in Tree Annotator with a 10% burn-in. Trees were viewed and edited in Figtree v.1.4.2. CIPRES Science Gateway was used to run jModelTest2 and BEAST (Miller *et al.* 2010, Darriba *et al.* 2015, Suchard *et al.* 2018).

Cross-species transmission analysis

To investigate viral sharing between hosts, we aligned the paramyxovirus sequences identified in this study with closely

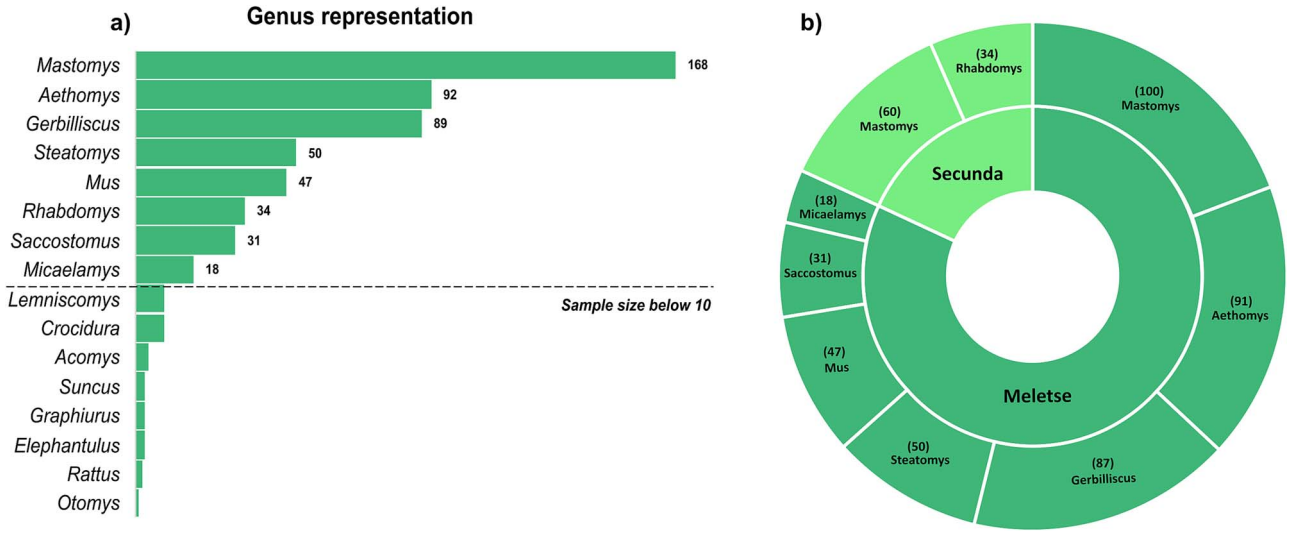

Figure 2. (a) Total sample sizes of rodent, shrew, and sengi genera for paramyxovirus and coronavirus biosurveillance in South Africa and (b) summary of the sample sizes per genus (only for genera where >10 individuals were collected) according to the location of longitudinal (Meletse) and semi-longitudinal (Secunda) sampling sites in South Africa.

related sequences from African countries in BioEdit sequence alignment editor (v.7.2.5) and constructed a phylogenetic tree in BEAST v1.10.4 (Suchard *et al.* 2018) using the best fitting model GTR as suggested by JModeltest2, with Gamma sites and empirical base frequencies. An asymmetric discrete trait substitution model was used with a strict clock and Bayesian Skyline coalescent tree to reconstruct ancestral states and run on CIPRES. An MCMC of 100M was evaluated to reach convergence in Tracer v1.7 (Rambaut *et al.* 2018) by achieving an ESS of >200. The Bayesian stochastic search variable selection log file was analysed in SPREAD3 v.9.7.1 (Bielejec *et al.* 2016) with a burn-in of 20% for the calculation of Bayes factors (BFs), of which BF > 3 was considered well-supported evidence, and a BF of between 10 and 100 was considered strong evidence for viral sharing with (BF > 100 being very highly supported) according to Faria *et al.* (2013).

Next-generation sequencing

Full genome sequencing was attempted on selected samples for genome recovery. RNA samples were submitted to the Biotechnology platform at the Agricultural Research Council for RNA quality assessment prior to MGIEasy RNA Library Prep kit (MGI Tech) and sequencing on the MGI DBNSEQ G400 platform. Sequencing data were analysed on the CZID platform (Kalantar *et al.* 2020) to assign reads to hits from public databases as well as Qiagen CLC Genomics Workbench 24.0 (https://digitalinsights.qiagen.com/) to clean and trim reads, and map reads/contigs to genetically similar reference genomes.

Results

A total of 563 non-volant small mammals from 16 genera were collected and sampled for viral biosurveillance, representing 3 sengi, 12 shrews, and 548 rodents. Between November 2014 and November 2017, 449 non-volant small mammals were sampled at Meletse in the Rooiberg region (Limpopo), 100 near Secunda town (Mpumalanga), with 14 from other opportunistic sites (Fig. 2, Supplementary Table S1).

Biosurveillance for paramyxoviruses

For the paramyxovirus biosurveillance, 11.9% ($n = 556$; 95% CI 9.18%–14.56%) of samples were positive for paramyxovirus RNA, with 11.8% in rodents and 16.6% in shrews. A positivity of 6.5% ($n = 246$; 95% CI 3.42%–9.59%) was reported from the kidney and 10.1% ($n = 545$; 95% CI 7.56%–12.62%) from gastrointestinal sample types. Two shrews and three rodents tested positive in both the kidney and gastrointestinal sample types. No sengi samples tested positive for viral RNA.

Viral RNA from four *Orthoparamyxoviridae* genera was detected, representing 20 putative viral species (Figs 3 and 4). A putative *Henipavirus* species was detected from two shrew individuals (*Crocidura mariquensis*) sampled from the same locality a year apart. This putative species phylogenetically clusters with Mojiang and Langya viruses that were detected in China from other rodent and shrew species, sharing a 72% to 75% nucleotide and 83% to 86% amino acid identity with these viruses. Viral sequences related to this known zoonotic genus were exclusively detected in shrew samples, despite the inclusion of a much larger rodent sample set.

Two putative viral species were detected within the *Morbillivirus* genus, with the closest phylogenetic relationship to Longquan *Berylmys bowersi* morbillivirus 1 described from a white-toothed rat in China. Only the *Gerbilliscus* and *Mastomys* host genera were associated with this viral genus, and viral sharing was observed between these two host genera, with detected sequences sharing 99%–100% nucleotide sequence identity. Notably, one putative viral species was circulating among these hosts from 2013 to 2014 northeast of Hoopstad, while the other was detected from 2016 to 2017 in rodents sampled in the Meletse region.

The *Narmovirus* genus was primarily linked to the *Mastomys* and *Aethomys* host genera and included two putative viral species identified in this study. Host genus specificity was observed for these viruses, although cross-species transmission was observed between *Mastomys* spp. and *Aethomys* and one *Gerbilliscus* individual. The host identity of the latter *Gerbilliscus* individual remains unverified, as it could not be confirmed with molecular barcoding (Fig. 4, Supplementary Table S1).

The highest viral diversity was described from the *Jeilongvirus* genus, representing 15 of the 20 putative viruses reported in

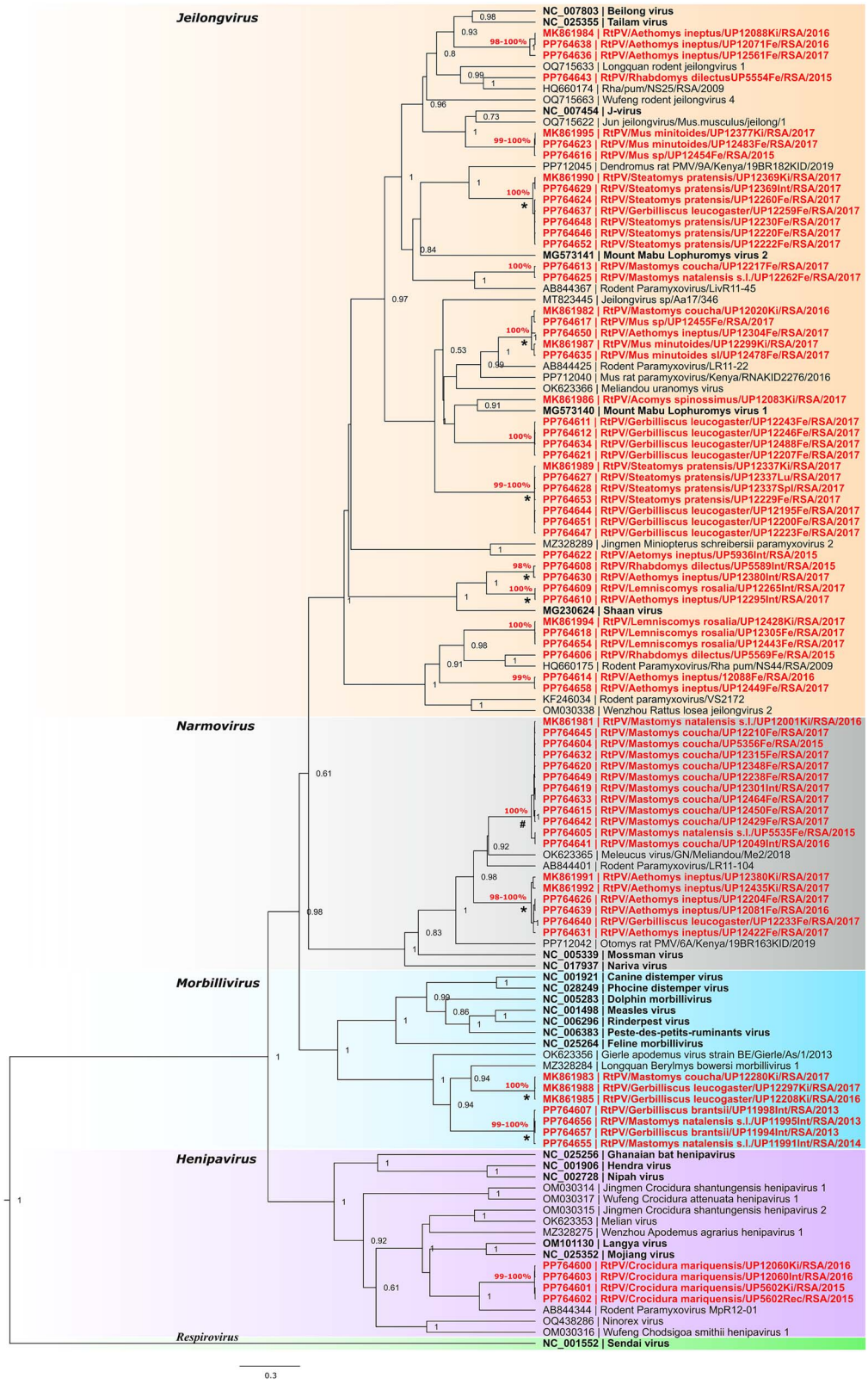

Figure 3. Paramyxovirus Bayesian phylogeny from a segment of the RNA-dependent RNA-polymerase gene region, with sequences in bold and red originated from this study, sequences in black and bold are from known species in specific genera; only posterior probabilities of greater than 0.5 are indicated and instances of cross-species transmission are indicated by a hash (#), while cross-genus transmission is indicated by an asterisk (*).

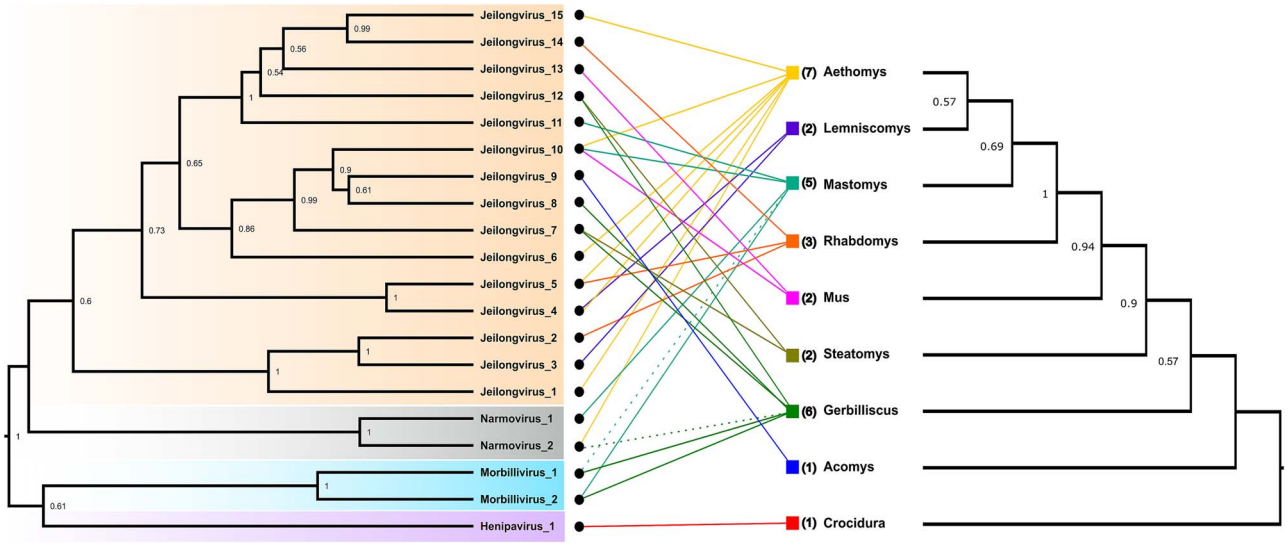

Figure 4. The association of 20 putative paramyxoviral species (left) and their non-volant small mammal hosts (right) are indicated, while genera are denoted by distinct coloured blocks; solid lines represent host–virus associations confirmed through molecular or morphological museum voucher identification and dashed lines indicate associations where host identification could not be confirmed. Numbers in brackets indicate the number of putative viral species associated with each host genus.

this study, with 8 associated host genera belonging to different rodent families and subfamilies (Fig. 3). Two of the putative viral species associated with *Rhabdomys pumilio* (UP5569 and UP5554) grouped phylogenetically with viral sequences previously reported from the same species sampled in South Africa in 2009 (Rha_pum/NS44 and NS25). In addition, a putative viral species was detected in *Mus* spp. sampled in 2015 and 2017, phylogenetically grouped with both J jeilongvirus (NC_007454) and Jun jeilongvirus (OQ715622)—both previously reported from the same rodent species. Collectively, these findings indicate an overall host specificity of rodents and paramyxoviruses. Two putative viral species, associated with the *Aethomys*, *Rhabdomys*, and *Lemniscomys* hosts, were most closely related to Shaan virus, a bat-borne jeilongvirus, sharing 76% nucleotide identity and between 86% and 87% on the amino acid level. Another viral sequence detected in an *Aethomys ineptus* individual (UP5936) was also most closely related to a bat-borne virus described from a *Miniopterus schreibersii* insectivorous bat (sharing an 80% nucleotide and 93% amino acid identity). Cross-genus transmission was recorded for five putative jeilongvirus species, with sequences detected in the different hosts sharing between 99% and 100% nucleotide identity (Figs 3 and 4). Viral sharing is predominantly observed between two host genera; however, one putative viral species was associated with three genera (*Mastomys*, *Mus*, and *Aethomys*), with the viral sequence first detected in *Mastomys* (UP12020) in the year preceding the detection in other host species.

Assessment of the longitudinal data collected from the Meletse site indicated no statistically significant difference in the positivity between males and females ($\chi^2_1 = 0.11$, $P = .745$). Viral excretion was observed throughout the year with no significant increase in viral excretion at any time point across the study ($\chi^2_{10} = 13.32$, $P = .207$). However, March (21.05%) and June (21.43%) exhibited relatively higher positivity rates, although no clear temporal pattern was observed. The increased rates may be attributed to larger sample sizes during these times for certain years (Supplementary Table S1) or could be as a result of nutritional stress due to increasing food scarcity during autumn (March) and winter (June) months. Positivity rates varied across genera, with higher rates observed in *Lemniscomys* (44.4%) and *Acomys* (25%) despite these not being the dominant species sampled from Meletse (Fig. 2). However, no statistically significant difference in positivity was found between host genera ($\chi^2_{12} = 18.73$, $P = .095$).

Biosurveillance for coronaviruses

From the gastrointestinal samples tested for coronaviruses, 1.79% ($n = 558$; 95% CI 0.86%–3.27%) was found to contain coronavirus RNA, including sequences belonging to the *Alphacoronavirus* and *Betacoronavirus* genera (Fig. 5). The coronaviruses were only identified among six rodent genera (*Aethomys*, *Gerbilliscus*, *Mastomys*, *Micaelamys*, *Rhabdomys*, and *Steatomys*) and primarily from 2016/2017 at Meletse in the Rooiberg region, with two others from 2015 in Secunda. The betacoronaviruses all belonged to the *Embecovirus* subgenus, with sequences originating from Meletse rodents (sharing 97%–100% nucleotide identity) clustering with other rodent sequences of the HKU24 diversity from China (Lau *et al.* 2015). One of the sequences originating from a *Rhabdomys* sp. from Secunda (UP5537) shared 94%–97.8% nucleotide identity with the sequences from Meletse. The *Mastomys* sp. sequence, UP5531, from Secunda, shared only 84%–85.2% nucleotide identity to the other identified sequences and 96% with the Murine hepatitis virus. Two alphacoronavirus sequences were identified, sharing 74.2% nucleotide identity. These rodent alphacoronaviruses (sharing only 65%–71% nt identity to other African rodent alphacoronaviruses) grouped with bat coronaviruses from the *Miniopterus* (sharing 95%–97.4% nt identity) and *Rhinolophus* (sharing 94.8%–98.7% nt identity) coronavirus clades sampled in South Africa and Kenya.

Genome sequencing, viral coinfections, and cross-species transmission

None of the attempts at sequencing complete viral genomes of detected coronaviruses or paramyxoviruses were successful, and this was primarily due to poor quality RNA that often did not pass quality, as well as concentration requirements for library preparation kits. Despite poor quality RNA, the library prepared for the putative henipavirus detected in the shrew genus *Crocidura* (UP12060) was sequenced, producing 33 million reads, though no

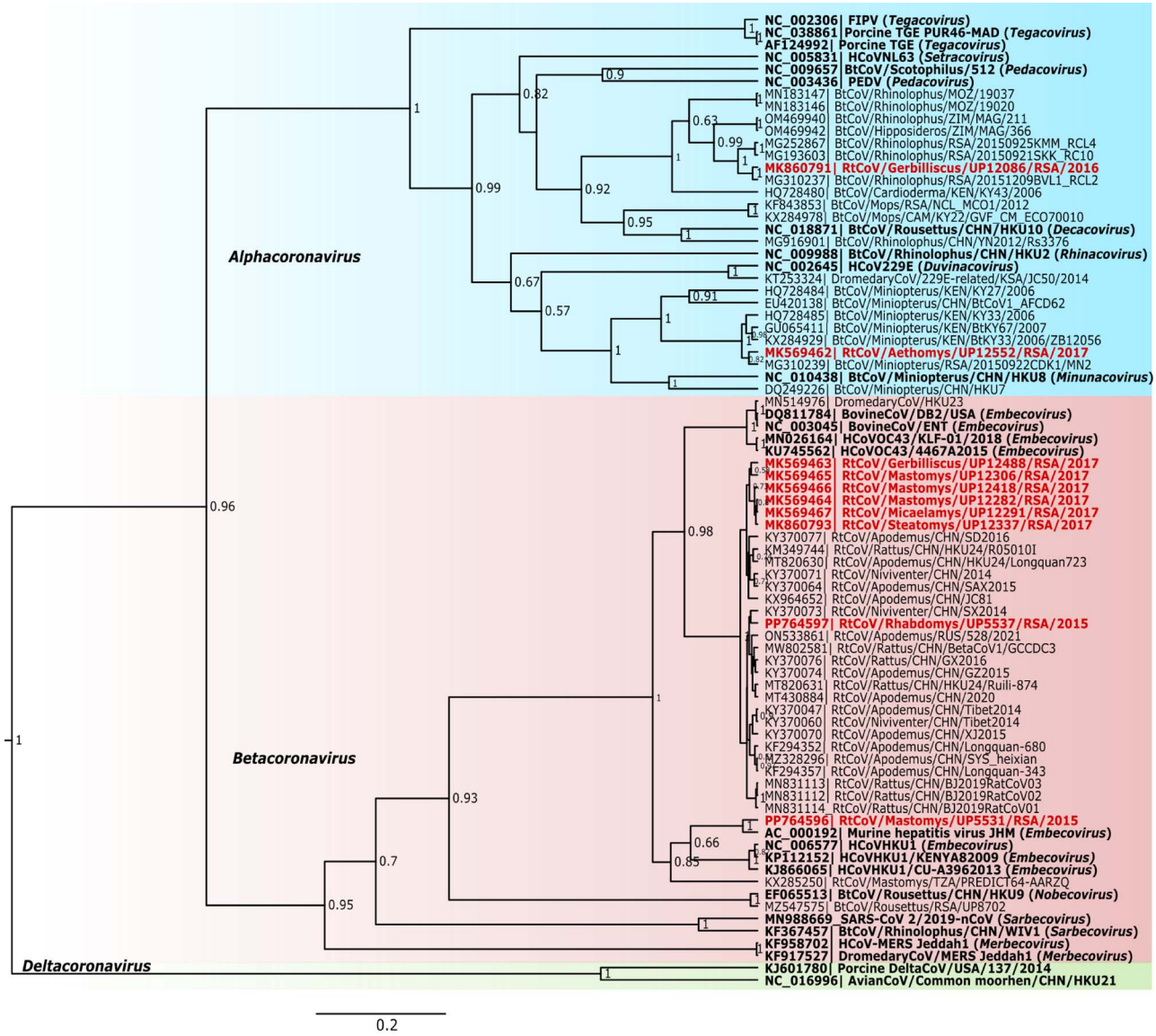

Figure 5. Coronavirus Bayesian phylogeny from a segment of the RNA-dependent RNA-polymerase gene region where sequences in bold and red originated from this study, sequences in black and bold are from known species in specific subgenera; only posterior probabilities of greater than 0.5 are indicated.

henipavirus reads were identified by either CZID or through read-based reference mapping in CLC.

Two *A. ineptus* individuals, for which both a gastrointestinal and kidney sample were tested, showed to be coinfected with two different paramyxoviruses. For UP12088, two distinct putative jeilongvirus species were detected, sharing a nucleotide identity of only 64%. For UP12380, a putative jeilongvirus was detected in the intestine, while a putative narmovirus was detected in the kidney of the individual (Fig. 3). Two rodents (UP12337—*Steatomys pratensis*, and UP12488—*Gerbilliscus leucogaster*), both sampled from the Meletse site in Limpopo in May and August 2017, were coinfected with a betacoronavirus and jeilongvirus (Supplementary Table S1). One of the individuals shedding a betacoronavirus (UP12291—*Micaelamys namaquensis*) was previously also found to be shedding arenavirus RNA (Geldenhuys *et al.* 2023).

Through ancestral state reconstruction (coronaviruses were excluded due to limited data), we identified nine Bayesian-supported intra-host virus transmission events—one intra-family, two intra-subfamily, and seven intra-genus (Supplementary Table S2). The evidence for the intra-family host switching from *Steatomys* (Nesomyidae) to *Gerbilliscus* (Muridae) was very highly supported with a BF (>2000; Fig. 6). In addition, significantly supported host switches evidenced by BFs above 100 (and strong posterior probabilities) include donors *Steatomys*, *Aethomys*, and *Mus*, with recipients *Gerbilliscus*, *Lemniscomys*, and *Mastomys*. Other well-supported host switches with BF between 23 and 58 include donors *Gerbilliscus*, *Aethomys*, and *Mus* to recipients *Mastomys*, *Rhabdomys*, and *Aethomys*, with lower supported BF (3–6) evidenced for viral sharing between *Mus*, *Mastomys*, and *Aethomys* to *Steatomys*, *Aethomys*, and *Mus* individuals (Fig. 6). For all assessed donor–recipient pairs, only one, between *Aethomys* and *Mus*, produced strong evidence of transmission in both directions, though the directionality from *Mus* to *Aethomys* had the strongest support (BF > 20).

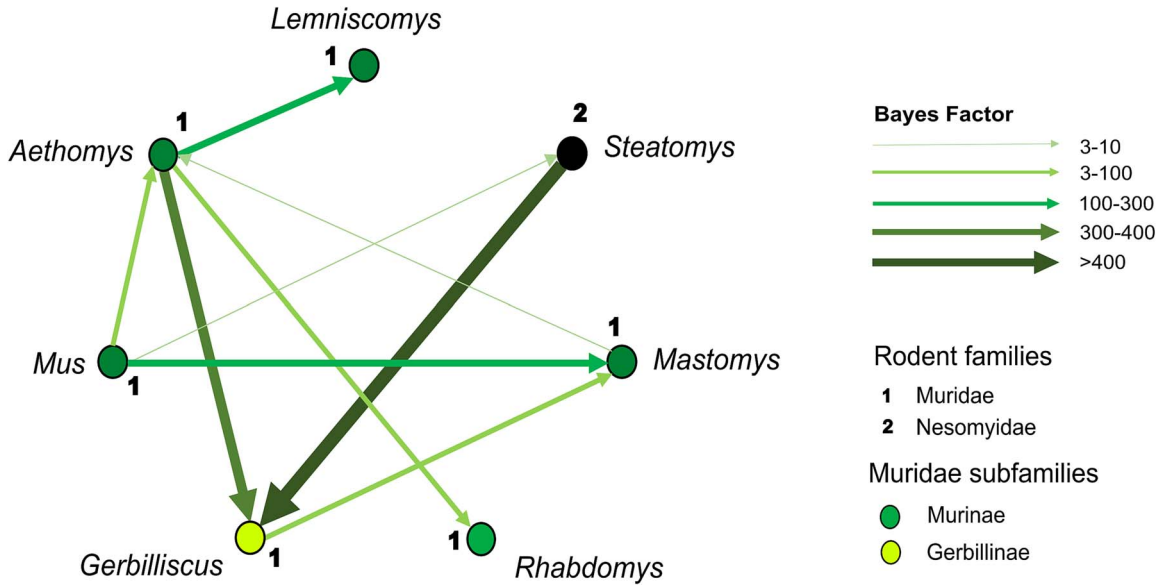

Figure 6. The rodent interfamily, subfamily, and genus-level transmission of orthoparamyxoviruses are indicated with directionality of virus–host switching as arrows; only host switches supported by a strong BF > 3 are shown with colour and thickness of the arrows representing different strengths of the BFs.

Discussion

Rodents and other non-volant small mammals play significant ecological and epidemiological roles as reservoirs and vectors of zoonotic pathogens. The ubiquity of some species to occur across a range of habitats, such as human-modified landscapes, positions these species at the interface of wildlife, livestock, and humans, creating ample opportunity for pathogen spillover. In this study, we explored the diversity and host association of paramyxoviruses and coronaviruses in non-volant small mammals from South Africa, and contributed to the growing knowledge regarding these virus–host associations.

As reported in rodent biosurveillance studies, a diverse range of paramyxoviruses are associated with these small mammals. Our findings align with this trend, detecting viral sequences related to four *Orthoparamyxovirinae* subfamilies. The overall prevalence of 11.8% observed in our study is lower than those reported in other regions, such as Zambia (21%) and North America (36%), where kidney and faecal material were targeted, respectively (Sasaki *et al.* 2014, Larsen *et al.* 2022). Notably, our results also revealed a lower prevalence in kidney samples compared to gastrointestinal samples, consistent with the differences observed in these studies supporting both systemic infection and viral shedding. However, species composition significantly influences overall prevalence across studies, as paramyxoviruses are more prevalent in some species than others. For instance, in our study, *Lemniscomys* (44.4%) and *Acomys* (25%) exhibited high prevalence rates despite small sample sizes. In comparison, a study from Germany sampling over a thousand *Myodes* individuals reported a comparatively low prevalence of 2.6% (Drexler *et al.* 2012).

Viruses within the *Henipavirus* genus are well-known for their zoonotic potential and have predominantly been associated with *Pteropus* fruit bat species (Halpin *et al.* 2011). However, increased surveillance in non-volant small mammals has highlighted the emerging role of shrews as natural hosts of henipaviruses (Caruso and Edwards 2023). Among the rodent-associated paramyxoviruses described to date, only Langya henipavirus has been directly linked to zoonotic spillover events, with shrew species playing the predominant role (Zhang *et al.* 2022). In the present study, the detection of a putative henipavirus exclusively in shrew samples, despite the significantly larger rodent sample sizes, further reinforces the key role of shrews as henipavirus hosts. The close genetic relationship of the South African shrew-associated henipavirus to Langya virus highlights the need for further investigation, including virus characterization and assessment of its pathogenicity.

Not many surveillance studies in Africa have investigated rodents for coronaviruses. Research from Ghana, Gabon, and Kenya (with sample sizes ranging from 293 to 617) did not identify circulating coronaviruses (Onyuok *et al.* 2019, Maganga *et al.* 2020, Suu-Ire *et al.* 2022, Wang *et al.* 2024), while surveillance in the Republic of Congo, Democratic Republic of Congo, and Cameroon (with larger sample sizes of between 1340 and 2700) reported prevalences of less than 1% (Kumakamba *et al.* 2021, Ntumvi *et al.* 2022). The latter studies reported novel alphacoronaviruses from rodent genera *Deomys* and *Malacomys* (not included in this study) as well as the shrew genus *Crocidura*. Here, we identified a less than 2% coronavirus prevalence among non-volant mammals. While comparable, it would suggest that the prevalence of coronaviruses reported from African rodents is generally very low. In addition to novel alphacoronaviruses, we also identified betacoronaviruses from the *Embecovirus* subgenus, with closest sequenced relatives from rodents in Asia (e.g. diversity related to HKU24) or global strains of Murine hepatitis virus. These findings indicate that targeted longitudinal surveillance increasing sample sizes per species would yield viruses from both genera but that the overall prevalence may still remain low. In addition, finding highly similar embecoviruses from five different rodent genera also suggests that this diversity of coronaviruses is not strictly restricted to specific hosts, though the limited data from this study precluded detailed investigation into viral sharing of embecoviruses. Our current efforts to characterize complete genomes have been unsuccessful, primarily due to poor RNA quality and low concentrations, though we are exploring alternative options with remaining sample material for both coronaviruses and paramyxoviruses.

Viral sharing among rodent genera remains underexplored, though this was previously documented in a paramyxovirus biosurveillance study conducted in Zambia (Sasaki *et al.* 2014). While the authors did not further explore these observations, their phylogenetic analyses revealed instances of viral sharing between *Crocidura* and *Rattus* for *Henipavirus*-related sequences; *Mastomys* and *Aethomys*, as well as *Mastomys* and *Rattus* for *Jeilongvirus*-related viruses; and between *Mastomys* and *Aethomys* for *Narmovirus*-related viruses. Our study corroborates these findings, reporting viral sharing between *Mastomys*, *Aethomys*, and *Mus* for a putative jeilongvirus, as well as the association of the *Narmovirus* genus with *Aethomys* and *Mastomys* species. Furthermore, the current study highlights the association of the *Morbillivirus* genus with *Mastomys* and *Gerbilliscus*, also demonstrating cross-subfamily viral sharing. This supports the findings of Sasaki *et al.* (2014) where closely related viral sequences were identified in the same rodent genera sampled in Zambia. Whether host characteristics or viral properties, such as broad receptor usage, drive these cross-species transmission events remains to be determined. Contact points for viral transmission likely occur at common food sources within overlapping habitats, where naïve species are exposed to infectious excreta.

Notably, the two rodent species identified as hosts to *Morbillivirus*-related viruses, *Gerbilliscus* and *Mastomys*, are well-known agricultural pests (Watson *et al.* 2016, Du Plessis *et al.* 2016a, Du Plessis *et al.* 2016b). The widespread presence of these species in human-transformed areas increases the likelihood of human–rodent interactions, elevating the risk of human exposure and potential infection with zoonotically transmitted paramyxoviruses associated with these hosts. Given that members of the *Morbillivirus* genus are already known for their broad host distribution, spanning humans, canids, and even aquatic species, the detection of multi-host rodent-borne viruses within this genus highlights their potential for spillover and zoonotic disease transmission (Libbey and Fujinami 2023).

The ecological complexity involving multiple natural host species likely supports intricate transmission networks, which can contribute to an increased prevalence of these viruses within the environment. Rodent population explosions, often driven by environmental, resource, or agricultural factors, further amplify this risk by increasing opportunities for contact between rodents, intermediate host species, and humans (Pauciullo *et al.* 2024). Viruses capable of infecting multiple hosts additionally enhance the transmission potential and likelihood of zoonotic disease spillover. This adaptability underscores the need for refined risk assessments and targeted mitigation strategies that account for the expanded host range, the influence of environmental factors on host population dynamics, and the non-specific, year-round viral excretion patterns. Furthermore, correctly identifying host species and understanding their ecological and epidemiological role as host species is fundamental for improving biosurveillance efforts and predicting future disease emergence. These findings highlight the importance of investigating the viral ecology of non-volant small mammals to inform strategies to evaluate and reduce zoonotic disease risk, especially in regions of high rodent–human interaction.

Acknowledgements

We would like to thank the landowners, Aquila Steel (Thabazimbi) Pty Ltd and Motjoli Resources, for permitting access to the property where sampling was conducted in Meletse, Limpopo. Thank you to Hannes de Wet and Christo Schoonwinkel for their assistance with the collection of the Hoopstad samples, and Daan Loock from Sasol Synfuels for the work conducted at the Secunda site. We would also like to acknowledge Hlengiwe Nkambule and Lebogang Matshaba for laboratory support and the staff and students at the Biosurveillance and Ecology of Emerging Zoonoses (BEEZ) research group for fieldwork assistance and sample collection.

Author contributions

Conceptualization, W.M., M.M., and M.G.; Sample collections, T.K., W.M., M.M., M.G., R.R., M.K., E.F.V.M., and L.H.S.; data generation, R.R., M.M., and M.G.; data analysis and collation, R.R., M.M., and M.G.; host taxonomic confirmation, T.K.; original draft preparation, M.M. and M.G.; review and editing, W.M., R.R., M.K., E.F.V.M., L.H.S., and T.K.; and funding acquisition, W.M. All authors have read and agreed to the published version of the manuscript.

Supplementary data

Supplementary data is available at *VEVOLU Journal* online.

Conflict of interest: The authors declare that there are no conflicts of interest.

Funding

This research was partially supported by the Grant or Cooperative Agreement Number [5 NU2GGH001874-02-00], funded by the Centers for Disease Control and Prevention. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the Centers for Disease Control and Prevention or the Department of Health and Human Services. This work was also supported in part by the National Research Foundation (NRF) of South Africa: the DSI-NRF South African Research Chair held by WM Grant No. 98339. The acknowledge the NRF for funding the equipment at the DNA Sanger sequencing facility, Faculty of Natural and Agricultural Sciences, University of Pretoria that was used to generate Sanger sequencing data presented in this work. The financial assistance of the National Research Foundation (NRF) towards this research is hereby acknowledged. Opinions expressed and conclusions arrived at are those of the authors and are not necessarily to be attributed to the NRF. The project or effort depicted was sponsored by the Department of the Defense, Defense Threat Reduction Agency (HDTRA1-20-1-0025).The content of the information does not necessarily reflect the position or the policy of the federal government, and no official endorsement should be inferred. Additional funding was obtained through the Sasol Agricultural Trust.

Data availability

The original data presented in the study are provided in the supplemental material associated with the publication. All genetic sequences generated in his study have been deposited in the National Center for Biotechnology Information (NCBI) GenBank database (available online at https://www.ncbi.nlm.nih.gov/genbank/) with GenBank accession numbers: Coronaviruses—MK569462 to MK569467, MK860791, MK860793, PP764596, and PP764597; Paramyxoviruses—MK861981 to MK861992, MK861994, MK861995, and PP764600 to PP764658; Host barcodes—PO785691, OP785692, and PQ602271 to PQ602316.

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