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Protocols

Detection of jeilongvirus circulation in Finnish urban rat populations by a novel pan-jeilong-RT-qPCR

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ABSTRACT

Paramyxoviruses can infect a variety of host species and cause infections among wildlife and humans. Some of these viruses can also cause severe zoonotic infections. Although new paramyxoviruses, such as jeilongviruses, are being recognized, little is known about their host species variation and pathogenesis. The current screening methods to detect paramyxoviruses are either laborious conventional PCR methods or costly next-generation sequencing methods. Therefore, we designed a new pan-jeilong-RT-qPCR assay to detect jeilong- and para-jeilongviruses from wildlife samples and screened rat, shrew, and bat samples from Finland and Latvia. With this new real-time PCR assay, we detected jeilongviruses in Finnish urban rat populations during 2020–2023 with a 13% positivity rate, including beilong virus (*Jeilongvirus beilongi*). This finding highlights the abundance of jeilongvirus in rats and the need for continued surveillance and more detailed characterization of these novel pathogens to evaluate the zoonotic potential of these viruses in rat and other rodent populations.

1. Introduction

Paramyxoviruses (PMVs; family *Paramyxoviridae),* and especially orthoparamyxoviruses (subfamily *Orthoparamyxovirinae*), include many viruses that infect different host species and have possible zoonotic properties. PMVs are negative-sense RNA viruses that can switch host species, creating a risk of zoonotic spillover (Kitchen et al., 2011). The known zoonotic PMVs include the deadly bat-borne Hendra and Nipah viruses, and the less severe shrew-borne Langya virus (Mahalingam et al., 2012; Eaton et al., 2006; Zhang et al., 2022).

The taxonomy of PMVs has gone through changes since new viruses, like the Shaan virus, with distinct genomic differences from other PMVs have been discovered. The new genus *Jeilongvirus* was initially called the Shaan-virus group, which included the bat-borne Shaan virus and rodent borne J- and Tailam viruses (Wu et al., 2016; Noh et al., 2018; Jang et al., 2020; Wells et al., 2022). Later, in 2023, jeilong- and para-jeilongviruses were separated as distinct genera by the International Committee on Taxonomy of Viruses (ICTV) to 32 jeilongvirus and 18

Abbreviations: BLAST, Basic Local Alignment Search Tool; CSIRO, Commonwealth Scientific and Industrial Research Organisation; EDTA, ethylenediaminetetraacetic acid; IDT, Integrated DNA Technologies; NCBI, National Center for Biotechnology Information; NGS, next-generation sequencing; PMV, paramyxovirus; RT, reverse transcription.

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parajeilongvirus species. In addition to the genetic differences between these viruses, the taxonomic separation is based on host species associations; parajeilongviruses are carried predominantly by bats, whereas jeilongviruses are carried primarily by rodents and shrews, although some jeilongviruses have also been detected in pinnipeds (Costa-Silva et al., 2025). Jeilongviruses have been identified across multiple continents, including Europe (Vanmechelen et al., 2020, 2022, 2021; Horemans et al., 2023a; Vanmechelen et al., 2018), Asia (Zhu et al., 2022; Su et al., 2023; Ch'ng et al., 2023; Xu et al., 2024a; Gan et al., 2024a; Natasha et al., 2024; Sata et al., 2024a), Africa (Mortlock et al., 2025a; Moonga et al., 2024a) and the Americas (Wells et al., 2022; Costa-Silva et al., 2025; DeRuyter et al., 2024a). Very few studies of orthoparamyxovirus screening have been conducted in Northern Europe (Pulkkinen et al., 2026). PMV screening efforts in Northern Europe have mainly targeted avian paramyxoviruses from the subfamily *Avulavirinae* in wild waterfowl samples (Lindh et al., 2008; Tolf et al., 2013). Furthermore, novel PMVs are constantly being recognized in different host species around the world, but the zoonotic risk of these recently discovered PMVs, like jeilongviruses, remains unknown.

The most recently recognized orthoparamyxovirus groups—jeilong- and parajeilongviruses— remain poorly understood, highlighting the need for more in-depth research. Much of the current knowledge of these viruses comes from the use of the pan-paramyxovirus nested-PCR assay developed by Tong et al (Tong et al., 2008)., which has been used to identify jeilong- and parajeilongviruses among other paramyxoviruses from a variety of sample materials, including different bat species (Wells et al., 2022) and rodents and shrews (Xu et al., 2024a; Natasha et al., 2024). Although other conventional PCR assays have been published (Vanmechelen et al., 2022; van Boheemen et al., 2012), techniques that use probe-based real-time PCR assays have not been established. Some researchers have used next-generation sequencing (NGS) with small (Vanmechelen et al., 2018) and large sample sets (Zhu et al., 2022; Ch'ng et al., 2023) as well as with virus cultivation (DeRuyter et al., 2024b; Sata et al., 2024b), but these methods are often resource-intensive and not accessible to many researchers, especially those in resource-limited settings.

In this study, we sought to design and set up a new screening tool based on real-time RT-PCR (RT-qPCR), that targets the PMV genera *Jeilongvirus* and *Parajeilongvirus*. We tested the performance of the pan-jeilong RT-qPCR by retrospectively screening Finnish and Latvian wildlife samples from shrews, rats, and bats.

2. Materials and methods

Under necessary permits, samples collected between 2019–2025 included shrew (N = 238) and rat (N = 158) tissue from Finland, as well as bat fecal samples from Finland (N = 108) and Latvia (N = 92). Full characteristics of the samples are described in Supplementary Table 1. The collection consisted of animals from both natural habitats and areas close to human settlements. The samples were screened for jeilong- and parajeilongviruses with the new pan-jeilong RT-qPCR assay. To confirm the positive findings and acquire PCR products for Sanger sequencing, conventional PCR assays were performed with pan-jeilong and beilong virus-specific primers. NGS was also used to acquire whole-genome sequences.

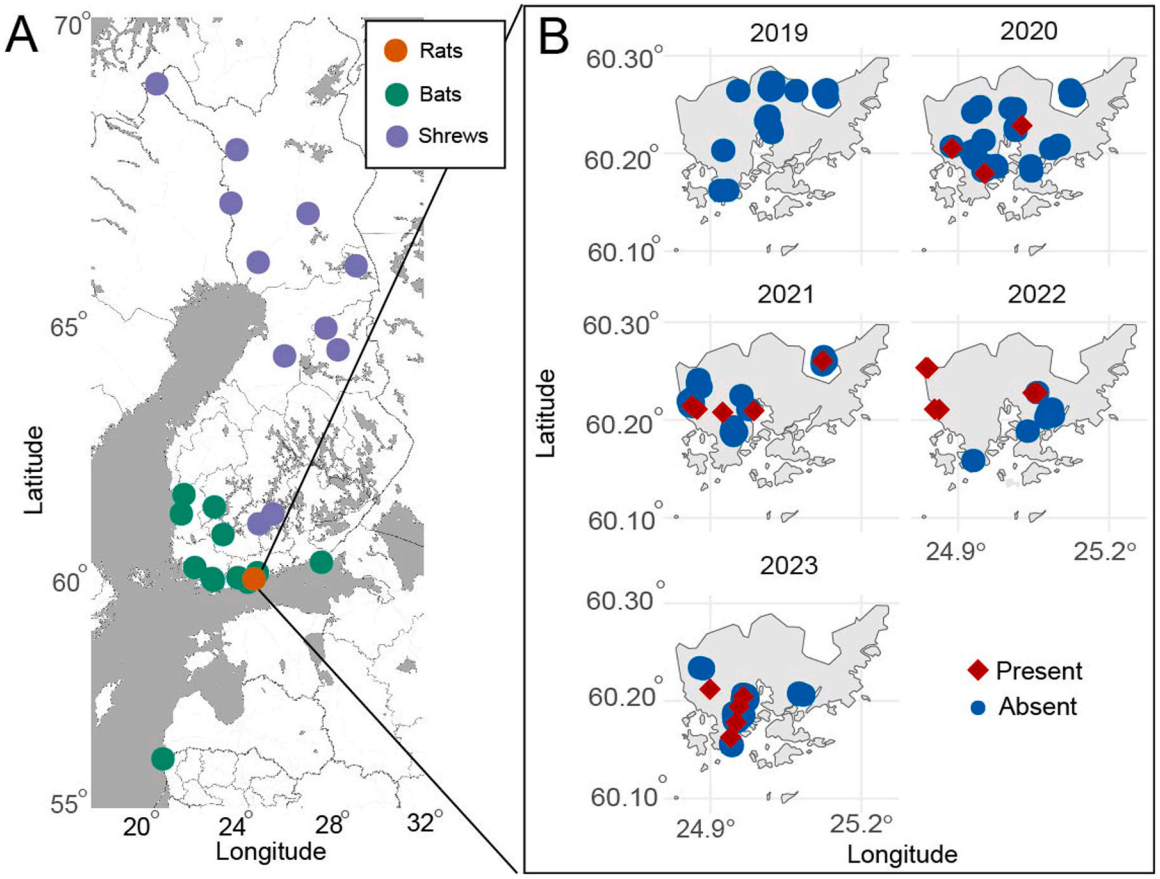

Fig. 1. Sample collection sites in Finland and Latvia (A). Location of bat faecal samples are indicated in green, shrew samples in purple, and rat samples in orange. Close-up maps of the city of Helsinki (B) indicate rat collection sites in 2019–2023. Jeilongvirus-positive and -negative rats are indicated with red diamonds and blue spheres, respectively.

2.1. Bat samples

The captured bats (N = 194) included *Eptesicus nilssonii* (N = 51), *Pipistrellus nathusii* (N = 26), and *Plecotus auritus* (N = 25) species in Finland (Fig. 1) from 2021 and 2022, and *Eptesicus nilssonii* (N = 1) and *Pipistrellus nathusii* (N = 91) in Latvia in 2020. Bats were captured using the trapping method (i.e., harp traps, mist nets, or hand capture) appropriate for the roost site. Bats were morphologically identified to species in the field, using taxonomic keys when necessary. Each bat was held in a paper bag for 10 min, or until a faecal sample was recovered, and then released. An additional six fecal samples were collected from underneath a bat box with a known *Pipistrellus nathusii* colony. All faecal samples were stored in RNAlater (QIAGEN, Hilden, Germany) and kept in −80°C until processed.

Samples were briefly centrifuged after thawing to room temperature to collect the pellet and to allow for removal of RNAlater without losing any sample material. Samples were washed with 500 µl of 0.5 M ethylenediaminetetraacetic acid (EDTA). Samples were incubated for 1 h, and centrifuged for 1 min at 12,000 × g. Following removal of the supernatant, 400 µl of PBS were added. The tubes were placed into a Tissuelyser for 3 min at 30 Hz.

RNA was extracted from 200 µl of homogenized faecal sample using the QIAamp 96 Virus QIAcube HT Kit (QIAGEN, Hilden, Germany) following the manufacturer's protocol. Faecal samples were processed using onboard lysis, which is recommended when chemical lysis is performed by the QIAcube robot.

2.2. Rat and shrew samples

Rat tissue samples (N = 158) were obtained from a subset of a previously described and used carcass sample set (Aivelo et al., 2024). A description of rat samples is listed in Supplementary File 1. Rats were collected between 2019 and 2023 from Helsinki, Finland (Fig. 1) by pest-control operators using multi-catch electric kill traps. The carcasses were initially stored at −20°C. After dissection, tissue samples were stored at −80°C. The rats did not present obvious signs of illnesses based on external observation.

Shrew samples (N = 238) were collected between 2024 and 2025 from various locations across Finland (Fig. 1). Shrews were caught as part of the national small mammal monitoring program. The caught animals were stored at −20°C. After dissection, tissue samples were stored at −80°C.

Rat kidney tissues and shrew lung and heart tissues were homogenized using MagNA Lyser, RNA was extracted with Invitrogen TRIzol (Thermo Fisher Scientific, Waltham, Massachusetts, USA).

2.3. Real-time RT-qPCR

Initial detection of jeilong- and parajeilongviruses was conducted using a new pan-jeilong-RT-qPCR. The primers and probes were designed by aligning full L gene sequences of jeilong- and parajeilongvirus from the National Center for Biotechnology Information (NCBI) databank with ClustalW (Larkin et al., 2007). A set of four forward primers, four reverse primers, and three probes (Table 1) were designed to detect a 100-bp long region from the L gene encoding RNA-dependent RNA polymerase by using Primer3-software (Untergasser et al., 2012) as an initial tool for designing, followed by manual modifications based on the alignment of different jeilong- and parajeilongvirus sequences listed in the Supplementary Table 5.

Pan-jeilong-RT-qPCRs were conducted using an AriaMx Real-Time PCR system and QuantStudio5 Real-Time PCR System. The PCR reaction consisted of 1 × TaqMan™ Fast Virus 1-Step Multiplex Master Mix (Applied Biosystems, Thermofisher scientific), 1 µl of template RNA, 0.4 µM of each primer, 0.2 µM of each probe and PCR-grade water for a final volume of 20 µl. PCR cycles included 15 min of reverse transcriptase at 50 °C, RT inactivation at 95°C for 20 s, and 45 cycles of denaturation and annealing at 95°C for 15 s and 60°C for 60 s.

Table 1
List of primers and probes in pan-jeilong RT-qPCR assay and their corresponding sequence in the genome of J-virus (AY900001.1).

Primer / probe	Sequence	Corresponding sequence (nt)
JEF4.1	5' -TTTATGCCWTTRACWCTAGATGA- 3'	13608–13630
JEF4.2	5' -TTTATGTCCTTSACAYTRGACGA- 3'	13608–13630
JEF5.1	5' -TTCATGCCTYTRACWCTGGATGA- 3'	13608–13630
JEF5.2	5' -TTTATGCCTCTTRCWCTWGATGA- 3'	13608–13630
JER3.1	5' -TCWCTAGRATAVACIGAGTCCCA- 3'	13683–13705
JER3.2	5' -TCHYTAGGATAHACAGAATCCCA - 3'	13683–13705
JER4.1	5' -TCTYTIGGGTAAACWGAATCCCA - 3'	13683–13705
JER4.2	5' -TAATCAGGRTAIRCTGAATCCCA - 3'	13683–13705
JEP4	Hex 5' - TGTAYATGAAAGAYAAAGCWT – 3' MGB-Eclipse	13642–13662
JEP5	Hex 5' -TGTATATGAARGAYAAAGCWC- 3' MGB-Eclipse	13642–13662
JEP6	Hex 5' -ATGAARGAYAAAGCWT- 3' MGB-Eclipse	13647–13662

2.4. Transcription to cDNA

A total of 20 rat tissue samples were positive with pan-jeilong-RT-qPCR. From these samples, an additional reverse transcription (RT) reaction from sample RNA to cDNA was conducted using a RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific). The RT reaction consisted of 4 µl of 5 × reaction buffer, 1 µl of random hexamer primer, 2 µl of dNTP mix (10 mM each), 1 µl of RiboLock RNase Inhibitor (20 U/µl), 1 µl of RevertAid RT (200 U/µl), 6 µl of PCR-grade water, and 5 µl of template RNA. The reaction was incubated at 25°C for 5 min for primer annealing, followed by RT at 60°C for 60 min, and enzyme inactivation at 70°C for 5 min.

2.5. Conventional PCRs for sequencing

A conventional PCR was conducted for the converted cDNAs of the pan-jeilong-RT-qPCR positive rat samples as a confirmatory tool to create a 300-bp PCR-product for Sanger sequencing. The protocol used forward primers from the pan-jeilon-RT-qPCR with a new reverse primer to achieve a longer PCR product. The first PCR cycle consisted of 1 × AllTaq Master Mix (Qiagen), 0.4 µM of PAN-JEF4 primers and PAN-JEF5 primers, 1 µM of JER-nested reverse primer (5' -TGCYCTCA-TYTTRTAWGTCAT- 3'), 2 µl of cDNA template, and PCR-grade water to a final volume of 25 µl. The PCR thermal cycling included hot start at 95°C for 2 min followed by two different 20-cycled amplification cycles; the first was conducted with denaturation at 95°C for 15 s, annealing at 40°C for 30 s, and extension at 72°C for 1 min with an increase in the annealing temperature by 0.5°C every cycle. The second amplification cycle was identical with a fixed annealing temperature at 50°C.

A second-round PCR (nested-PCR) was performed to amplify the PCR product yield. The nested-PCR consisted of the same PCR reaction conditions as described above with 2 µl of the first-round PCR product instead of cDNA template. The thermal cycling conditions were the same, except the annealing temperature was fixed at 53°C for the last 20 cycles of amplification. The nested-PCR products were run on a 2% agarose gel to visualize the products, which were sent to Sanger sequencing.

Sanger sequencing revealed beilong viruses in our samples. To detect which of the pan-jeilong-RT-qPCR positive samples contained beilong virus nucleic acids, a beilong virus-specific conventional PCR (beilong-PCR) was designed and performed. The beilong-PCR reaction consisted of 1 × AllTaq Master Mix (Qiagen), 1 µM BEI-F primer (5' -TTTATGC-CACTAACTCTTGAT- 3'), 1 µM BEI-R primer (5' -TGCCCTCATCTTG-TAAGTCAT- 3'), 2 µl of cDNA as a template, and PCR-grade water to reach a final volume of 25 µl. The thermal cycling consisted of hot start

at 95°C for 2 min followed by 40 cycles of denaturation at 95°C for 5 s, annealing at 45°C for 15 s and extension at 72°C for 10 s. The beilong-PCR products were visualized by running them in 2% agarose gels. In total, 10 samples showed amplification of a 300-bp beilong-PCR product, which were sent to Sanger sequencing.

2.6. Plasmid controls

For the setup and validation of pan-jeilong-RT-qPCR, as well as for use as positive controls during the screenings, we used commercial plasmid constructs from Integrated DNA Technologies (IDT) with partial genomes of some jeilong- and parajeilongviruses (Table 2). To determine analytical sensitivity and repeatability of a pan-jeilong-RT-qPCR, a limit of detection (LOD), efficiency and intra-assay repeatability was conducted for J-virus using quantified plasmid control (minimum of 10 parallel reactions). A negative panel of samples was also tested to determine the specificity (Table 3).

2.7. Sequencing

Sanger sequencing of the 300-bp PCR products (nested-PCR and beilong-PCR) was conducted by BIDGEN (Institute of Biotechnology, Helsinki Institute of Life Science, University of Helsinki) using an Applied Biosystems BigDye Terminator v3.1 Cycle Sequencing Kit (Part No. 4336921) with Applied Biosystems ABI3130XL Genetic Analyzer (16-capillaries).

For NGS sequencing, RNA sequencing libraries were prepared and sequenced by Novogene Europe (Novogene Co., Ltd.) using a NovaSeq X platform with paired-end 150-bp reads.

The raw sequence data were quality-filtered with fastp, and host-specific sequence reads were removed by assembling the reads against human (*Homo sapiens*), mouse (*Mus musculus*), and rat (*Rattus norvegicus*) reference genomes (accession numbers GCF_000001405.40, GCF_000001635.27, and GCF_036323735.1, respectively) using BWA-MEM. Thereafter, the host-filtered reads were de novo assembled with Megahit, followed by homology search using SANSparallel and DIAMONDp against UniProt/TrEMB database implemented in the LazyPipe3 pipeline (Weinstein et al., 2025).

2.8. Phylogenetic analysis

For the nearly whole-genome sequence data of beilong virus, a similarity plot analysis was performed using SimPlot++ (Samson et al., 2022). The analysis was performed using a Jukes-Cantor model with 200-bp window length and 20-bp steps.

For the construction of phylogenetic trees, sequences derived from NCBI databank were aligned with ClustalW (Larkin et al., 2007). Maximum-likelihood trees were constructed by finding the best fitting model with IQTREE ModelFinder (Kalyaanamoorthy et al., 2017) and built with IQTREE (Quang et al, n.d) with 1000 bootstrap steps. Trees were visualized using iTOL (Letunic and Bork, 2024).

Table 3
Cross-reactivity test, a 10-fold dilution series of J-virus plasmid and other plasmid controls of different jeilong- and parajeilongviruses with the pan-jeilong-RT-qPCR.

Sample	Result (average Ct; parallel reactions)
J-virus plasmid $1/10^2$ -dilution	pos (20.1)
J-virus plasmid $1/10^3$ -dilution	pos (23.4)
J-virus plasmid $1/10^4$ -dilution	pos (25.4)
J-virus plasmid $1/10^5$ -dilution	pos (29.9)
J-virus plasmid $1/10^6$ -dilution	pos (33.1)
J-virus plasmid $1/10^7$ -dilution	pos (37.1)
J-virus plasmid $1/10^8$ -dilution	neg (9/10 No Ct; one pos 39.8)
Tailam virus plasmid $1/10^3$	pos (28.1)
Bat paramyxovirus plasmid $1/10^3$	pos (32.6)
Shaan virus plasmid $1/10^3$	pos (30.6)
Wenzhou Myotis laniger paramyxovirus plasmid	pos (26.3)
Morbillivirus canis RNA	neg (No Ct)
Morbillivirus hominis RNA	neg (No Ct)
Henipavirus hendraense RNA	neg (No Ct)
Henipavirus nipahense RNA	neg (No Ct)
Orthohantavirus puumalaense RNA	neg (No Ct)
LCMV RNA	neg (No Ct)

Results with Ct-value < 40 are considered positive.

2.9. Statistical test of virus infection in rats

To test whether virus infections in rats are related to sex and age of the rats, and whether the probability of infection prevalence varied by year and season, a set of generalized linear mixed models with virus infection status (infected/not infected) as response variable were conducted. Four virus negative juveniles were excluded from the analysis, as their sex was not determined at the time of sampling. Thus, the final analysis included 154 rats, of which 20 were infected. In the first model, sex (female/male) and maturity status (juvenile/mature) were used as explanatory fixed effects. Sampling year and location were included as random factors to account for possible spatial and temporal dependency. To test differences among years, a second set of models with year as fixed factor and location as random factor were fitted. A separate model was fitted with each year set as the reference (intercept) to derive pairwise contrasts. Finally, a third set of models where four quarters of the calendar year (January-March, April-June, July-September, October-December) were used as a fixed factor and location as a random effect were fitted. A separate model was fitted with each quarter as the reference (intercept) to obtain pairwise comparisons between the quarters. To obtain a posterior probability distribution with 1000 samples, we ran four independent Markov chain Monte Carlo (MCMC) chains for 1 875 000 iterations. The first 625 000 samples were

Table 2
Positive and negative controls for setup and validation of the pan-jeilong-RT-qPCR and conventional PCR protocol.

Name	NCBI GenBank code	Sample type	Area from the viral genome	Origin	control type
Jeilongvirus queenslandense (J-virus)	AY900001.1	Plasmid	13512–14112	IDT	positive
Jeilongvirus tailamense (Tailam virus)	NC_025355.1	Plasmid	13587–14187	IDT	positive
Parajeilongvirus hainanense (Bat paramyxovirus)	OR439358.1	Plasmid	1250–1750[a]	IDT	positive
Parajeilongvirus miniopteri (Shaan virus)	MG230624.1	Plasmid	12320–12861	IDT	positive
Parajeilongvirus zhejiangense (Wenzhou Myotis laniger paramyxovirus)	OM030336.1	Plasmid	10550–11049	IDT	positive
Henipavirus hendranense (Hendra virus)		RNA		CSIRO	negative
Henipavirus nipahense (Nipah virus)		RNA		CSIRO	negative
LCMV		RNA		UH	negative
Morbillivirus canis (Canine distemper virus)		RNA		UH	negative
Morbillivirus hominis (Measles)		RNA		UH	negative
Orthohantavirus puumalaense (Puumalavirus)		RNA		UH	negative

[a] Nucleotide location of the L-gene of the virus, only a partial genome sequence is available at the NCBI GeneBank.
CSIRO = Commonwealth Scientific and Industrial Research Organisation, Australia, IDT = Integrated DNA Technologies, LCMV = Lymphocytic choriomeningitis virus, UH = University of Helsinki

discarded as transit, remaining samples were thinned by 5000, and the last 250 samples were saved. Models were deemed converged when visual inspection showed proper mixing of the chains. Statistical analyses were performed in R (version 4.5.1) using a Bayesian interphase with package hmsc (version 1.1.13.) (Ovaskainen and Abrego, 2020).

3. Results

3.1. Validation of the pan-jeilong-RT-qPCR

The pan-jeilong-RT-qPCR was validated using a 10-fold dilution series of different plasmid controls and a panel of negative-control RNAs (Table 3). All jeilong- and parajeilongvirus plasmid constructs showed clear curves and detection on RT-qPCR; this was not observed with the negative panel (Table 3; 6 out of 6, 100%). The calculated analytical sensitivity with J-virus plasmid was estimated with SPSS Probit 30.0.0.0 (IBM). With 95% confidence interval, the limit of detection was 387 copies per PCR reaction with a range of variation of 278–1513. At copy level of 1167 per PCR reaction (10 parallel reactions), the intra-assay repeatability was average of 37.1 Ct, standard deviation (STDEV) of 0.51 and coefficient variation (CV) percent of 1.4. The efficiency of the RT-qPCR (standard curve) was 97% with slope value of −3.39 and coefficient correlation R^2 of 0.99 (Fig. 3).

The sensitivity of the pan-jeilong-RT-qPCR and the confirmatory conventional PCR were compared by using a 10-fold dilution series of J-virus plasmid. RT-qPCR detected the J-virus plasmid still at 1167 copies per PCR reaction (10^{-7}), while the conventional nested PCR showed clear amplification visualized on agarose gel at 11670 copies per PCR reaction (Fig. 2). The sensitivity of beilong-PCR was not tested, as this was designed based on our findings and is specific for beilong virus (*Jeilongvirus beilongi*); our controls were not suitable for the assessment.

3.2. Jeilongvirus screening

Of the 158 rat kidney samples screened, 20 tested positive with the pan-jeilong-RT-qPCR (Table 4, Fig. 1). Nineteen of these samples had Ct-values < 40, ranging from 29.76 to 39.4, while one sample had a Ct-value of 41.16 (Supplementary Table 4). To test jeilongvirus positivity in other tissue materials from positive rats, 16 corresponding liver samples were also screened: all tested negative with the pan-jeilong-RT-qPCR.

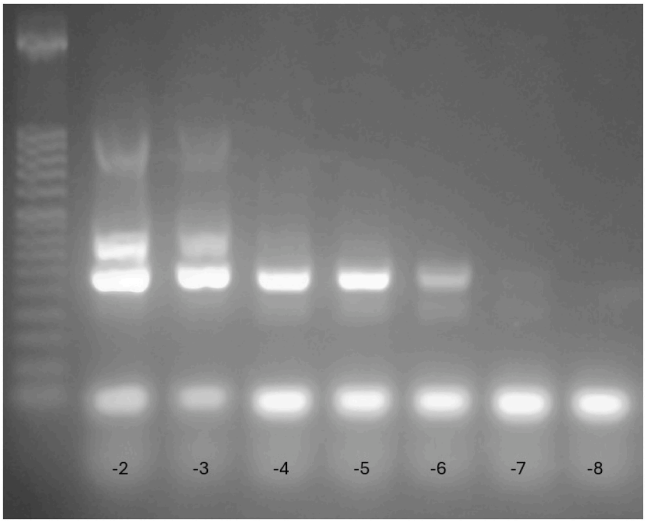

Fig. 2. 10-fold dilution series of J-virus plasmid amplified with conventional nested PCR with 50-bp DNA ladder. The $1/10^6$ dilution at copy number of 11670 shows a clear amplification. The fade bands at the $1/10^7$ dilution are also visible (copy number 1167), but this would not be considered as positive.

Table 4
Description of rat samples and jeilongvirus positivity with pan-jeilong-RT-qPCR. The proportion of positive samples is given with 95% confidence intervals.

Characteristics	Tested positive/all	% with 95% CI
Sex		
female	11/66	16.7 (9.6–27.4)
male	9/88	10.2 (5.5–18.3)
unknown	0/4	0 (0.0–49.0)
Maturity		
juvenile	5/86	5.8 (2.5–12.9))
adult	15/72	20.8 (13.1–31.6)
gravid	3/7	42.9 (15.8–75.0)
lactating	1/1	100.0 (20.6–100.0)
Year		
2019	0/27	0.0 (0.0–12.5)
2020	3/30	10. 0 (3.5–25.6)
2021	5/40	12.5 (5.5–26.1)
2022	6/14	42.9 (21.4–67.4)
2023	6/47	12.8 (6.0–25.2)
Collection period		
January-March	7/59	11.9 (5.9–22.5)
April-June	10/48	20.8 (11.7–34.3)
July-September	2/18	11.1 (3.1–32.8)
October-December	1/33	3.0 (0.5–15.3)
Total	20/158	12.7 (8.4–18.7)

As a confirmatory test for the pan-jeilong RT-qPCR positive samples, a conventional pan-jeilong nested-PCR was performed. This nested-PCR was designed to amplify a longer fragment from the same genomic region using an additional reverse primer. It specifically targets different jeilongvirus species, as the positive findings were detected in rat samples and not in bat samples. From analysed positive samples, 8 showed clearly visible amplification on agarose gel, from which 2 were successfully Sanger sequenced. Based on the BLAST annotation (NCBI), the sequences showed high similarity with known beilong virus (*Jeilongvirus beilongi*) sequences. Therefore, we designed specific primers targeting beilong virus only and conducted a conventional beilong-PCR for all positive samples. Of these, 10 showed clear amplification on agarose gels and all were Sanger sequenced. All sequences had high similarities (89.77–98.44%) with existing beilong virus sequences in the NCBI nucleotide databank. This beilong-PCR also confirmed the positivity of one sample with high Ct-value (41.16). We were unable to retrieve any sequences for 10 pan-jeilong-RT-qPCR positive samples.

All shrew lung and heart samples (N = 238) and bat faecal samples (N = 194) were negative with the pan-jeilong-RT-qPCR.

3.3. NGS and phylogenetic analysis

To retrieve whole-genome sequences, NGS with NovaSeq X platform (Novogene Europe) with a non-targeted metagenomic approach was performed for 4 pan-jeilong-RT-qPCR positive RNA samples (BEI-23, BEI-27, BEI-82 and BEI-129). The samples were individually selected from the positives based on their low Ct-values on qPCR and strong amplification with conventional pan-jeilong-PCR. However, due to the high level of RNA fragmentation in our samples, sequencing was successful for only one of the samples (BEI-23). For this sample, data analysis revealed a nearly full beilong virus sequence (19 282 nucleotides). The sequence showed high similarities (96.59%) with other beilong virus strains based on BLAST annotation (NCBI). A similarity plot was generated comparing two other beilong virus sequences (OK623358.1, OQ715595.1) and *Jeilongvirus oujiangense* (MZ328277) sequences against a reference beilong virus sequence (DQ100461.1) (Fig. 4). The analyses confirmed the high similarity with other beilong virus strains. Our beilong virus strain had all known open reading frames (ORFs) typical for beilong virus (Fig. 4). The maximum-likelihood phylogenetic tree (Fig. 5) also demonstrated close genetic relationship with other beilong virus strains for the BEI-23 strain, with short branches and lack of subclustering. All shorter sequences (274 bp) from

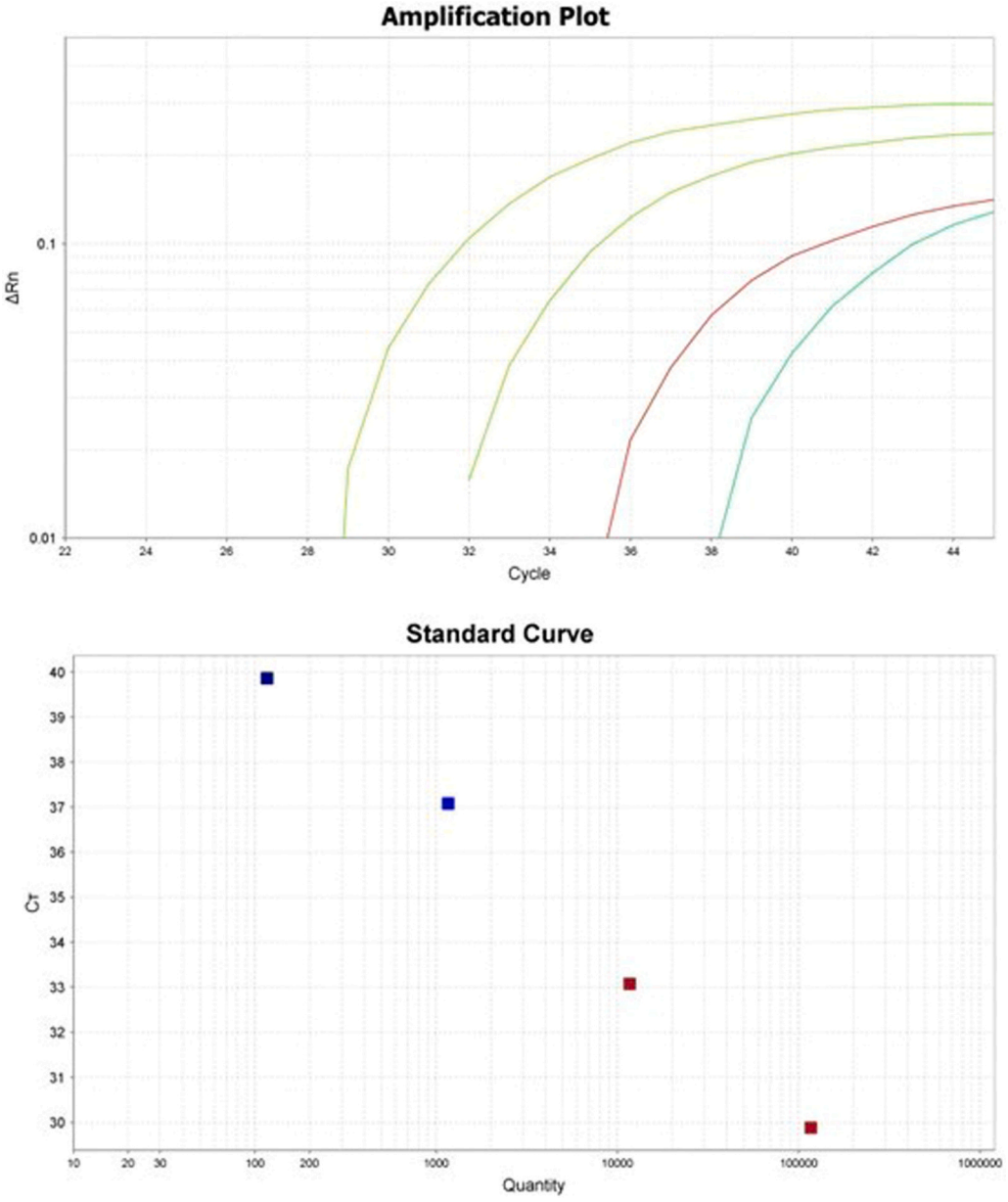

Fig. 3. Examples of amplification plot and standard curve from Quantstudio Desing and Analyses Software v1.6.1. Quantitative values of standard curve are 116.7, 1167, 11670, and 116700 copies per PCR reaction.

beilong-PCR distributed together with other beilong virus strains (Fig. 5).

In addition to beilong virus, our NGS approach retrieved sequences of rat hepatitis C, rat parvovirus, pegivirus, and fesa-like virus from the BEI-23 rat kidney sample. A short description of these sequences is provided in Supplementary Table 2.

3.4. Factors related to the infection in rats

Infection was more often found in mature rats compared to juveniles, whereas there was no difference between sexes (Table 5). Infections were more common in 2022 than in other years, except 2023 (Table 5) based on 95% posterior support. Across quarters, there was lower infection prevalence from October to December than from January to March (posterior mean −1.06, 95% [-2.1, −0.14], Supplementary table 3), but there was no posterior support for differences between other quarter pairs (Supplementary table 3).

4. Discussion

To make PMV screening studies more efficient and targeted to the jeilong- and parajeilongvirus groups, we designed and validated a new pan-jeilong-RT-qPCR assay. This real-time PCR is time efficient, as it eliminates the need for multiple PCR cycles and gel electrophoresis and produces results that are easy to interpret. Therefore, it can be used to screen a large number of samples. Once positive samples are detected with RT-qPCR, either NGS or conventional PCR reactions can be used to achieve longer PCR products for Sanger sequencing. However, it is notable that when using a probe-based real-time PCR assay, the sensitivity is restricted to the sufficient probe binding with the target sequence. As a pan-assay, the LOD for this assay is higher than what it would be with specific qPCR with a single target. Therefore, this method is useful when screening samples with high viral load, and possible complex matrixes with PCR inhibitors may affect the results. To further test this new pan-jeilong-RT-qPCR method, we screened wildlife samples from Finland and Latvia.

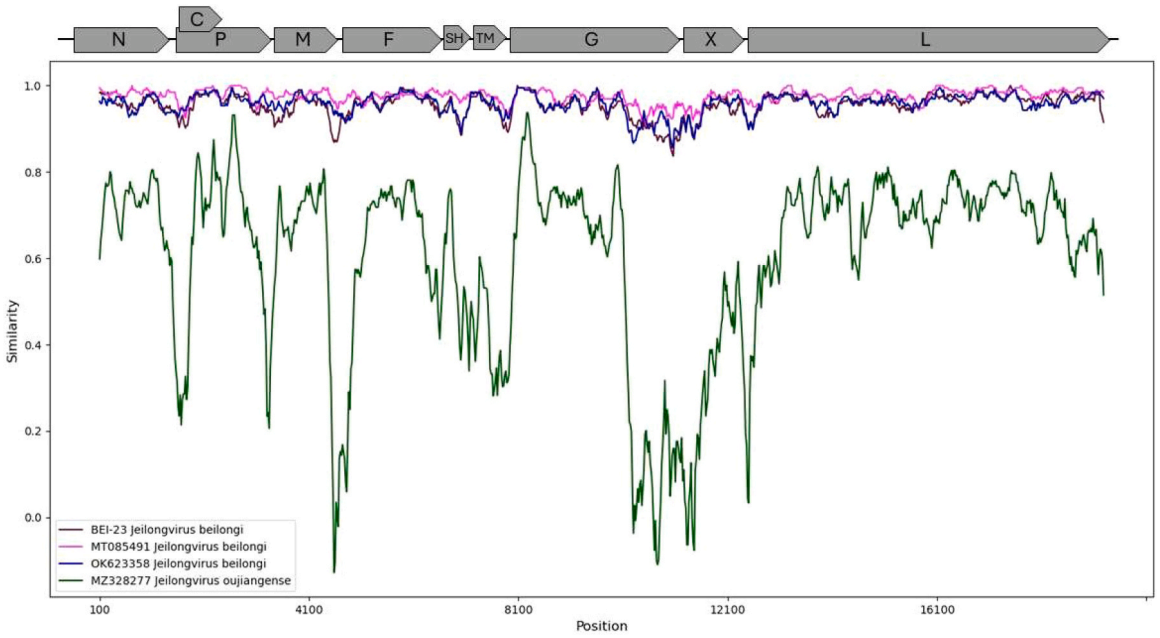

Fig. 4. SimPlot analysis of beilong virus strains BEI-23, OK623358.1 and OQ715595.1, and *Jeilongvirus oujiangense* MZ328277 against the reference beilong virus strain DQ100461.1 and the open reading frame structure of BEI-23 strain. The similarity plot analysis was performed with SimPlot+ + (Samson et al., 2022) using a Jukes-cantor model with 200-bp window and 20-bp steps.

Our screening revealed a continuous circulation of jeilongviruses, including beilong viruses (genus *Jeilongvirus*), in urban rat colonies in Helsinki. We detected the presence of jeilongviruses among urban rat populations between 2020 and 2023, with a 13% (20/158) positivity rate. From these, half (N = 10) were confirmed beilong virus-positive, while for the other half we were not able to retrieve sequence data. The lacking sequence data for some of the positive samples could have been caused by the RNA fragmentation in our samples and the sensitivity differences between the RT-qPCR and nested-PCR. For half of the positive samples without sequence data, the obtained Ct-values were 37.98 or over, which might result in difficulties with conventional PCRs and NGS (Supplementary Table 4). The observed distribution of jeilongvirus in rats suggests that the virus spreads across urban habitats in Helsinki, with a higher prevalence among mature individuals than in juveniles. The higher prevalence among adults may be due to chronic or persistent infection, which is common among other rodent-borne viruses (Bernshtein et al., 1999). Interestingly, the prevalence of jeilongviruses among pregnant (3/7) and lactating (1/1) rats were double compared to other adult rats, which might suggest immunosuppression and physiological stress during the pregnancy. This could possibly lead to an activation of a latent infection, further on virus secretion and perinatal transmission. However, as the sampling material was not produced systematically, and the numbers of pregnant animals in our sample collection are relatively low, this hypothesis should be addressed in further research. Because the sampling strategy relied on pest control, the sampling was more opportunistic than systematic. The sampling efforts focused on locations with high rat densities. As rats are neophobic and capable of learning to avoid previously used traps, the trapped animals may not fully represent the population structure. Additionally, the size limitation of the tunnel of the smart box kill-traps might also favour the capture of smaller, juvenile individuals. These factors may introduce bias in the rat sample material, limiting the ability to draw conclusions about jeilongvirus circulation across the rat population in the Helsinki area. Thus, while our results show ongoing circulation across the city, it does not allow for estimation of virus prevalence.

Phylogenetic analysis of the obtained complete coding sequence beilong virus sequence revealed high similarity with beilong virus strains found in brown rats from Belgium and China (Fig. 4, Fig. 6) and in Asian house rats (*Rattus tanezumi*) and striped field mice (*Apodemus agrarius*) from China (Fig. 6). The shorter sequences from all beilong-positive samples cluster with other known beilong virus strains (Fig. 5). The close genomic resemblance of the Finnish beilong virus sequence to the sequences from Belgium and China could implicate a global distribution of beilong virus via brown rats as an invasive species.

As a limitation of this study, all sample materials varied (i.e., faeces, lungs, heart, and kidney tissues) between species. In addition, different sample types may act differently in PCRs due to the, i.e. inhibitors, resulting in false negative results. This is a limitation of this study as different sample types have not been possible to be validated before PCR screening. Because samples from bats and shrews were collected retrospectively from sample archives, we did not have any kidney tissue samples available from these animals. In the future, kidney tissue samples should be studied to obtain data about the positivity for jeilong- and parajeilongviruses, as it has been observed an optimal tissue material for paramyxovirus screenings (Haring et al., 2024). In addition, the negative results of bat faecal samples might be caused due to methodological limitations, as a complex matrix type can affect to the sensitivity of the pan-jeilong-RT-qPCR. To date, detection of parajeilongviruses in Finland or Lativa have not been recorded, therefore we cannot discriminate between methodological limitations and prevalence of parajeilongviruses in the region to be the cause of our negative screening result.

Bats harbour many different PMVs (Drexler et al., 2012; Maganga et al., 2014; Kurth et al., 2012), but as new PMVs are being recognized, it is clear that rodents and shrews are also notable hosts for these viruses (Horemans et al., 2023a; Ch'ng et al., 2023; Horemans et al., 2023b; Mortlock et al., 2025b; Moonga et al., 2024b; Gan et al., 2024b; Xu et al., 2024b). Although transmission routes of jeilong- and parajeilongviruses have not been studied extensively, these viruses have been detected especially in kidneys (Vanmechelen et al., 2022, 2018; Natasha et al., 2024; DeRuyter et al., 2024b; Haring et al., 2024; Xu et al., 2024b), in addition to other organs like spleen, lungs and liver (DeRuyter et al., 2024b; Moonga et al., 2024b; Gan et al., 2024b). Zoonotic PMVs, like Hendra virus and Nipah virus, are secreted in the urine and saliva of fruit bats (*Pteropus* spp.) (Chua et al., 2002; Reynes et al., 2005;

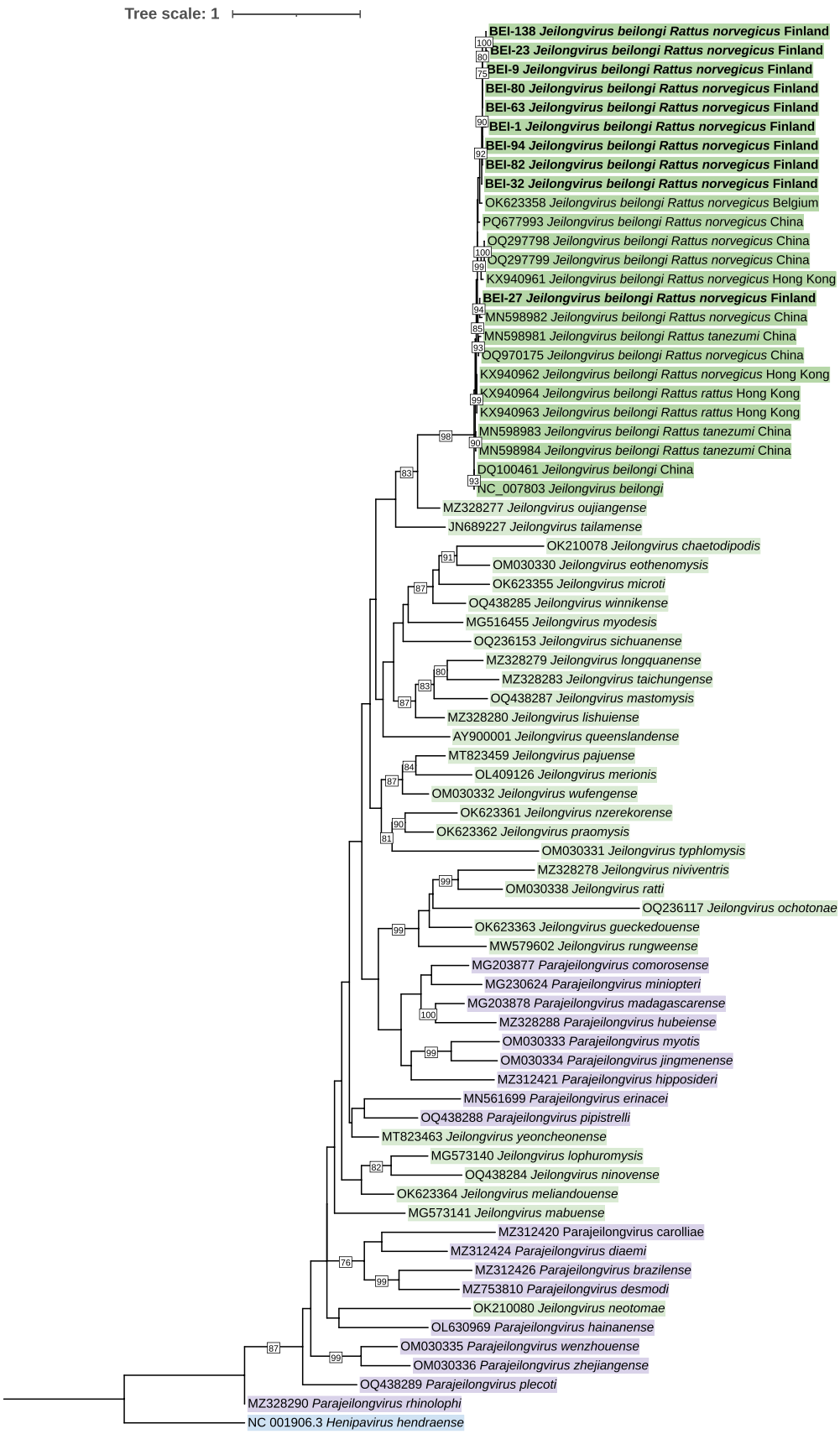

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Fig. 5. A maximum-likelihood tree of detected beilong virus strains (dark green, bold), other beilong virus strains from BLAST annotation (dark green), and official jeilongvirus (light green) and parajeilongvirus (lilac) strains from ICTV taxonomy. The tree is rooted with *Henipavirus hendraense* sequence (blue). The tree was constructed using 274-bp sequences. The best fitting model was estimated by using IQTREE ModelFinder (Kalyaanamoorthy et al., 2017) and constructed with IQTREE (Minh et al., 2020) using GTR+F+I+G4 model with 1000 bootstrap replicates and visualized with iTOL (Letunic and Bork, 2024). Bootstrap values ≥ 75 are shown.

Table 5
Regression coefficients for models testing whether infections were related to rat sex or maturity and varied by years. Posterior mean estimates with statistical support based on the 95% central credible interval are bolded.

Model	Parameter	Posterior mean	95% Credible interval
The effect of sex and maturity	Intercept	-1,48	[-2.24, −0.79]
	Sex: Male	-0,27	[-0.8, 0.27]
	Maturity: Mature	**0,83**	**[0.19, 1.49]**
Comparing years to 2019	Intercept	-1,74	[-2.68, −1]
	2020	0,32	[-0.71, 1.36]
	2021	0,50	[-0.5, 1.52]
	2022	**1,43**	**[0.46, 2.59]**
	2023	0,63	[-0.4, 1.66]
Comparing years to 2020	Intercept	-1,29	[-1.91, −0.73]
	2019	**-1,48**	**[-3.4, −0.07]**
	2021	0,07	[-0.72, 0.83]
	2022	**1,01**	**[0.15, 1.97]**
	2023	0,19	[-0.61, 0.98]
Comparing years to 2021	Intercept	-1,17	[-1.75, −0.66]
	2019	**-1,51**	**[-3.55, −0.24]**
	2020	-0,22	[-1.11, 0.6]
	2022	**0,93**	**[0.04, 1.81]**
	2023	0,08	[-0.67, 0.83]
Comparing years to 2022	Intercept	-0,49	[-1.22, 0.18]
	2019	**-1,99**	**[-4.15, −0.57]**
	2020	-0,86	[-1.79, 0.09]
	2021	-0,69	[-1.51, 0.19]
	2023	-0,58	[-1.41, 0.35]
Comparing years to 2023	Intercept	-1,09	[-1.63, −0.62]
	2019	**-1,52**	**[-3.46, −0.16]**
	2020	-0,29	[-1.13, 0.5]
	2021	-0,13	[-0.85, 0.55]
	2022	0,83	[-0.07, 1.68]

Wacharapluesadee et al., 2005; Halpin et al., 2011), which is also the predicted transmission route of these viruses to domesticated animals and humans. Nipah virus spreads from fruit bats to humans via contaminated fruit palm sap, which is infested with either the droppings of the animals or partially eaten fruits contaminated with animal saliva (Yeasmin et al., 2025). This transmission route could resemble the spread of jeilong- and parajeilongviruses, but further research is needed.

The detection of jeilongvirus nucleic acids in the rat kidney tissues suggests that these viruses might be secreted in the urine and thus could enter the environment. In urban settings, encountering rat excrement is more likely than contact with the animal itself, posing a possible transmission route and spillover via faeces and urine. However, further studies are needed to determine whether jeilongvirus is actively secreted in nature and whether the urine is infectious. Similarly, the potential for virus secretion in rat faeces and saliva should be investigated when evaluating the risk of possible spillover to other species. Unfortunately, we did not have these sample materials available for screening purposes in this study. The first discovery of beilong virus cDNA was from human kidney mesangial cell line, however clear indications for infection caused by beilong virus using serology and immunostaining were absent (Li et al., 2006). Another rodent-borne jeilongvirus, Gainesville rodent jeilongvirus 1, has been shown to infect human and non-human primate cells *in vitro* (DeRuyter et al., 2024b), proving its ability to replicate in human cells. Despite the fact that there are no described human clinical diseases caused by jeilongviruses, these findings underline the need for more comprehensive research to better understand the infectivity of these viruses for other species, including humans.

In Helsinki, there has been a growing number of brown rat populations (Aivelo T., *pers.comm.*). These rat populations have previously shown to carry zoonotic pathogens like *Hymenolepis nana*, rat hepatitis E virus, *Campylobacter jejuni*, and *Leptospira interrogans*, even though the prevalence of these pathogens was relatively low compared with other European cities (Aivelo et al., 2024). To our knowledge, similar jeilongvirus screening of urban rat populations has not been conducted elsewhere in Europe, preventing comparisons of jeilongvirus prevalence between Helsinki and other European cities.

In China, beilong viruses have been detected, and thought to be widely spread, in not only rat populations, but also in other rodent species of the *Muridae* family as well as in shrews (Vanmechelen et al., 2022; Gan et al., 2024b; Chen et al., 2020). In Helsinki, rats often share habitats with yellow-necked mice *Apodemus flavicollis*. Because beilong virus has also been detected in *Apodemus* species in addition to other species of *Muridae*, *Cricetidae*, *Sciuridae*, and *Soricidae* (Chen et al., 2020), we hypothesize that mice and shrew populations inhabiting the Helsinki area might also carry beilong virus.

A growing number of new PMVs are being discovered, including jeilong- and parajeilongviruses. The pan-jeilong RT-qPCR described here allows initial detection of these viruses without the need for nested PCR, making it easy to implement in resource-limited settings and suitable for screening large sample collections. Using this method, we detected jeilongvirus circulation in Finnish urban rat populations. To continue our work, we will screen additional rodent and shrew species particularly in Helsinki area, using kidney tissues and excreta to better understand the viral distribution in animals, as well as possible transmission routes via faeces and urine.

CRediT authorship contribution statement

Jääskeläinen Anne J: Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Thomas Lilley:** Writing – review & editing, Resources. **Tarja Sironen:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Nina Suomalainen:** Writing – review & editing. **Tuomas Aivelo:** Writing – review & editing, Visualization, Resources. **Meierhofer Melissa B:** Writing – review & editing, Resources. **Suvi Sallinen:** Writing – review & editing, Resources, Formal analysis, Data curation. **Viktor Zöldi:** Writing – review & editing, Resources. **Heikki Henttonen:** Writing – review & editing, Resources. **Teemu Smura:** Writing – review & editing. **Tuure Kinnunen:** Writing – review & editing, Supervision. **Ella Sippola:** Writing – original draft, Resources. **Otso Huitu:** Writing – review & editing, Resources. **Emilia Pulkkinen:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Elena Sgarabotto:** Writing – review & editing.

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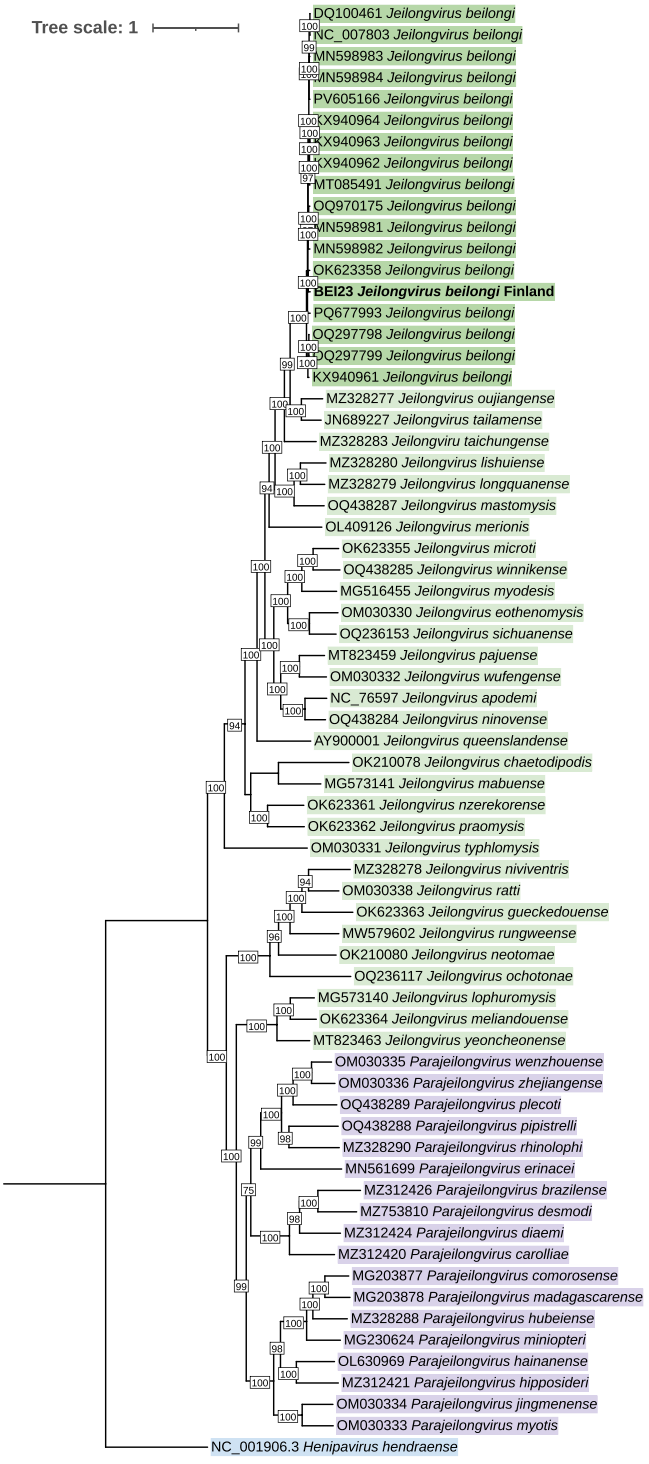

Fig. 6. A maximum-likelihood tree of the whole genome of BEI-23 beilong virus strain (dark green, bold). Other beilong virus strains from NCBI databank are shown in dark green, and official jeilongvirus (light green) and parajeilongvirus (lilac) strains from ICTV taxonomy. The tree is rooted with *Henipavirus hendraense* sequence (blue). The best fitting tree was estimated by using IQTREE ModelFinder and constructed with IQTREE using GTR+F+I+R5 model with 1000 bootstrap replicates and visualized with iTOL (Letunic and Bork, 2024). The tree is midpoint rooted, and bootstrap values ≥ 75 are shown.

collection and analysis, decision to publish, or preparation of the manuscript.

Research was conducted with the following research permit: 159/HUS/151/2022 20-HUS/283/2025 (HUS Diagnostic Center, Helsinki, Finland).

Bat samples were collected under the following permits: UUDELY/3244/2020, PIRELY/2232/2020, VARELY/3985/2021, KASELY/476/2020.

Shrew samples were collected under the following permits: VN/4576/2018, MH 2215/2024, EPOELY/1012/2024. These permits allow for the capture of protected species.

We did not handle live rats. Rats were killed following national best practices in pest management in line with the Finnish Animal Welfare Act and based on the principle of integrated pest management. Rats are an unprotected species according to the Finnish Hunting Act, thus no specific permits are required.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jviromet.2026.115391.

Data Availability

Data will be made available on request.

References

Aivelo, T., Alburkat, H., Suomalainen, N., Kukowski, R., Heikkinen, P., Oksanen, A., et al., 2024. Potentially zoonotic pathogens and parasites in opportunistically sourced urban brown rats (Rattus norvegicus) in and around Helsinki, Finland, 2018–2023. Eur. Surveill. 29. https://doi.org/10.2807/1560-7917.ES.2024.29.40.2400031.

Bernshtein, A.D., Apekina, N.S., Mikhailova, T.V., Myasnikov, Y.A., Khlyap, L.A., Korotkov, Y.S., et al., 1999. Dynamics of Puumala hantavirus infection in naturally infected bank voles (Clethrinomys glareolus). Arch. Virol. 144, 2415–2428. https://doi.org/10.1007/s007050050654.

van Boheemen, S., Bestebroer, T.M., Verhagen, J.H., Osterhaus, A.D.M.E., Pas, S.D., Herfst, S., et al., 2012. A family-wide RT-PCR assay for detection of paramyxoviruses and application to a large-scale surveillance study. PLoS One 7, e34961. https://doi.org/10.1371/JOURNAL.PONE.0034961.

Chen, J.J., Zhang, X.A., Fan, H., Jiang, F.C., Jin, M.Z., Dai, K., et al., 2020. Distribution and characteristics of Beilong virus among wild rodents and shrews in China. Infect. Genet. Evol. 85. https://doi.org/10.1016/j.meegid.2020.104454.

Chua, K.B., Lek Koh, C., Hooi, P.S., Wee, K.F., Khong, J.H., Chua, B.H., et al., 2002. Isolation of Nipah virus from Malaysian Island flying-foxes. Microbes Infect. 4, 145–151. https://doi.org/10.1016/S1286-4579(01)01522-2.

Ch'ng, L., Low, D.H.W., Borthwick, S.A., Zhang, R., Ong, Z.A., Su, Y.C.F., et al., 2023. Evolution and ecology of Jeilongvirus among wild rodents and shrews in Singapore. One Health Outlook 5. https://doi.org/10.1186/S42522-023-00094-1.

Costa-Silva, S., Ewbank, A.C., Duarte-Benvenuto, A., Sacristán, C., Soares, R.M., Sánchez-Sarmiento, A.M., et al., 2025. Novel paramyxovirus in wild pinnipeds, Brazil. Vet. Res Commun. 49, 1–7. https://doi.org/10.1007/S11259-025-10799-5/FIGURES/1.

DeRuyter, E., Subramaniam, K., Wisely, S.M., Morris, J.G., Lednicky, J.A., 2024a. A novel Jeilongvirus from Florida, USA, has a broad host cell tropism including human and

non-human primate cells. Pathogens 13. https://doi.org/10.3390/PATHOGENS13100831.
DeRuyter, E., Subramaniam, K., Wisely, S.M., Morris, J.G., Lednicky, J.A., 2024b. A Novel Jeilongvirus from Florida, USA, has a broad host cell tropism including human and non-human primate cells. Pathogens 13. https://doi.org/10.3390/PATHOGENS13100831.
Drexler, J.F., Corman, V.M., Müller, M.A., Maganga, G.D., Vallo, P., Binger, T., et al., 2012. Bats host major mammalian paramyxoviruses. Nat. Commun. 3. https://doi.org/10.1038/NCOMMS1796.
Eaton, B.T., Broder, C.C., Middleton, D., Wang, L.F., 2006. Hendra and Nipah viruses: different and dangerous. Nat. Rev. Microbiol. 4, 23–35. https://doi.org/10.1038/NRMICRO1323.
Gan, M., Hu, B., Ding, Q., Zhang, N., Wei, J., Nie, T., et al., 2024a. Discovery and characterization of novel jeilongviruses in wild rodents from Hubei, China. Virol. J. 21. https://doi.org/10.1186/S12985-024-02417-8.
Gan, M., Hu, B., Ding, Q., Zhang, N., Wei, J., Nie, T., et al., 2024b. Discovery and characterization of novel jeilongviruses in wild rodents from Hubei, China. Virol. J. 21, 146. https://doi.org/10.1186/s12985-024-02417-8.
Halpin, K., Hyatt, A.D., Fogarty, R., Middleton, D., Bingham, J., Epstein, J.H., et al., 2011. Pteropid bats are confirmed as the reservoir hosts of henipaviruses: a comprehensive experimental study of virus transmission. Am. J. Trop. Med. Hyg. 85, 946–951. https://doi.org/10.4269/AJTMH.2011.10-0567.
Haring, V.C., Litz, B., Jacob, J., Brecht, M., Bauswein, M., Sehl-Ewert, J., et al., 2024. Detection of novel orthoparamyxoviruses, orthonairoviruses and an orthohepevirus in European white-toothed shrews. Micro Genom. 10, 001275. https://doi.org/10.1099/MGEN.0.001275.
Horemans, M., Van Bets, J., Joly Maes, T., Maes, P., Vanmechelen, B., 2023b. Discovery and genome characterization of six new orthoparamyxoviruses in small Belgian mammals. Virus Evol. 9. https://doi.org/10.1093/VE/VEAD065.
Horemans, M., Van Bets, J., Maes, J., Maes, P., Vanmechelen, B., 2023a. Discovery and genome characterization of six new orthoparamyxoviruses in small Belgian mammals. Virus Evol. https://doi.org/10.1093/ve/vead065.
Jang, S.S., Noh, J.Y., Lo, V.T., Choi, Y.G., Yoon, S.W., Jeong, D.G., et al., 2020. The epidemiological characteristics of the Korean Bat Paramyxovirus between 2016 and 2019, 2020;8:844 Microorganisms 8, 844. https://doi.org/10.3390/microorganisms8060844.
Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K.F., Von Haeseler, A., Jermiin, L.S., 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. Nat. Methods 14, 587. https://doi.org/10.1038/NMETH.4285.
Kitchen, A., Shackelton, L.A., Holmes, E.C., 2011. Family level phylogenies reveal modes of macroevolution in RNA viruses. Proc. Natl. Acad. Sci. USA 108, 238–243. https://doi.org/10.1073/PNAS.1011090108.
Kurth, A., Kohl, C., Brinkmann, A., Ebinger, A., Harper, J.A., Wang, L.F., et al., 2012. Novel paramyxoviruses in free-ranging European bats. PLoS One 7. https://doi.org/10.1371/journal.pone.0038688.
Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., Mcgettigan, P.A., McWilliam, H., et al., 2007. Clustal W and Clustal X version 2.0. Bioinformatics 23, 2947–2948. https://doi.org/10.1093/BIOINFORMATICS/BTM404.
Letunic, I., Bork, P., 2024. Interactive Tree of Life (iTOL) v6: recent updates to the phylogenetic tree display and annotation tool. Nucleic Acids Res. 52, W78–W82. https://doi.org/10.1093/NAR/GKAE268.
Lindh, E., Huovilainen, A., Rätti, O., Ek-Kommonen, C., Sironen, T., Huhtamo, E., et al., 2008. Orthomyxo-, paramyxo- and flavivirus infections in wild waterfowl in Finland. Virol. J. 5, 35. https://doi.org/10.1186/1743-422X-5-35.
Li, Z., Yu, M., Zhang, H., Magoffin, D.E., Jack, P.J.M., Hyatt, A., et al., 2006. Beilong virus, a novel paramyxovirus with the largest genome of non-segmented negative-stranded RNA viruses. Virology 346, 219–228. https://doi.org/10.1016/J.VIROL.2005.10.039.
Maganga, G.D., Bourgarel, M., Nkoghe, J.O., N'Dilimabaka, N., Drosten, C., Paupy, C., et al., 2014. Identification of an unclassified paramyxovirus in Coleura afra: a potential case of host specificity. PLoS One 9. https://doi.org/10.1371/JOURNAL.PONE.0115588.
Mahalingam, S., Herrero, L.J., Playford, E.G., Spann, K., Herring, B., Rolph, M.S., et al., 2012. Hendra virus: an emerging paramyxovirus in Australia. Lancet Infect. Dis. 12, 799–807. https://doi.org/10.1016/S1473-3099(12)70158-5.
Minh, B.Q., Schmidt, H.A., Chernomor, O., Schrempf, D., Woodhams, M.D., Von Haeseler, A., et al., 2020. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. Mol. Biol. Evol. 37, 1530–1534. https://doi.org/10.1093/MOLBEV/MSAA015.
Moonga, L.C., Chipinga, J., Collins, J.P., Kapoor, V., Saasa, N., Nalubamba, K.S., et al., 2024a. Application of a sensitive capture sequencing approach to reservoir surveillance detects novel viruses in zambian wild rodents. Viruses 16, 1754. https://doi.org/10.3390/V16111754/S1.
Moonga, L.C., Chipinga, J., Collins, J.P., Kapoor, V., Saasa, N., Nalubamba, K.S., et al., 2024b. Application of a sensitive capture sequencing approach to reservoir surveillance detects novel viruses in zambian wild rodents. Viruses 16. https://doi.org/10.3390/V16111754.
Mortlock, M., Geldenhuys, M., Keith, M., Rademan, R., Swanepoel, L.H., Von Maltitz, E. F., et al., 2025a. Paramyxo- and coronavirus diversity and host associations in non-volant small mammals: evidence of viral sharing. Virus Evol. 11. https://doi.org/10.1093/VE/VEAF041.
Mortlock, M., Geldenhuys, M., Keith, M., Rademan, R., Swanepoel, L.H., Von Maltitz, E. F., et al., 2025b. Paramyxo- and coronavirus diversity and host associations in non-volant small mammals: evidence of viral sharing. Virus Evol. 11. https://doi.org/10.1093/VE/VEAF041.
Natasha, A., Pye, S.E., Cho, S.H., Pangestu, H.S., Park, J., Park, K., et al., 2024. Molecular detection and genomic characterization of Samak Micromys paramyxovirus-1 and -2 in Micromys minutus, Republic of Korea. Virol. J. 21, 255. https://doi.org/10.1186/S12985-024-02532-6.
Noh, J.Y., Jeong, D.G., Yoon, S.W., Kim, J.H., Choi, Y.G., Kang, S.Y., et al., 2018. Isolation and characterization of novel bat paramyxovirus B16-40 potentially belonging to the proposed genus Shaanvirus. Sci. Rep. 8. https://doi.org/10.1038/S41598-018-30319-7.
Ovaskainen, O., Abrego, N., 2020. Joint Species Distribution Modelling: With Applications in R. Cambridge University Press, Cambridge. https://doi.org/10.1017/9781108591720.
Pulkkinen, E., Jackson, R., Joensuu, R., Korhonen, E.M., Masika, M.M., Anzala, O., et al., 2026. New parajeilongviruses detected in bats but not in humans: assays for screening and diagnostic purposes, 2026;171 Arch. Virol. 171 (2), 55. https://doi.org/10.1007/s00705-025-06520-1.
Quang B., Schmidt H.A., Chernomor O., Schrempf D., Woodhams M.D., Von Haeseler A., et al. IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era (n.d). https://doi.org/10.1093/molbev/msaa015.
Reynes, J.M., Counor, D., Ong, S., Faure, C., Seng, V., Molia, S., et al., 2005. Nipah virus in lyle's flying foxes, Cambodia - Volume 11, Number 7—July 2005 - emerging infectious diseases journal - CDC. Emerg. Infect. Dis. 11, 1042–1047. https://doi.org/10.3201/EID1107.041350.
Samson, S., Lord, É., Makarenkov, V., 2022. SimPlot++: a python application for representing sequence similarity and detecting recombination. Bioinformatics 38, 3118–3120. https://doi.org/10.1093/BIOINFORMATICS/BTAC287.
Sata, S., Kojima, I., Esaki, M., Funakoshi, K., Kajihara, M., Hirano, S., et al., 2024a. The First Isolation and Characterization of Bat Jeilongviruses in Japan. Transbound. Emerg. Dis. 2024, 5530007. https://doi.org/10.1155/TBED/5530007.
Sata, S., Kojima, I., Esaki, M., Funakoshi, K., Kajihara, M., Hirano, S., et al., 2024b. The First Isolation and Characterization of Bat Jeilongviruses in Japan. Transbound. Emerg. Dis. 2024, 5530007. https://doi.org/10.1155/TBED/5530007.
Su, H., Wang, Y., Han, Y., Jin, Q., Yang, F., Wu, Z., 2023. Discovery and characterization of novel paramyxoviruses from bat samples in China. Virol. Sin. 38, 198–207. https://doi.org/10.1016/J.VIRS.2023.01.002.
Tolf, C., Wille, M., Haidar, A.K., Avril, A., Zohari, S., Waldenström, J., 2013. Prevalence of avian paramyxovirus type 1 in Mallards during autumn migration in the western Baltic Sea region. Virol. J. 10. https://doi.org/10.1186/1743-422X-10-285.
Tong, S., Chern, S.W.W., Li, Y., Pallansch, M.A., Anderson, L.J., 2008. Sensitive and broadly reactive reverse transcription-PCR assays to detect novel paramyxoviruses. J. Clin. Microbiol. 46, 2652–2658. https://doi.org/10.1128/JCM.00192-08.
Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B.C., Remm, M., et al., 2012. Primer3—new capabilities and interfaces. e115 Nucleic Acids Res. 40, e115. https://doi.org/10.1093/nar/gks596.
Vanmechelen, B., Bletsa, M., Laenen, L., Lopes, A.R., Vergote, V., Beller, L., et al., 2018. Discovery and genome characterization of three new Jeilongviruses, a lineage of paramyxoviruses characterized by their unique membrane proteins. BMC Genom. 19. https://doi.org/10.1186/s12864-018-4995-0.
Vanmechelen, B., Meurs, S., Horemans, M., Loosen, A., Maes, T.J., Laenen, L., et al., 2022. The characterization of multiple novel paramyxoviruses highlights the diverse nature of the subfamily Orthoparamyxovirinae. Virus Evol. 8. https://doi.org/10.1093/VE/VEAC061.
Vanmechelen, B., Meurs, S., Zisi, Z., Goüy de Bellocq, J., Bletsa, M., Lemey, P., et al., 2021. Genome sequence of ruloma virus, a novel paramyxovirus clustering basally to members of the Genus Jeilongvirus. Microbiol Resour. Announc 10. https://doi.org/10.1128/MRA.00325-21.
Vanmechelen, B., Vergote, V., Merino, M., Verbeken, E., Maes, P., 2020. Common occurrence of Belerina virus, a novel paramyxovirus found in Belgian hedgehogs. Sci. Rep. 10. https://doi.org/10.1038/S41598-020-76419-1.
Wacharapluesadee, S., Lumlertdacha, B., Boongird, K., Wanghongsa, S., Chanhome, L., Rollin, P., et al., 2005. Bat Nipah Virus, Thailand - Volume 11, Number 12—December 2005 - Emerging Infectious Diseases journal - CDC. Emerg. Infect. Dis. 11, 1949–1951. https://doi.org/10.3201/EID1112.050613.
Weinstein, I., Vapalahti, O., Kant, R., Smura, T., 2025. Lazypipe3: customizable virome analysis pipeline enabling fast and sensitive virus discovery from NGS data, 04.29.651217. BioRxiv 2025. https://doi.org/10.1101/2025.04.29.651217.
Wells, H.L., Loh, E., Nava, A., Solorio, M.R., Lee, M.H., Lee, J., et al., 2022. Classification of new morbillivirus and jeilongvirus sequences from bats sampled in Brazil and Malaysia. Arch. Virol. 167, 1977–1987. https://doi.org/10.1007/S00705-022-05500-Z.
Wu, Z., Yang, L., Ren, X., He, G., Zhang, J., Yang, J., et al., 2016. Deciphering the bat virome catalog to better understand the ecological diversity of bat viruses and the bat origin of emerging infectious diseases, 2015;10 ISME J. 10 (3), 609–620. https://doi.org/10.1038/ismej.2015.138.
Xu, J.-L., Chen, J.-T., Hu, B., Guo, W.-W., Guo, J.-J., Xiong, C.-R., et al., 2024a. Discovery and genetic characterization of novel paramyxoviruses from small mammals in Hubei Province. Cent. China. https://doi.org/10.1099/mgen.0.001229.
Xu, J., Le, Chen, J.T., Hu, B., Guo, W.W., Guo, J.J., Xiong, C.R., et al., 2024b. Discovery and genetic characterization of novel paramyxoviruses from small mammals in Hubei Province, Central China. Micro Genom. 10. https://doi.org/10.1099/MGEN.0.001229.
Yeasmin, D., Hossain, M.M., Haider, S., Rahman, M., Hassan, M.Z., 2025. The deadly drink: Nipah virus transmission through date palm sap, cultural practices and the

evolution of behavioral interventions in Bangladesh over two decades. J. Infect. Public Health 18, 102949. https://doi.org/10.1016/J.JIPH.2025.102949.

Zhang, X.-A., Li, H., Jiang, F.-C., Zhu, F., Zhang, Y.-F., Chen, J.-J., et al., 2022. A zoonotic henipavirus in febrile patients in China. N. Engl. J. Med 387, 470–472. https://doi.org/10.1056/NEJMC2202705.

Zhu, W., Huang, Y., Yu, X., Chen, H., Li, D., Zhou, L., et al., 2022. Discovery and evolutionary analysis of a novel bat-borne paramyxovirus. Viruses 14. https://doi.org/10.3390/V14020288.