

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a Confirmed

- ☐ ☒ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- ☐ ☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- ☐ ☒ The statistical test(s) used AND whether they are one- or two-sided
Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- ☐ ☒ A description of all covariates tested
- ☐ ☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- ☐ ☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- ☐ ☒ For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
Give P values as exact values whenever suitable.
- ☐ ☒ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- ☒ ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- ☒ ☐ Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection

GISAID background sequences: AY.39 and BF.1 sequences used as phylogenetic background are GISAID-sourced and cannot be redistributed here. Acknowledgment tables for contributing laboratories are in Supplemental Files 3–5 of the manuscript.

White Tailed Deer sequences: Four AY.39 white-tailed deer sequences were provided by the USDA National Veterinary Services Laboratories and are included in the GenBank submission.

To process, validate, and analyze our genomic data, we assembled consensus viral sequences from raw reads using commercial DNASTar SeqMan NGen v14.0.1 paired with open-source IRMA v0.6.7. We structurally validated these sequences via VADR, designated viral lineages using Pangolin v3.1.1, and aligned them against the Wuhan-Hu-1 reference using Nextclade v3.3.1 for clade assignment and mutation tracking.

Data analysis

After applying an open-source smot v0.17.4 python toolkit to execute monophyletic down-sampling, we inferred maximum-likelihood trees via IQ-TREE v2.4.7, evaluated temporal signals using TempEst v1.5.3, and pruned genetic outliers using TreeTime v0.10.1. We then modeled time-scaled evolutionary dynamics and host-specific local clock rates within BEAST v1.10.5 using the BEAGLE 3 high-performance library, verified MCMC chain convergence with Tracer v1.7.2, and summarized distributions into maximum clade credibility trees using TreeAnnotator v1.10.4 and TreeStat v1.10.4. For downstream analyses, we utilized the R statistical environment, performing multivariate analyses of variance and group variance tests via the `adonis2()` and `betadisper()` functions in the `vegan` v2.7.3 package, cataloging mutations through the Coronapp web application, and generating visualizations via `ggtree` v3.13.1 and `ggplot2`. Finally, we developed and ran custom Python scripts to bridge these tools by trimming uninformative non-coding regions and masking systematically unstable nucleotide positions across our alignments before tree building; all custom code, configuration files, and analytical scripts developed for this study are publicly accessible in our GitHub Respository (<https://github.com/acrespo-virevol/sars-cov2-US-mink-surveillance>).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

All complete SARS-CoV-2 genomic consensus sequences from this study are deposited in the NCBI GenBank database, with accession codes PQ594496 to PQ594788. Epidemiological data and reference sequences for human and animal backgrounds were obtained from publicly accessible repositories, including the Johns Hopkins University Center for Systems Science and Engineering COVID-19 data repository via outbreak.info and the GISAID database. The custom Python scripts, R script configurations, and downstream analytical code used for phylogenetic and mutational modeling are openly available without restrictions at <https://github.com/acrespo-virevol/sars-cov2-US-mink-surveillance>. There are no restrictions on data access.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender

No human subjects were included in this study.

Reporting on race, ethnicity, or other socially relevant groupings

No human subjects were included in this study.

Population characteristics

No human subjects were included in this study.

Recruitment

No human subjects were included in this study.

Ethics oversight

No human subjects were included in this study.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☐ Life sciences ☐ Behavioural & social sciences ☒ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description

This study aimed to characterize the transmission dynamics, evolutionary trajectories, and potential reservoir establishment of SARS-CoV-2 across U.S. mink farms during 2022–2023. To achieve this, we deployed a longitudinal surveillance design combining active serological screening of mink populations with whole-genome sequencing and host-specific phylogenetic modeling of viral isolates from farmed mink, local humans, and nearby wildlife.

Research sample

Our research sample comprised commercially farmed American mink (*Neogale vison*) of varying sexes and ages across participating U.S. facilities sampled during 2022–2023. Because mink susceptible to rapid SARS-CoV-2 propagation, this species was selected to represent evaluate whether these animals function as viral reservoirs.

Sampling strategy

To assess viral circulation safely, our longitudinal sampling strategy utilized a collection of 15,903 mink, achieving the highest sample size obtainable without causing any disruption to normal, daily farm operations. This ample sample size is statistically and

	epidemiologically sufficient to study the rapid viral transmission dynamics in mink.
Data collection	On each participating farm, staff selected a cohort of 250 mink to follow longitudinally from July 2022 through the pelting harvest season in November–December 2022. The biological samples and associated metadata were collected by farm personnel on provided datasheets using pens.
Timing and spatial scale	The longitudinal study was conducted across the United States over a multi-month period, initiating surveillance in July 2022 and concluding at the end of the pelting harvest season in November–December 2022. This timing and national spatial scale allowed the tracking of viral dynamics across geographically distinct farms.
Data exclusions	SARS-CoV-2 sequencing was only attempted for samples with RT-PCR cycle threshold values of 34.0 or lower. This was a predetermined exclusion criteria to not waste resources on samples that do not typically have enough viral material for successful sequencing.
Reproducibility	To ensure rigorous reproducibility, our study design utilized a structured longitudinal sampling framework with standardized collection protocols that can be systematically replicated to monitor viral evolution over time in other agricultural or wildlife settings.
Randomization	Randomization was not performed or not applicable because SARS-CoV-2 infection status was unknown in all samples prior to testing. All samples were extracted in batches without any specific efforts toward randomization.
Blinding	Serology results were analyzed and scored by individuals blinded to the sample identification until after the data were completed.
Did the study involve field work?	☒ Yes ☐ No

Field work, collection and transport

Field conditions	Samples were collected on mink farms during the autumn and winter months. Sample collection in barns or sheds, which provided cover from wind and rainfall.
Location	USA
Access & import/export	We established a collaborative network to access participating facilities and harvest biological samples responsibly. Our on-farm surveillance protocol operated through a formal cooperative agreement backed and monitored by USDA APHIS. Direct coordination and farm logistics were codeveloped alongside Fur Commission USA, establishing trusted public-private operational boundaries that allowed us to work with commercial mink populations safely and transparently. The physical collection of nasal swabs and serological samples was conducted by trained farm staff.
Disturbance	No additional disturbances were made for the purpose of this study

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals	This study did not involve any laboratory animals.
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Wild animals	Wild white-tailed deer were harvested by hunters as part of state managed hunting seasons and not for the purposes of this study. Sample collection was opportunistic from these harvested wild deer and was conducted postmortem.
Reporting on sex	The selection of animal sex for sampling was dependent entirely on the individual animals designated by the farm staff at each facility. Because the longitudinal cohort was established based on operational choices made by personnel during normal handling, the male-to-female ratio in our sample directly reflects the baseline herd management distributions of the participating farms.
Field-collected samples	All animals involved in the study remained under the direct care of farm staff and were managed according to normal farm practices. The only alterations to their standard daily care and routine husbandry were the sample collection procedures explicitly outlined in this study.
Ethics oversight	Sample collection from the mink was conducted in accordance with the animal use protocol approved by The Ohio State University Institutional Animal Care and Use Committee. Sample collection from deer was conducted postmortem, which was deemed exempt from Institutional Animal Care and Use Committee oversight.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

Seed stocks	No plants were included in this study.
Novel plant genotypes	No plants were included in this study.
Authentication	No plants were included in this study.