Lack of detection of SARS-CoV-2 in British wildlife 2020-21 and first description of a stoat (*Mustela erminea) Minacovirus*

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1.1 Keywords

Coronavirus, Mustelid, Cricetid rodent, *Minacovirus*, Stoat, SARS-CoV-2

1.2 Repositories:

The *Minacovirus* sequence assembled from this study has been deposited in the NCBI GenBank database under accession number OP933726. In addition, Illumina read datasets generated have been submitted under Bioproject accession number PRJNA897822, SRA accession numbers SAMN3158039, SAMN31580331, SAMN31580344 and Biosample accession numbers SRS15672848-50.

2. Abstract

Repeat spill over of SARS-CoV-2 into new hosts has highlighted the critical role of cross species transmission of coronaviruses and establishment of new reservoirs of virus in pandemic and epizootic spread of coronaviruses. Species particularly susceptible to SARS-CoV-2 spill-over include Mustelidae (mink, ferrets and related animals) and cricetid rodents (hamsters and related animals). These predispositions led us to screen British wildlife with sarbecovirus specific qPCR and pan coronavirus PCR assays for SARS-CoV-2 using samples collected during the human pandemic to establish if widespread spill-over was occurring. Fourteen wildlife species (n=402) were tested, including : 2 Red Foxes (*Vulpes vulpes*), 101 Badgers (*Meles meles*), 2 wild American Mink (*Neovison vison*), 41 Pine Marten (*Martes martes*), 2 Weasels (*Mustela nivalis*), 7 Stoats (*Mustela erminea*), 108 Water Voles (*Arvicola amphibius*), 39 Bank voles (*Myodes glareolous*), 10 Field Voles (*Microtus agrestis*), 15 Wood Mice (*Apodemus sylvaticus*), 1 Common Shrew (*Sorex aranaeus*), 2 Pygmy Shrews (*Sorex minutus*), 2 Hedgehogs *(Erinaceus europaeus*) and 75 Eurasian Otters (*Lutra lutra*). No cases of SARS-CoV-2 were detected in any animals, however a novel minacovirus related to mink and ferret alphacoronaviruses was detected in stoats recently introduced to the Orkney Islands. This group of viruses is of interest due to pathogenicity in ferrets. The impact of this virus on the health of stoat populations remains to be established.

3. Introduction

Coronaviruses are a large and diverse group of enveloped RNA viruses spread found in diverse vertebrate hosts. Well-studied mammalian hosts, such as humans and domestic dogs, have multiple coronaviruses of several different subfamilies, although most of those of concern in mammals are members of the *Alpha*- and *Betacoronavirus* genera (Woo, Lau et al. 2012). The SARS-CoV-2 pandemic is thought to have arisen via a spill over from horseshoe bats (the natural hosts of the *Betacoronavirus* subgenus sarbecoviruses), with human infection likely via a 'liaison host' such as the racoon dog (*Nyctereutes procyonoides*) or Malayan pangolin (*Manis javanica*) (Holmes, Goldstein et al. 2021, Crits-Christoph, Gangavarapu et al. 2023, Liu, Lin et al. 2023).

The overwhelming scale of the global human pandemic led to repeated spill overs and onward transmission into other mammalian species such as domestic cats (*Felis cattus*) (Hosie, Hofmann-Lehmann et al. 2021, Goletic, Goletic et al. 2022, Jairak, Chamsai et al. 2022), farmed American mink (*Neogale vison*) (Oreshkova, Molenaar et al. 2020, Domańska-Blicharz, Orłowska et al. 2021, Eckstrand, Baldwin et al. 2021, Hammer, Quaade et al. 2021, Wasniewski, Boué et al. 2023) and

Syrian hamsters (*Mesocricetus auratus*) (Kok, Wong et al. 2022, Yen, Sit et al. 2022), and the establishment of a new reservoir in North American white tailed deer (*Odocoileus virginianus*) (Hale, Dennis et al. 2022, Kuchipudi, Surendran-Nair et al. 2022). A wide range of other species are either able to be infected experimentally or have been subjects of sporadic case reports of SARS-CoV-2 infection or seroconversion including: cricetid rodents (a variety of hamster species: *Phodopus campbelli, Cricetulus griseus, Phodopus sungorus, Phodopus roborovskii, Mesocricetus auratus,* deer mice: *Peromyscus maniculatus nebrascensis, P. m. rufinus* , *P. m. sonoriensis, P. m. bairdii, P. polionotus subgriseus, P. californicus,* bushy tailed wood rats *Neotoma cinerea* and bank voles *Myodes glareolus*), felids (lion *Panthera leo*, puma *Puma concolor*, tiger *Panthera tigris*, leopard *Panthera pardus*, fishing cat *Prionailurus viverrinus,* Eurasian lynx *Lynx lynx*, Canada lynx *Lynx canadensis* , snow leopard *Panthera uncia*), other small carnivores and mustelids (dogs *Canis lupus familiaris*, racoon dogs *Nyctereutes procyonoides*, ferrets *Mustela furo*, red foxes *Vulpes vulpes*, racoons *Procyon lotor*, skunks *Mephitis mephitis*, marten *Martes martes*, badger *Meles meles*, spotted hyena *Crocuta crocuta*, otters *Lutra lutra*, coati *Nasua nasua*, Asian small clawed otter *Aonyx cinereus*, binturong *Arctictis binturong*), primates (African green monkeys *Chlorocebus aethiops*, rhesus macaques *Macacca mulatta*, baboons *Papio hamadryas*, marmosets *Callithrix jacchus*, gorilla *Gorilla gorilla*, squirrel monkeys *Saimiri spp.*), bats (Egyptian fruit bats *Rousettus aegyptiacus*), Chinese tree shrews *Tupaia belangeri chinensis*, cattle *Bos taurus*, mule deer *Odocoileus hemionus*, rabbits *Oryctolagus cuniculus*, big hairy armadillo *Chaetophractus villosus* , Virginia opossum *Didelphis virginiana*, Eastern grey squirrel *Sciurus carolinensis*, hippopotamus *Hippopotamus amphibius*, giant anteater *Myrmecophaga tridactyla*, West Indian Manatee *Trichechus manatus manatus*, (Nielsen, Alvarez et al. 2023)

SARS-CoV-2 infection in Muridae such as house mice (*Mus musculus*) and brown rats (*Rattus norvegicus*) may depend on the virus strain: initial studies with the original (Wuhan) strains of the virus failed to infect them (Dinnon, Leist et al. 2020, Shuai, Chan et al. 2021) and field studies failed to demonstrate evidence of infection in wild populations (27 *M.musculus and 97 R.Norvegicus)*. Later variants did however cause infection in laboratory studies (Gu, Chen et al. 2020, Shuai, Chan et al. 2021, Halfmann, Iida et al. 2022, Zhang, Cui et al. 2022) and there has been at least one field report of infection of rats (Wang, Lenoch et al. 2023).

This study focused on species of wild animals present in Great Britain that were assessed to be of higher risk for SARS-CoV-2 spillover in 2021, when the study was begun. These included, horseshoe bats (subject of a separate report ((Apaa, Withers et al. 2023)) mustelids, small carnivores and cricetid rodents.

Thus far, no infection with SARS-CoV-2 has been reported in Horseshoe bats (*Rhinopholus* spp) in Britain or mainland Europe (Orłowska, Smreczak et al. 2022, Sander, Moreira-Soto et al. 2022, Apaa, Withers et al. 2023) although other related coronaviruses have been detected in these species. Nor, in contrast with N. America, has the virus been detected in European deer (Holding, Otter et al. 2022, Moreira-Soto, Walzer et al. 2022, Wernike, Fischer et al. 2022). Wild animal surveillance studies in mainland Europe have indicated sporadic detection in wild mustelids, including by qPCR in wild American mink (*N.vison)*, particularly near farmed mink outbreaks, and one otter (*L. lutra*) (Aguiló-Gisbert, Padilla-Blanco et al. 2021, Padilla-Blanco, Aguiló-Gisbert et al. 2022, Sikkema,

Begeman et al. 2022). Serological evidence of exposure has been described in 3/14 pine martens (*M. martes*) and 2/10 badgers (*M. meles*) (Davoust, Guérin et al. 2022), but other studies found no evidence of infection in 48 polecats (*Mustela putorius*), 163 badgers or cricetid and murid rodents (694 *M. glareolus*, 2 *Microtus arvalis*, 27 *M. musculus*, 97 *R. norvegicus* and 8 *Apodemus* species) (Wernike, Drewes et al. 2022, Carmona, Burgos et al. 2023, Zamperin, Festa et al. 2023).

4. Methods

4.1 Sample collection

A total of 402 animals from 14 species (Table 1) were collected through a network of wildlife researchers and volunteers engaged in wildlife conservation, monitoring or pest control. The majority of samples were collected during the human covid pandemic, with the exception of a small number of historical otter samples. The species targeted were primarily mustelids (Otters *L. lutra*, Badgers *M. meles*, Mink, Pine Martens *M. martes*, Weasels *Mustela nivalis*, Stoats *M. erminea*) or cricetid rodents (Water Voles *Arvicola amphibius*, Field Voles *Microtus agrestis*, Bank Voles *M. glareolus*) with a small number of other species collected opportunistically (Hedgehogs *Erinaceus europaeus*, Common Shrews *Sorex araneus*, Pygmy Shrews *S. minutus*, Red Foxes *V. vulpes*, Wood Mice *Apodemus sylvaticus*). Ethical approval was granted by the University of Nottingham School of Veterinary Medicine and Science Committee for Animal Research and Ethics (CARE), and the University of Sussex Animal Welfare and Ethical Review Board.

Lung samples were taken from postmortem cadavers submitted to the Cardiff Otter Project (otters) or found dead badgers submitted for tuberculosis monitoring to the University of Nottingham (badgers tested for covid were all confirmed culture-negative for *Mycobacterium tuberculosis* complex infections). Mink and Stoat samples (oronasal and rectal swabs from cadavers) were provided from programmes for invasive species control and were from animals that were live trapped and euthanased (Mink) or lethally trapped (Stoats). Rodent samples were faecal samples from animals live trapped in Longworth or Elliot traps for population monitoring (faecal samples taken from traps or latrine/burrow sites), and from a small number of captive Water Voles from a licenced breeding and release programme. A small number of lung samples were taken from animals found dead. Weasel, Hedgehog, and Shrew samples were from a small number of animals caught as bycatch in Longworth or Elliot traps. Pine Marten samples were all faecal samples (scat) from environmental monitoring. Samples were collected from a variety of British locations (Supplementary Information). Collectors were solicited by social media, notices in game and hunting organisation newsletters, letters in the *Veterinary Record* and contact networks of wildlife organisations working with study participants and were provided with sampling packs including gloves, collection and shipping material and instructions. Samples were collected into RNAlater® before either storage at -20°C or direct shipping to The University of Nottingham, depending on the capacity of the collectors.

Table 1: Species and Sample Type Screened for SARS-CoV-2

Species	Oral Swab	Rectal swab	Lung sample	Faecal sample	Total number of animals	Sample collection dates
Mustelids						
Otter	-	-	75	-	75	Jan 2020-April 2022 (51 samples) April 2017-Dec 2019 (25 samples)
Badger	-	-	101	-	101	April 2021-April 2022
Mink	1	1	-	-	2	Aug 2021-June2022
Pine Marten	-	-	-	41	41	March 2020- Oct 2021
Weasel	-	-	2	-	2	Sept 2021
Stoat	7	7	-	-	7	Dec 2021
Cricetid Rodents						
Water Vole	-	-	-	108	108	August-Nov 2021
Bank Vole	-	-	7	32	39	Jan-Feb 2022
Field Vole	-	-	5	5	10	Nov 2021-Feb 2022
Other species						
Wood Mouse	-	-	-	10	10	July 2021-Feb 2022
Common Shrew	-	-	-	1	1	Jan 2022
Pygmy Shrew	-	-	-	2	2	Jan 2022
Hedgehog	-	-	-	2	2	Nov 2022

Red Fox	2	2	-	-	2	April 2022
Total					402	

143

144 4.2 RNA extraction, reverse transcriptase (RT) and RNA-dependent RNA polymerase 145 (RDRP) gene coronaviruses generic conventional PCR and envelope gene 146 sarbecovirus-specific real-time PCR

147 RNA extraction from lung tissue, faecal samples, rectal and oronasal swabs, and cell culture
148 supernatant as positive control, was carried out using the Macherey-Nagel RNA tissue extraction kit
149 as per manufacturer's instructions. The Wuhan SARS-CoV-2 strain positive control sample used
150 throughout this study was kindly donated by Dr Christopher Coleman (Division of Infection,
151 Immunity and Microbes, School of Life Sciences, University of Nottingham, UK). RT was performed in
152 two steps, using M-MLV-RT and random hexamer primers (Promega) as per manufacturer's
153 instructions. All cDNA products were stored at -20 °C for conventional PCR.

154 A generic pan-coronavirus PCR assay published by (Woo, Lau et al. 2005) was used to amplify a 440
155 bp fragment of the coronavirus RDRP gene using Q5® Hot Start High-Fidelity DNA Polymerase (New
156 England Biolabs cat no: M0493S). Primers were: F: GGTTGGGACTATCCTAAGTGTGA and R:
157 CCATCATCAGATAGAATCATCATA. PCR products were purified using the Nucleospin® extract II kit
158 (Macherey-Nagel) according to manufacturer's instructions and were Sanger sequenced (Eurofins
159 UK).

160 Real-time PCR was carried out using the Promega GoTaq® Probe 1-Step RT-qPCR System (Promega)
161 with Sarbecovirus-specific envelope gene primers from bat RNA samples as published by (Corman,
162 Landt et al. 2020). Primers and probes were: F: ACAGGTACGTTAATAGTTAATAGCGT Probe: FAM-
163 ACACTAGCCATCCTTACTGCGCTTCG-BBQ R: ATATTGCAGCAGTACGCACACA

164 RNA and cDNA quality control was assessed via partial amplification of 108 bp of the beta actin gene
165 using a published conventional PCR protocol (Fischer, Freuling et al. 2014). Primers were F:
166 CAGCACAATGAAGATCAAGATCATC and R: CGGACTCATCGTACTCCTGCTT

167 4.3 High throughput sequencing and genome analyses

168 RNA sequencing was performed on positive samples by Novogene UK, using the Illumina NovaSeq
169 6000 platform. Quality filtering and trimming to remove adapters, duplicates and low quality reads
170 was achieved using fastp v0.23.1 (Chen, Zhou et al. 2018). Kraken2 v2.1.2 was used for taxonomic
171 classification reads against the Kraken2 viral Refseq database (Wood, Lu et al. 2019) (retrieved on 9th
172 June 2022). Reads were assembled using the coronaSPAdes option in SPAdes genome assembler
173 v3.15.4 (Meleshko, Hajirasouliha et al. 2021) using default parameters. While CheckV v1.0.1, a fully
174 automated command-line pipeline, was used for identification and quality assessment of contigs,
175 contigs were also queried against the NCBI custom BLASTN (v2.12.0) viral database (Altschul, Gish et
176 al. 1990) (retrieved on 3rd July 2022).

177 Assembled contigs were indexed, and those that were classified and assessed as complete
178 alphacoronavirus genomes were extracted for downstream analysis using SAMtools v1.16.1 faidx
179 option (Danecek, Bonfield et al. 2021). Assembled genomes were annotated in Geneious Prime®
180 (v.2022.2.2) using NCBI coronavirus reference sequences for minacoviruses and tegacoviruses.

181 4.4 Phylogenetic analysis

182 Complete coronavirus genomes, extracted RDRP, spike, and nucleocapsid nucleotide sequences
183 from alphacoronavirus genomes assembled in this study, and a total of 22 reference
184 alphacoronavirus genomes (all *Minacovirus* full genomes available and a selection of Refseq or well
185 characterised full length isolates of tegacoviruses with the reference sequence of porcine epidemic
186 diarrhoea virus, PEDV, as an outgroup, supplementary information) were downloaded from NCBI,
187 aligned using Mafft v7.490 (Katoh and Standley 2013). Maximum likelihood phylogenetic trees were
188 reconstructed based on complete coronavirus genomes, and four different genes using IQ-TREE
189 v2.0.7 (Minh, Schmidt et al. 2020), using 1000 ultrafast bootstrap approximations using UFBoot2
190 within IQ-TREE v2.0.7 to evaluate branch support (Hoang, Chernomor et al. 2018). The ModelFinder
191 function within IQ-Tree was used to select the best fitting nucleotide substitution model for
192 phylogenetic reconstruction (Kalyaanamoorthy, Minh et al. 2017). Phylogenetic trees were visualized
193 and annotated in FigTree v1.4.4 (https://github.com/rambaut/figtree/). Using the same approach
194 trees were also similarly constructed for individual genes (25 Spike and Nucleocapsid genes as the S
195 genes of this group of viruses are known to recombine) and for all available *Minacovirus* partial gene
196 fragments of RDRP (RNA dependent RNA polymerase) and Spike (where there are a lot more
197 sequences available than for other parts of the genome).

198 5. Results

199 No animal sample tested positive on the *Sarbecovirus*-specific E gene qPCR.

200 Four (out of seven) stoat rectal swab samples (57%) tested positive on the pancoronavirus PCR.
201 Sanger sequencing of PCR products indicated that these were alphacoronaviruses of the *Minacovirus*
202 group. No oral swab samples tested positive from the same animals. All stoat samples in this study
203 were from the same population, and were sourced from the Orkney Islands stoat eradication
204 programme (https://www.nature.scot/professional-advice/land-and-sea-management/managing-
205 wildlife/orkney-native-wildlife-project) and are from the same recently introduced and heavily
206 bottlenecked population . All bar one sample in this study were from adult animals with a mix of
207 male and females. Positive samples were from 1 adult male, 1 juvenile female and 2 adult females.

208 5.1 Illumina sequencing Taxonomic classification, genome assembly

209 Taxonomic classification using Kraken2 identified reads assigned to other viral operational
210 taxonomic units, however, only reads classified to the Coronaviridae viral family are reported in this
211 study. De novo assembly of datasets from the stoats yielded one full length coronavirus contig of
212 28.1kb (100% quality, 99.9% completeness) and two partial contigs (7.5kb, 27% quality, 26.6%
213 completeness and 27.9kb, 99% quality and 99.1% completeness) from a further two samples. The
214 remaining sample did not yield any coronavirus contigs. The sequences had closest homology to

alphacoronaviruses of the *Minacovirus* group. The complete full genome sequence of the most complete contig has been deposited in Genbank (accession number OP933726).

5.2 Genome annotation and organization

Genome annotation demonstrated a typical *Alphacoronavirus* genome organisation consisting of 5' and 3' UTRs, a large ORF1ab, encoding sixteen non-structural peptides (nsp1-16) making up about 2/3 of the viral genome, and genes encoding four structural proteins: the spike (S), membrane (M), envelope (E) and nucleocapsid (N) (Figure 1). Accessory proteins included open reading frames with homology to ORF 3c and 7b of feline coronavirus isolates. A number of smaller potential open reading frames within these regions were also present, possibly corresponding to other accessory proteins with homology to the ORF3 or 7 of other *Minacovirus* and *Tegacovirus* isolates

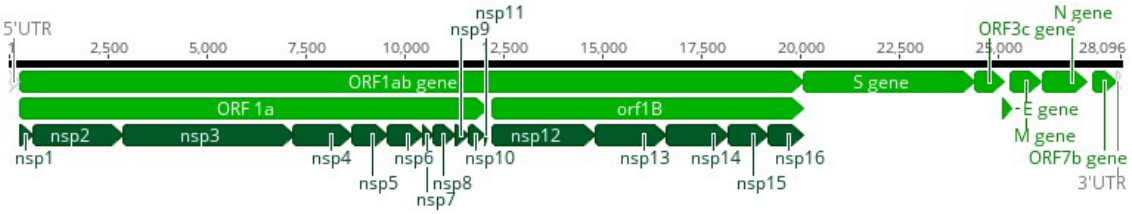

Figure 1: Genome organisation of the stoat sequences derived from this study . Open reading frames are shown in light green, UTR's in grey and regions with homology to non-structural proteins derived from feline coronavirus ORF1ab are marked in dark green.

5.3 Phylogenetic analysis

Results from the maximum likelihood phylogenetic trees drawn using all available complete *Minacovirus* genomes and selected reference sequences for tegacoviruses, demonstrated that the stoat sequence is most closely clustered with ferret isolates (Figure 2). This relationship is consistent across analysis of individual genes (Spike, Nucleocapsid full genes) and in larger phylogenetic trees of all partial fragments of *Minacovirus* RDRP and Spike available in the NCBI database(Supplementary information).

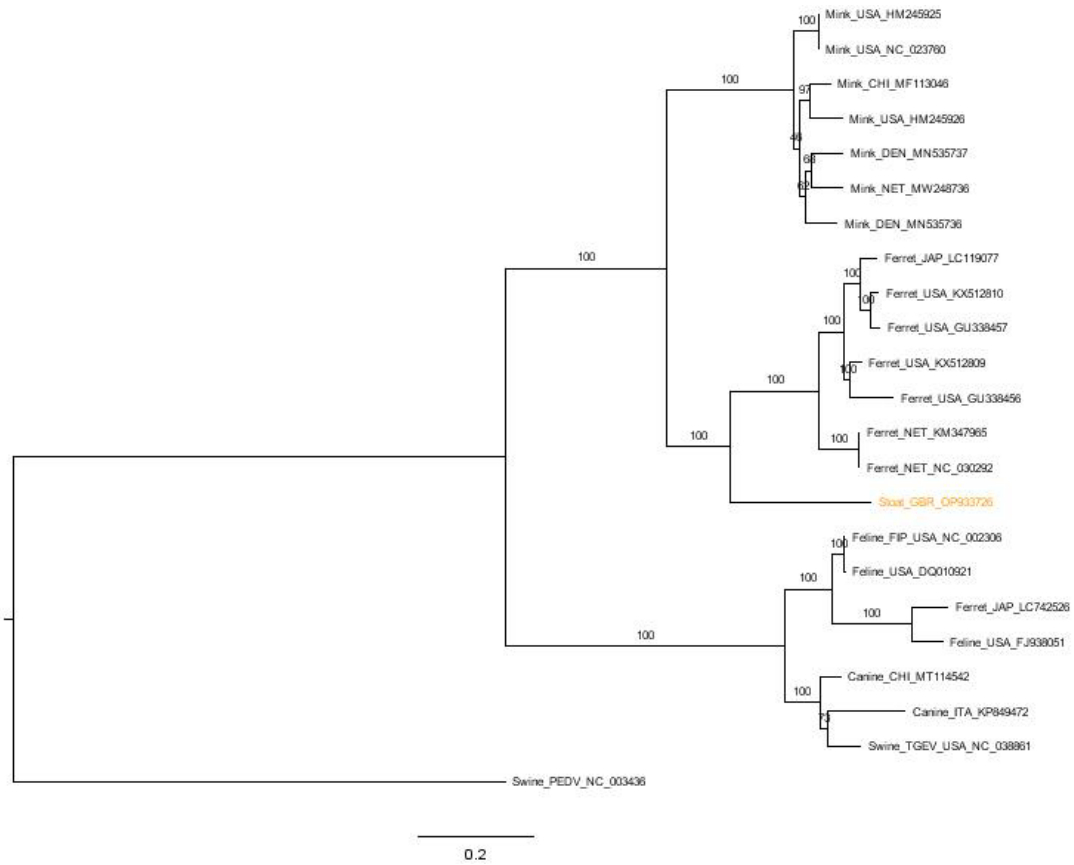

Figure 2: Maximum likelihood phylogenetic tree of full genomes of minacoviruses and tegacoviruses constructed with 1000 bootstrap approximation, rooted on the PEDV coronavirus reference sequence. Twenty three full genomes were included. The sequence from this study is marked in orange. Sequences are named with species of origin, name of virus (if applicable) and a three letter code for geographic origin (GBR=Great Britain, USA= United States of American, CHI=China, JAP=Japan, NET=Netherlands, DEN=Denmark) and Genbank ID. Bootstrap values shown on nodes

6. Discussion

This study found no evidence of widespread circulation of SARs-CoV-2 in our opportunistically gathered British wild carnivores and cricetid rodents in 2021-22. The study was designed to detect an incidence rate of 5% based on our previous studies of alphacoronaviruses in British rodents (Tsoleridis, Onianwa et al. 2016) with a target of 73 samples per species calculated with the online Epitools sample size calculator (Epitools 2020). For some species including Otters and Badgers, for which there were ongoing post mortem studies of found dead (largely road kill) animals (Sandoval Barron, Swift et al. 2018, Swift, Barron et al. 2021, O'Rourke, Hynes et al. 2022, Thomas, Hailer et al. 2022), this sample size target was readily achieved. This target was also achieved for Water Voles facilitated by a network of wildlife monitoring and captive breeding for re-introduction (Kirkland and Farré 2021). We were however reliant on volunteer submitters for other species and did not achieve

258 the target levels of fresh samples for, e.g., hedgehogs, foxes, martens and other species. We were
259 also restricted in the type of sample collected for most species as it was primarily non-invasive
260 (faeces or postmortem rectal and oronasal swabs) that could be collected by network members.
261 There remains a possibility that our study did not detect low level circulation of SARS-CoV-2 in the
262 species tested if infection is spatially restricted, that we targeted the wrong tissue sample type or
263 that our samples were too degraded to detect virus with RNA based methods. This is inherent to the
264 study design of a study such as this one seeking to provide the basis for a preliminary assessment
265 with have scope to implement a spatially stratified study.

266 The detection of another coronavirus in this study, however, indicates that the methods used and
267 sample preservation were adequate for viral detection, at least at high prevalence, even in small
268 populations of samples. Our study is also in line with other European wildlife studies indicating
269 absence of widespread SARS-CoV-2 circulation in wild small carnivores and rodents, including wild
270 American Mink (Davoust, Guérin et al. 2022, Keller, Peter et al. 2022, Sikkema, Begeman et al. 2022,
271 Villanueva-Saz, Giner et al. 2022, Wernike, Drewes et al. 2022, Carmona, Burgos et al. 2023,
272 Zamperin, Festa et al. 2023). Detection of SARS-CoV-2 in one Eurasian River Otter in lung tissue and
273 nasal swabs and detection of a Eurasian Badger specific coronavirus in lung tissue from similar post
274 mortem monitoring programmes indicates that lungs are an appropriate target tissue for
275 coronavirus monitoring in those species (Padilla-Blanco, Aguiló-Gisbert et al. 2022, Zamperin, Festa
276 et al. 2023). SARS-CoV-2 is also readily detected in faecal samples in laboratory studies of rodents
277 and carnivore species as well as field studies of farmed mink (Griffin, Chan et al. 2021, Adney,
278 Lovaglio et al. 2022, Wolters, de Rooij et al. 2022, Wurtzer, Lacote et al. 2022), indicating faecal
279 sample screening is also an appropriate sample for coronavirus monitoring. Faecal samples or rectal
280 swabs are also among the easiest and most acceptable samples for volunteer submitters to collect
281 safely promoting their use in disease surveillance.

282 The stoat alphacoronavirus reported in this study is a novel finding. The stoats all came from an
283 eradication programme on the Orkney Islands, an archipelago off the northeastern tip of mainland
284 Great Britain. They are not native to the islands having been first found in 2010, and cause
285 considerable negative impact on breeding bird populations (Project 2023). Despite the small number
286 of samples collected (7) more than half of them (4) were PCR positive on rectal swabs, suggesting
287 that the prevalence of this virus in this population is high. Wider studies of this population and
288 mainland populations would be warranted to gauge the prevalence of the virus in the larger (and
289 source) populations.

290 The novel virus we identified in Orkney stoats is a member of the *Minacovirus* subgroup of the
291 Genus *Alphacoronavirus,* and clusters closely to mink and ferret coronaviruses. The ferret and mink
292 viruses have a well described pathogenicity, causing diarrhoea and sometimes a systemic disease
293 syndrome, similar to feline infectious peritonitis in cats (Wise, Kiupel et al. 2010, Vlasova, Halpin et
294 al. 2011, Terada, Minami et al. 2014, Autieri, Miller et al. 2015, Lescano, Quevedo et al. 2015, Doria-
295 Torra, Vidaña et al. 2016, Lamers, Smits et al. 2016, Minami, Kuroda et al. 2016, Wills, Beaufrère et
296 al. 2018). Wider studies of the pathogenesis of this virus in stoats and the impact of this on the
297 species would be warranted. There are multiple reports of the ferret viruses in this group
298 recombining with each other (Lamers, Smits et al. 2016, Minami, Kuroda et al. 2016, Xu 2020) and

299 multiple reports of farmed mink infected with SARS-CoV-2 also being co-infected with minacoviruses
300 at a high prevalence rate (Ip, Griffin et al. 2021, Kwok, de Rooij et al. 2021, Wasniewski, Boué et al.
301 2023). While recombination between SARS-CoV-2 and minacoviruses has not been observed it is a
302 possibility that warrants monitoring, particularly in farmed mink outbreaks.

303 Of interest the tegacoviruses (the most closely related alphacoronavirus clade to the minacoviruses)
304 are known for readily recombining and jumping host species, with canine, feline and porcine
305 recombinants in this group reported in multiple separate events (Wang, Ma et al. 2006, Decaro, Mari
306 et al. 2010, Ntafis, Mari et al. 2011, Licitra, Duhamel et al. 2014, Chen, Liu et al. 2019, Pratelli,
307 Tempesta et al. 2022). Canine coronaviruses have also been reported multiple times in multiple
308 locations in people with respiratory disease (Lednicky, Tagliamonte et al. 2022, Vlasova, Diaz et al.
309 2022, Vlasova, Toh et al. 2022), making this group of viruses of concern for recombination and cross
310 species transmission potential and warranting monitoring.

311 Sequences in the *Tegacovirus* group have also been reported from racoon dogs (*Nyctereutes*
312 *procyonoides)* (Wang, Ma et al. 2006, Wang, Tian et al. 2022), suggesting that there could be cross-
313 species transmission between racoon dogs, domestic cats, dogs, pigs and people. This is of particular
314 concern as this racoon dogs are known to be able to be infected with and transmit SARS-CoV-2 and
315 are one of the main suspects for the origin of the SARS-CoV-2 outbreak in humans (Freuling,
316 Breithaupt et al. 2020, Crits-Christoph, Gangavarapu et al. 2023, Rao, Parthasarathy et al. 2023).

317 The *Minacovirus* sequences isolated to date are all associated with mustelids of the genus *Mustela*,
318 subfamily Mustelinae (ferrets, mink, stoats). However there are relatively few studies of
319 coronaviruses in mustelids. That is beginning to be rectified with the publication of SARs-CoV-2
320 monitoring studies, with a possible *Gammacoronavirus* identified in Chinese ferret badgers
321 (*Melogale moschata*) and, most recently, isolates of a possibly new Genus, *Epsioloncoronavirus, in*
322 Italian badgers (Dong, Liu et al. 2007, Zamperin, Festa et al. 2023). The potential host and geographic
323 ranges of these viruses remain unknown.

324 Overall this study adds to a growing picture of a lack of SARS-CoV-2 circulation in wild European
325 mammals. It has however reported a novel alpha coronavirus of the *Minacovirus* sub-genus in an
326 island population of stoats, adding much needed information on alphacoronavirus diversity in
327 mustelids. The wider disease impacts and epidemiology of this virus in this species is however
328 unknown and requires further study.

329

330 # 7. Author statements

331

332 ## 7.1 Conflicts of interest

333 The authors declare that there are no conflicts of interest

334

7.2 Funding information

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7.3 Ethical approval

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