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Ancient origin and genetic segregation of canine circovirus infecting arctic foxes (*Vulpes lagopus*) in Svalbard and red foxes (*Vulpes vulpes*) in Northern Norway

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13

14 **Ancient origin and genetic segregation of canine circovirus infecting arctic foxes (*Vulpes***
15 ***lagopus*) in Svalbard and red foxes (*Vulpes vulpes*) in Northern Norway**

16

17 **Running title:** Canine circovirus in arctic foxes and red foxes

18

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37 **Abstract**

38 Canine circovirus (CanineCV) is a relatively new viral species, belonging to the family
39 *Circoviridae*, whose pathogenic role is still uncertain. Since its first description in one domestic dog
40 in 2011 from the USA, several reports have been documenting its distribution worldwide. Recently,
41 CanineCV was also detected in wild animals such as wolves, foxes and badgers. In order to
42 investigate the presence and the genetic characteristics of CanineCV in foxes of Arctic and Sub-
43 Arctic regions, the presence of CanineCV DNA in internal organs (liver and spleen) of 51 arctic
44 foxes (*Vulpes lagopus*) from Svalbard archipelago and 59 red foxes (*Vulpes vulpes*) from Northern
45 Norway, sampled from 1996 to 2001 and from 2014 to 2018, respectively, was screened by real-
46 time PCR. CanineCV was detected in 11/51 arctic foxes and in 10/59 red foxes, backdating the
47 circulation of the virus at least to 1996 in the arctic fox population. The complete genome of 14
48 identified CanineCV was sequenced and analysed showing an identity higher than 80.8% with the
49 reference strains available to date. According to the species demarcation threshold of 80% genome-
50 wide nucleotide sequence identity for members of the family *Circoviridae* provided by International
51 Committee on Taxonomy of Viruses (ICTV), all the CanineCV belong to a single species.
52 Phylogenetic analysis revealed that all the CanineCV were subdivided into five main clusters with
53 one including only CanineCV identified in foxes. Furthermore, CanineCV identified in arctic foxes
54 and red foxes formed two distinct lineages. From these data we hypothesize that the viral
55 transmission did not occur between the two species of foxes as a consequence of the lack of contact
56 between the two hosts or that the virus acquired mutations in the time elapsed between the
57 samplings.

58

59 **Keywords**

60 Arctic, arctic fox, Canine circovirus, Norway, red fox, Svalbard

61 **1. Introduction**

62 Circoviruses are non-enveloped viruses belonging to the genus *Circovirus* of the family
63 *Circoviridae*, and contain a small, circular single-stranded DNA genome of approximately 2000
64 nucleotides (nt) in length (Breitbart et al., 2017). These viruses have an ambisense genome
65 organisation with two major and inversely arranged open reading frames (ORFs) encoding for the
66 replicase associated protein (Rep) and the capsid protein (Cap) (Kotsias et al., 2019). Canine
67 circovirus (CanineCV) was first identified in serum from healthy domestic dogs in 2011 in the USA
68 (Kapoor et al., 2012). Since then, the virus has been reported in dogs from several countries with a
69 prevalence ranging from 3.8% to more than 30% (Anderson et al., 2017; Decaro et al., 2014;
70 Dowgier et al., 2017; Gentil et al., 2017; Hsu et al., 2016; Li et al., 2013; Niu et al., 2020; Piewbang
71 et al., 2018; Sun et al., 2019; Thaiwong et al., 2016; Zaccaria et al., 2016). Unlike the presence of
72 CanineCV in dogs worldwide, little is known about the actual distribution of CanineCV in wild
73 species. However, a few studies reported CanineCV in wolves, foxes and badgers with variable
74 prevalence depending on the population studied (i.e. healthy versus unhealthy individuals), the
75 diagnostic method used for virus detection, the type of biological sample tested and the geographic
76 origin of samples (Bexton et al., 2015; De Arcangeli et al., 2020; Zaccaria et al., 2016). CanineCV
77 infection is associated with a certain clinical state characterised by vasculitis, haemorrhage and
78 enteritis in dogs (Anderson et al., 2017; Dowgier et al., 2017; Gentil et al., 2017; Kotsias et al.,
79 2019; Li et al., 2013) and encephalitis in foxes (Bexton et al., 2015). Moreover, an association
80 between CanineCV and canine parvovirus (CPV-2) infection in dogs has been reported (Anderson
81 et al., 2017; Dowgier et al., 2017; Thaiwong et al., 2016; Zaccaria et al., 2016). To date, few data on
82 the genetic variability of CanineCV infecting wild canids are available compared to the numerous
83 genomic sequences of CanineCV obtained from domestic dogs that have allowed to propose
84 different distinct phylogenetic clades for this virus (Niu et al., 2020; Sun et al., 2019). In addition,
85 despite the recent discovery, the virus may have circulated among carnivorous populations much

86 longer and testing biological samples of domestic and wild canids taken before 2011 could help
87 clarify this aspect.

88 The aim of this study was to investigate the presence and the genetic characteristics of
89 CanineCV in arctic foxes (*Vulpes lagopus*) from the Svalbard archipelago, Norway, and red foxes
90 (*Vulpes vulpes*) from Northern Norway (mainland) sampled from 1996 to 2018.

91

92 **2. Materials and Methods**

93 **2.1. Study sites and sampling**

94 Fifty-one arctic foxes from the High-Arctic Svalbard archipelago were included in this study
95 (Figure 1A, TableS1). Arctic foxes, 25 females, 24 males and two animals with unidentified gender,
96 were caught by local trappers using baited traps during the annual harvest at Spitsbergen, Svalbard,
97 Norway (76–81°N, 15–25°E). Samples were collected during the trapping seasons (November 1st –
98 March 15th) from the period 1996-2001. Carcasses were allocated to six sampling sites (Figure 1B)
99 and frozen before they were delivered to Norwegian Polar Institute for laboratory measurements
100 and sampling. Tissue samples were obtained from liver (n=47) and/or spleen (n=49).

101 Fifty-nine red foxes from the northernmost part of the Norwegian mainland, Finnmark County,
102 were included in this study (Figure 1A, TableS1). Red foxes, 28 females and 31 males, were shot as
103 part of legal culling programs in Finnmark County during the period 2014-2018. Red foxes were
104 collected from two geographical regions: the Varanger Peninsula (70.4 °N, 29.5 °E), located at the
105 border between the Sub-Arctic and the Low-Arctic zone, and the mountain region around the lake
106 Iešjávri (69.6 °N, 24.4 °E; Figure 1C). Carcasses were frozen at -20 °C until laboratory
107 measurements and sampling of liver (n=58) and/or spleen (n=58) at UiT The Arctic University of
108 Norway.

109 Age was determined by counting the cementum annuli of a sectioned canine tooth (Grue &
110 Jensen, 1976). Age ranged between one and 11 years (median 1) for arctic foxes and between one
111 and eight years (median 2) for red foxes.

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2.2. Detection of the CanineCV DNA

DNA extraction from tissue samples of 25-50 mg was carried out by using the Maxwell 16 Tissue DNA Purification Kit (Promega) and the automatic extractor Maxwell 16 System (Promega) according to the manufacturer's instructions. Extracted DNA was stored at -20 °C until use. The presence of CanineCV DNA was screened by using a SYBR Green Real-time polymerase chain reaction (qPCR) targeting a highly conserved fragment of 132 nt in the intergenic region (IR) between the 3' ends of the two major ORFs, with the primers CanineCV 909-931 qPCR-For (5'-CTGAAAGATAAAGGCCTCTCGCT-3') and CanineCV 1020-1040 qPCR-Rev (5'-AGGGGGGTGAACAGGTAAACG-3') (De Arcangeli et al. 2020). The reaction was performed using the PowerUp SYBR Green master mix (Thermo Fisher Scientific) in a total volume of 20 µL containing 0.3 µM of each primer, 2X Master Mix and 2 µL DNA in the StepOnePlus qPCR system (Thermo Fisher Scientific). The thermal cycling consisted of 95 °C for 5 min, followed by 45 cycles of 95 °C for 15 s and 60 °C for 1 min. Melting experiment for the evaluation of the specificity of the reaction was performed after the last extension step by a continuous increment from 55 °C to 99 °C and specific melting temperature ranged from 93.2 °C to 93.6 °C. CanineCV DNA copies number determination was carried out by absolute quantification using the standard curve method. Serial 10-fold dilutions of a plasmid (pCR4 plasmid; Life Technologies) containing one copy of the CanineCV target sequence were used as external standards for the construction of the assay standard curve by plotting the plasmid copy number against the corresponding threshold cycle values. The limit of detection (LOD) of the reaction was determined based on the highest dilution of recombinant plasmid possible to amplify with good reproducibility and was found to be five copies/µL. The DNA samples and standards were repeated within each run in duplicate. A no template control, consisting of ultrapure water, underwent analysis simultaneously. Samples showing an exponential increase in the fluorescence curve, a target DNA amount greater than or equal to the LOD and a specific melting peak in both replicates were considered positive.

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139 ***2.3. Amplification and sequencing of the CanineCV complete genome***

140 The complete genome of CanineCV was amplified from positive samples by integrating
141 rolling circle amplification (RCA) and end-point PCR methods (De Arcangeli et al., 2020). The
142 RCA was performed to increase the amount of circular DNA using the TempliPhi 100 amplification
143 kit (GE Healthcare) following the manufacturer's instructions. Briefly, after an incubation at 95 °C
144 for 3 min of a mix containing 10 µL of Sample buffer and 5 µL of sample DNA, 10 µL of a second
145 mix containing TempliPhi Reaction buffer, TempliPhi Enzyme Mix and dNTPs were added to each
146 samples, incubated at 30 °C for 16 h and inactivated at 65 °C for 10 min. A positive control DNA
147 supplied by the manufacturer and a no template control, consisting of ultrapure water, were added.
148 Subsequently, two overlapping regions of the viral genome were amplified by end-point PCR using
149 two sets of primers, respectively: CanineCV_1.020-1.040_For (5'-
150 CGTTTACCTGTTACCCCCCT-3') – CanineCV_909-931_Rev (5'-
151 AGCGAGAGGCCTTTATCTTTCAG-3') targeting a region of 1932 nt and CanineCV_3'-3'_For
152 (5'-ATGGTGGGATGGCTACGATG-3') – CanineCV_3'-3'_Rev (5'-
153 CAAGGAAGAGGGAATGCTACAAG-3') targeting a region of 936 nt (De Arcangeli et al., 2020).
154 A proofreading DNA polymerase (Phusion Hot Start II High-Fidelity DNA Polymerase, Thermo
155 Fisher Scientific) was used. The reactions were performed in a total volume of 50 µL containing 0.5
156 µM of each primer, 5X HF buffer, 2.5mM dNTP, 2U/µL Phusion Hot Start II DNA Polymerase and
157 5 µL of RCA product. The thermal cycling consisted of an initial denaturation at 98 °C for 30 s
158 followed by 35 cycles of denaturation at 98 °C for 10 s, annealing at 65.3 °C or 63.7 °C (for the first
159 and the second set of primers mentioned above, respectively) for 30 s and elongation at 72 °C for 1
160 min, followed by a final elongation step at 72°C for 10 min. A DNA extract of a CanineCV positive
161 sample was used as positive control (09-10F/2011, GenBank ID: MH454599, De Arcangeli et al.,
162 2020). A no template control, consisting of ultrapure water, underwent analysis simultaneously.
163 PCR products (5 µL) were separated by electrophoresis in a 1.0% agarose gel in TAE buffer and

164 visualised by UV light after staining with SYBR Safe DNA Gel Stain (Bio-Rad Laboratories).
165 Amplicons of the expected size were considered positive, purified using the QIAquick PCR
166 Purification Kit (QIAGEN) according to the manufacturer's instructions and directly sequenced by
167 Sanger method (BioFab Research) using both forward and reverse primers. For foxes that showed
168 CanineCV specific qPCR products in more than one organ, the amplicon that showed the highest
169 amount of target DNA and no non-specific products, was sequenced. The complete CanineCV
170 genome sequences obtained in this study are openly available in INSDC database
171 (<http://www.insdc.org/>; ID: MT180077-MT180090).

172

173 ***2.4. Sequence analysis***

174 Complete viral genomes were assembled, aligned with 96 reference sequences of CanineCV
175 from GenBank (Table S2) using the ClustalW method implemented in BioEdit 7.2.5 and translated
176 into amino acid sequences. Phylogeny was carried out on complete genome nucleotide sequences
177 and concatenated Rep and Cap amino acid sequences using the software MEGA X version 10.1.7
178 (Kumar et al., 2018). Phylogenetic trees were constructed using Maximum Likelihood method and
179 the best-fit model of nucleotide substitution was determined for both alignments using the Find Best
180 DNA/Protein Model function implemented in MEGA X. The General Time Reversible (GTR)
181 model with gamma distribution and invariable sites resulted optimal for the nucleotide alignment,
182 while the Jones-Taylor-Thornton (JTT) model with gamma distribution and invariable sites resulted
183 optimal for the amino acid alignment. The robustness of individual nodes on the phylogeny was
184 estimated using 1000 bootstrap replicates and bootstrap values >70 were indicated at the
185 corresponding node.

186

187 **3. Results**

188 ***3.1. Detection of CanineCV DNA***

Eleven out of 51 arctic foxes and 10/59 red foxes tested positive for CanineCV DNA.

CanineCV specific amplicons were generated from both sampled organs (liver and spleen) from nine of the 11 positive arctic foxes and five of the 10 positive red foxes. From the remaining foxes, such amplicons were generated from the spleen, except for one arctic fox which tested positive on the liver sample only. The overall median quantity of CanineCV DNA copies per microliter of spleen and liver DNA extract was 2.3×10^4 (range 7.7×10^1 - 1.7×10^6) in arctic foxes and 5.1×10^2 (range 6.7×10^1 and 3.3×10^5) in red foxes. For each fox species, the median amount of viral DNA detected in the extracts of the two tissues was of the same order of magnitude. Signalment data and positivity to CanineCV DNA of the foxes included in this study are reported in Table 1 (summary) and in Table S1 (detailed per animal).

3.2. Full-genome characterization

The complete CanineCV genome sequence was obtained from 8 arctic foxes and 6 red foxes. The genome of the 14 CanineCV sequenced in this study was 2063 nt in length. Genome characteristics include two major ORFs, ORF1 (912 nt) on the virion strand and ORF2 (813 nt) on the complementary strand of the replicative form, encoding for the Rep (303 amino acids) and the Cap (270 amino acids), respectively. Two intergenic noncoding regions of 135 and 203 nt in length, respectively, were located between the start and stop codons of the two major ORFs. The 5' IR, located between the start codons, contains a characteristic stem-loop structure with a conserved 9 nt motif (TAGTATTAC) for initiation of the rolling cycle replication.

Nucleotide alignment between the complete genomic sequences of CanineCV obtained in this study and reference strains showed an overall nucleotide identity ranging from 80.8 to 100%. All the sequences obtained in this study were different from each other and distinguishable in two groups: one composed by CanineCV identified in arctic foxes and one composed by CanineCV identified in red foxes, since they had a greater nucleotide identity within the groups (97.7-99.8% and 94.3-99.3%, respectively) rather than between the two groups (91.9-93.9%)..

215 The two unrooted phylogenetic trees constructed with complete genome nucleotide
216 sequences (Figure 2) and with concatenated amino acid sequences of predicted Rep and Cap protein
217 (Figure 3) showed a well distinguishable clustering of the CanineCV nucleotide sequences into five
218 groups. Group 1 included CanineCV identified in dogs, wolves and a badger from Europe, USA
219 and China. The groups 2, 3 and 4 includes CanineCV identified in dogs from Asia, with the
220 exception of virus 09-10F/2011, which was identified in a red fox in Italy in 2011 (GenBank ID:
221 MH454599; De Arcangeli et al., 2020) and allocated to group 4. Group 5 was only composed by
222 CanineCV identified in foxes, including the sequences obtained in this study, and showed a well
223 distinct origin in particular in the amino acidic tree (Figure 3). In this latter group, two lineages
224 composed by CanineCV identified in arctic foxes and in red foxes, respectively, were evidenced
225 and further distinguishable for geographic origin and years of sampling. Only in the nucleotide tree
226 the CanineCV 55590, identified in a red fox in Croatia in 2014 (GenBank ID: KP941114, Lojkić et
227 al., 2016), formed a distinct lineage within group 5 (Figure 2). The CanineCV obtained from a dog
228 in the USA in 2011 (GenBank ID: KC241983, Li et al., 2013) did not cluster in any of the five
229 groups, neither when comparing the nucleotide sequences nor the amino acid sequences. The
230 nucleotide identity values calculated for the CanineCV sequences belonging to the same cluster and
231 between different clusters identified in the phylogenetic analysis are reported in Table 2.

232

233 4. Discussion

234 In this study, we investigated the spread of CanineCV infection in arctic foxes from the
235 Svalbard archipelago, Norway, and red foxes from mainland Northern Norway (1996 to 2018), and
236 analysed the complete genome of the identified viruses. The CanineCV DNA was detected in liver
237 and/or spleen samples of 11/51 arctic foxes and in 10/59 red foxes. A median quantity of viral
238 DNA greater in tissues of arctic foxes than in red foxes was detected. Nevertheless, the
239 impossibility of correlate the number of CanineCV DNA copies detected per microliter of extract to
240 the exact amount of extracted tissue and the extreme variability of the time elapsed from the death

241 of the foxes to the sampling, with variable degradation of the tissues, do not allow to draw
242 conclusions. Further studies, as a controlled inoculation study, are needed to better evaluate this
243 aspect. The detection of CanineCV DNA in arctic foxes sampled between 1996 and 2001 represent
244 the first report for this wild species and suggest that the virus circulated in arctic foxes at least
245 fifteen years before its first discovery in domestic dog sera in the USA (Kapoor et al., 2012).
246 Accordingly, we suggest that wild carnivores may have harboured an ancestor of CanineCV, as
247 speculated by numerous studies on CPV-2, a similar small single-stranded DNA virus, in which
248 wild hosts played a key role in the emergence of infection in dogs (Allison et al., 2012, 2013;
249 Frölich et al., 2005; Truyen et al., 1995). The number of red foxes tested positive for CanineCV
250 DNA in this study (10/59) is lower than reported in United Kingdom (7/15 in healthy red foxes and
251 13/17 in red foxes with neurologic signs, Bexton et al. 2015) and higher than reported in Italy by De
252 Arcangeli et al. (1/32, 2020) and Zaccaria et al. (0/24, 2016). These discrepancies could be due to a
253 different viral spread in the geographic areas investigated, but also to the health status of the
254 sampled animals or the type of biological samples tested in the different studies. Indeed, serum,
255 faeces and several internal organs (spleen, tonsil, lymph nodes, liver, intestine, lung, kidney and
256 brain) samples have been tested in foxes for the molecular detection of CanineCV infection (Bexton
257 et al., 2015; De Arcangeli et al., 2020; Zaccaria et al., 2016), but the limited knowledge available to
258 date on the pathogenesis and clinical manifestations of the virus in wild hosts make it difficult to
259 choose the optimal organ sample for viral DNA detection.

260 From the complete genome alignment carried out in this study, all the CanineCV sequences
261 identified in dogs, foxes, wolves and badgers showed a nucleotide identity ≥ 80.8 %. According to
262 the species demarcation threshold of 80% genome-wide nucleotide sequence identity for members
263 of the family *Circoviridae* provided by Breitbart et al. (2017) and Rosario et al. (2017), this result
264 confirms the existence of a unique circovirus species (nominally canine circovirus), infecting dogs,
265 foxes, wolves and badgers and including the viruses detected in this study.

Phylogeny provided evidence of cluster formation of the CanineCV sequences into five groups, except for CanineCV UCD3-478 which could represent an intermediate sequence between groups 4 and 5 or the only virus to date sequenced of a further sixth group. Four of the five groups (named 1 to 4) correspond to the genotypes proposed by Niu et al. (2020). A fifth group, the number 5, composed only by CanineCV identified in foxes, included the viruses detected in this study, confirming a general clustering on geographic and/or host basis (Niu et al., 2020). In particular, the existence of a group of viruses infecting foxes, phylogenetically distinguishable from those infecting dogs, wolves and badgers, was evidenced as previously suggested by Zaccaria et al. (2016). However, not all the CanineCV detected in foxes clustered together in group 5, indeed the CanineCV 09-10F/2011 identified in a red fox clustered in group 4 with other viruses identified in dogs. Therefore, the CanineCV sequences do not group strictly on the basis of the host species in which they were identified. Further studies would be needed to investigate the susceptibility of domestic and wild carnivores to genetically different CanineCV with experimental or in vitro infections. Within group 5, the CanineCV identified in both arctic foxes and red foxes formed two distinct lineages. This genetic distance could be explained by the geographical segregation of the two fox species populations investigated in this study. Indeed, red foxes from the Norwegian mainland do not get in direct contact with arctic foxes in the archipelago of Svalbard (direct line distance is 835 km over the Barents Sea, that is not covered by sea ice), making a viral transmission between the two hosts at those locations impossible. Possible routes of transmission were also absent between arctic foxes in Svalbard and red foxes from Great Britain and Croatia in which the other CanineCV included in this group were identified. Nevertheless, indirect viral transmission between the two species populations could be possible through migration of arctic foxes to Svalbard from other arctic regions in which red foxes are present by using the sea ice as platform (Geffen et al., 2007; Henttonen et al., 2001; Mørk et al., 2011; Norén et al. 2011) or through domestic dogs (including sled dogs) brought from the Norwegian mainland to the Svalbard archipelago. Another hypothesis that may explain the sequence diversity that emerged between the CanineCV identified

292 in arctic foxes and red foxes, respectively, is the acquisition of mutations during the time elapsed
293 between the sampling of the two species.

294

295 **5. Conclusions**

296 The present study reports the detection of CanineCV DNA in arctic foxes from the Svalbard
297 archipelago, Norway, since 1996, fifteen years before the first report in domestic dogs in USA.
298 Sequences analysis showed that CanineCV identified in arctic foxes from Svalbard and red foxes
299 from mainland Norway represent two distinct lineages in a well distinct phylogenetic group,
300 separated from other CanineCV. Currently, very little is known about the pathogenic role of
301 CanineCV in wild carnivores and the possible transmission of the virus between wild animals and
302 domestic dogs. Continuous epidemiological surveillance is therefore needed to understand the
303 importance and evolution of CanineCV in wild animals and to characterise the potential pathogenic
304 impact CanineCV may have on dogs and on wild carnivore species and populations.

305

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312 Institute, for graphical design of maps.

313

314 **Authors' contributions**

315 LU wrote the original manuscript draft. LU and AB performed tests acquiring the sequence
316 data. MT, DE, EF collected samples and data. LU, MT and AB analysed the data. MT, MB and AB

317 conceived and designed the study and critically revised the manuscript. All authors read,
318 commented on and approved the final drafts of the manuscript.

319

320 **Ethical Statement**

321 The authors confirm that the ethical policies of the journal, as noted on the journal's author
322 guidelines page, have been adhered to. No ethical approval was required as sample collection was
323 obtained from dead animals and no life animal handling was performed in order to conduct the
324 present study.

325

326 **Conflict of Interest Statement**

327 The authors have no financial or personal interests that could influence or bias the content
328 of this article. The authors declare that they have no competing interests. All authors have seen and
329 approved the manuscript.

330

331 **Data Availability Statement**

332 The data that support the findings of this study are openly available in INSDC
333 (<http://www.insdc.org/>; ID: MT180077-MT180090).

334

335 **Supporting Information**

336 Additional supporting information may be found online in the Supporting Information
337 section at the end of the article.

338

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430 **Table 1** Signalment data and positivity to canine circovirus DNA of the arctic foxes (*Vulpes*
431 *lagopus*) and red foxes (*Vulpes vulpes*) included in this study

| Variables | Arctic foxes | | | Red foxes | | |
|-------------------------------|--------------|----------|----------|-----------|----------|---------|
| | Positive | Negative | Total | Positive | Negative | Total |
| Number of foxes | 11 | 40 | 51 | 10 | 49 | 59 |
| Sex | | | | | | |
| Male | 7 | 17 | 24 | 6 | 25 | 31 |
| Female | 4 | 21 | 25 | 4 | 24 | 28 |
| NA | - | 2 | 2 | - | - | - |
| Geographical origin | | | | | | |
| Svalbard archipelago (Norway) | | | | | | |
| Adventdalen | - | 1 | 1 | - | - | - |
| Austfjordnes | 3 | 8 | 11 | - | - | - |
| Bjonehamna | 1 | - | 1 | - | - | - |
| Bjørndalen | - | 1 | 1 | - | - | - |
| Coles Bay | 1 | - | 1 | - | - | - |
| Colesdalen/Grumant | 3 | 5 | 8 | - | - | - |
| Flowerdalen | - | 1 | 1 | - | - | - |
| Fredheim | - | 1 | 1 | - | - | - |
| Janssonhaugen | 1 | 2 | 3 | - | - | - |
| Kapp Wijk | 1 | 11 | 12 | - | - | - |
| Koslodalen | - | 1 | 1 | - | - | - |
| Kapp Murdoch | - | 1 | 1 | - | - | - |
| Nordenskiöld Land | - | 5 | 5 | - | - | - |
| Reindalen | - | 1 | 1 | - | - | - |
| Templet | 1 | - | 1 | - | - | - |
| NA | - | 2 | 2 | - | - | - |
| Finnmark County (Norway) | | | | | | |
| Iešjávri | - | - | - | 7 | 19 | 26 |
| Varanger Peninsula | - | - | - | 3 | 30 | 33 |
| Year of sampling | | | | | | |
| 1996 | 1 | 3 | 4 | - | - | - |
| 1997 | 6 | 23 | 29 | - | - | - |
| 1998 | 1 | 2 | 3 | - | - | - |
| 1999 | 3 | 11 | 14 | - | - | - |
| 2001 | - | 1 | 1 | - | - | - |
| 2014 | - | - | - | 2 | 13 | 15 |
| 2015 | - | - | - | 2 | 10 | 12 |
| 2016 | - | - | - | 1 | 9 | 10 |
| 2017 | - | - | - | 3 | 10 | 13 |
| 2018 | - | - | - | 2 | 7 | 9 |
| Age (years) [†] | 1 [1-3] | 1 [1-11] | 1 [1-11] | 2 [1-3] | 2 [1-8] | 2 [1-8] |

432 ^{††}Data are reported as median [minimum value – maximum value].

433 Note: NA = not available.

434 **Table 2** Nucleotide identities (%) among complete genome sequences (2063-2064 nt) belonging to
 435 the clusters identified in the phylogenetic analysis. The sequences used for alignment are shown in
 436 the Table S2

437

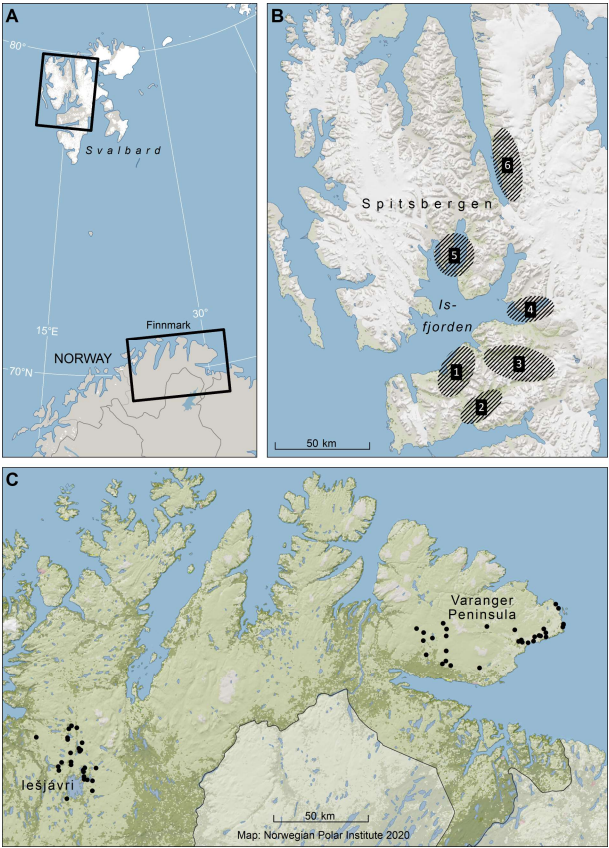
| | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 | AF | RF | UCD3-478 |
|-----------------|----------------|----------------|----------------|----------------|----------------|-----------|-----------|-----------------|
| Group 1 | 93.5-100 | 83.8-87.3 | 88.7-90.4 | 85.9-92.6 | 82.5-84.9 | 82.6-84.7 | 82.5-84.9 | 84.6-86.8 |
| Group 2 | 83.8-87.3 | 89.1-99.9 | 85.1-90.1 | 84.8-90.6 | 80.8-83.6 | 81.2-83 | 80.8-83.6 | 81.9-84.6 |
| Group 3 | 88.7-90.4 | 85.1-90.1 | 96.4-99.8 | 85.9-89.8 | 82.1-83.8 | 82.4-83.4 | 82.1-83.8 | 84.3-84.5 |
| Group 4 | 85.9-92.6 | 84.8-90.6 | 85.9-89.8 | 92.4-99.9 | 84.4-86.6 | 84.4-85.8 | 84.6-86.6 | 88.8-90.9 |
| Group 5 | 82.5-84.9 | 80.8-83.6 | 82.1-83.8 | 84.4-86.6 | 91.9-99.8 | - | - | 88.8-89.9 |
| AF | 82.6-84.7 | 81.2-83 | 82.4-83.4 | 84.4-85.8 | - | 97.7-99.8 | 91.9-93.9 | 88.9-89.1 |
| RF | 82.5-84.9 | 80.8-83.6 | 82.1-83.8 | 84.6-86.6 | - | 91.9-93.9 | 94.3-99.3 | 88.9-89.9 |
| UCD3-478 | 84.6-86.8 | 81.9-84.6 | 84.3-84.5 | 88.8-90.9 | 88.8-89.9 | 88.9-89.1 | 88.9-89.9 | 100 |

438 Data are expressed as range from the minimum identity value to the maximum identity value.

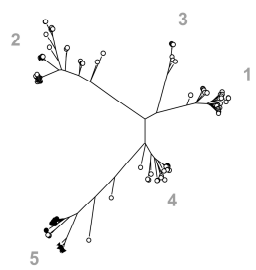
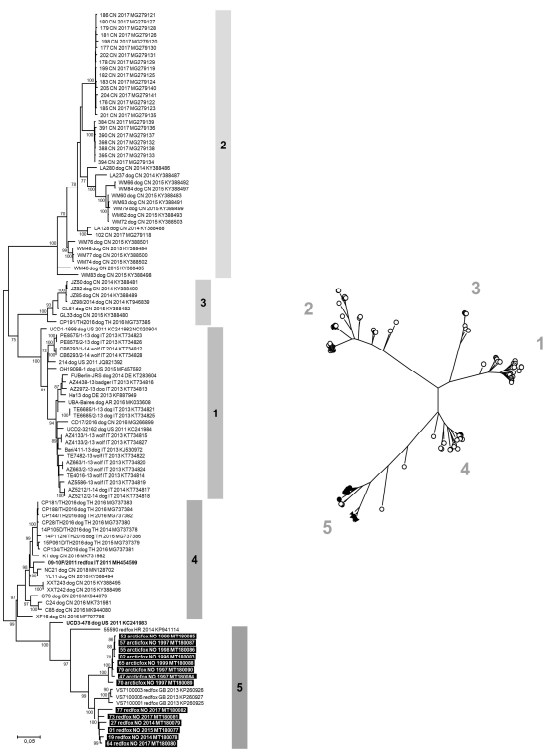
439 Nucleotide identities among sequences of CanineCV identified in this study in arctic foxes and red
 440 foxes and sequences of CanineCV within group 5 have not been reported because they are
 441 themselves included in group 5.

442 Note: AF = CanineCV identified in this study in arctic foxes; RF = CanineCV identified in this
 443 study in red foxes.

444 Fig. 1. Sampling locations. (A) Arctic foxes (*Vulpes lagopus*) were sampled on Spitsbergen,
 445 Svalbard, and red foxes (*Vulpes vulpes*) in Finnmark County, mainland Norway. (B) Arctic foxes
 446 were sampled from six areas on Spitsbergen, Svalbard. Area 1: including Bjørndalen, Coles Bay,
 447 Colesdalen and Grumant; Area 2: including Reindalen; Area 3: including Adventdalen,
 448 Flowerdalen, Janssonhaugen, Koslodalen and Nordenskiöld Land; Area 4: including Bjonehamna,
 449 Fredheim, Kapp Murdoch and Templet; Area 5: including Kapp Wijk and Area 6: including
 450 Austfjordnes. (C) Red foxes were sampled in Low-Arctic ecosystems of Varanger Peninsula and in
 451 the Sub-Arctic ecosystems in the mountain region around the lake Iešjávri, Finnmark County,
 452 Norway.

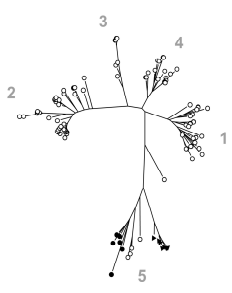
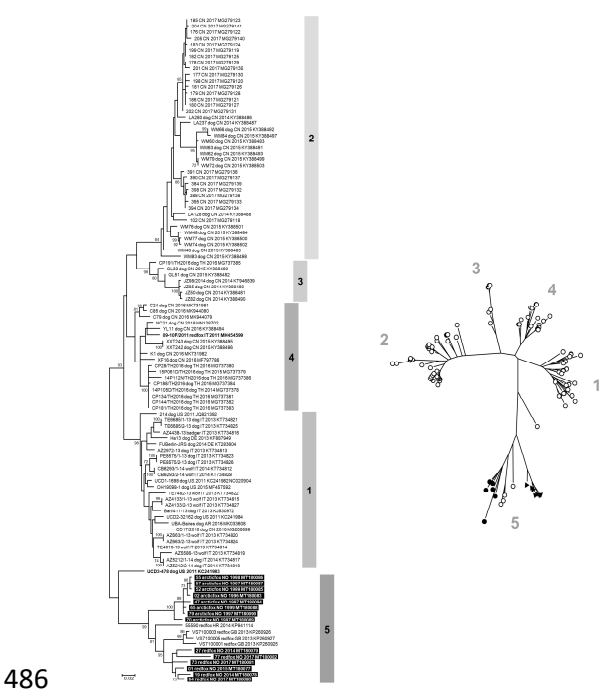


454 Fig. 2. Unrooted phylogenetic tree based on the complete genome nucleotide sequences of canine
455 circovirus (CanineCV) obtained in this study and reference strains in the GenBank database (Table
456 S2). The best-fit model of nucleotide substitution was determined using the Find Best DNA/Protein
457 Model function implemented in MEGA X version 10.1.7. General Time Reversible (GTR) model
458 with gamma distribution and invariable sites resulted optimal for the sequence data. The
459 evolutionary distances were computed using the Maximum Likelihood method. Statistical support
460 was provided by bootstrapping with 1000 replicates. Bootstrap values greater than 70% are
461 indicated on the respective branches. The scale bars indicate the estimated numbers of nucleotide
462 substitutions. Highlighted in black: Sequences generated in this study. In bold: Sequence of
463 CanineCV 09-10F/2011 (MH454599) and CanineCV UCD3-478 (KC241983). On the left a
464 traditional rectangular branch style of the tree. On the right a radiation branch style of the tree
465 (black triangles: CanineCV identified in arctic foxes in this study, black circles: CanineCV
466 identified in red foxes in this study, Empty circles: CanineCV reference sequences). Numbers in
467 grey are the groups evidenced in this study and from 1 to 4 correspond to genotypes proposed by
468 Niu et al. (2020).



469

470 Fig. 3. Unrooted phylogenetic tree constructed with the multiple gene approach: concatenated
471 amino acid sequences of the replicase associated protein (Rep) and the capsid protein (Cap) of
472 canine circovirus (CanineCV) generated in this study and reference sequences available from
473 GenBank (Table S2). The best-fit model of nucleotide substitution was determined using the Find
474 Best DNA/Protein Model function implemented in MEGA X version 10.1.7. Jones-Taylor-
475 Thornton (JTT) model with gamma distribution and invariable sites resulted optimal for the
476 sequence data. Phylogenetic tree was constructed using Maximum Likelihood method and bootstrap
477 values were determined by 1000 replicates to assess the confidence level of each branch pattern.
478 Bootstrap values greater than 70% are indicated on the respective branches. The scale bars indicate
479 the estimated numbers of amino acid substitutions. Highlighted in black: Sequences generated in
480 this study. In bold: Sequence of the CanineCV 09-10F/2011 (MH454599) and CanineCV UCD3-
481 478 (KC241983). On the left a traditional rectangular branch style of the tree. On the right a
482 radiation branch style of the tree (black triangles: CanineCV identified in arctic foxes in this study,
483 black circles: CanineCV identified in red foxes in this study, Empty circles: CanineCV reference
484 sequences). Numbers in grey are the groups evidenced in this study and from 1 to 4 correspond to
485 genotypes proposed by Niu et al. (2020).



486