Prevalence, distribution and molecular epidemiology of Aleutian mink disease virus, mink enteritis virus and other parvoviruses in wild mink of British Columbia

Final report, July 2021

Lay summary.

Our group studies the ecology of parvoviruses infecting carnivores. We are interested in how these viruses move across lands and host species and how they evolve and adapt to new hosts and environments. In this study, we evaluated two different viruses belonging to two different but closely related genera, the skunk amdoparvovirus (genus *Amdoparvovirus*) and the fox newlavirus (genus *Protoparvovirus*), both discovered in our lab. The skunk amdoparvovirus primarily infects skunks but it has been found in mink as well and is likely capable of infecting other carnivores. This virus has been detected across North America, both in the U.S. and Canada, and a very specific spatial segregation of strains was observed such that viruses from the east were very divergent from those of the west and we observed marked differences between those in the northern and southern parts of the west as well. This is a very different behavior compared to its close relative Aleutian mink disease virus, which was the subject of our previous investigations, and which shows no geographic differentiation, a phenomenon linked to mink farming and animal import-export. The fox newlavirus, on the contrary, was found to be species-specific as it reached high prevalence (~40%) in fox populations of Newfoundland and Labrador but was not detected in any other tested carnivores, including coyotes, dogs, lynx, mink, marten, ermine, and seals. This is also a very different behavior compared to its close relative canine parvovirus 2, which our group investigated in the past, and which showed no host specificity and was found in a vast range of carnivore species. Differently from the skunk amdoparvovirus, we did not observe geographic differences in the distribution of fox newlavirus strains, although we observed a very high genetic diversity. In conclusion, there is a great variety of parvoviruses of carnivores, and they all show different ecology and behavior. Since many carnivore populations have never been investigated, it is very certain there are still more carnivore parvoviruses to be discovered.

Research objective.

The main goal of this research was to investigate the distribution of mink parvoviruses in mainland BC and compare this to the results we previously obtained from islands in BC. This study had the broader scope of acquiring an understanding of the ecology, host ranges and geographic distributions of carnivore parvoviruses. The research objective focused specifically on mink pathogens, such as the Aleutian mink disease viruses (AMDV and AMDV-2)[1,2], the skunk amdoparvovirus (SKAV)[3], and the mink enteritis virus (MEV)[4], and the potential threat viruses in the wildlife pose to farmed animals. An additional objective of our research was to identify novel parvoviruses of carnivores that can pose an unanticipated threat to mustelids, both farmed and wild.

Results.

Due to the COVID-19 pandemic and the consequent containment measures, it was impossible to collect the samples as planned in the grant proposal. Furthermore, the lockdown and the consequent closure of universities and research facilities drastically reduced laboratory access and the planned experiments could not be performed. Efforts were, therefore, focused on completing analyses with data collected during previous research, studying the molecular epidemiology of amdoparvoviruses of skunks using already available samples, and performing virus discovery on samples that were previously collected from carnivores.

1. Skunk amdoparvovirus phylogeography

A collaborative study was initiated with the research group of Dr. Patricia Pesavento at the University of California, Davis, to investigate the phylogeography of SKAVs throughout North America. SKAV is a virus of skunks that can infect mink[3,5], although the extent of the distribution of this virus in mink in not known.

During this study, 29 complete genomes were sequenced from samples collected from striped skunks in several locations of North America, including British Columbia (BC, N=10), Ontario (ON, N=7), Nova Scotia (NS, N=1), California (CA, N=7), New Hampshire (NH, N=2), and Maine (MA, N=2).

Maximum-likelihood phylogenetic analysis performed with the non-structural protein 1 (NS1) of these viruses and using AMDV and AMDV-2 as the outgroup revealed a clear geographic segregation of strains. Specifically, SKAV sequences formed 3 bootstrap-supported groups, one corresponding to the southwest (California), one including sequences from the northwest (BC), and one corresponding to the eastern viruses (ON, NS, NH, MA) (Figure 1). Furthermore, within this last group, sequences clustered together according to sampling location. This is a very different scenario compared to what we previously observed for AMDV[1] and probably reflects the fact that SKAVs evolved into independent lineages in geographically separated wild animal populations and the lack of viral dispersion through animal trading. In fact, we previously hypothesized that the lack of geographic segregation of AMDV strains was due to the practice of importing and exporting animals between distant locations and subsequent virus dispersal into the environment[1].

Our results show that SKAV, although capable of infecting mink[3], has so far not spread in mink farms and the genetic differences between strains simply reflect local differentiation. Additionally, in the occasion of a SKAV outbreak in mink farms, this specific signature would make it very easy to locate the origin of the introduction and to study the evolutionary dynamics of this virus, should it be introduced into the wildlife of new geographic areas.

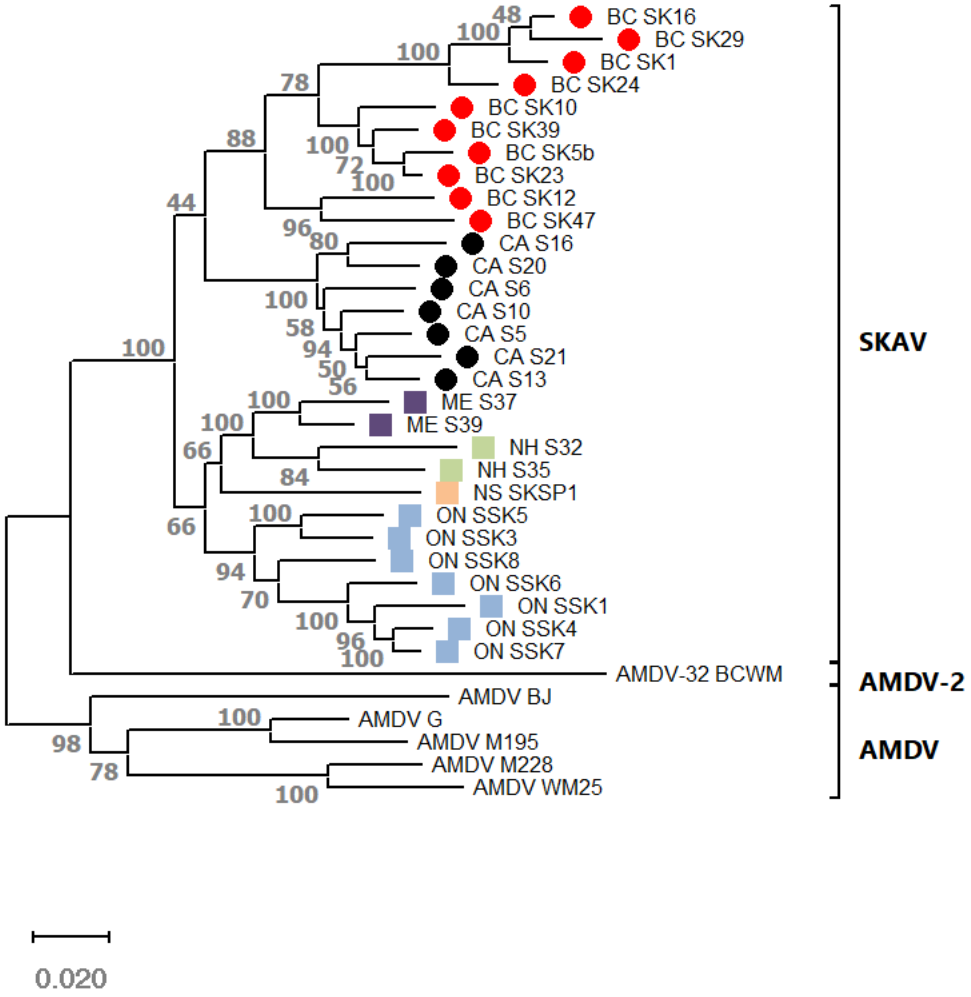

Figure 1. Maximum-likelihood phylogenetic analysis of SKAV sequences from various North American locations. *Sequences of AMDV and AMDV-2 were used as the outgroup. Strains are labelled according to the collection site: full circles represent the west (BC in red and California in black), while full squares represent the east (Maine in purple, New Hampshire in green, Nova Scotia in Orange, and Ontario in blue).*

2. *Parvovirus discovery*

In an effort to identify previously uncharacterized viruses that could pose a threat to wild and farmed mink, the VidION virus discovery method[6] was applied to fecal samples (rectal swabs or feces) collected from wild carnivores in BC and Newfoundland and Labrador. In detail, 10 rectal swabs from BC martens (*Martes americana*), 10 rectal swabs from BC wolves (*Canis lupus*), and 68 fecal samples from red and Arctic foxes (*Vulpes vulpes* and *Vulpes lagopus*) from Labrador were investigated and a novel fox parvovirus was discovered. The novel virus, which we named Newlavirus (Newfoundland and Labrador virus) was fully molecularly characterized, its relationship with other viruses investigated, and its epidemiology and host distribution was studied.

Six complete genomes were obtained from different Newlavirus strains, and these showed the typical organization of parvoviruses with two main gene cassettes coding for the non-structural and capsid

proteins. Splicing signals typical of parvoviruses were also identified (Figure 2A). Additionally, molecular motifs typical of parvovirus proteins were found in the NS1, including the rolling circle replication (RCR) motif II and III and the four helicase Walker domains, and of the capsid protein, including the two phospholipase A2 domains (Calcium binding site and catalytic domain) and the glycine-rich domain (Figure 2B)[7].

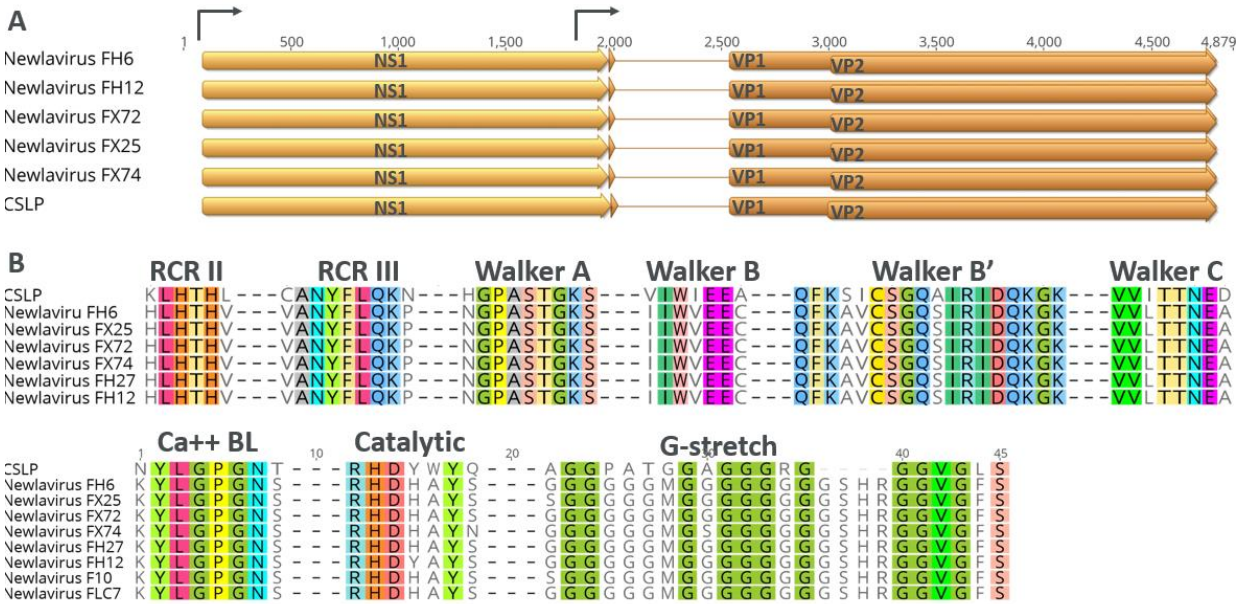

Figure 2. Genome organization of Newlavirus. A. *Schematic genome representations of the novel parvovirus (strains FH6, FX25, FX72, FX74, FH27, and FH12) compared to the closely related California sea lion parvovirus (CSLP). Hypothetical non-structural (NS) and structural (VP) proteins are also indicated. The arrows represent putative promoters.* ***B.*** *Conserved rolling circle replication (RCR) and Walker motifs typical of parvoviral SF3 helicases (top) and conserved phospholipase A2 (Ca++ BL: calcium binding loop; Catalytic: catalytic site) and poly-glycine scratch typical of parvoviral VP proteins (bottom).*

Phylogenetic analysis of the conserved NS1 helicase domain of Newlavirus and members of the genus *Protoparvovirus* revealed that the novel virus belongs to this genus, but it is distantly related to other species (38.7-54.1% NS1 amino acid identity) (Figure 3). Given the ICTV rules for species and genera assignments[8], which have an NS1 amino acid identity cut-off of 85% and 30% for species and genus, respectively, Newlavirus has to be considered a novel species within the genus *Protoparvovirus*.

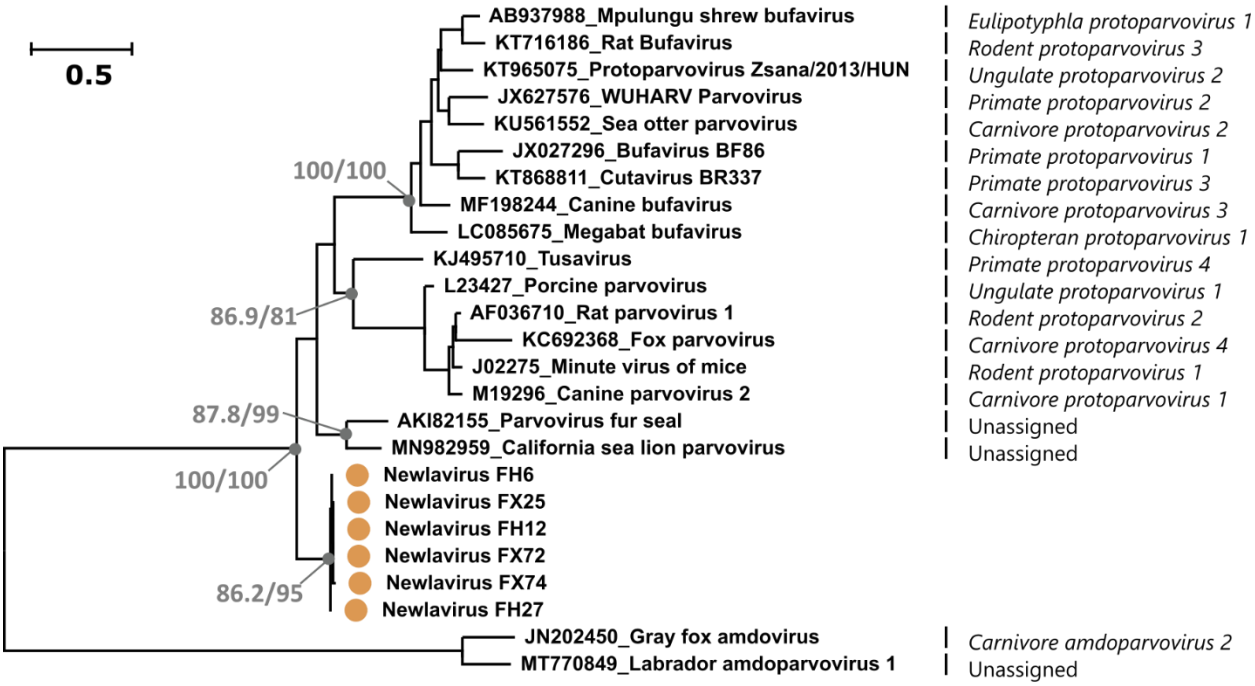

Figure 3. Phylogenetic analysis of Newlavirus. *The maximum-likelihood phylogenetic tree was built with the NS1 helicase domain of representative members of each known species of the genus* Protoparvovirus *plus two fox amdoparvoviruses as the outgroup. The viruses identified in this study are indicated by orange dots. Species names are indicated on the right.*

The host distribution of Newlavirus was investigated by screening 729 samples collected from 613 carnivores, including canids, felids, and mustelids. The tested samples included paired tissues and fecal material. Interestingly, the virus was only found in foxes and in both red foxes and Arctic foxes (Table 1). This suggests that Newlavirus is a virus of foxes and may not infect other carnivores, although a larger number of samples should be tested to confirm these data.

Table 1. Animals positive for Newlavirus. NA: not available.

Animal	Overall	Fecal samples	Tissue samples
Mink, N=47	0/47 (0%)	NA	0/47 (0%)
Marten, N=146	0/146 (0%)	NA	0/146 (0%)
Ermine, N=17	0/17 (0%)	NA	0/17 (0%)
Seal, N=18	0/18 (0%)	0/18 (0%)	NA
Lynx, N=58	0/58 (0%)	NA	0/58 (0%)
Coyote, N=92	0/92 (0%)	0/40 (0%)	0/87 (0%)
Dog, N=48	0/48 (0%)	0/48 (0%)	NA
Fox, N=187	76/187 (40.6%)	24/80 (30.0%)	61/179 (34.1%)

In foxes, the virus was significantly more prevalent in both fecal samples (24/80, 30.0%) and spleen (59/152, 38.8%) than lymph nodes (2/37, 5.4%), $p<0.002$, and only 27.3% of the positive animals had the virus in both organs and in feces. These results indicate that the virus causes active infection in foxes, has intestinal tropism, and either causes mild symptomatology or is shed for a long time after remission.

The virus was widespread in both Newfoundland and Labrador, and viral prevalence was similar in all considered regions, except for the area of Happy Valley Goose Bay (Table 2). However, samples from this location were mostly lymph nodes and a lower viral prevalence could be the result of a sampling bias.

Table 1. Newlavirus prevalence in Newfoundland and Labrador.

Location	N. positive (%)
Newfoundland - Total, N=50	22 (44)
Newfoundland – Avalon Peninsula, N=34	14 (43.8)
Newfoundland – Northern Peninsula, N=14	7 (50.0)
Labrador - Total, N=137	54 (39.4)
Labrador - Nain, N=9	5 (55.6)
Labrador – Hopedale, N=29	15 (51.7)
Labrador – Labrador City, N=48	24 (50)
Labrador – Happy Valley Goose Bay, N=51	10 (19.6)

All investigated wild mink were from the Avalon peninsula. In this region, 43.8% of investigated foxes were Newlavirus-positive so we can conclude that there is likely no cross cross-species transmission of Newlavirus from foxes to mink. This is intriguing since other investigated parvoviruses, like MEV or amdoparvoviruses, do frequently infect multiple carnivores[9,10].

To better clarify the molecular epidemiology of this virus, a ~700 nt fragment of the VP1 gene was amplified and sequenced from 53 positive samples. Among the sequenced samples, 18 (34%) showed the presence of more than one virus, indicated by the presence of several double peaks in the electropherogram. Among the other 37 amplified strains, we could identify 13 different clades defined as bootstrap-supported groups of sequences characterized by at least 90% pairwise identity (Figure 4). Although in most cases samples from Newfoundland and Labrador were included within the same groups, within the clade with the highest number of sequences (clade 1) we could observe a clear separation between sequences from Newfoundland and those from Labrador. These results indicate the presence of high genetic variability and partial geographic segregation of strains, indicating that the viruses have been circulating in the fox population for a long time and that viruses have been exchanged between foxes of the two regions.

In conclusion, Newlavirus is a novel virus of foxes, characterized by high genetic diversity and high prevalence. These characteristics indicate that this virus likely circulates in other parts of North America and future investigations should elucidate its distribution. Our results show that this virus might be restricted to the fox reservoir, and it is not, therefore, a potential threat for wild and farmed mink. However, these results need be confirmed by studies involving a higher number of samples.

3. Completion of previous work

Data and sequence analysis of the results obtained during the study investigating AMDV and MEV in wildlife of insular BC resulted in an article published in the journal *Pathogens* (see below). Furthermore, a study about the characterization of two novel carnivore amdoparvoviruses (Labrador amdoparvoviruses 1 and 2, LaAV-1 and -2) and the mechanisms behind amdoparvoviral cross-species transmission was published in *Virus Evolution* (see below). LaAV-1 is a virus that can be transmitted between foxes and mustelids and as such can be considered a potential threat for the mink farming industry. This study also reports for the first time the identification of AMDV in lynx and foxes.

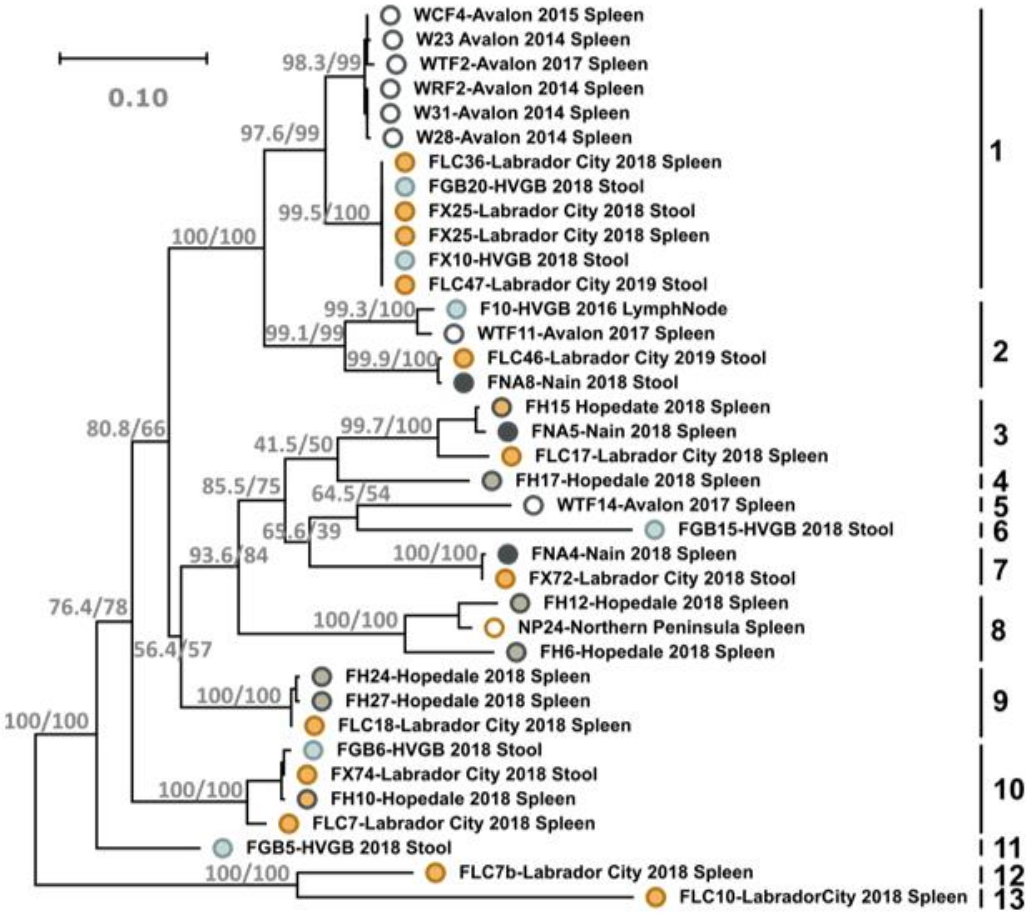

Figure 3. Phylogenetic analysis of Newlavirus strains from Newfoundland and Labrador. *The maximum-likelihood phylogenetic tree was built with a fragment of the VP1 gene (approximately 700 nt). Identified viral clades are indicated on the right and strains are indicated with colored labels corresponding to the sampling locations.*

Publications.

In the years 2020 and 2021 we produced five manuscripts that acknowledge funding from the JMRC, three of which have been published and two of which are in preparation for submission.

M. Canuti, M. Todd, P. Monteiro, K. Van Osch, R. Weir, H. Schwantje, A.P. Britton, A.S. Lang; **Ecology and infection dynamics of multi-host amdoparvoviral and protoparvoviral carnivore pathogens**. *Pathogens* 2020, 9:124

J.J. Pénzes, M. Söderlund-Venermo, M. Canuti, A.M. Eis-Hübinger, J. Hughes, S.F. Cotmore, B. Harrach; **Reorganizing the family *Parvoviridae*: a revised taxonomy independent of the canonical approach based on host association**. *Archives of Virology*, 2020, 165(9):2133-2146

M. Canuti, E. McDonald, S.M. Graham, B. Rodrigues, É. Bouchard, R. Neville, M. Pitcher, H.G. Whitney, H.D. Marshall, A.S. Lang; **Multi-host distribution of known and novel carnivore amdoparvoviruses**. *Virus Evolution*, 2020, 6(2):veaa072

M. Canuti, É. Bouchard, B. Rodrigues, H.G. Whitney, S.C. Dufour, A.S. Lang, J.T.P. Verhoeven; **Newlavirus, a novel highly prevalent and highly diverse protoparvovirus of foxes**. In preparation for submission to *Viruses*

C.E. Alex, M. Canuti, M. Schlesinger, K.A. Jackson, D. Needle, A.S. Lang, P. Pesavento: **Genetic diversity of skunk amdoparvoviruses infecting striped skunks (*Mephitis mephitis*) from the United States and Canada**. In preparation for submission to *Transboundary and Emerging Diseases*

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8 Pénzes JJ, Söderlund-Venermo M, Canuti M, *et al.* Reorganizing the family *Parvoviridae*: a revised taxonomy independent of the canonical approach based on host association. *Arch Virol* 2020; **165**: 2133–46.

9 Canuti M, Todd M, Monteiro P, *et al.* Ecology and infection dynamics of multi-host amdoparvoviral and protoparvoviral carnivore pathogens. *Pathogens* 2020; **9**: 124.

10 Canuti M, McDonald E, Graham SM, *et al.* Multi-host dispersal of known and novel carnivore amdoparvoviruses. *Virus Evolution* 2020; **6**. DOI:10.1093/ve/veaa072.