

Ecology and infection dynamics of multi-host amdoparvoviral and protoparvoviral carnivore pathogens

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Table S1. Summary details for animals screened in this study.

		American mink	Pacific marten	Ermine	River otter	Total
Total		77	130	27	22	256
Sex						
	Female	21	60	6	10	97
	Male	56	70	21	12	159
Age						
	Juvenile	11	65	2	8	86
	Adult	66	65	10	14	155
	Unknown	/ ¹	/	15	/	15
Location						
	Vancouver Island	76	107	21	22	226
	Haida Gwaii, Graham Island	/	9	3	/	12
	Haida Gwaii, Moresby Island	/	14	/	/	14
	Minstrel Island	/	/	3	/	3
	Quadra Island	1	/	/	/	1
Death year						
	2002-2015	/	/	18	/	18
	2016	/	79	1	/	80
	2017	21	37	/	/	58
	2018	40	14	2	/	56
	2019	16	/	6	22	44
Body condition						
	Excellent	6	4	/	1	11
	Good	32	40	6	3	81
	Fair	29	73	14	15	131
	Poor	10	13	7	3	33

¹ A / indicates no animals were present in that category

Table S2. Parvovirus screening results for mink.

	AMDV			Protoparvoviruses			Co-infection
	Positive N (%)	Negative N (%)	p	Positive N (%)	Negative N (%)	p	
TOTAL (N=77)	32 (41.6)	45 (58.4)		5 (6.5)	72 (93.5)		
2017 (N=21)	8 (38.1)	13 (61.9)	0.7-0.9	1 (4.8)	20 (95.2)	0.07-0.7	X
2018 (N=40)	17 (42.5)	23 (57.5)		1 (2.5)	39 (97.5)		
2019 (N=16)	7 (43.8)	9 (56.2)		3 (18.8)	13 (81.2)		
South-east VI (N=60)	24 (40)	36 (60)	<0.02	4 (6.7)	56 (93.3)		X
Cowichan River Valley (N=6)	1 (16.7)	5 (83.3)*		2 (33.3)	4 (66.7)		
North Cowichan (N=7)	3 (75)	4 (25)		2 (28.6)	5 (71.4)		
Mesachie Lake (N=18)	7 (38.9)	11 (61.1)*		0 (0)	18 (100)		
Copper Canyon (N=11)	6 (54.5)	5 (45.5)		0 (0)	11 (100)		
Ladysmith-Mckay Peak (N=14)	5 (35.7)	9 (64.3)*		0 (0)	14 (100)		
Coombs/Blueberry Road (N=4)	2 (50)	2 (50)		0 (0)	4 (100)		
South-west VI (3)	0 (0)	3 (100)		0 (0)	3 (100)		
French Creek Headwaters (N=3)	0 (0)	3 (100)*		0 (0)	3 (100)		
Middle-island VI (N=9)	8 (88.9)	1 (11.1)		0 (0)	9 (100)		
Great Central Lake (N=9)	8 (88.9)	1 (11.1)		0 (0)	9 (100)		
North-east VI (N=4)	0 (0)	4 (100)		1 (33.3)	3 (66.7)		
Campbell River (N=4)	0 (0)	4 (100)*		1 (33.3)	3 (66.7)		
Quadra Island (N=1)	0 (0)	1 (100)		0 (0)	1 (100)		
Female (N=21)	8 (38.1)	13 (61.9)	0.7	0 (0)	21 (100)	0.2	X
Male (N=56)	24 (42.9)	32 (57.1)		5 (8.9)	51 (91.1)		
Adult (N=66)	30 (45.5)	36 (54.5)	0.1	5 (7.6)	61 (92.4)	0.5	X
Juvenile (N=11)	2 (18.2)	9 (81.8)		0 (0)	11 (100)		
Excellent (N=6)	2 (40)	4 (60)	0.1-0.8	1 (20)	5 (80)	0.07-0.9	X
Good (N=32)	11 (34.4)	21 (65.6)		4 (12.5)	28 (87.5)		
Fair (N=29)	16 (55.2)	13 (44.8)		0 (0)	29 (100)		
Poor (N=10)	3 (30)	7 (70)		0 (0)	10 (100)		
Enlarged spleen (N=8)	2 (25)	6 (75)	0.4				
Non-enlarged spleen (N=69)	30 (43.5)	39 (56.5)					
	Average (SD)	Average (SD)		Average (SD)	Average (SD)		
Weight (kg)	0.73 (0.23)	0.71 (0.26)	0.7	0.99 (0.20)	0.70 (0.24)	0.01	0.89
Length (mm)	558.03 (42.66)	537.93 (65.05)	0.1	509.60 (114.49)	548.83 (51.80)	0.1	576.00
Chest girth (mm)	158.84 (23.34)	159.27 (24.57)	0.9	173.60 (16.21)	158.08 (24.12)	0.2	179.00

* Prevalence was significantly lower than in Great Central Lake area.

Table S3. Parvovirus screening results for Vancouver Island Pacific martens.

	AMDV			Protoparvoviruses		
	Positive N (%)	Negative N (%)	p	Positive N (%)	Negative N (%)	p
TOTAL (N=107)	4 (3.7)	103 (96.3)		1 (0.9)	106 (99.1)	
2016 (N=76)	3 (3.9)	73 (96.1)	0.9	1 (1.3)	75 (98.7)	0.7
2017 (N=31)	1 (3.2)	30 (96.8)		0 (0)	31 (100)	
South-east VI (N=47)	0 (0)	47 (100)		1 (2.1)	46 (97.9)	
Mesachie Lake (N=18)	0 (0)	18 (100)		0 (0)	18 (100)	
Copper Canyon (N=14)	0 (0)	14 (100)		1 (7.1)	13 (92.9)	
Ladysmith-Mckay Peak (N=15)	0 (0)	15 (100)		0 (0)	15 (100)	
Middle-island VI (N=5)	1 (20)	4 (80)		0 (0)	5 (100)	
Upper Taylor River (N=5)	1 (20)	4 (80)		0 (0)	5 (100)	
North-west VI (N=17)	0 (0)	17 (100)		0 (0)	17 (100)	
Fair Harbour area (N=17)	0 (0)	17 (100)		0 (0)	17 (100)	
North-east VI (N=38)	3 (7.9)	35 (92.1)		0 (0)	38 (100)	
Rupert Main (N=9)	1 (11.1)	8 (88.9)		0 (0)	9 (100)	
Coho Road (N=5)	0 (0)	5 (100)		0 (0)	5 (100)	
West Main (N=6)	0 (0)	6 (100)		0 (0)	6 (100)	
Cluxewe Road (N=2)	1 (50)	1 (50)		0 (0)	2 (100)	
Alice main (N=2)	1 (50)	1 (50)		0 (0)	2 (100)	
Others (N=14)	0 (0)	14 (100)		0 (0)	14 (100)	
Female (N=57)	2 (3.5)	55 (96.5)	0.9	1 (1.8)	56 (98.2)	0.5
Male (N=50)	2 (4)	48 (96)		0 (0)	50 (100)	
Adult (N=49)	2 (4.1)	47 (95.9)	0.9	0 (0)	49 (100)	0.5
Juvenile (N=58)	2 (3.4)	56 (96.6)		1 (1.7)	57 (98.3)	
Excellent (N=3)	0 (0)	3 (100)	0.8->0.9	0 (0)	3 (100)	0.7->0.9
Good (N=27)	1 (3.7)	26 (96.3)		0 (0)	27 (100)	
Fair (N=70)	3 (4.3)	67 (95.7)		1 (1.4)	69 (98.6)	
Poor (N=7)	0 (0)	7 (100)		0 (0)	7 (100)	
	Average (SD)	Average (SD)		Average (SD)		
Weight (kg)	0.75 (0.14)	0.80 (0.17)	0.6	0.72	0.8 (0.17)	0.6
Length (mm)	534.50 (109.85)	550.10 (82.61)	0.7	566	549.35 (83.56)	0.8
Chest girth (mm)	149.50 (22.17)	147.50 (14.15)	0.8	129	147.75 (14.33)	0.2

Table S4. Protoparvovirus screening results for Haida Gwaii Pacific martens.

	Protoparvoviruses		
	Positive N (%)	Negative N (%)	p
TOTAL (N=23)	1 (4.3)	22 (95.7)	
2016 (N=3)	0 (0)	3 (100)	
2017 (N=6)	1 (16.7)	5 (83.3)	0.3-0.7
2018 (N=14)	0 (0)	14 (100)	
Haida Gwaii, Graham Island (N=9)	1 (11.1)	8 (88.9)	
Haida Gwaii, Moresby Island (N=14)	0 (0)	14 (100)	0.4
Female (N=3)	0 (0)	3 (100)	
Male (N=20)	1 (5)	19 (95)	0.9
Adult (N=16)	0 (0)	16 (100)	
Juvenile (N=7)	1 (14.3)	6 (85.7)	0.3
Excellent (N=1)	0 (0)	1 (100)	
Good (N=13)	0 (0)	13 (100)	
Fair (N=3)	0 (0)	3 (100)	0.3-0.9
Poor (N=6)	1 (16.7)	5 (83.3)	
	Average (SD)		
Weight (kg)	0.77	1.08 (0.29)	0.3
Length (mm)	570	550.32 (129.12)	0.9
Chest girth (mm)	193	184.82 (33.66)	0.8

Table S5. Protoparvovirus screening results for river otters.

	Protoparvoviruses		
	Positive N (%)	Negative N (%)	p
TOTAL (N=22)	7 (31.8)	15 (68.)	
2019 (N=22)	7 (31.8)	15 (68.2)	
South-east VI (N=14)	5 (35.7)	9 (64.3)	
North Cowichan (N=9)	4 (80)	5 (20)	
Duncan Hatchery (N=2)	0 (0)	2 (100)	
Cowichan River Valley (N=3)	1 (33.3)	2 (67.7)	
South-west VI (N=8)	2 (25)	6 (75)	
Sooke to Jordan River (N=8)	2 (25)	6 (75)	
Female (N=10)	4 (40)	6 (60)	
Male (N=12)	3 (25)	9 (75)	0.5
Adult (N=14)	2 (14.3)	12 (85.7)	
Juvenile (N=8)	5 (62.5)	3 (37.5)	0.04
Excellent (N=1)	0 (0)	1 (100)	
Good (N=3)	1 (33.3)	2 (66.7)	
Fair (N=15)	5 (33.3)	10 (66.7)	0.1-0.8
Poor (N=3)	1 (33.3)	2 (66.7)	
	Average (SD)	Average (SD)	
Weight (kg)	7.92 (1.74)	9.61 (2.07)	0.08
Length (mm)	1194.29 (53.03)	1249.20 (79.46)	0.1
Chest girth (mm)	430.43 (47.45)	446.87 (39.02)	0.4

Table S6. Percentage identity (1 - p-distance) within and between AMDV taxa identified in insular British Columbia.

Within		Between							
Taxa		Clade 1	MAVI71	MAVI78	Clade 2	Clade 3	Clade 4	Clade 5	Clade 6
Clade 1	99.1								
MAVI71	/	95.1							
MAVI78	/	94.5	95.3						
Clade 2	99.2	94.7	96	96.6					
Clade 3	98.3	93.8	94.3	95.8	97				
Clade 4	99.3	93.6	93.6	95.3	95.9	97			
Clade 5	99.8	93.2	93.6	94.9	95.4	95.8	96.1		
Clade 6	97.1	91.5	91.8	92.3	93.7	94	93.7	94.4	
Clade 7	99.4	94.2	94.1	95.1	95.9	95.8	95.1	95.4	93.7

Table S7. Amino acid mutations in VP2 of the prototype strains (FPV, CPV, CPV-2a, CPV-2b and CPV-2c) and of the viruses characterized in this study.

Residue	FPV	Study FPV	CPV	Study CPV	CPV-2a	CPV-2b	CPV-2c
80	K	K	R	R	R	R	R
87	M	M	M	L	L	L	L
93	K	K	N	N	N	N	N
101	I/T	T	I	T	T	T	T
103	V	V	A	A	A	A	A
305	D	D	D	H	Y	Y	Y
323	D	D	N	N	N	N	N
426	N	N	N	N	N	D	E
564	N	N	S	S	S	S	S
568	A	A	G	G	G	G	G

Figure S1. Phylogenetic analysis of the complete amdoparvoviral NS1 gene. The phylogenetic tree was obtained with the neighbor-joining method [1], based on the Tamura 3-parameter model [2], identified as the best-fitting model after the model test analysis, using MEGA 7 [3]. The outcome of the bootstrap analysis [4] is shown next to the nodes. Strains identified in BC are labelled with circles colored corresponding to the host in which they were identified (black for VI mink, blue for farmed mainland BC mink, red for martens, orange for BC skunks). A subtree derived from the main tree showing the strains obtained in this study is shown on the right.

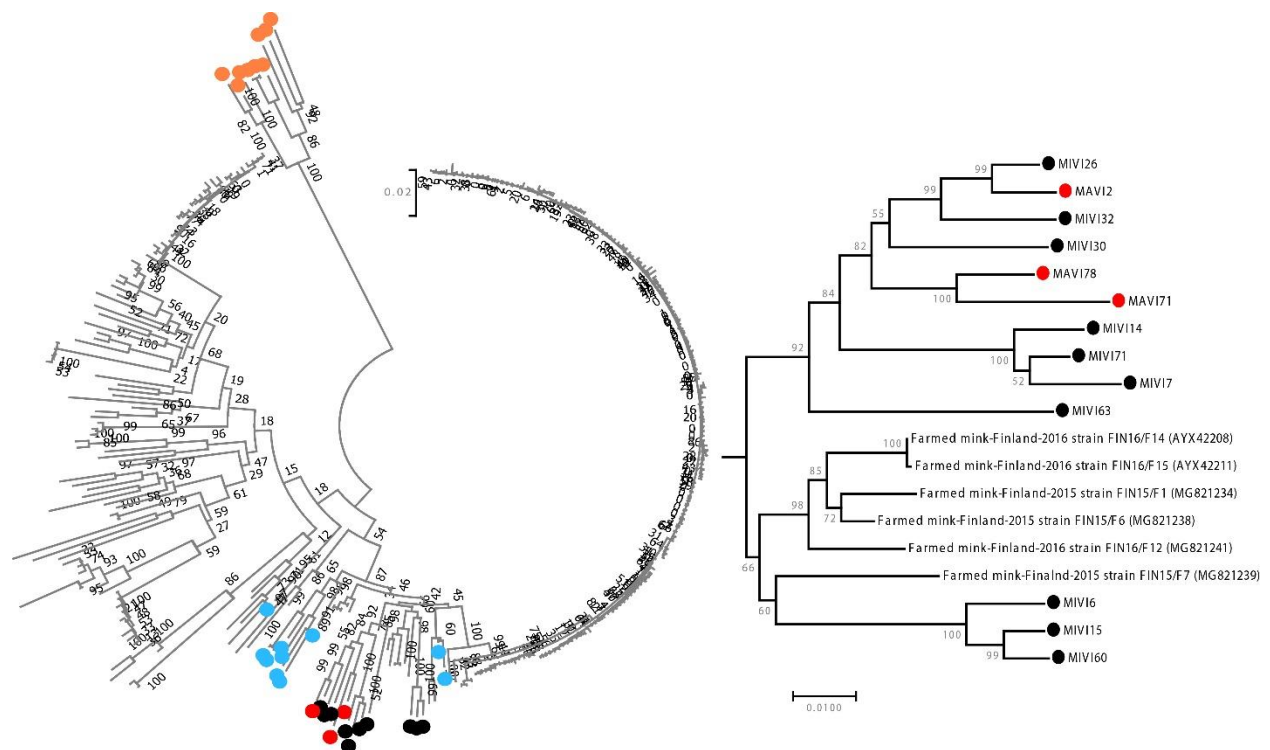


Figure S2. Phylogenetic analysis of the complete amdoparvoviral VP2 gene. The phylogenetic tree was obtained with the neighbor-joining method [1], based on the Tamura Nei model [5], identified as the best-fitting model after the model test analysis, using MEGA 7 [3]. The outcome of the bootstrap analysis [4] is shown next to the nodes. Strains identified in BC are labelled with circles colored corresponding to the host in which they were identified (black for VI mink, red for martens, orange for BC skunks). A subtree derived from the main tree showing the strains obtained in this study is shown on the right.

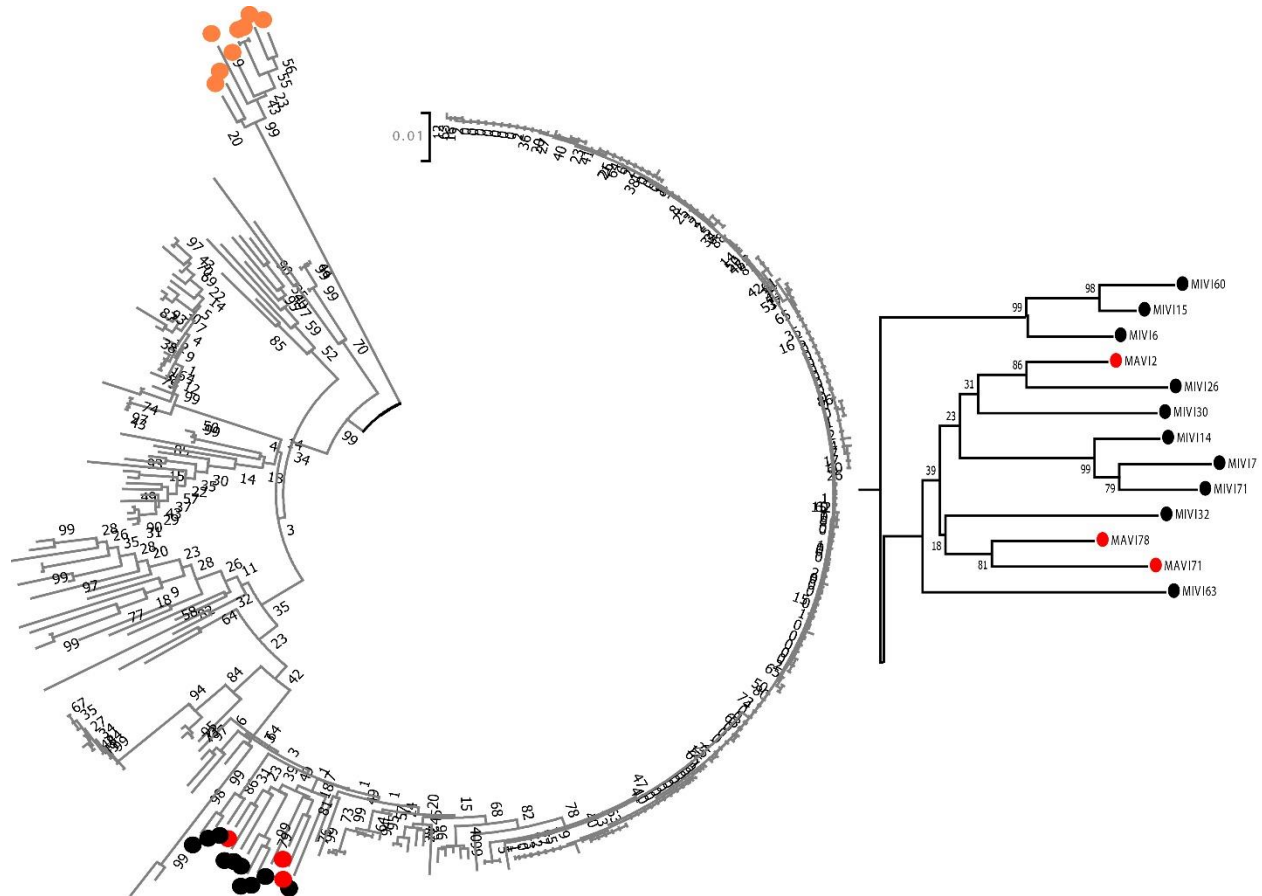


Figure S3. Phylogenetic analysis of the complete protoparvoviral NS1 gene. The phylogenetic tree was obtained with the neighbor-joining method [1], based on the Tajima-Nei model [6], identified as the best-fitting model after the model test analysis, using MEGA 7 [3]. The outcome of the bootstrap analysis [4] is shown next to the nodes. Strains identified in BC are labelled with circles colored corresponding to the host in which they were identified (black for otters, red for mink, purple for VI marten, orange for HG marten, blue for raccoons). Subtrees derived from the main tree showing the strains obtained in this study are shown on the left. Subtrees are indicated by letters that indicate the location of these branches on the main tree.

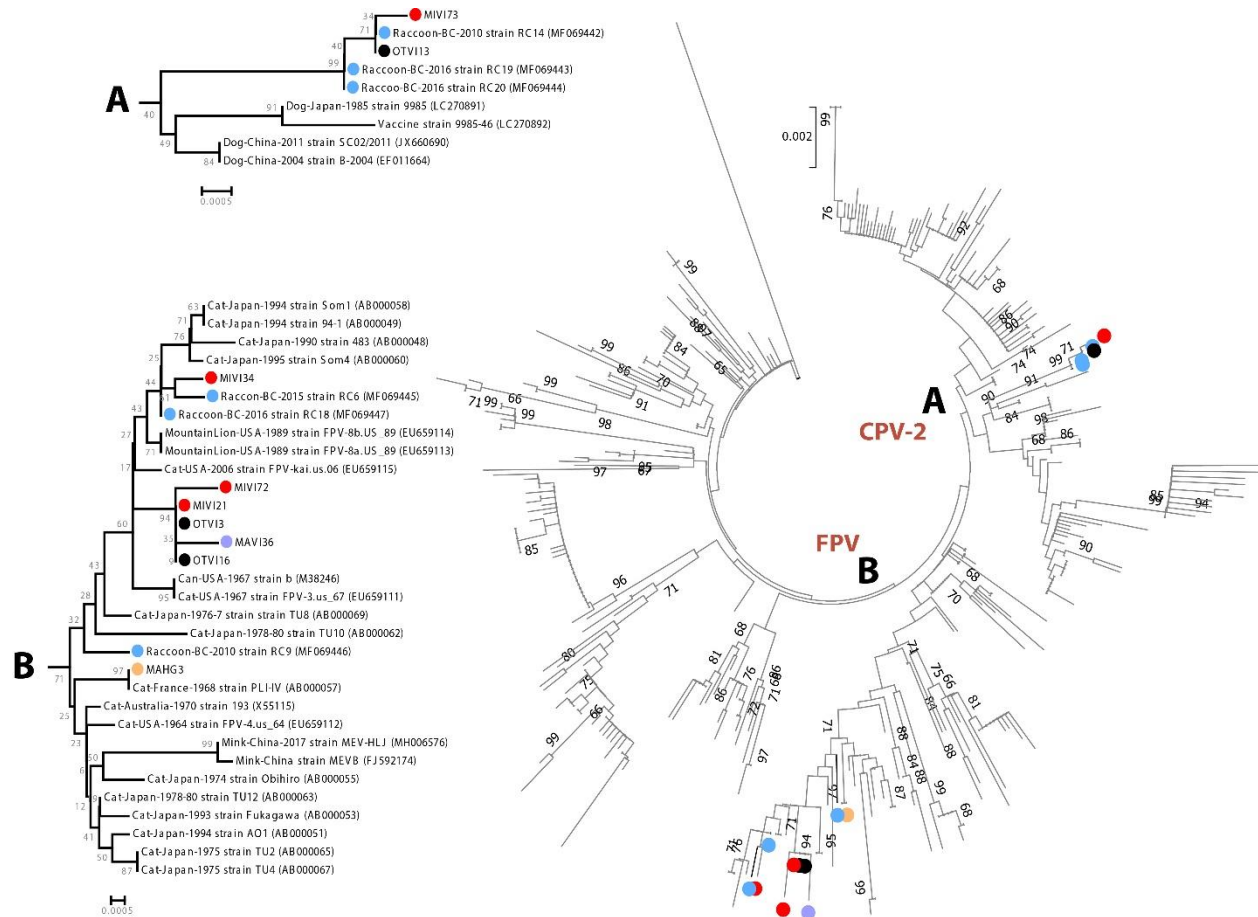
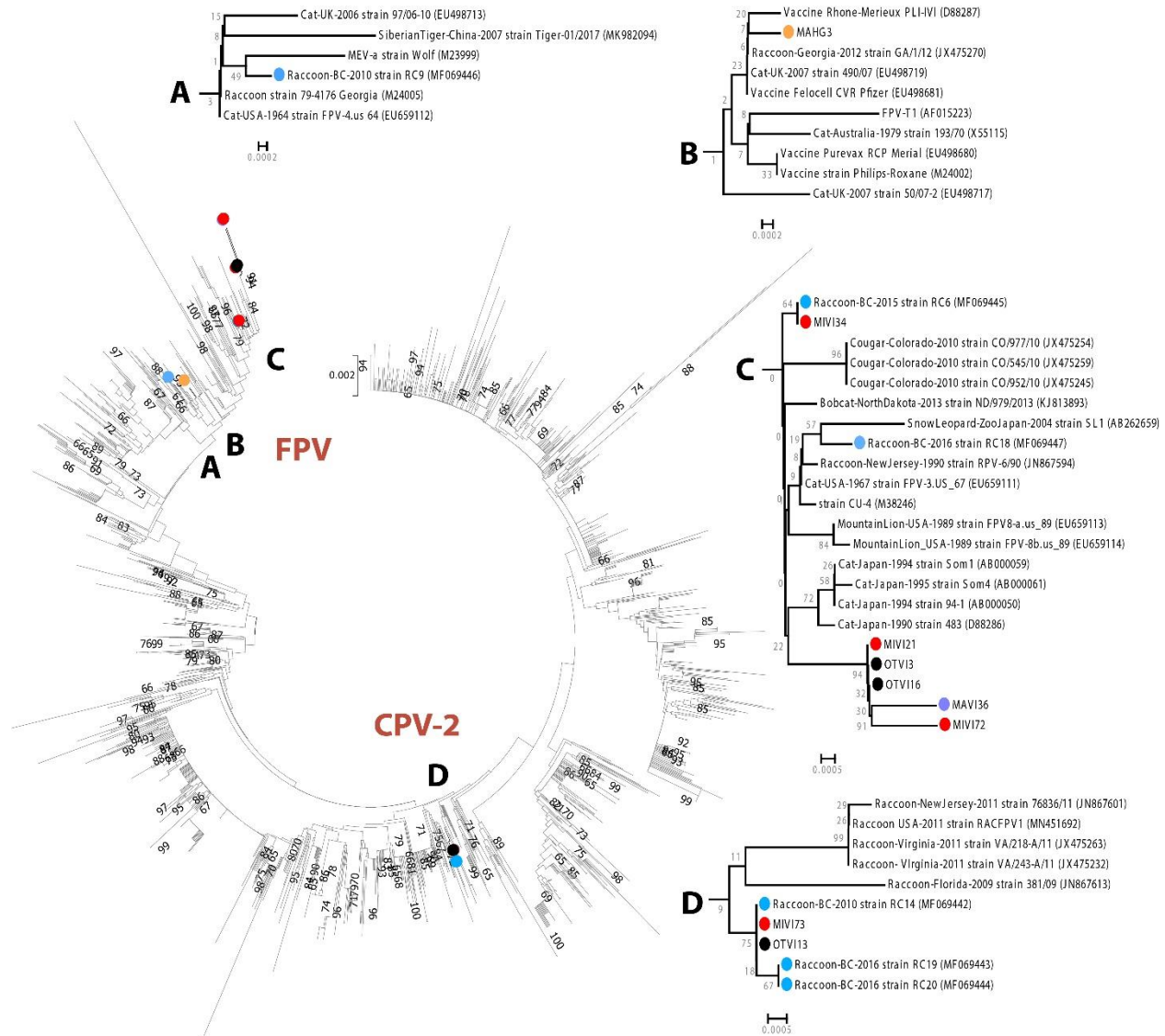


Figure S4. Phylogenetic analysis of the complete protoparvoviral VP2 gene. The phylogenetic tree was obtained with the neighbor-joining method [1], based on the Tamura 3-parameters model [2], identified as the best-fitting model after the model test analysis, using MEGA 7 [3]. The outcome of the bootstrap analysis [4] is shown next to the nodes. Strains identified in BC are labelled with circles colored corresponding to the host in which they were identified (black for otters, red for mink, purple for VI martens, orange for HG marten, blue for raccoons). Subtrees derived from the main tree showing the strains obtained in this study are shown on the top and on the right. Subtrees are indicated by letters that indicate the location of these branches on the main tree.



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