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**TITLE:** Genetic characterization of Carnivore Parvoviruses in Spanish wildlife reveals domestic dog and cat-related sequences

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1 **Genetic characterization of Carnivore Parvoviruses in Spanish**  
2 **wildlife reveals domestic dog and cat-related sequences**

3

4 **Running title:**

5 Carnivore parvovirus infection in Spanish wildlife

6

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38

39           **Summary**

40           The impact of carnivore parvovirus infection on wild populations is not yet understood;  
41           disease signs are mainly developed in pups and assessing the health of litters in wild  
42           carnivores has big limitations. This study aims to shed light on the virus dynamics among  
43           wild carnivores thanks to the analysis of 213 samples collected between 1994 and 2013 in  
44           wild ecosystems from Spain. We determined the presence of carnivore parvovirus DNA by  
45           real-time PCR and sequenced the *vp2* gen from 22 positive samples to characterize the  
46           strains and to perform phylogenetic analysis.

47           The presence of carnivore parvovirus DNA was confirmed in 18% of the samples, with  
48           a higher prevalence detected in wolves (*Canis lupus signatus*, 70%). Fourteen sequences  
49           belonging to nine wolves, three Eurasian badgers (*Meles meles*), a common genet (*Genetta*  
50           *genetta*) and a European wildcat (*Felis silvestris*) were classified as canine parvovirus 2c  
51           (CPV-2c); five sequences from three wolves, a red fox (*Vulpes vulpes*) and a stone marten  
52           (*Martes foina*) as CPV-2b; and three sequences from a badger, a genet and a stone marten  
53           as feline parvovirus (FPV). This was the first report of a wildcat infected with a canine strain.  
54           Sequences described in this study were identical or very close related to others previously  
55           found in domestic carnivores from distant countries, suggesting that cross-species  
56           transmission takes place and that the parvovirus epidemiology in Spain, as elsewhere, could  
57           be influenced by global factors.

58

59           *Keywords:* Carnivore parvovirus, conservation, epidemiology, multi-host, wildcat,  
60           Iberian wolf

61

62           **1. Introduction**

63           Canine and feline parvoviruses (CPV and FPV) belong to the newly assigned species  
64           *Carnivore protoparvovirus-1*, commonly known as carnivore parvoviruses (Cotmore et al.,  
65           2014). Even though CPV and FPV are antigenic variants of the same virus species and  
66           share a genome homology of 98% (Reed, Jones, & Miller, 1988), there are evolutionary and  
67           epidemiological differences between them. While FPV existed from, at least, the beginning  
68           of the 20th Century (Verge & Christoforoni, 1928) and remained without noticeable changes  
69           during the last decades; CPV emerged in dog populations in the 1970s and underwent  
70           notable antigenic drift. The first antigenic variant or strain of CPV was named CPV-2 and it  
71           was rapid replaced globally by CPV-2a in 1980. Four years later arose the first reports of  
72           CPV-2b in the USA and, after twelve more years, CPV-2c was discovered in Europe. Whilst  
73           CPV-2 is differentiated from the rest of the CPV variants by a few amino acid substitutions,  
74           2a, 2b and 2c variants are differentiated by the residue 426 from the VP2 protein (Barker &  
75           Parrish, 2001; Decaro et al., 2007; Parrish et al., 1991). The constant antigenic change of  
76           CPV allowed the achievement of a wide host range distribution thanks to a high rate of  
77           nucleotide substitution (about  $10^{-4}$  substitutions per site per year) (Shackelton, Parrish,  
78           Truyen, & Holmes, 2005). By contrast, the FPV clade has maintained a constant host range  
79           distribution and varies at slower rates (Shackelton et al., 2005).

80           The host range of CPV involves almost all wild and domestic carnivore species studied  
81           but it is more commonly described in domestic dogs (*Canis lupus familiaris*) and cats (*Felis*  
82           *silvestris catus*). FPV infects a wide variety of carnivores as well, except canid species  
83           including wolves and domestic dogs (Allison et al., 2014; Ikeda et al., 2002; Steinel, Parrish,  
84           Bloom, & Truyen, 2001). Transmission between wild and domestic carnivores is supported  
85           by a number of molecular studies that showed, based on sequencing of the VP2 protein, that  
86           wild and domestic carnivores shared identical or closely related parvoviruses (Duarte et al.,  
87           2009, 2013; Mendenhall et al., 2016; Steinel, Munson, Van Vuuren, & Truyen, 2000). .

88 Pathologic and epidemiologic characteristics of carnivore parvoviruses are complex  
89 and the consequences of infection at an individual and a population level cannot be easily  
90 predicted. Even though these viruses are present in almost all carnivore populations tested,  
91 under certain circumstances, epidemic waves could trigger declines in naïve wild populations  
92 as happened, for example, in wolves (*Canis lupus*) in North America (Mech, Goyal, Paul, &  
93 Newton, 2008). The severity of disease signs varies from sub-clinical, acute to lethal,  
94 depending on a series of factors involving host age, host immunity, and virus strain. Signs  
95 are mainly developed in pups (Parrish, 1995) and assessing the health of litters in wild  
96 carnivores has big limitations. For all these reasons, the impact of carnivore parvoviruses in  
97 wild ecosystems is poorly understood.

98 The VP2 protein forms most of the capsid structure and determines the antigenic  
99 properties of the virus strain. Molecular studies sequencing the *vp2* gene allow strain  
100 characterization and phylogenetic associations and have been widely used in domestic  
101 carnivore investigations (Miranda & Thompson, 2016). In wild carnivores, however,  
102 serological studies have been more widely performed but due to cross-reactivity, this  
103 technique raises big limitations such as the strain determination (Harrison et al., 2004; Steinel  
104 et al., 2001). Therefore, to deepen our understanding of parvoviruses in wild ecosystems,  
105 further molecular studies are needed. Non-invasive sampling techniques are crucial and  
106 spleen samples collected from wild carnivore carcasses, which harbour high viral loads, have  
107 a great potential for this purpose (Allison et al., 2013; Decaro, Martella, et al., 2007).

108 The Iberian Peninsula has the richest carnivore diversity in Western Europe with  
109 sixteen native species belonging to six different families (Palomo, Gisbert, & Blanco, 2007)  
110 and it offers an appropriate environment to study parvoviruses epidemiology in wild  
111 ecosystems.

112           The aim of this study was to describe for the first time the prevalence of parvovirus  
113 infection in wild carnivores in Spain and characterize the circulating strains. Using molecular  
114 characterization of positive cases, we also aimed to analyse the relationship with viruses from  
115 other wild and domestic carnivores elsewhere and infer phylogenetic associations. Our  
116 ultimate goal was to better understand the virus dynamics of carnivore parvoviruses among  
117 wild carnivores.

118

## 119           **2. Materials and methods**

### 120           *2.1. Sampling*

121           Spleen samples from 213 free-ranging carnivores belonging to the *Mustelidae* (n=104)  
122 *Canidae* (n=79), *Viverridae* (n=27), *Felidae* (n=2) and *Procyonidae* (n=1) families were  
123 collected in 1994-2013 from different regions of Spain (detailed in *Table 1 and Figure 1*) and  
124 stored at -20°C. All samples were taken opportunistically (no animal was killed for  
125 investigative purposes), with permission from the regional administrations of Galicia,  
126 Asturias, Catalonia and the Balearic Islands. Most sampled animals were found dead in  
127 forested areas near human habitation, usually road kill, except 20 Iberian wolves (*Canis lupus*  
128 *signatus*), which were legally shot in remote forested areas.

129

### 130           *2.2. Molecular analysis*

131           Approximately 50mg of tissue were used for DNA extraction. After mechanical  
132 maceration, DNA was purified using the pressure filtration method QuickGene® DNA tissue  
133 kit S (FujiFilm Lifescience, Tokyo, Japan), following the manufacturer's instructions. Purified  
134 DNA was stored at -80°C until further use.

135           Screening for parvovirus-positive samples (case ascertainment) was achieved through  
136 a previously described real-time PCR, which targeted a conserved region of 163 nucleotides

137 of the *vp2* gene (Decaro et al., 2005). Molecular characterization was achieved through the  
138 amplification and sequencing of nearly the whole *vp2* gene (Truyen, Evermann, Vieler, &  
139 Parrish, 1996). Two nested PCRs amplified a 1746 nucleotide segment. The external PCR  
140 amplified a 2401 nucleotide segment and was performed by combining the primers VPF and  
141 M5mod (Mochizuki, Horiuchi, & Hiragi, 1996; Steinel et al., 2000); whereas the internal PCR  
142 was conducted using the primers P1 and VPR (Battilani et al., 2001; Mochizuki, San Gabriel,  
143 Nakatani, Yoshida, & Harasawa, 1993) (*Table 2*). The temperature profile for the external  
144 PCR was set at 94°C for 5', followed by 45 cycles: 94°C for 30", 55°C for 30" and 72°C for  
145 2'30", with a final extension of 72°C for 7'. The internal PCR was set at 94°C for 5', followed  
146 by 40 cycles: 94°C for 15", 52°C for 15" and 72°C for 2', with a final extension of 72°C for 7'.  
147 PCR products were sequenced with eight different primers (detailed in *Table 2*).

148 A commercial CPV-2 vaccine containing CPV-2 (Nobivac® Puppy DP, MSD Animal  
149 Health, Carbajosa de la Sagrada, Spain) was used as a positive control in each assay. Non-  
150 template controls were also included in each assay to assure that the samples were not  
151 contaminated. Consistency in the generated results ruled out contamination.

### 152 2.3. *Phylogenetic analysis*

153 Nucleotide sequences were aligned with the ClustalW method and translated into  
154 putative amino acid sequences using the MEGA 6.0® software (Tamura, Dudley, Nei, &  
155 Kumar, 2007). Specific amino acids were used for the classification as FPV, CPV-2a, CPV-  
156 2b and CPV-2c strains (Buonavoglia et al., 2001; Parrish, 1999; Truyen et al., 1996) and  
157 further comparison of the obtained sequences with the GenBank® database was performed.  
158 Nucleotide and amino acid sequence identity of sequences of 1746bp were calculated using  
159 the online software SIAS®, Sequence Identity and Similarity  
160 (<http://imed.med.ucm.es/Tools/sias.html>).



161 A maximum-likelihood phylogenetic tree was inferred based on nucleotide sequences  
162 and the reliability of the obtained tree was evaluated with the bootstrap method based on  
163 1000 replicates (Tamura et al., 2007). The median-joining network was performed using  
164 SplitsTree4<sup>®</sup> software (Huson & Bryant, 2006). This algorithm better represents the existence  
165 of haplotypes shared by different species (Ohneiser, Hills, Cave, Passmore, & Dunowska,  
166 2015) and, compared with phylogenetic trees, is more reliable when intraspecific phylogenies  
167 are studied. The recombination between taxa, the persistence of ancestral alleles and the  
168 presence of multiple descendants from single ancestors give rise to reticulated and  
169 multifurcating patterns in relationships (Lapointe, 2000).

170 Statistical analyses were developed using the traditional Clopper-Pearson confidence  
171 interval (95% confidence level) for prevalence calculations. QGIS<sup>®</sup> Geographic Information  
172 System Software (<https://qgis.org/es/site/>) was used for the representation of sample  
173 locations.

174

### 175 **3. Results**

176 Real-time PCR confirmed carnivore parvovirus infection in 39 samples (18.3%, C.I.  
177 13.6–24.1) belonging to eight different species (detailed in *Table 1*). Infection was detected  
178 in all species in which sample size was above three. The greatest prevalence was detected  
179 in wolves, with 67.6% of positives (C.I.= 50.2–82.0) of positives. Given that wolf samples  
180 were obtained from different regions, no statistical inference regarding species-related  
181 differences could be performed. Excluding wolves, the remaining carnivores had a  
182 prevalence of 7.95% (C.I. 4.4–12).

183 Nineteen sequences of 1746 bp, one of 1678 bp and two of 600 bp were described.  
184 Fourteen of these were classified as CPV-2c, five as CPV-2b and three as FPV. CPV-2c was  
185 described in nine wolves, three Eurasian badgers (*Meles meles*), a European wildcat (*Felis*

186 *silvestris silvestris*) and a genet; CPV-2b in three wolves, a red fox and a stone marten; and  
187 FPV in a badger, a genet and a stone marten (*Martes foina*). Therefore, 63% of the identified  
188 sequences corresponded to CPV-2c, 23% to CPV-2b and 14% to FPV. However, when wolf  
189 samples were excluded, given that this species is refractory to FPV infection (Allison et al.,  
190 2014), FPV accounted for 30% of the cases.

191 Sequences differed in 48 nucleotide positions, 17 of which were coding mutations  
192 (Table 3). Comparison among the CPV sequences from this study showed nucleotide  
193 identities of 99.5-100% (mean 99.8, SD 0.12) and amino acid identities of 99.5-100% (mean  
194 99.8, SD 0.13). The two FPV sequences described showed a nucleotide identity of 99.82%  
195 and were translated into the same amino acid sequence type (aaST H). Three sequences of  
196 only 1678 and 600 bp were excluded from the identities calculation.

197

198 Within aaST A, there were 5 nucleotide sequences with synonymous substitutions  
199 (nucleotide sequence types 1, 6, 9, 10 and 13), with ntST9 predominating. aaST A  
200 belonged to the CPV-2c clade and was described in nine different hosts analysed between  
201 2001 and 2013 (five wolves, three badgers and a wildcat) (Table 4).

202 Two additional amino acid sequences types were shared by different host species:  
203 aaST F, belonging to the CPV-2b clade, described in two wolves and a red fox (*Vulpes*  
204 *vulpes*) from 2007-2013; and aaST H, belonging to the FPV strain, and described in a stone  
205 marten and a badger from 2002 and 2013.

206 Comparisons with other sequences published in the GenBank® showed that ntST9 was  
207 identical to other 16 nucleotide sequences described in dogs from different countries and  
208 years, such as, for example, France-2005 and Uruguay-2011 (GenBank® accession numbers  
209 KM457142 and DQ025942).

210 Three additional amino acid sequence types showed 100% identity with other  
211 sequences; aaST F, which was identical to a sequence described in a domestic dog from  
212 France-2008 and in a cougar (*Puma concolor*) from U.S.A.-2011 (DQ0255991 and  
213 JX475250); aaST H, identical to others described in cats from Japan-2000 and Portugal-2008  
214 (AB054227 and KT240130); and aaST A, identical to sequences in dogs from France-2005,  
215 Italy-2008 and Uruguay-2011 (DQ025942, FJ005206 and KM457142). The rest of the amino  
216 acid sequences types obtained in this study (aaSTs B, G, C, D and E) were reported for the  
217 first time (detailed in Tables 3 and 4).

218 This subtyping coincides with the phylogenetic network and tree, which clearly clusters  
219 the different sequences of FPV and CPV (*Figures 2 and 3*). A group of CPV-2b (ntST-2 and  
220 ntST-5 belonging to aaST F; and ntST4 belonging aaST G) was clustered near the CPV-2c  
221 clade. The aaST F, classified as CPV-2c, differed only in one amino acid mutation  
222 (Asp426Glu) within aaST A. This clade, previously named as "new CPV-2b" also includes  
223 sequences previously published from wildlife in USA and Portugal (Allison et al., 2013; Duarte  
224 et al., 2013). The network also showed a common CPV-2c haplotype, shared by a domestic  
225 dog, wild canids as well as Mustelidae and Felidae members.

226

#### 227 **4. Discussion**

228 Results from this study provide insights into the presence and distribution of different  
229 strains of parvoviruses among Iberian wild carnivores. Further, we adapted a highly efficient  
230 nested PCR, which enabled the sequencing of nearly the complete *vp2* gene from half of the  
231 positive samples, often in an advanced decomposition state.

232 The high prevalence of CPV in the small and disperse populations of wolves (67.6%)  
233 is compatible with a sustained onward transmission event from domestic dogs. Further,  
234 comparisons with the GenBank® showed identical nucleotide sequences in domestic dogs

235 from Europe, North and South America. Because infection often 'spills overs' from more  
236 abundant hosts (Woodroffe, 1999), our results highlight the role of dogs as a probable source  
237 of infection for wolves, which could get infected through scent communication, predation,  
238 coprophagy, or fomites (Llaneza, García, & López-Bao, 2014; Zarnke et al., 2001).

239 The prevalence of infection in small carnivores, excluding wolves, was 7.95% and the  
240 predominant virus strain CPV (70% of the described sequences). Surprisingly, in Portugal  
241 the prevalence was 63% , being FPV the most prevalent strain (87%) (Duarte et al., 2013).  
242 The lack of standardization in molecular techniques and the opportunistic nature of the  
243 sampling hinder the comparisons among different studies. However, these important  
244 discrepancies in prevalence and strain types could indicate that epidemiology is strongly  
245 influenced by spatial factors that vary across the Iberian Peninsula.

246 Historically, residue 426 has been used to define CPV-2a, 2b and 2c clades  
247 (Buonavoglia et al., 2001) although this classification is increasingly controversial.  
248 Sequences described here showed a high similarity even though they were classified in  
249 different CPV clades. For example, aaST A and aaST F, which belonged to the CPV-2c and  
250 2b clades respectively, were differentiated by only residue 426. This could be in part  
251 explained because residue 426 presented the major variability among all sites in this study.

252 The predominance of the Glu residue at position 426 (CPV-2c), reported in 63% of all  
253 the sequences described, suggested that it is the most prevalent genotype circulating among  
254 Spanish wild carnivores in 1994-2013. The oldest CPV-2c sequence was obtained from a  
255 wildcat in 2001; this is five years after the first report of CPV-2c in the literature (Decaro,  
256 Desario, et al., 2007), and five years before the first detection of CPV-2c in Spain (Decaro et  
257 al., 2006). CPV-2c appears to be the most recent clade of carnivore parvoviruses (Miranda  
258 & Thompson, 2016) and it might replace older clades in Spanish wild ecosystems.

259           New host-virus associations have been described in this study, which could be  
260 explained by the wide host range of carnivore parvoviruses (Steinel et al., 2001). To the best  
261 of our knowledge, this is the first time that CPV-2c variant has been reported in wildcats and  
262 the first time that FPV has been reported in stone martens. Pathogenicity of CPV-2c in captive  
263 felines is similar to the pathogenicity described by FPV infection and could compromise the  
264 immune system (Nakamura et al., 2001) and therefore, the susceptibility of wildcats to CPV-  
265 2c infection could be relevant in feline conservation.

266           Genetic variability among our sequences was low. Only eight coding mutations were  
267 spread among our CPV sequences, but they were not consistent with a concrete species,  
268 region or time period. Further, identical CPV and FPV nucleotide sequences were described  
269 in different host species belonging to three different families (*Canidae*, *Felidae* and  
270 *Mustelidae*), indicating that mutations in the *vp2* domain may not be necessary for the jump  
271 among sympatric species, as was reported in other wild ecosystems (Calatayud et al., 2019).

272

273           In conclusion, carnivore parvovirus infection appears to be widespread among Spanish  
274 wildlife, with CPV-2c being the most prevalent clade. We detected very low genetic variability  
275 among the VP2 fragments analyzed and identical sequences were infecting different species  
276 suggesting the existence of cross-species events. Further, identical sequences were  
277 previously described in domestic carnivores in USA, Europe and Asia, indicating that  
278 domestic carnivores may be a source of infection and that parvoviruses epidemiology in  
279 Spain may be influenced by global factors such as domestic dog international trade. The  
280 consequences of these infections in Spanish wildlife are unknown and further assessment  
281 would be needed to understand the global scenario.

282

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297

## 298 **6. Conflict of interest statement**

299 Conflicts of interest: none.

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303

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444

445

446 **TABLES**

447

448 **Table 1**

	Region				Total	Positives	Prevalence (95% C.I.)
	Asturias	Galicia	Catalonia	Balearic Islands			
Canidae							
Wolf	20	17			37	25	67.6% (50.2–82.0)
Red fox			42		42	1	2.4% (0.1–12.6)
Felidae							
European Wildcat			2		2	1	
Viverridae							
Common genet			21	6	27	3	11.1% (2.4–29.2)
Eurasian badger			68		68	6	8.8% (3.3–18.2)
Pine marten				23	23	1	4.3% (0.1–21.9)
Stone marten			9		9	2	22.2% (2.8–60)
European mink			1		1	0	
Least weasel				2	2	0	
American mink			1		1	0	
Coati				1	1	0	
<b>Overall</b>	<b>20</b>	<b>17</b>	<b>144</b>	<b>32</b>	<b>213</b>	<b>39</b>	<b>18.3% (13.6–24.1)</b>

449

450 Origin of the samples and prevalence of infection. Species represented by a sample  
451 size lower than 3 were excluded from the species-specific prevalence calculation.

452 **Table 2**

Primer	Sequence (5' to 3')	Binding site*	Sense	Ref.
VPF	ATGGCACCTCCGGCAAAGA	2285-2303	+	(38)
VPR	TTTCTAGGTGCTAGTTGAG	5285-5302	-	
P1	ATGAGTGATGGAGCAGTTC	2786-2804	+	(18)
P2	TCATCTAAAGCCATGTTTC	3066-3084	-	
VPM	TGGAGGTAAAACAGGAATT	4093-4111	+	
P3	CCATTTCTAAATTCTTTG	3650-3667	+	
P4	AAGTCAGTATCAAATTCTT	4200-4218	-	
Parvo 2	TCAGAATCTGCTACTCAGCCACCA	3245-3268	+	(39)
Parvo 3	ACCAACCACCCACACCATAACAAC	4924-4947	-	
Primer F	TGGAAGTAGTGGCACACCAA	3454-3473	+	(40)
Probe	6FAM-CAGGTGATGAATTTGCTACAGG-BHQ1	3555-3576	+	
Primer R	AAATGGTGGTAAGCCCAATG	3636-3655	-	
M5mod	ATAACAAACCTTCTAAATCCTATATCAAAT	4681-4709	-	(17)

453

454 Primers used during the present study

455 \* Binding site calculated with respect to the reference sequence CPV-N (Genbank

456 Accession Number M19296)

457



458 **Table 3**

aaSTs <sup>†</sup>	N <sup>‡</sup>	80	83	87	93	103	191	232	256	297	300	305	323	361	426	564	568	579
A	9	R	V	L	N	A	R	I	R	A	G	Y	N	R	E	S	G	A
B	1	.	F	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C	1	.	.	.	.	.	I	.	.	.	.	.	.	.	.	.	.	P
D	1	.	.	.	.	.	.	.	.	.	.	.	.	Q	.	.	.	.
E	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	H
F	3	.	.	.	.	.	.	.	.	.	.	.	.	.	D	.	.	.
G	1	.	.	.	.	.	.	.	K	.	.	.	.	.	D	.	.	.
H	2	K	.	M	K	V	.	V	.	S	A	D	D	.	N	N	A	.

459

460 Variable amino acid positions among the sequences described in this study. <sup>†</sup>aaSTs,

461 amino acid sequence types; <sup>‡</sup>N, number of samples sharing identical amino acid

462 sequence types (ST).

463

464 **Table 4**

Strain	Ref.	ntST <sup>‡</sup>	aaST <sup>†</sup>	Year	Species	Origin	GenBank <sup>®</sup> accession number	Max. amino acid identity (Species/country)
CPV-2c	150	1	A	2011	Wolf	Galicia	KP682511	100% (Dog/France, Italy, Uruguay)
	154	9		2012	Wolf	Galicia	KP682523	
	172	9		2012	Wolf	Asturias	KP692527	
	309	9		2011	Badger	Catalonia	KP682519	
	426	9		2007	Badger	Catalonia	KP682524	
	441	9		2001	Wildcat	Catalonia	KP682522	
	171	6		2013	Wolf	Asturias	KP682516	
	417	10		2009	Badger	Catalonia	KP682521	
	169	13		2011	Wolf	Asturias	KP682529	
	163	3	B	2012	Wolf	Asturias	KP682513	99.8% (Dog/France, Italy, Uruguay)
	173	7	C	2013	Wolf	Asturias	KP682517	99.8% (Dog/France, Italy, Uruguay)
	279	8	D	Not known	Genet	Catalonia	KP682518	99.8% (Dog/France, Italy, Uruguay)
160 <sup>§</sup>	n.a.	n.a.	2012	Wolf	Galicia	KP682529	99.8% (Dog/Italy, Uruguay, Stone marten/Portugal)	
170	12	E	2011	Wolf	Asturias	KP682528	99.8% (Dog/France, Italy, Uruguay)	
CPV-2b	158	2	F	2012	Wolf	Galicia	KP682512	100% (Dog/France, Puma/USA)
	167	5		2013	Wolf	Asturias	KP682515	
	434	11		2007	Red fox	Catalonia	KP682525	
	166	4	G	2013	Wolf	Asturias	KP682514	99.8% (Dog/Italy, Taiwan, USA)
	439 <sup>¶</sup>	n.a.	n.a.	1994	Stone marten	Catalonia	-	-
FPV	298	14	H	2002	Stone marten	Catalonia	KP682526	100% (Cat/Japan, Portugal)
	351	15		2013	Badger	Catalonia	KP682520	
	291 <sup>¶</sup>	n.a.	n.a.	Not known	Genet	Catalonia	-	-

465

466 Parvovirus sequences identified in the present study.

467 † Amino acid sequence type.

468 ‡ Nucleotide sequence type.

469 § Only 1678 bp obtained. Not included in the tree.

470 ¶ Only about 600 bp obtained. Not included in the tree. n.a.: not applicable.

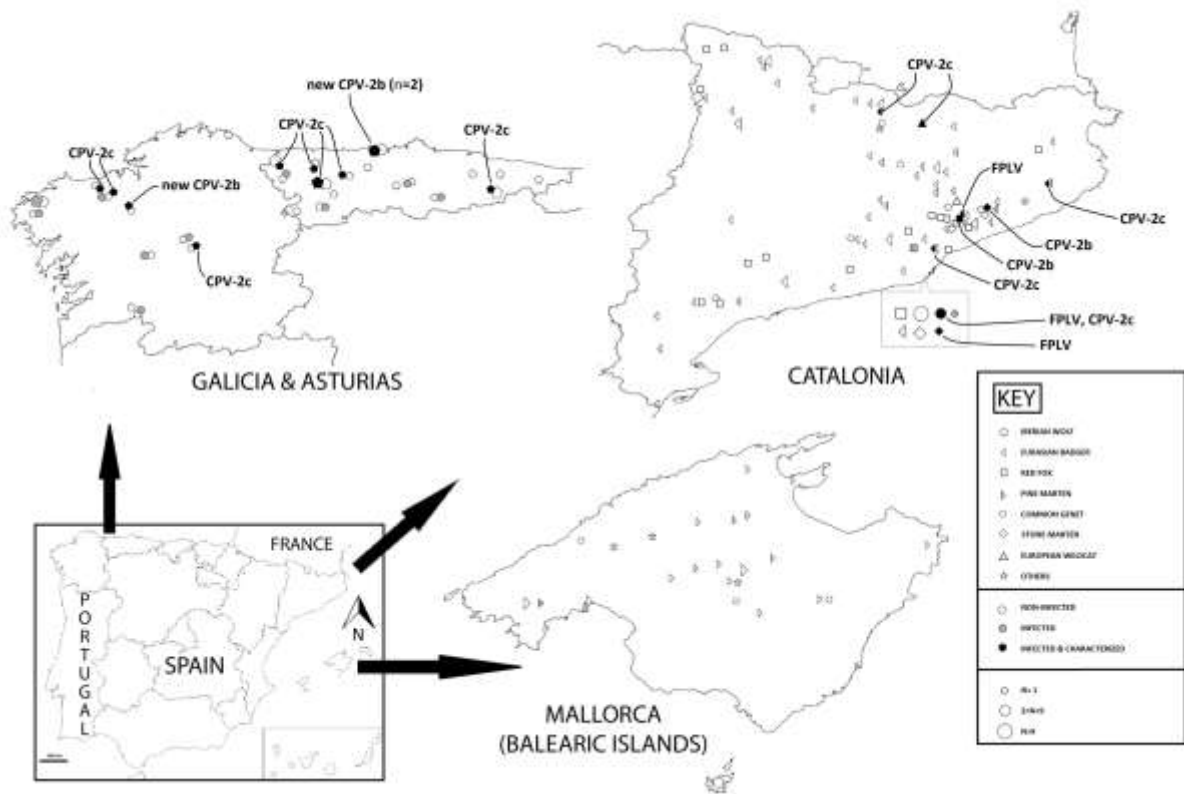
471

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473

474 **Figures**

475



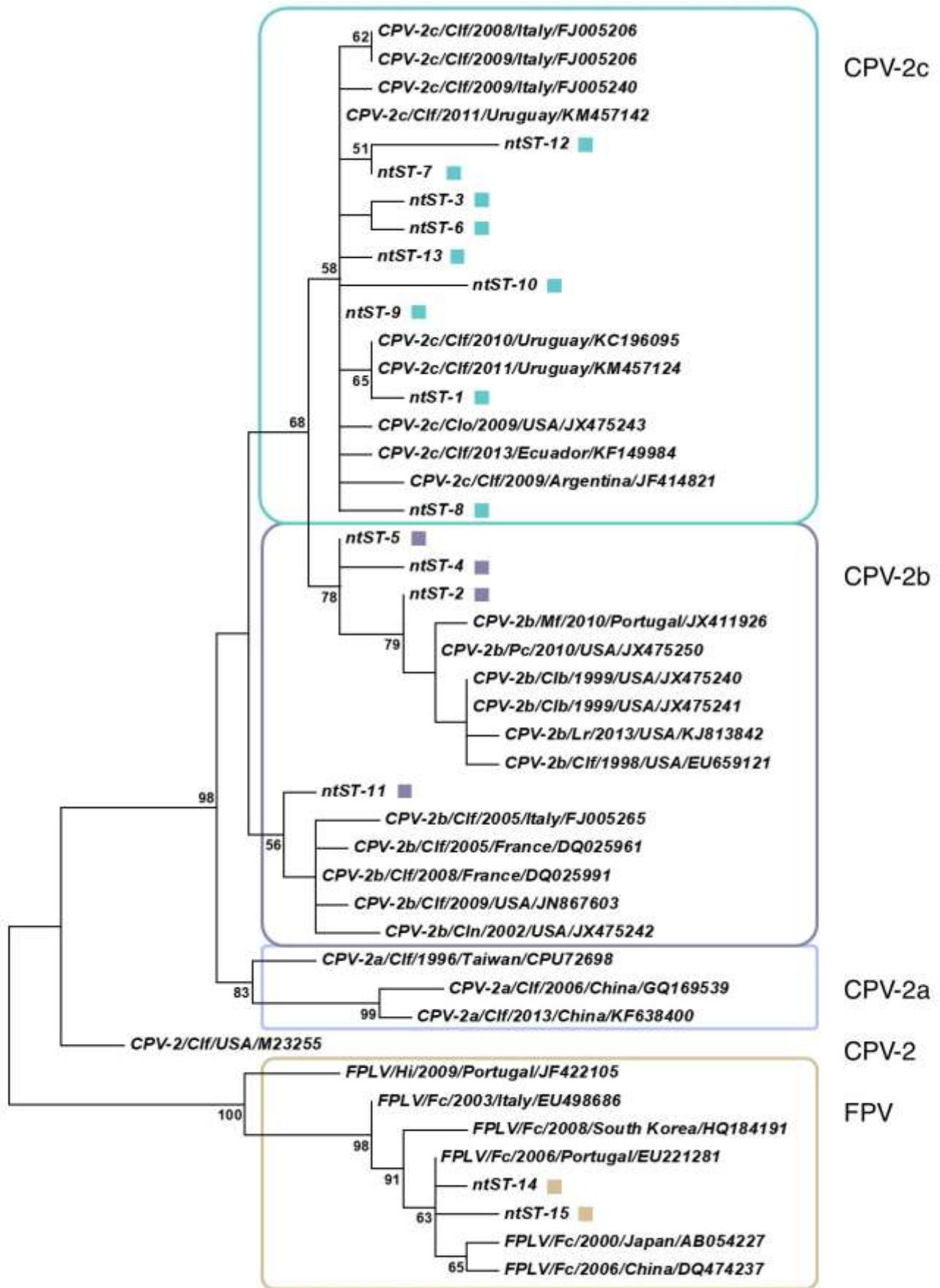
476

477

478 **Fig. 1. Geographical distribution of samples included for detection of parvovirus infection in**  
479 **wild carnivores in Spain.**

480 White dots: negative; grey dots: positive; black dots: positive and characterized (strain is indicated).

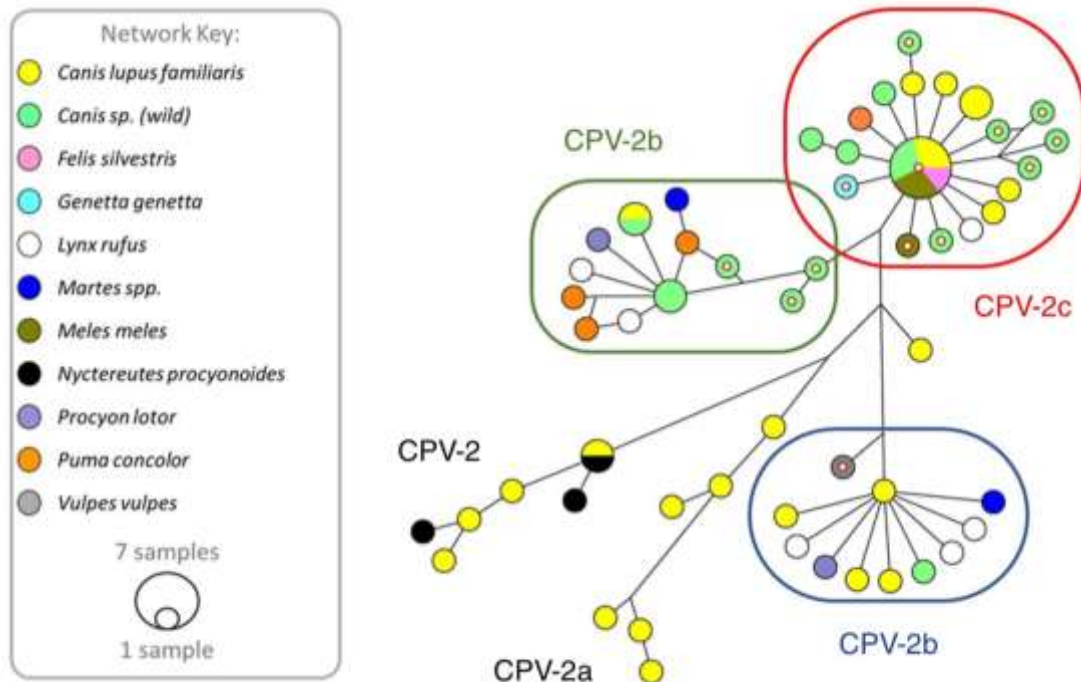
481



483 **Figure 2. Phylogenetic tree constructed from the VP2 gene nucleotide sequences of parvovirus**  
484 **strains in this study and in other parts of the world.**

485 Sequences obtained in this study are indicated by their nucleotide sequence type (ntST) and  
486 highlighted with a square. The rest of sequences are numbered with their GenBank accession number.  
487 Clb: *Canis lupus baileyi*; Clf: *Canis lupus familiaris*; Cln: *Canis lupus nubilus*; Clo: *Canis lupus*  
488 *occidentalis*; Cls: *Canis lupus signatus*; Fc: *Felis catus*; Fs: *Felis silvestris*; Hi: *Herpestes ichneumon*;  
489 Gg: *Genetta genetta*; Lr: *Lynx rufus*; Mf: *Martes foina*; Mm: *Meles meles*; Pc: *Puma concolor*; Vv:  
490 *Vulpes vulpes*. Bootstrap values less than 50% were omitted in the tree.

491



492

493 **Fig. 3. Median-joining network from selected VP2 gene nucleotide sequences**

494 Network from 74 selected VP2 gene nucleotide sequences of parvovirus strains obtained in this study  
495 (marked with a circle inside) and in other parts of the world. Each circle represents one haplotype.

496