

1 **TITLE**

2 Phylogenomic insights to the origin and spread of Phocine Distemper Virus in European harbour
3 seals in 1988 and 2002

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28 **RUNNING HEAD**

29 Phocine Distemper Virus in harbour seals

30

31 **ABSTRACT**

32 The 1988 and 2002 Phocine Distemper Virus (PDV) outbreaks in European harbour seals (*Phoca*
33 *vitulina*) are among the largest mass mortality events recorded in marine mammals. Despite its large
34 impact on harbour seal population numbers, and three decades of studies, many questions regarding
35 the spread and temporal origin of PDV remain unanswered. Here, we sequenced and analysed 7,123
36 base pairs of the PDV genome, including the coding and non-coding regions of the entire P, M, F
37 and H genes in tissues from 44 harbour seals to shed new light on the origin and spread of PDV in
38 1988 and 2002. The phylogenetic analyses trace the origin of the PDV strain causing the 1988
39 outbreak to between May 1987 and April 1988, while the origin of the strain causing the 2002
40 outbreak can be traced back to between June 2001 and May 2002. The analyses further point to
41 several independent introductions of PDV in 1988, possibly linked to a southward mass
42 immigration of harp seals in the winter and spring of 1987-1988. The vector for the 2002 outbreak
43 is unknown, but the epidemiological analyses suggest that the subsequent spread of PDV from the
44 epicentre in Kattegat, Denmark, to haul-out sites in the North Sea through several independent
45 introductions.

46

47 **KEYWORDS**

48 Morbillivirus; epidemic; viral phylogeny; virus evolution, wildlife pathogen

49 INTRODUCTION

50 Over the past few decades, several epidemics have been recorded in marine mammal populations
51 around the globe (Jo *et al.*, 2018; Duignan *et al.*, 2014). The pathogens responsible for these
52 epidemics have mainly been identified as Influenza A (Krog *et al.*, 2015) or viruses belonging to
53 the morbillivirus genus such as various cetacean morbilliviruses (Kennedy *et al.*, 1989; Domingo *et*
54 *al.*, 1990; Aguilar & Antonio Raga 1993; Birkun *et al.*, 1999; Lipscomb *et al.*, 1996), Canine
55 Distemper Virus (CDV) (Mamaev *et al.*, 1995) and Phocine Distemper Virus (PDV) (Dietz *et al.*,
56 1989a; Curran *et al.*, 1990; Harding *et al.*, 2002; Härkönen *et al.*, 2006). The morbillivirus genus is
57 a group of highly infectious viruses from the *Paramyxoviridae* family. The genus also includes the
58 Measles Virus (MeV) and the now eradicated Rinderpest (RPV) that have caused severe disease
59 outbreaks among humans and cattle respectively, resulting in suffering and economic losses in
60 affected populations (Furuse *et al.*, 2010; Roeder *et al.*, 2013). Morbillivirus infection occurs
61 through the respiratory route where the virus replicates in macrophages and dendritic cells (Rijks *et*
62 *al.*, 2012). Members of the genus have frequently caused epidemic disease in previously unexposed
63 populations, and during the past century interspecies infections have been reported on several
64 occasions (Mamaev *et al.*, 1995; Goldstein *et al.*, 2009; Roelke-Parker *et al.*, 1996). Thus, new
65 zoonotic diseases could potentially emerge from wildlife reservoirs of morbilliviruses.

66

67 The PDV epidemics in 1988 and 2002 are among the largest mass mortality events ever recorded in
68 marine mammals, resulting in the death of over 23,000 harbour seals in 1988 and approximately
69 30,000 harbour seals in 2002 (Dietz *et al.*, 1989a; Harding *et al.*, 2002; Härkönen *et al.*, 2006). The
70 two outbreaks share similarities with respect to the timing, duration and location of the first cases,
71 occurring early in spring on the island of Anholt in central Kattegat, Denmark. From there, the virus
72 seems to have spread to harbour seal colonies across northern Europe both to neighbouring haul-out

73 sites and via large jumps in location (Härkönen *et al.*, 2006). The sudden appearance and spread of
74 the previously undescribed morbillivirus among European harbour seals in 1988 immediately
75 sparked investigations into the virus' origin and mechanisms of transmission and spread. Antibodies
76 against morbillivirus have subsequently been found in samples collected from harp seals
77 (*Pagophilus groenlandicus*) and ringed seals (*Pusa hispida*) from Greenland and Svalbard waters
78 prior to the European outbreak in 1988, suggesting that the virus is circulating in Arctic seals (Dietz
79 *et al.*, 1989b). In contrast, the virus seems not to be circulating in European harbour seals. PDV
80 antibodies were not detected in harbour seals in these waters prior to the 1988 outbreak, and the
81 proportion of the European population carrying antibodies declined steadily after each outbreak,
82 dropping from more than 50% in 2003 to less than 10% in 2007 (Bodewes *et al.*, 2013; Ludes-
83 Wehrmeister *et al.*, 2016; Pomeroy *et al.*, 2005). It has been hypothesized that the unusual mass
84 migration of harp seals in the winter and spring 1987/1988 lead to the introduction of the virus to
85 the European harbour seals in 1988 (Haug & Nilssen, 1995; Nilssen *et al.*, 1998), but the exact
86 timing of these events have never been investigated, and a vector for the 2002 epidemic has not
87 been proposed. Moreover, while the long-distance transmission events of PDV among harbour seal
88 colonies was initially suggested to be aided by the less susceptible and more mobile grey seal
89 (*Haliobalchus grypus*) (Härkönen *et al.*, 2006; McConnell *et al.*, 1999) more recent tagging data
90 indicate that while harbour seals are, in general, philopatric, individual seals occasionally undertake
91 long-distance movements (Dietz *et al.*, 2013; Reijnders *et al.*, 2010; Tougaard *et al.* 2008; Aarts *et*
92 *al.*, 2016). Therefore, dispersing harbour seals could have served as a vector for spreading the virus
93 across Europe.

94

95 Thus, despite nearly three decades of research on PDV, a number of fundamental questions remain
96 to be addressed regarding the origin and evolution of PDV, the diversity and relationship of

97 circulating PDV strains, the source and spread of PDV, and the mechanisms of virus transmission.
98 Here, we sequenced a continuous 7,123 bp sequence of the PDV genome, including the complete
99 coding and non-coding regions of the viral P, M, F and H genes directly from harbour seal samples
100 collected throughout the geographic and temporal progression of the 1988 and 2002 outbreaks to
101 provide the first detailed epidemiological assessment of the emergence and spread of PDV among
102 harbour seals in northern Europe.

103

104 **MATERIALS & METHODS**

105 *Sampling*

106 Tissue samples from harbour seals were collected at haul-out sites along the coasts of Denmark,
107 Sweden, Germany, the Netherlands, England and Scotland during the 1988 and 2002 outbreaks
108 (Supplementary Table 1). Unfortunately high quality samples (e.g. lung tissue stored in RNAlater at
109 -80°C) were only available animals that died in Sweden during the 2002 outbreak. Thus we tested
110 32 liver and muscle tissue samples collected at locations in Denmark and Germany in 1988, and 67
111 spleen, lung and muscle tissue samples from 2002, covering haul-out sites in most Northern Europe.
112 To increase the likelihood of detecting and sequencing the PDV virus, preference was given to
113 samples collected from carcasses that were fresh at the time of collection. Furthermore, we
114 attempted to increase the phylogenomic inference by selecting samples representing as broad a
115 geographical and temporal range as possible, prioritizing samples from early, mid and late stages of
116 each outbreak.

117

118 *Virus screening and sequencing*

119 Viral RNA was extracted from spleen, lung, liver or muscle tissue using the Thermo Scientific
120 KingFisher™ Cell and Tissue DNA Kit. The extractions were first tested for the presence of viral

121 RNA by RT-PCR runs with the H5 (5'-AGATGATATCTTTCCTCC-3') and H6 (5'-
122 ATCCATATGAGTTGCTCC-3') primers. Different sequencing methods were attempted to
123 generate full genomes, but ultimately only primer walking followed by standard Sanger sequencing
124 of four genes was possible. Primer pairs designed for sequencing the PDV genome (de Vries *et al.*
125 2013) were used to amplify and sequence 13 separate, but overlapping segments of 7,123 bp of the
126 PDV genome, covering the complete sequence from position 1,744 to 8,915 (excluding stop
127 codons), including the phosphoprotein (P), the unglycosylated matrix protein (M), the fusion
128 protein (F), and the attachment protein hemagglutinin (H) (Supplementary Table 2 and
129 Supplementary Figure 1). RT-PCRs were performed with the QIAGEN OneStep RT-PCR kit with
130 cycling conditions of 30 min at 50 °C, 10 min at 95 °C, 40-38 cycles of 1 min at 95 °C, 1 min at 55-
131 65 °C and 1 min at 72 °C (Supplementary Table 3) and a final step of elongation for 10 min at 72
132 °C followed by 4 °C. The total volume for each reaction was 25 µl and consisted of 5 µl QIAGEN
133 OneStep RT-PCR Buffer, 1µl dNTP (10 mM), 1.5 µl of each primer (10 µM), 1 µl QIAGEN
134 OneStep RT-PCR Enzyme Mix, 12.5 µl H₂O and 2.5 µl RNA template. PCR products were purified
135 and sequenced by Sanger sequencing at MacroGen Europe (Amsterdam, the Netherlands).

136

137 *Sequence preparation*

138 The raw sequences were assembled and examined for errors in Geneious version 9.1.2 (Kearse *et*
139 *al.*, 2012) (Supplementary Table 4). All ambiguous base calls and singletons (i.e. mutations only
140 occurring in a single sample) were checked by re-sequencing. Low signal regions at the beginning
141 and end of each sequence were removed, and two bases (TT) were deleted before the M gene
142 (position 1,651-1,652) and the H gene (position 5,302-5,303) to place the coding regions in the
143 correct reading frame. The resulting sequences were mapped to the PDV reference genome from
144 1988 (NCBI GenBank accession no. KC802221; de Vries *et al.*, 2013) using MUSCLE (Edgar,

145 2004) to obtain a continuous 7,123 bp sequence, spanning the coding and non-coding regions of the
146 viral P, M, F and H genes. Three different alignments were generated; one including sequences
147 from the 1988 outbreak (eight sequences), one including the 2002 outbreak sequences (36
148 sequences) and one of all sequences combined from the two outbreaks (44 sequences).

149

150 *Substitution saturation and recombination analyses*

151 The full data set was tested for the absence of substitution saturation using the entropy-based index
152 in DAMBE (Xia et al. 2003; Xia, 2013) (Supplementary Table 5). The results showed no indication
153 of substitution saturation among the sequences. Likewise, no evidence of recombination was found
154 using GARD recombination analysis under the HKY85 nucleotide evolution model implemented in
155 Datamonkey (www.datamonkey.org) (Hasegawa *et al.*, 1985; Pond *et al.*, 2006; Delport *et al.*,
156 2010).

157

158 *Phylogenetic analyses*

159 Phylogenetic trees and divergence times were estimated by using the Bayesian Markov chain Monte
160 Carlo (MCMC) method in the BEAST 2.4.6 software package (Bouckaert *et al.*, 2014). Sequence
161 alignments were partitioned into coding regions, 1st, 2nd and 3rd codon positions and non-coding
162 intergenic regions to account for the expected variation in the substitution rates among in this region
163 of the sequences (Lio & Goldman, 1998). The data were analysed using the HKY substitution
164 model (Hasegawa *et al.*, 1985) determined as the best fit model based on the final weight of three
165 criteria; AIC (Akaike, 1973), BIC (Akaike, 1973) and DT (Minin *et al.*, 2003) estimated in
166 JModeltest (Posada, 2008) (Supplementary Table 6). The evolution of the partitions was described
167 by the Gamma site model thereby allowing the substitution rates of each site to correspond to a
168 continuous distribution of different rates among sites (Haug, 1990). Runs were carried out under the

169 assumption of a coalescent constant population growth model for the dataset, and a coalescent
170 exponential population growth model for the separate 1988 and 2002 datasets, respectively, using a
171 relaxed uncorrelated lognormal molecular clock in all analyses (Drummond *et al.*, 2006)
172 (Supplementary Table 7 and 8). The tree was calibrated by the inclusion of sampling dates (tip
173 dates), specified in years. Four independent runs were conducted with a chain length of 20,000,000
174 iterations, with sampling of the tree parameters for every 2,000th iteration and a burn-in of 10 %.
175 The results of each individual run were visually inspected in Tracer v1.6.0 for convergence and to
176 check that the effective sample size (ESS) values were > 200. Subsequently, the files produced from
177 independent runs of the MCMC yielding similar results were merged into one tree and log file using
178 LogCombiner v2.4.6 (Bouckaert *et al.*, 2014) with 50% sampling frequency and a burn-in of 10%.
179 The tree files were summarized in maximum clade credibility trees in TreeAnnotator and viewed in
180 Figtree v1.4.3 (Rambaut 2016). The time to the most recent common ancestor (TMRCA) of the
181 combined 1988 and 2002 dataset was estimated as a mean value with its 95% high-probability
182 density (HPD) interval. To account for uncertainties in branch lengths and topology the final tree
183 was constructed using Densitree (Bouckaert & Heled, 2014).

184

185 **RESULTS AND DISCUSSION**

186 *Sequencing PDV from the 1988 and 2002 outbreaks*

187 A total of 99 harbour seal samples were screened from the outbreaks in 1988 ($n = 32$) and 2002 ($n =$
188 67) from which 7,123 base pairs of the PDV genome (position 1,744-8,915, excluding stop codons)
189 was successfully amplified and sequenced from 44 samples; eight from 1988 and 36 from 2002
190 (Figure 1; Supplementary Table 1; Supplementary Table 4). In total 112 polymorphic sites were
191 found in the combined 1988 and 2002 dataset, 16 polymorphic sites (including 11 singletons)
192 occurred in the sequences from the 1988 samples, and 57 polymorphic sites (32 singletons)

193 occurred in the sequences from the 2002 samples (Supplementary Tables 9 and 10). The
194 amplification and sequencing success was higher for samples from 2002 than 1988, and within the
195 2002 samples success was higher for samples that had been stored in RNAlater at -80°C compared
196 to storage in ethanol or frozen dry at -20°C. Nevertheless, our results illustrate that PDV virus can
197 be extracted and sequenced from infected seals even after three decades of suboptimal sample
198 storage.

199

200 *A winter origin of the 1988 and 2002 PDV strains*

201 The origin of the virus sequenced from the 1988 epidemic was estimated to be mid-November
202 1987, with a 95% probability interval ranging from mid-May 1987 to early April 1988 (Table 1).
203 This estimate correlates well with the timing of observed mass migrations of harp seals from the
204 Barents Sea south along the coasts of Norway and into the North Sea in the winter of 1987-1988
205 (Haug & Nilssen, 1995). The migration event is widely believed to have introduced PDV to the
206 previously unexposed European harbour seals (Haug & Nilssen, 1995; Nilssen *et al.*, 1998; Dietz *et*
207 *al.*, 1989a,b; Härkönen *et al.*, 2006), and was presumably caused by the collapse of the Barents Sea
208 capelin (*Mallotus villosus*) and herring (*Clupea harengus*) stocks (Haug & Nilssen, 1995).
209 Although the morbillivirus itself has not been detected in Arctic seals (Kreutzer *et al.*, 2008)
210 morbillivirus specific antibodies have been found in Greenlandic harp seals and ringed seals
211 sampled between 1984 and 1987 (Dietz *et al.*, 1989b), as well as in Barents Sea harp seal samples
212 collected between 1987 and 1989 (Markussen & Have, 1992). Markussen and Have (1992)
213 documented that the percentage of harp seals with antibodies increased from 1987 to 1989,
214 indicating that a large proportion of the Barents Sea seals carried a morbillivirus before and during
215 the European epidemic in 1988. Harp seals are migratory and even discrete populations have
216 overlapping ranges during part of the year (Øien & Øritsland, 1995; Folkow *et al.*, 2004),

217 supporting the possibility of periodical encounters and intraspecific and interspecific disease
218 transmissions across the Arctic.

219

220 The origin of the virus sequenced from the 2002 epidemic was estimated to be mid-January 2002,
221 with a 95% probability interval ranging from late June 2001 to early May 2002 (Table 1). Thus, like
222 the 1988 outbreak, the 2002 PDV strain can be traced back to the preceding winter. However,
223 unlike the 1988 outbreak, there was no obvious candidate for the introduction of PDV in 2002. The
224 phylogenetic analyses presented here strongly suggest that the 2002 strain did not originate directly
225 from the 1988 strain (Figure 2B). This is also supported by previous analyses of shorter PDV
226 sequence fragments (Nielsen *et al.*, 2009; Earle *et al.*, 2011), as well as an absence of virus in
227 European harbour seals screened between the two outbreaks (Ludes-Wehrmeister *et al.*, 2016;
228 Bodewes *et al.*, 2013). PDV neutralizing antibodies have been detected in harp seals, ringed seals,
229 hooded seals (*Cystophora cristata*) and polar bears (*Ursus maritimus*) sampled between 1988 and
230 1996 in the waters surrounding Greenland, the Canadian Arctic and North America (Nielsen *et al.*,
231 1997; Cattet *et al.*, 2004). PDV positive harbour seals, grey seals and harp seals were also detected
232 along the North Atlantic coast of North America between 2004-2007 (Siembieda *et al.*, 2017). Thus
233 there are several potential candidate species that could have been reservoir hosts or carriers. Similar
234 to 1988, the Barents Sea capelin and herring stock experienced declines in the years just before the
235 2002 epidemic (Loeng & Drinkwater 2007); however, this collapse did not result in a new mass
236 migration of harp seals as occurred in 1998. Nevertheless, the virus could have been carried and
237 transmitted by single migrating individuals as harp seals tagged in Canada were observed to visit
238 the North Sea between 2001 and 2002 (Kreutzer *et al.*, 2008).

239

240

241 *Multiple introductions of PDV in 1988*

242 In addition to supporting the hypothesised introduction of PDV by harp seals in the winter of 1987-
243 88, the phylogenetic analyses of the 1988 data provide new insight to the emergence and
244 subsequent pattern of spread among harbour seals. In the beginning of March 1988, the viral strains
245 split into two clades; one clade containing sequences from central Kattegat and a second clade
246 containing sequences from the Wadden Sea and the Limfjord. The analysis of the 1988 data in
247 isolation – as well as the combined 1988-2002 data – places a sample (#42524) collected in the
248 Limfjord on 22 July 1988 at the base of the tree (Supplementary Figure 2). Interestingly, while the
249 viral sequence from sample #42524 contains mutations that are unique to the 1988 outbreak, it
250 diverges from the rest of the 1988 sequences by six mutations (Supplementary Table 9). The
251 presence of these mutations was confirmed by forward and reverse sequencing and Phred scores of
252 > 30, and they do not appear to be artefacts caused by DNA damage and deamination as only three
253 of the six substitutions are C-T or G-A. Moreover, another sample from the Limfjord (#42523) –
254 which was omitted from the full phylogenetic analyses because full sequences only could be
255 obtained for positions 3,910-4,535, 5,027-6,204 and 6,715-8,916, respectively – also contained four
256 of the six unique mutations found in sample #42524. The basal phylogenetic placement of the
257 Limfjord sequence indicates that the 1988 outbreak among European harbour seals consisted of not
258 just a single, but several introductions of related PDV strains. The observed rise in the number of
259 harbour seal deaths along the Danish west coast by February 1988 (Danielsen *et al.*, 1988; Dietz *et*
260 *al.*, 1989a) might have been caused by such an introduction, independent of the full-scale outbreak
261 two months later in Kattegat. This peculiar Limfjord strain could represent an early and partly
262 unsuccessful variant of the virus, later enhanced in the form of the second, more virulent and
263 widespread strain. Further clarification on the role of harp seals as vectors of the 1988 outbreak, as
264 well as the potential introduction, evolution, pathogenicity and circulation of several PDV strains

265 throughout this outbreak, would require the successful retrieval and comparison of viral RNA from
266 European harbour seals and Barents Sea harp seals collected before, during and after the 1988
267 outbreak.

268

269 *Spread of PDV in 2002 through multiple transmission events*

270 In 2002, the first harbour seal mortalities were observed on the island of Anholt in Kattegat on May
271 4th, and by May 30th, sick seals had been reported along the Danish and Swedish coasts of Kattegat
272 and Skagerrak (Härkönen *et al.*, 2006). On June 16th the first cases were reported from Vlieland in
273 the Dutch Wadden Sea (Härkönen *et al.*, 2006), and by July 18th the index case of England was
274 recorded in the Wash area (Lawson & Jepson, 2003), 800-1000 km from Kattegat. Intriguingly, sick
275 seals were not observed in the geographically intermediate German and Danish Wadden Sea and
276 the Limfjord before mid-September (Härkönen *et al.*, 2006). Thus, based on the observed sequence
277 of mortalities, the predominant hypothesis has been that the virus made a long-distance jump from
278 the Kattegat epicentre to localities in the Dutch Wadden Sea from where it spread in two waves:
279 west to England, Belgium and France, and east to the German and Danish Wadden Sea before
280 going north along the coast to the Limfjord (Härkönen *et al.*, 2006).

281

282 The phylogenetic analysis presented here supports a Kattegat epicentre of the PDV outbreak in
283 2002, but offers new insights into the subsequent long-distance spread among European harbour
284 seal colonies (Figure 2A; Supplementary Figure 3). The most basal sample of the phylogenetic tree
285 is from Kattegat, which by early May splits into branches containing samples from Kattegat,
286 Skagerrak, Øresund and southwestern Baltic, in agreement with the observed spatio-temporal
287 pattern of emergence. In contrast, the phylogenetic analysis does not agree with the presumed
288 scenario in which harbour seal colonies outside of the epicentre were infected by one long-distance

289 transmission event from Kattegat to the Dutch Wadden Sea and further to other North Sea colonies.
290 Rather, based on the phylogenetic analyses we hypothesise that PDV arrived in southern England,
291 the Wadden Sea and the Limfjord, respectively, through at least three independent long-distance
292 transmission events from the Kattegat epicentre (Figure 2C). The integrity of each of these
293 subclades is supported by posterior values above 0.8, but the positioning of samples within
294 subclades, as well as the relationship among subclades is poorly resolved (Figure 2A;
295 Supplementary Figure 3).

296
297 The finding of multiple transmission events poses the question of who the likely carriers of PDV
298 among harbour seal colonies are. As harbour seals show strong site fidelity throughout the summer
299 (Dietz *et al.*, 2013) and exhibit fine-scale population genetic structuring (Olsen *et al.*, 2014), it has
300 previously been assumed that the more mobile and less susceptible grey seal acted as carrier of
301 PDV between geographically distant haul-out sites (Hammond *et al.*, 2005; Pomeroy *et al.*, 2005;
302 Härkönen *et al.*, 2006). However, while grey seals did haul-out on Anholt in 2002, the numbers
303 were likely no more than 5-10 animals, several orders of magnitude lower than the 800-1000
304 harbour seals hauling out. Thus, although it only takes one infected animal to start an epidemic, the
305 likelihood of transmission from harbour seal to harbour seal is substantially greater than from grey
306 seal to harbour seal. Moreover, recent tagging and genetic studies indicate that especially juvenile
307 harbour seals undertake movements across much greater distances than previously reported, and
308 thus potentially could have played a larger role in transporting PDV longer distances. For instance,
309 Wadden Sea harbour seals have been shown to undertake foraging trips of a couple of hundred
310 kilometres into the North Sea (Dietz *et al.*, 2013; Tougaard *et al.*, 2008; Aarts *et al.*, 2016), and
311 recent population genetic analyses suggested movements among the seals in southern England,
312 France and the Dutch Wadden Sea (Olsen *et al.*, 2017). Likewise, tagging of harbour seals in the

313 fall and early winter of 2016 provided the first documented movements from a haul-out in southern
314 Kattegat to the central Limfjord (Rune Dietz and collaborators, unpublished) by an animal that has
315 now been genetically determined to originate from the Limfjord (Morten Tange Olsen,
316 unpublished). Thus, while grey seals cannot be ruled out, we hypothesise that both short- and long-
317 distance PDV transmissions in 2002 could have been facilitated by harbour seal movements.
318 Although beyond the scope of the present analysis, additional insights into the dispersal of seals and
319 transmission of PDV could be obtained by integration of genetic, epidemiological and tagging data
320 (Swinton *et al.*, 1998; Hall *et al.*, 2006).

321

322 *New insights to PDV virus evolution*

323 The mean substitution rate of the full data set – including coding and non-coding regions – was
324 found to be 6.73×10^{-4} sub/site/yr, while the substitution rate estimates for the sequences of each
325 outbreak were 2.28×10^{-3} sub/site/yr and 1.58×10^{-3} sub/site/yr for 1988 and 2002, respectively. The
326 results correspond well with the rates of 10^{-3} to 10^{-4} sub/site/yr reported for other morbilliviruses
327 (Furuse *et al.*, 2010; Hanada *et al.*, 2004; Xu *et al.*, 2014). The substitution rates estimated at gene
328 level did not differ much and were found to be between 8.03×10^{-4} to 2.2×10^{-4} for the full data set,
329 5.75×10^{-3} to 4.62×10^{-3} for the 1988 sequences and 5.72×10^{-3} to 1.53×10^{-3} for the 2002 sequences
330 (Table 2). In the full dataset, the F, M and H genes had similar substitution rates with overlapping
331 HPD intervals, whereas the substitution rate of the P gene (2.2×10^{-4}) was substantially lower.
332 Interestingly, while the P gene had the lowest substitution rate, it had the highest proportion of non-
333 synonymous substitutions (64%), followed by the F gene (50%) and H gene (48%), and finally the
334 M gene (23%). The P gene encodes for three different proteins; P (1,524 bp), V (899 bp) and C
335 (525 bp), which use different reading frames of the same nucleotide sequence, and high sequence
336 variability has previously been documented in MeV (Bankamp *et al.*, 2008; Beaty & Lee, 2016;

337 Devaux & Cattaneo, 2004). Nine amino acid changes were found in the reading frame of the P
338 protein, which is a part of the polymerase complex and is essential for viral RNA replication
339 (Bankamp *et al.*, 2011). Six amino acid changes were found in the reading frame of the V protein
340 and two amino acid changes were found in the reading frame of the C protein. The V and C proteins
341 both play important roles in the inhibition of interferons (Nanda & Baron 2006; Chinnakannan *et*
342 *al.*, 2014) and influence the antiviral host response. Additionally, the C protein is believed to affect
343 the transcription of mRNA of the viral genes (Baron & Barrett 2000) and this could explain why the
344 percentage of changes is lower than the V protein. H and F genes code for the hemagglutinin
345 glycoprotein and the fusion protein, respectively, allowing the virus to attach and fuse with the host
346 cell membrane (Wild *et al.*, 1991). The two genes are known targets for the virus-neutralizing
347 antibodies (Xu *et al.*, 2014), and would be expected to contain a high amount of genetic variation,
348 which our study also suggests. The substitution rate of the H gene for MeV have previously been
349 estimated to be 6.44×10^{-4} sub/site/yr (Furuse *et al.*, 2010) – an estimate which corresponds well to
350 our estimate of 5.6×10^{-4} for PDV. The M gene codes for the matrix protein and is considered to be
351 relatively conserved (Beaty & Lee, 2016), which the relatively low proportion of non-synonymous
352 substitutions estimated for PDV also suggests.

353

354 *Perspectives*

355 Wildlife pathogens, such as PDV and other morbilliviruses, represent a major risk for -epidemic
356 outbreaks resulting in extensive die offs in naïve populations and species. The past centuries'
357 human activities and extensive utilization of natural resources in terrestrial and marine
358 environments have led to substantial habitat alteration, loss of biodiversity and climatic changes
359 (Jones *et al.*, 2008). As a consequence, wildlife species shift their geographical ranges, increasing
360 the likelihood of disease transmissions and outbreaks within and among naïve species and

361 populations (Karesh *et al.*, 2012). Indeed, the mass migration of harp seals hypothesised to have
362 brought PDV to the naïve harbour seal population in 1988 was caused by a collapse of capelin and
363 herring stocks following intense human exploitation (Haug & Nilssen, 1995). Such outbreaks could
364 lead to ecological or economic losses and have huge consequences for already exposed or
365 endangered species (Osterhaus *et al.*, 1997; Thorne & Williams, 1988). Further work should focus
366 on understanding the dynamics of pathogen emergence and spread, including the co-evolutionary
367 dynamics of intra- and interspecific pathogen transmissions.

368

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370

371 **AUTHOR CONTRIBUTIONS**

372 IS and MTO conceived and designed the study; MTO, TH, KH, US, RD, JT and AH provided
373 funding; TH, KH, US, KL, RD, JT, AG, LWH, EC and AH collected and curated the samples; IS
374 conducted the lab work and phylogenetic analyses; IS and MTO drafted the manuscript. All authors
375 provided input to the manuscript and approved the final version.

376

377 **CONFLICT OF INTEREST**

378 The authors declare no conflict of interest.

379

380 **DATA ACCESSIBILITY**

381 The edited PDV sequences will be made available on NCBI GenBank upon acceptance of the
382 manuscript.

383

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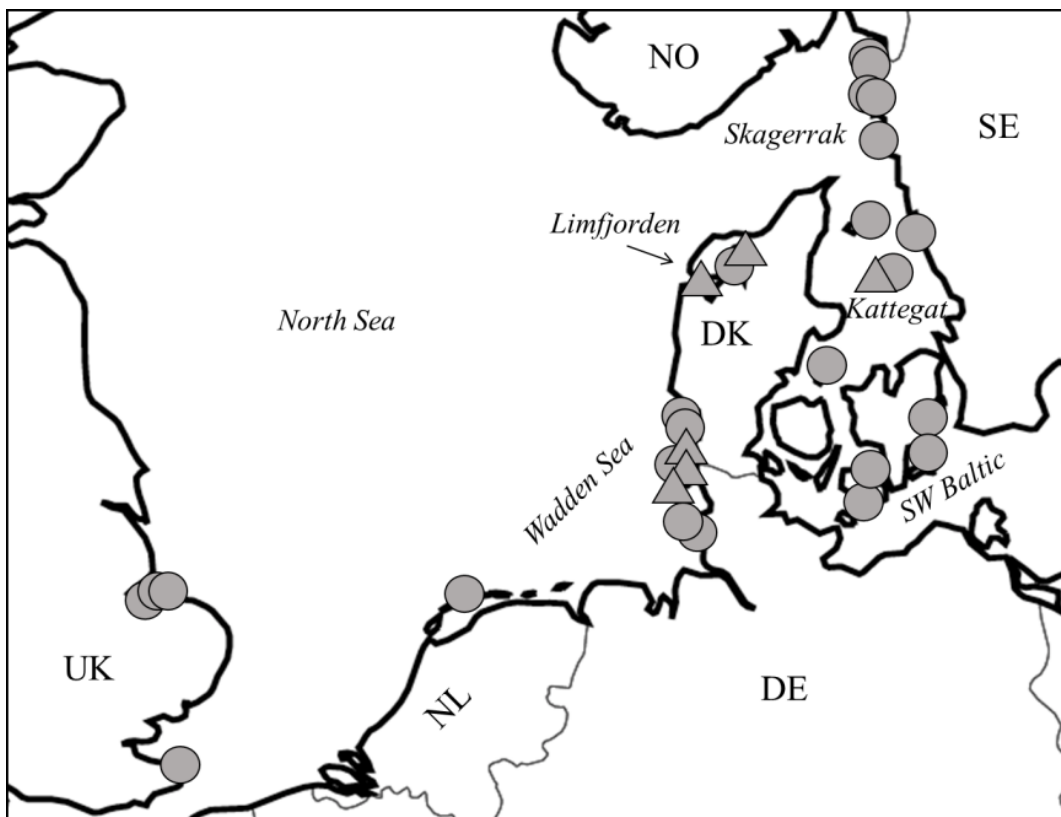
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574 **FIGURES AND TABLES**

575

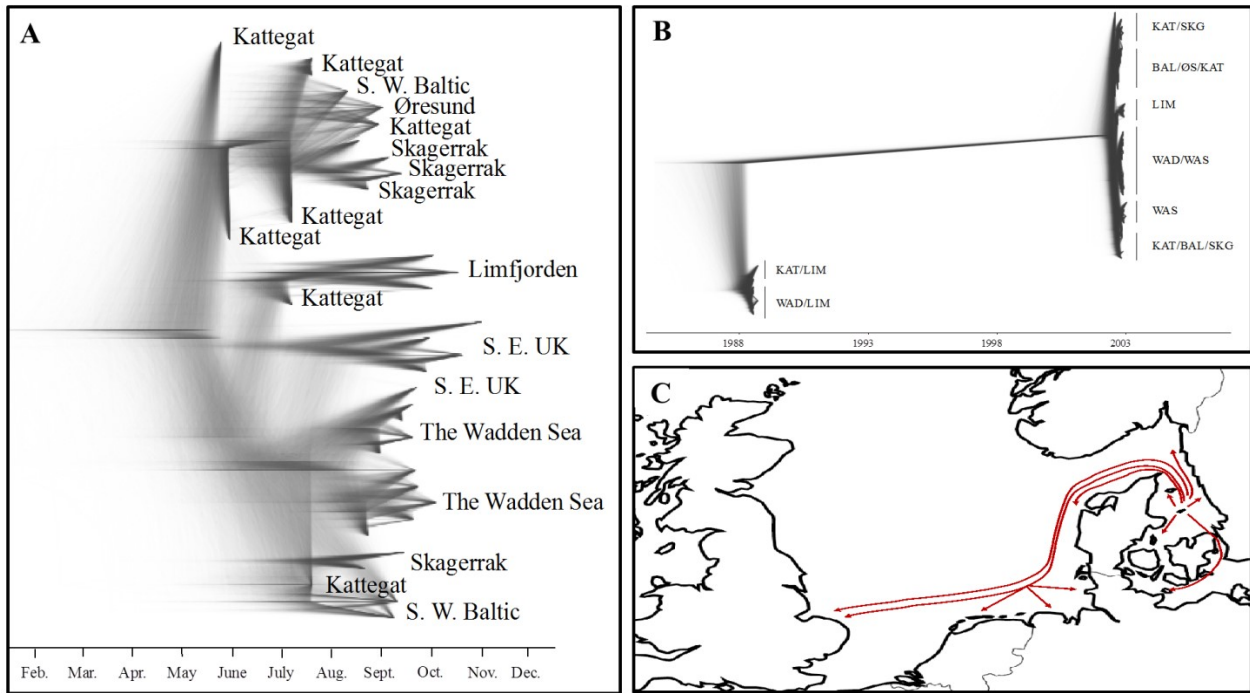


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577 **Figure 1** Spatial and temporal origin of the 44 harbour seals and PDV sequences used in the
578 phylogenetic analysis. Triangles = Samples from 1988 collected in Kattegat (N=2), Limfjorden
579 (N=2) and the German Wadden Sea (N=4). Circles = Samples from 2002 collected in Kattegat
580 (N=11), Skagerrak (N=6), the Baltic (N=3), Limfjorden (N=3), the Danish Wadden Sea (N=4), the
581 German Wadden Sea (N=4), the Dutch Wadden Sea (N=1) and the southeastern coast of England
582 (N=5).

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Figure 2 Phylogenetic relationship and hypothesised spread of PDV among European harbour seals. A) Bayesian phylogenetic analysis of 36 PDV sequences obtained from harbour seals sampled during the 2002 outbreak and sequenced in 7,123bp (position 1,744-8,915) of the viral P, M, F and H genes. The analysis provides strong support for Kattegat as the origin of the outbreak, with subsequent independent pulses of spread to harbour seal localities throughout northern Europe. Uncertainties in the tree topology are visualized as a smear around the tree nodes and branches. B) Phylogenetic analysis of the combined data set of 44 samples from 1988 and 2002 supporting the independent origin of the strain leading to the 2002 outbreak. C) A novel hypothesis for the spread of PDV during 2002 from the Kattegat epicentre to other European harbour seal localities through a series of independent transmission events.

604 **Table 1** Summary statistics for the Bayesian phylogenetic analyses of the sequences from the PDV outbreaks in European harbour seal
 605 populations

Data set	uclid.stdev	Coefficient of variance	Rate of evolution sub/site/yr	Tree height	Growth rate	TMRCA date [95 % HPD]	
						1988	2002
PDVall	0.71	0.75	6.73*10 ⁻⁴	15.54 = 15 y 198 d		25/11-1987	7/1-2002
(constant growth)	[0.41, 1.04]	[0.4, 1.15]	[5.04*10 ⁻⁴ , 8.41*10 ⁻⁴]	[14.62, 17.02]	-	[10/6-87, 3/4-88]	[28/7-01, 30/4-02]
PDV1988	3.74*10 ⁻³	4.09*10 ⁻³	2.42*10 ⁻⁶	0.01			
(exp. growth)	0.20	0.19	2.28*10 ⁻³	0.38	12.97		
(exp. growth)	[6.52*10 ⁻¹⁰ , 0.69]	[5.61*10 ⁻¹⁰ , 0.64]	[4.54*10 ⁻⁴ , 4.22*10 ⁻³]	[9.19, 0.73]	[1.76, 23.91]	-	-
PDV2002	2.55*10 ⁻³	2.31*10 ⁻³	1.55*10 ⁻⁵	3.17*10 ⁻³	0.09		
(exp. growth)	0.23	0.23	1.58*10 ⁻³	0.56 = 203,16 d	8.48		
(exp. growth)	[8.15*10 ⁻⁹ , 0.62]	[8.12*10 ⁻⁹ , 0.63]	[1.03*10 ⁻³ , 2.18*10 ⁻³]	[0.46, 0.68]	[4.84, 11.96]	-	-
	2.40*10 ⁻³	2.47*10 ⁻³	5.49*10 ⁻⁶	1,18*10 ⁻³	0.03		

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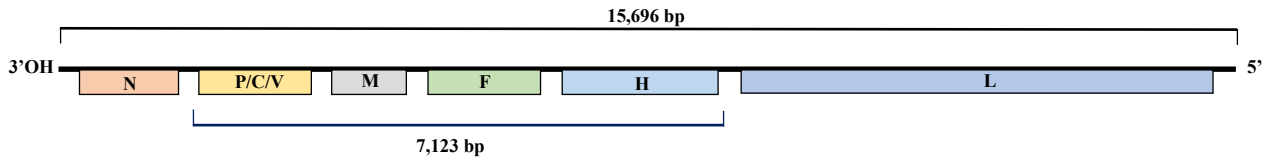
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Table 2 The number of genetic differences, amino acid changes and the substitution rates for the F, H, M and P genes calculated using an uncorrelated relaxed clock. The M gene from the 1988 data set contained no substitutions and thus no substitution rate was calculated.

Gene	Lenght	Number of mutations			Amino acid changes			Substitution rate		
		PDVall	PDV1988	PDV2002	PDVall	PDV1988	PDV2002	PDVall	PDV1988	PDV2002
F	1,893 (2,206)	28	4	15	0.50	0	0.66	8.03*10 ⁻⁴ [4.44*10 ⁻⁴ , 1.16*10 ⁻³] 4.08*10 ⁻⁴	4.62*10 ⁻³ [4.52*10 ⁻⁴ , 9.82*10 ⁻³] 3.4*10 ⁻⁵	3.96*10 ⁻³ [1.3*10 ⁻³ , 7.08*10 ⁻³] 5.35*10 ⁻⁵
H	1,821 (1,857)	31	5	12	0.48	0.40	0.50	5.6*10 ⁻⁴ [2.56*10 ⁻⁴ , 8.96*10 ⁻⁴] 5.11*10 ⁻⁶	4.75*10 ⁻³ [2.44*10 ⁻⁴ , 0.01] 4.76*10 ⁻⁵	2.19*10 ⁻³ [4.89*10 ⁻⁴ , 4.37*10 ⁻³] 3.88*10 ⁻⁵
M	1,005 (1,447)	13	0	10	0.23	0	0.20	5.73*10 ⁻⁴ [1.9*10 ⁻⁴ , 1.01E*10 ⁻³] 6.74*10 ⁻⁶	-	5.72*10 ⁻³ [1.57*10 ⁻³ , 0.01] 6.70*10 ⁻⁵
P	1,521 (1,653)	14	4	8	0.64	0.75	0.75	2.2*10 ⁻⁴ [6.26*10 ⁻⁵ , 4.24*10 ⁻⁴] 2.51*10 ⁻⁶	5.71*10 ⁻³ [1.89*10 ⁻⁴ , 0.01] 5.97*10 ⁻⁵	1.53*10 ⁻³ [3.79*10 ⁻⁴ , 2.94*10 ⁻³] 1.25*10 ⁻⁵

1 **SUPPLEMENTARY MATERIAL**

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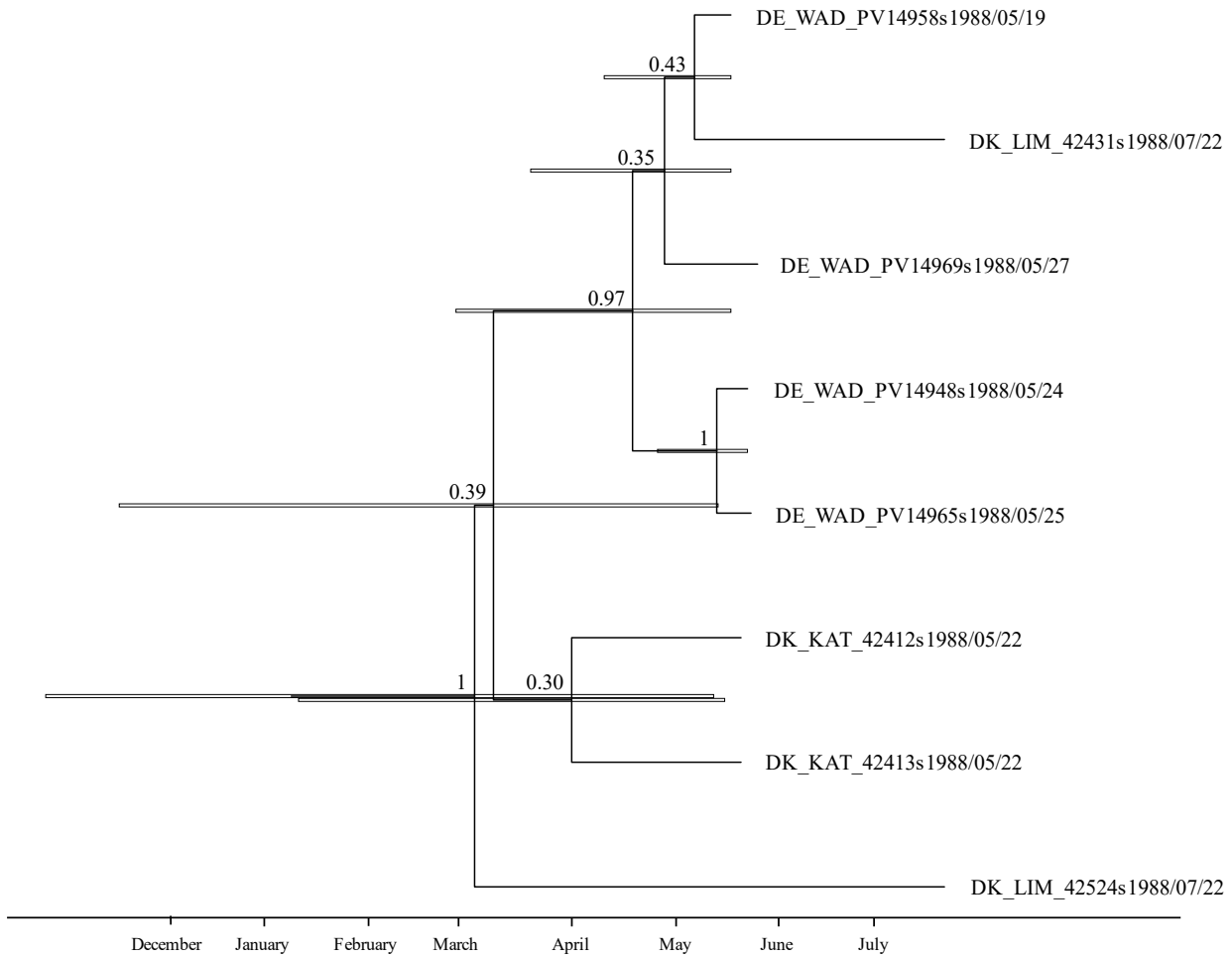
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6 **Supplementary Figure 1** A schematic drawing of the genome of Phocine Distemper Virus (PDV)

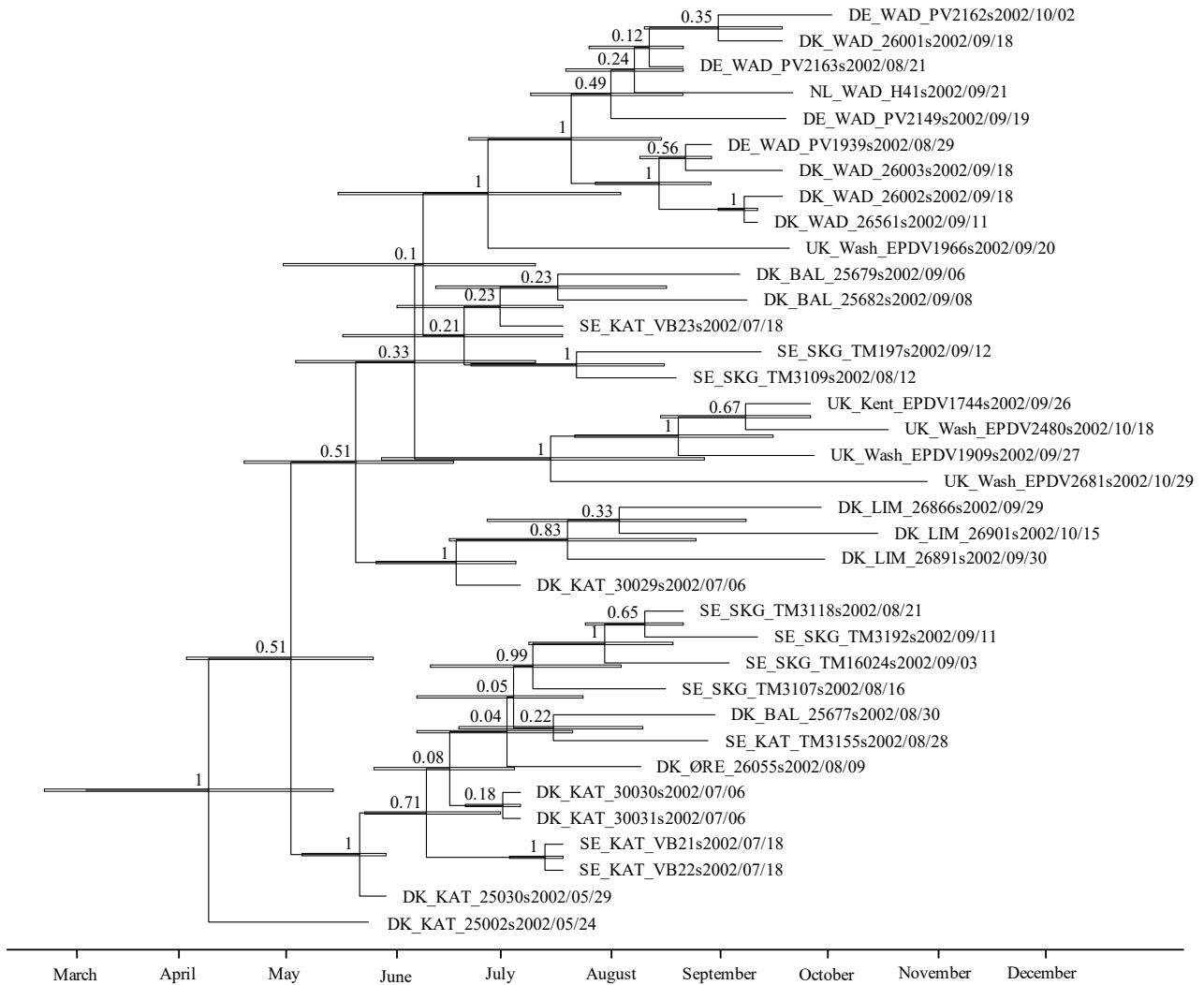
7 including the six non-overlapping genes; N (position 108-1,679), P (position 1,801-3,324), M
8 (position 3,432-4,439), F (position 5,028-6,923), H (position 7,079-8,902) and L (position 9,036-
9 15,590) coding for at least eight proteins as depicted (de Vries *et al.*, 2013) sequenced in this study
10 (position 1,744-8,915).

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16 **Supplementary Figure 2** Phylogenetic relationship among eight PDV strains sequenced from
17 harbour seals infected during the 1988 epidemic. The analysis suggests at least two independent
18 introductions of the PDV strain to Limfjorden (DK_LIM_42524; 1988/07/22) and Kattegat
19 (DK_KAT_42412; 1988/05/22), respectively. Node bars are shown as the 95 % interval of the node
20 heights thereby indicating their uncertainty.



Supplementary Figure 3 Phylogenetic relationship among PDV strains sequenced from harbour seals infected during the 2002 epidemic. The analysis provides strong support for Kattegat as the origin of the outbreak (DK_KAT_25002; 2002/05/24), with subsequent pulses of spread to harbour seal localities in Skagerrak (SKG), SW Baltic (BAL), the Danish German and Dutch Wadden Sea (WAD), Limfjorden (LIM) and the Wash (UK_Wash). Node bars are shown as the 95 % interval of the node heights thereby indicating their uncertainty.

Supplementary Table 1 Summary of tissue type, storage conditions and the number of samples extracted and included in the analyses.

Country	Tissue type		Storage temperature		Sample storage		Number of samples		Number of samples included in the data set	
	1988	2002	1988	2002	1988	2002	1988	2002	1988	2002
Denmark	Liver	Spleen/lung	-20 °C	-20 °C	Organs in bags	Organs in bags	27	28	4	16
England	-	Spleen/lung	-	-20 °C/-80 °C	-	-	-	17		5
Germany	Muscle	Spleen/lung	-20 °C	-20 °C	Organs in bags	Organs in bags	5	5	4	4
The Netherlands	-	Muscle	-	-20 °C	-	-	-	2		1
Scotland	-	Spleen/lung	-	-20 °C	-	-	-	4		0
Sweden	-	Spleen	-	-20 °C/-80 °C	-	Tubes RNAlater	-	11		10
Total							32	67	8	36

Supplementary Table 2 Table of primers used to amplify the fragments sequenced. The primers were compiled from several previous studies and combined to construct the complete genome sequence of PDV (de Vries et al., 2013).

Primer	Sequence	Position _{start}	Position _{end}	Coverage _{base pairs}	Gene
4	5'-TCAGCTAAGTTCTAAAAGACATTGC-3' 3'-CAGTTAGATGAAGCATTTCCTTCGG-5'	1,672	2,303	631	P
5	5'-GAAGGAAGAGAGGGAAGCCTTGATG-3' 3'-GTTTTTGTGATCGAGTCAATCTCTCC-5'	2,224	2,854	630	P
6	5'-TGAGGATAATCAATCAATACTTAGC-3' 3'-TGAGTCCTAAGTTTTTTGTAATTGC-5'	2,778	3,407	629	P
7	5'-ACCAATGCCTTTGTTAGTAATCAGG-3' 3'-TCGAAATCAAACATTCCGCGAGGG-5'	3,333	3,974	641	P/M
8	5'-GTAGTGACATGAGTATTACTAGAC-3' 3'-AACAGACTAAAGCTAATTAATGCTG-5'	3,9	4,549	649	M
9	5'-TAATGATATAGACTGAAGCACTCAC-3' 3'-TTCAGGGTTTAGAAATGGTGAATCG-5'	4,464	5,108	644	M/F
10	5'-ATGACTCGAGTCAAGAACTCCCAG-3' 3'-CAGCAGTTGCTACACCTAGAGCTGC-5'	5,028	5,655	627	F
11	5'-GGGATCAGGTAGAAGACAGAGGCG-3' 3'-GGGACAGTAGTGTACCATTCTGTG-5'	5,582	6,217	635	F
12	5'-GGGTTGTCGTACATAGATTGGAGGC-3' 3'-ATACCGGGAGTGCAATGAGGGTGCC-5'	6,148	6,777	629	F
13	5'-AATCCTCTGATCAAATCCTTGACAC-3' 3'-ATCCTTGACTTGATGATGCACTGCC-5'	6,703	7,348	645	F/H
14	5'-AAAAAGCAATTTGGAGTTCAATAAG-3' 3'-GATAATCCTGTAGTTTGTGTTTGG-5'	7,27	7,9	630	H
H5/H6	5'-AGATGATATCTTTCCTCC-3' 3'-ATCCATATGAGTTGCTCC-5'	7,615	8,065	451	H
15	5'-GATTCGTGTCTTTGAGATTGGATTCC-3' 3'-CCATTTTTAGGTGGAATTGTTAGCC-5'	7,819	8,448	629	H
16	5'-ATAATATTTAACGGTGATGGGATGG-3' 3'-AAACAAGGGAAGAAACACTTACCG-5'	8,366	8,972	606	H

Supplementary Table 3 RT-PCR conditions for the 14 different primer sets used in this study.

Cycles	1	1	40	/	38	x	(1	1	1)	1	1
Time (min)	30	15					1	1	1	10	∞
Primer 4 (°C)	50	95	x				94	64	72	72	4
Primer 5 (°C)	50	95	x				94	64	72	72	4
Primer 6 (°C)	50	95	x				94	63	72	72	4
Primer 7 (°C)	50	95			x		94	65	72	72	4
Primer 8 (°C)	50	95	x				94	63	72	72	4
Primer 9 (°C)	50	96	x				94	62	72	72	4
Primer 10 (°C)	50	95	x				94	62	72	72	4
Primer 11 (°C)	50	95	x				94	62	72	72	4
Primer 12 (°C)	50	95	x				94	62	72	72	4
Primer 13 (°C)	50	95	x				94	62	72	72	4
Primer 14 (°C)	50	95	x				94	58	72	72	4
(°C)	50	95	x				94	55	72	72	4
Primer 15 (°C)	50	95	x				94	58	72	72	4
Primer 16 (°C)	50	95			x		94	64	72	72	4

Supplementary Table 4 The final sample set of PDV sequences covering the coding and non-coding regions of the P, M, F and H genes of samples collected in 2002 and 1988. Numbers 1-4 indicate identical sequences. * Note that viral RNA was not successfully sequenced with primer 6 for the samples 42412 and 42413 and a fragment from a German sample was used to fill the gap (see Table 9).

Sample	Species	Location	Area	Date	Tissue
25002	Harbour seal	Læsø	DK-KAT	24. May 2002	(Liver)
25030	⁴ Harbour seal	Anholt	DK-KAT	29. May 2002	Spleen
30029	Harbour seal	Samsø	DK-KAT	6. July 2002	Spleen
30030	⁴ Harbour seal	Samsø	DK-KAT	6. July 2002	Spleen
30031	⁴ Harbour seal	Samsø	DK-KAT	6. July 2002	Spleen
VB21	⁵ Harbour seal	Prästaskär	SE-KAT	18. July 2002	Spleen
VB22	⁵ Harbour seal	Flatbåden	SE-KAT	18. July 2002	Spleen
VB23	Harbour seal	Prästaskär	SE-KAT	18. July 2002	Spleen
TM3155	Harbour seal	Luseskär	SE-KAT	28. August 2002	Spleen
TM197	Harbour seal	Väderöarna	SE-SKG	12. September 2002	Spleen
TM3107	Harbour seal	Sälvik	SE-SKG	16. August 2002	Spleen
TM3109	Harbour seal	Koster	SE-SKG	19. August 2002	Spleen
TM3118	Harbour seal	Lilla Sockna	SE-SKG	21. August 2002	Spleen
TM3192	Harbour seal	Tjämnö	SE-SKG	11. September 2002	Spleen
TM16024	Harbour seal	Ramsökälven	SE-SKG	3. September 2002	Spleen
26055	Harbour seal	Karlslande	DK-ØRE	9. August 2002	Spleen
25679	Harbour seal	Møn	DK-BAL	6. September 2002	Spleen
25682	Harbour seal	Rødsand	DK-BAL	8. September 2002	Spleen
25677	Harbour seal	Kippinge	DK-BAL	30. August 2002	Spleen
H41	Harbour seal	Ameland	NL-WAD	9. September 2002	Muscle
Pv 1939	Harbour seal	Eiderstedt	DE-WAD	29. August 2002	Spleen/lung
Pv 2149	Harbour seal	g	DE-WAD	19. September 2002	Spleen/lung
Pv 2162	Harbour seal	Sylt	DE-WAD	2. October 2002	Spleen
Pv 2163	² Harbour seal	Eiderstedt	DE-WAD	21. August 2002	Spleen
26001	² Harbour seal	(North)	DK-WAD	18. September 2002	Spleen
26002	¹ Harbour seal	(North)	DK-WAD	18. September 2002	Spleen
26003	Harbour seal	(North)	DK-WAD	18. September 2002	Spleen
26561	¹ Harbour seal	Koresand	DK-WAD	11. September 2002	Spleen
26866	Harbour seal	Blinde Røn	DK-LIM	29. September 2002	Spleen
26891	Harbour seal	Blinde Røn	DK-LIM	30. September 2002	Spleen
26901	Harbour seal	Blinde Røn	DK-LIM	15. October 2002	Spleen

Supplementary Table 4 (continued)

Sample	Species	Location	Area	Date	Tissue
EPDV2681	Harbour seal	Wash	UK-Wash	29. September 2002	Spleen/lung
EPDV2480	Harbour seal	Wash	UK-Wash	18. October 2002	Spleen/lung
EPDV1966	Harbour seal	Wash	UK-Wash	20. September 2002	Spleen/lung
EPDV1909	Harbour seal	Wash	UK-Wash	27. September 2002	Spleen/lung
EPDV1744	Harbour seal	Kent	UK-Wash	26. September 2002	Spleen/lung
PV14965	Harbour seal	Wadden Sea	DE-WAD	25. May 1988	Muscle/blubber
PV14958	Harbour seal	Wadden Sea	DE-WAD	19. May 1988	Muscle/blubber
PV14948	Harbour seal	Wadden Sea	DE-WAD	24. May 1988	Muscle/blubber
PV14969	Harbour seal	Wadden Sea	DE-WAD	27. May 1988	Muscle/blubber
42524	Harbour seal	Limfjorden	DK-LIM	22. July 1988	Liver
42531	Harbour seal	Limfjorden	DK-LIM	22. July 1988	Liver
42412 *	Harbour seal	Anholt	DK-KAT	22. May 1988	Liver
42413 *	Harbour seal	Anholt	DK-KAT	22. May 1988	Liver

Supplementary Table 5 Results of the substitution saturation tests of the alignment of PDV sequences from 1988 and 2002. No substitution saturation is detected if the Iss is significantly lower than Iss.c (Xia et al. 2003).

Codon(s)	Iss	Iss.c
Codon position 1 and 2	0.0117	0.8026
Codon position 3	0.0027	0.7909

Supplementary Table 6 Results of the substitution model tests conducted in JModeltest (Posada 2008). The score is the weights of AIC, AICc, BIC and DT and the model of the highest weight is the model which is best fitted for the given alignment.

Alignment	AIC		AICc		BIC		DT	
	Model	Score	Model	Score	Model	Score	Model	Score
PDVall	TVM + I	0.164	TPM1uf +I	0.165	HKY + I	0.307	HKY + I	0.016
PDV1988	TPM1uf	0.302	TPM1uf	0.304	HKY	0.487	HKY	0.057
PDV2002	TPM1uf +I	0.137	TPM1uf +I	0.138	HKY	0.731	HKY	0.031

Supplementary Table 7 General priors applied to the parameters in BEAUTi.

Parameter	Distribution	Initial value	Interval
gammaShape.s:1stpos	Exponential	1	$[-\infty, \infty]$
gammasShape.s:2ndpos	Exponential	1	$[-\infty, \infty]$
gammasShape.s:3rdpos	Exponential	1	$[-\infty, \infty]$
gammasShape.s:coding	Exponential	1	$[-\infty, \infty]$
gammasShape.s:noncoding	Exponential	1	$[-\infty, \infty]$
Kappa.s:1stpos	Log normal	2	$[0,0, \infty]$
Kappa.s:2ndpos	Log normal	2	$[0,0, \infty]$
Kappa.s:3rdpos	Log normal	2	$[0,0, \infty]$
Kappa.s:coding	Log normal	2	$[0,0, \infty]$
Kappa.s:noncoding	Log normal	2	$[0,0, \infty]$

Supplementary Table 8 Priors applied for the different tests of the coalescence constant and exponential population growth.

Parameter	Distribution	Initial value	Interval
ePopSize.t.tree	Gamma	1	$[0,001, 1000]$
ClockRate.c:clock	Uniform	1	$[-\infty, \infty]$
uclMean.c:clock (PDVall)	Uniform	0.0014	$[0, 0.1]$
uclMean.c:clock (1988 and 2002)	Uniform	4.329E-4	$[0, 0.1]$
GrowthRate.t.tree	Laplace Distribution	3.0E-4	$[-\infty, \infty]$

Supplementary Table 9 Mutations found in the sequences from samples collected in 1988. The parentheses indicate the positions where a fragment from a German sample was used to fill gaps. Results from an additional sample (42523) which was not fully sequenced was included and non-sequenced areas marked with -.

Samples	Locality	1,868 (Q = 52)	2,251 (Q = 52)	2,481 (Q = 55)	3,131 (Q = 55)	3,357 (Q = 52)	4,562 (Q = 52)
PV14965	Germany	C	A	C	G	G	G
PV14958	Germany	C	A	C	G	G	G
PV14948	Germany	C	A	C	G	A	G
PV14969	Germany	C	G	C	G	G	A
42524	Limfjorden	C	A	T	A	G	G
42523	Limfjorden	-	-	-	-	-	-
42531	Limfjorden	C	A	C	G	G	G
42412	Anholt	A	A	C	G	(G)	(G)
42413	Anholt	C	A	C	G	(G)	(G)

Samples	Locality	4,869 (Q = 54)	4,978 (Q = 59)	5,126 (Q = 30)	5,202	5,816	5,891
PV14965	Germany	A	C	C	C	T	C
PV14958	Germany	A	C	C	C	T	C
PV14948	Germany	A	C	C	C	T	C
PV14969	Germany	A	C	C	C	T	C
42524	Limfjorden	A	C	C	T	G	T
42523	Limfjorden	-	-	C	T	G	T
42531	Limfjorden	C	C	T	C	T	C
42412	Anholt	A	C	C	C	T	T
42413	Anholt	A	T	C	C	T	T

Samples	Locality	7,414	7,444	7,602 (Q = 52)	8,023	8,201 (Q = 52)
PV14965	Germany	T	A	T	C	A
PV14958	Germany	T	A	T	A	A
PV14948	Germany	T	A	T	C	A
PV14969	Germany	T	A	T	A	A
42524	Limfjorden	C	G	T	A	A
42523	Limfjorden	C	G	T	A	A
42531	Limfjorden	T	A	T	A	A
42412	Anholt	T	A	G	A	A
42413	Anholt	T	A	T	A	G

Supplementary Table 10 The mutations (≥ 2) of the PDV2002 data set. Samples are listed with a number indicating the clade to which they belong to in the tree.

Sample	Area	1,792 (P gene)	1,795 (P gene)	2,152 (P gene)	2,473 (P gene)
0_25002	DK-KAT	T	T	G	T
1_25030	⁴ DK-KAT	T	T	G	C
1_30030	⁴ DK-KAT	T	T	G	C
1_30031	⁴ DK-KAT	T	T	G	C
1_VB21	³ SE-KAT	T	T	G	C
1_VB22	³ SE-KAT	T	T	G	C
1_TM3155	SE-KAT	T	T	G	C
1_TM3107	SE-SKG	T	C	G	C
1_TM3118	SE-SKG	T	C	G	C
1_TM3192	SE-SKG	T	C	G	C
1_TM16024	SE-SKG	T	C	G	C
1_26055	DK-ØRE	T	T	G	C
1_25677	DK-BAL	T	T	G	C
2_26866	DK-LIM	T	T	G	T
2_26891	DK-LIM	T	T	G	T
2_26901	DK-LIM	T	T	G	T
2_30029	DK-KAT	T	T	G	T
3_EPDV2681	UK-Wash	T	T	G	T
3_EPDV2480	UK-Wash	T	T	G	T
3_EPDV1909	UK-Wash	T	T	G	T
3_EPDV1744	UK-Wash	T	T	G	T
4_EPDV1966	UK-Wash	T	T	G	T
4_H41	NL-WAD	T	T	G	T
4_Pv 1939	DE-WAD	C	T	G	T
4_Pv 2149	DE-WAD	T	T	G	T
4_Pv 2162	DE-WAD	T	T	G	T
4_Pv 2163	² DE-WAD	T	T	G	T
4_26001	² DK-WAD	T	T	G	T
4_26002	¹ DK-WAD	C	T	A	T
4_26003	DK-WAD	C	T	G	T
4_26561	¹ DK-WAD	C	T	A	T
5_VB23	SE-KAT	T	T	G	T
5_TM197	SE-SKG	T	T	G	T
5_TM3109	SE-SKG	T	T	G	T
5_25679	DK-BAL	T	T	G	T
5_25682	DK-BAL	T	T	G	T

Sample	Area	3,614 (M gene)	4,010 (M gene)	4,698 (Non c. M gene)	4,747 (Non c. M gene)
0_25002	DK-KAT	T	C	T	A
1_25030	⁴ DK-KAT	T	C	T	A
1_30030	⁴ DK-KAT	T	C	T	A
1_30031	⁴ DK-KAT	T	C	T	A
1_VB21	³ SE-KAT	T	C	C	A
1_VB22	³ SE-KAT	T	C	T	A
1_TM3155	SE-KAT	T	C	C	A
1_TM3107	SE-SKG	T	C	T	A
1_TM3118	SE-SKG	T	C	T	A
1_TM3192	SE-SKG	T	C	T	A
1_TM16024	SE-SKG	T	C	T	A
1_26055	DK-ØRE	T	C	T	A
1_25677	DK-BAL	T	C	T	A
2_26866	DK-LIM	T	C	T	A
2_26891	DK-LIM	T	C	T	A
2_26901	DK-LIM	T	C	T	A
2_30029	DK-KAT	T	C	T	A
3_EPDV2681	UK-Wash	C	C	T	A
3_EPDV2480	UK-Wash	C	C	T	A
3_EPDV1909	UK-Wash	C	C	T	A
3_EPDV1744	UK-Wash	C	C	T	A
4_EPDV1966	UK-Wash	T	T	T	G
4_H41	NL-WAD	T	T	T	A
4_Pv 1939	DE-WAD	T	T	T	A
4_Pv 2149	DE-WAD	T	T	T	G
4_Pv 2162	DE-WAD	T	T	T	A
4_Pv 2163	² DE-WAD	T	T	T	A
4_26001	² DK-WAD	T	T	T	A
4_26002	¹ DK-WAD	T	T	T	A
4_26003	DK-WAD	T	T	T	A
4_26561	¹ DK-WAD	T	T	T	A
5_VB23	SE-KAT	T	C	T	A
5_TM197	SE-SKG	T	C	T	A
5_TM3109	SE-SKG	T	C	T	A
5_25679	DK-BAL	T	C	T	A
5_25682	DK-BAL	T	C	T	A

Sample	Area	5,064 (F gene)	5,266 (F gene)	5,306 (F gene)	6,893 (F gene)
0_25002	DK-KAT	C	T	A	G
1_25030	⁴ DK-KAT	C	T	A	G
1_30030	⁴ DK-KAT	C	T	A	G
1_30031	⁴ DK-KAT	C	T	A	G
1_VB21	³ SE-KAT	C	T	A	G
1_VB22	³ SE-KAT	C	T	A	G
1_TM3155	SE-KAT	C	T	A	G
1_TM3107	SE-SKG	C	T	A	G
1_TM3118	SE-SKG	T	T	A	G
1_TM3192	SE-SKG	T	T	A	G
1_TM16024	SE-SKG	T	T	A	G
1_26055	DK-ØRE	C	T	A	G
1_25677	DK-BAL	C	T	A	G
2_26866	DK-LIM	C	T	A	G
2_26891	DK-LIM	C	T	A	G
2_26901	DK-LIM	C	T	A	G
2_30029	DK-KAT	C	T	A	G
3_EPDV2681	UK-Wash	C	T	A	G
3_EPDV2480	UK-Wash	C	T	A	A
3_EPDV1909	UK-Wash	C	T	A	A
3_EPDV1744	UK-Wash	C	T	A	A
4_EPDV1966	UK-Wash	C	T	A	G
4_H41	NL-WAD	C	T	A	G
4_Pv 1939	DE-WAD	C	G	A	G
4_Pv 2149	DE-WAD	C	T	A	G
4_Pv 2162	DE-WAD	C	T	A	G
4_Pv 2163	² DE-WAD	C	T	A	G
4_26001	² DK-WAD	C	T	A	G
4_26002	¹ DK-WAD	C	G	G	G
4_26003	DK-WAD	C	G	A	G
4_26561	¹ DK-WAD	C	G	G	G
5_VB23	SE-KAT	C	T	A	G
5_TM197	SE-SKG	T	T	A	G
5_TM3109	SE-SKG	C	T	A	G
5_25679	DK-BAL	C	T	A	G
5_25682	DK-BAL	C	T	A	G