Journal of General Virology (2010), **91**, 1560–1576 **DOI** 10.1099/vir.0.018374-0

The genome of pseudocowpoxvirus: comparison of a reindeer isolate and a reference strain

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Parapoxviruses (PPV), of the family *Poxviridae*, cause a pustular cutaneous disease in sheep and goats (orf virus, ORFV) and cattle (pseudocowpoxvirus, PCPV and bovine papular stomatitis virus, BPSV). Here, we present the first genomic sequence of a reference strain of PCPV (VR634) along with the genomic sequence of a PPV (F00.120R) isolated in Finland from reindeer (*Rangifer tarandus tarandus*). The F00.120R and VR634 genomes are 135 and 145 kb in length and contain 131 and 134 putative genes, respectively, with their genome organization being similar to that of other PPVs. The predicted proteins of F00.120R and VR634 have an average amino acid sequence identity of over 95 %, whereas they share only 88 and 73 % amino acid identity with the ORFV and BPSV proteomes, respectively. The most notable differences were found near the genome termini. F00.120R lacks six and VR634 lacks three genes seen near the right terminus of other PPVs. Four genes at the left end of F00.120R and one in the middle of both genomes appear to be fragmented paralogues of other genes within the genome. VR634 has larger than expected inverted terminal repeats possibly as a result of genomic rearrangements. The high G+C content (64 %) of these two viruses along with amino acid sequence comparisons and whole genome phylogenetic analyses confirm the classification of PCPV as a separate species within the genus *Parapoxvirus* and verify that the virus responsible for an outbreak of contagious stomatitis in reindeer over the winter of 1999–2000 can be classified as PCPV.

Received 13 November 2009
Accepted 22 January 2010

INTRODUCTION

In Finland, contagious pustular stomatitis of reindeer has been recognized for many years as an important disease particularly in the southern parts of the reindeer herding area. It occurs typically during the winter months when the weather conditions are severe and is characterized by ulcerative skin lesions and erosions in the mouth. This makes normal grazing difficult and ultimately leads to starvation in affected animals. The aetiological agent has been identified as a parapoxvirus (PPV). It was demonstrated that two outbreaks of disease, one in 1992–1993 and one in 1999–2000, were apparently caused by different species of PPV (Tikkanen *et al.*, 2004). The former, more severe outbreak with approximately 400 dead and about 2800 affected reindeer, was caused by a virus closely related to orf virus (ORFV), whereas the latter outbreak was caused by a virus showing notable partial sequence similarity to pseudocowpoxvirus (PCPV). This raised the question as to whether there is a new PPV species specific for reindeer or if the disease is caused by either ORFV or PCPV according to whichever virus they encounter.

The family *Poxviridae* consist of over 80 known or tentative species infecting both vertebrates (subfamily Chordopoxvirinae) and insects (subfamily Entomopoxvirinae). The chordopoxviruses are further divided into eight genera based on their morphology, host range and also on their antigenic

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The GenBank/EMBL/DDBJ accession numbers for the genome sequences of F00.120R and VR634 are GQ329669 and GQ329670, respectively.

and genetic relatedness (Moss, 2001). There are currently four classified members of the genus *Parapoxvirus*; ORFV infects both sheep and goats worldwide, whereas PCPV and bovine papular stomatitis virus (BPSV) infect cattle (Haig & Mercer, 1998). The fourth member of the genus, parapoxvirus of red deer in New Zealand (PVNZ), has only ever been isolated from red deer in New Zealand (Robinson & Mercer, 1995). To date, the genomes of at least one member of each *Chordopoxvirus* genus have been sequenced allowing comparative genome analysis of these viruses. This has revealed that the linear double-stranded DNA poxvirus genomes vary in length from approximately 134 to 365 kb, but that both gene content and order are well conserved in the central regions, whereas the terminal regions are considerably more variable (Upton *et al.*, 2003; McLysaght *et al.*, 2003; Gubser *et al.*, 2004, Mercer *et al.*, 2006).

Of the PPVs, only the genomes of ORFV, the type species of the genus *Parapoxvirus*, and BPSV have been fully sequenced (Delhon *et al.*, 2004; Mercer *et al.*, 2006). Despite the fact that these genomes are amongst the smallest of the poxviruses (134–139 kb with 132 putative genes) and that their G+C contents are considerably higher than in most other poxviruses, 88 of their genes were found to be conserved across all vertebrate poxviruses. These 'core' genes are generally responsible for genome replication, transcription and virus assembly and are useful for inferring phylogenetic relationships between the poxviruses. The genes located at the end of the genome, that have important roles in virulence and viral–host interactions, can be genus or even species specific (Delhon *et al.*, 2004; Mercer *et al.*, 2006).

To understand the relationship between the virus causing disease in Finnish reindeer and the other PPV, we determined the genomic sequence of a virus (F00.120R) isolated from the outbreak of contagious stomatitis in 1999–2000 together with that of a reference strain of PCPV (VR634). This enabled us, for the first time, to compare three established species of PPV, determine their genetic relatedness and to recognize the potential risk of corralling reindeer and cattle together.

RESULTS AND DISCUSSION

Genomic features and predicted coding regions of the F00.120R and VR634 genomes

The individual F00.120R and VR634 sequences were assembled into contiguous sequences of 133 169 and 145 289 bp, respectively. The 145 289 bp sequence of VR634 does not contain bases outside of the terminal *Bam*HI sites described previously (Ueda *et al.*, 2003). The terminal regions of both genomes contain inverted terminal repeats (ITR), a feature common to all poxviruses (Mercer *et al.*, 1987; Fraser *et al.*, 1990; Massung *et al.*, 1995). The ITR sequence reported here for F00.120R was 2064 bp in length, but does not include the uncloned terminal region, including the characteristic terminal hairpin loop, of the virus. Restriction fragment and Southern blot analyses were used to predict that the full ITR sequence is approximately 2.8 kb in length (results not shown) giving a total estimated length of 134.6 kb for F00.120R, which is consistent with other PPVs (Table 1). The ITR region of VR634 was larger at 14.9 kb. However, this is most probably due to duplication and translocation of sequence from the right terminus of the genome to the left terminus of the genome, a process which is often accompanied by deletion of sequence at the left terminus of the genome. This is a common phenomenon in poxviruses that have been passaged in cell culture and has been reported previously for ORFV (Fleming *et al.*, 1995; Cottone *et al.*, 1998; McInnes *et al.*, 2001). One open reading frame (ORF) encoding a predicted protein of 150 aa was found entirely within the F00.120R ITR and therefore is repeated at both ends of the genome (ORF 001/134). A similar ORF was found in the VR634 ITR sequence (89 % identical) and also in both ORFV (ORFV 001/134, 83 % identical) and BPSV (BPSV 001/134, 58 % identical). It is not known what the function of this ORF might be, but in ORFV it has been shown to be transcribed early in infection (Fraser *et al.*, 1990).

The G+C content of F00.120R and VR634 genomes are 64 and 65 %, respectively, which is consistent with the high G+C content characteristic for PPVs (Table 1). The previous G+C content analyses of three ORFV strains, BPSV and one species from each genus of mammalian poxviruses have shown that the two PPV species possess a similar G+C profile that differs from other poxviruses (Mercer *et al.*, 2006). The most notable feature of the profile is also seen in F00.120R and VR634; in the regions of ORFs 102–104 (nt 107403–109054 of F00.120R) and 109–110 (nt 112003–113005 of F00.120R) the G+C content is similar but exceptionally low (on average 42 and 47 %, respectively) despite a high variation in the specific nucleotide sequence. Our moving average analysis of G+C content of the F00.120R and VR634 genomes support the results of Mercer *et al.* (2006) that suggested a distinctive, genus-specific G+C profile typical for PPVs (Fig. 1) and thus support the classification of F00.120R and VR634 as PPVs.

The sequence between the ITRs was annotated using ORFV NZ2 as a template. Initially, ORFs encoding proteins of 60 or more amino acids were ascribed, but this was later extended to include shorter ORFs. The likelihood of the ORFs corresponding to genuine poxvirus genes was assessed by BLASTP comparisons of the predicted amino acid sequences with the GenBank database and poxvirus proteins in particular. The genes were predicted on the basis of their localization within the genome, the presence of promoter-like sequences or early gene transcription termination signals (T5NT), the size of the predicted protein and by its similarity with proteins previously described in poxviruses. From these analyses, 131 ORFs were predicted in F00.120R, including one which appears

Table 1. Origin and summary of genomic sequence data of six PPV strains

Strain	PCPV		ORFV			BPSV
	F00.120R	VR634	NZ2	IA82	SA00	AR02
Isolation source and history	Reindeer oral lesion material from 1999 to 2000 pustular stomatitis epidemic in Finland. Passaged nine times in bovine oesophagus cells	Lesion material from human after contact with cows having papular lesions in their udders. Passaged several times in cell culture	Sheep scab material isolated in New Zealand. Prior to inoculation to sheep, virus had been plaque purified twice in primary bovine testis cells	Nasal secretion material from sheep lamb isolated in Iowa, USA, in 1982 and passaged in ovine fetal turbinate cells	Scab material from a goat kid with severe, proliferative dermatitis isolated in Texas, USA, and propagated in Madin–Darby ovine kidney cells	Bovine calf oral lesion material isolated in Arkansas, USA, and passaged in primary lamb kidney cells
Reference(s)	Tikkanen *et al.* (2004)	Friedman-Kien *et al.* (1963)	Robinson *et al.* (1982); Mercer *et al.* (1987)	Delhon *et al.* (2004)	Guo *et al.* (2003)	Delhon *et al.* (2004)
Genome size	134600 bp	145289 bp	137820 bp	137241 bp	139962 bp	134431 bp
ITR size	2800 bp	14909 bp	3389 bp	3092 bp	3936 bp	1161 bp
Genome G+C mol%	64.1	65.0	64.3	64.3	63.4	64.5
No. predicted genes	131	134	132	132	132	133
GenBank accession. no.	GQ329669	GQ329670	DQ184476	AY386263	AY386264	AY386265

twice by virtue of its presence in the ITR, and 134 ORFs were predicted in VR634 (116 single-copy ORFs plus nine that are present at both ends due to the duplication and translocation event described above). Consistent with the other poxvirus genomes, ORFs in F00.120R and VR634 were, for the majority, non-overlapping (Moss, 2001). F00.120R and VR634 genomes have orthologues of all 88 ORFV genes (Delhon *et al.*, 2004; Mercer *et al.*, 2006), which constitute the conserved core region of chordopoxvirus genomes (Upton *et al.*, 2003; Gubser *et al.*, 2004).

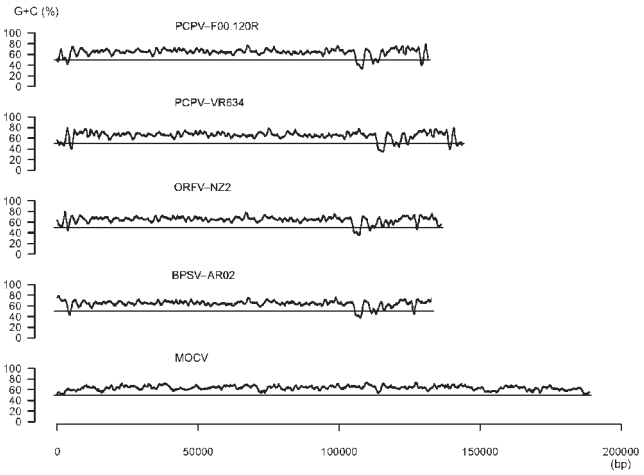

Fig. 1. G+C genome profiles of PCPV strains F00.120R and VR634, ORFV strain NZ2, BPSV strain AR02 and Molluscum contagiosum virus (MOCV) (GenBank accession no. U60315) (Senkevich *et al.*, 1996). Each trace represents the percentage of G+C content of the viral genomes. Traces are drawn to the scale (bp) at the bottom of the figure and arbitrarily aligned at their left ends. The G+C contents were determined by using a moving average method with a 1000 bp window with the program R 2.9.0 (R Development Core Team, 2008).

Of the 131 genes in F00.120R four, found close to the left terminus (001.6, 001.9, 004.6 and 004.8) and one near the central part of the genome (073.5) (Fig. 2, Table 2), appear to be remnants of genes paralogous to those found elsewhere in the genome. Whether or not these ORFs encode functional proteins has yet to be determined. ORF 073.5 has likely arisen from duplication of ORF 073 (amino acid identity approx. 49 %) and similarly, ORFs 004.6 and 004.8 are likely to have originated from duplication of ORF 005 (amino acid identities approx. 36 and 34 %, respectively). Genetic recombination is known to occur between poxvirus genomes (Gershon *et al.*, 1989; Moss, 2001). Intriguingly, the region between ORF 001 and 004.6 shows evidence of potential gene translocation and interspecies recombination. This region, containing ORFs 001.3, 001.6 and 001.9, shows the greatest sequence similarities to the region between ORFs 128 and 123 of BPSV. These include substantial predicted amino acid sequence similarities between 001.3 and BPSV 127, and between 001.9 and BPSV 124 as well as non-coding nucleotide similarities between the region around 001.6 and BPSV 126/125. In addition, the upstream promoter region of ORF 001.9 and its first six codons are similar to the corresponding sequences of ORF 132.5 of F00.120R/VR634 and ORF 002 of ORFV. Together these observations raise the possibility that the region containing BPSV genes 124–127 may have recombined into the PCPV genome around

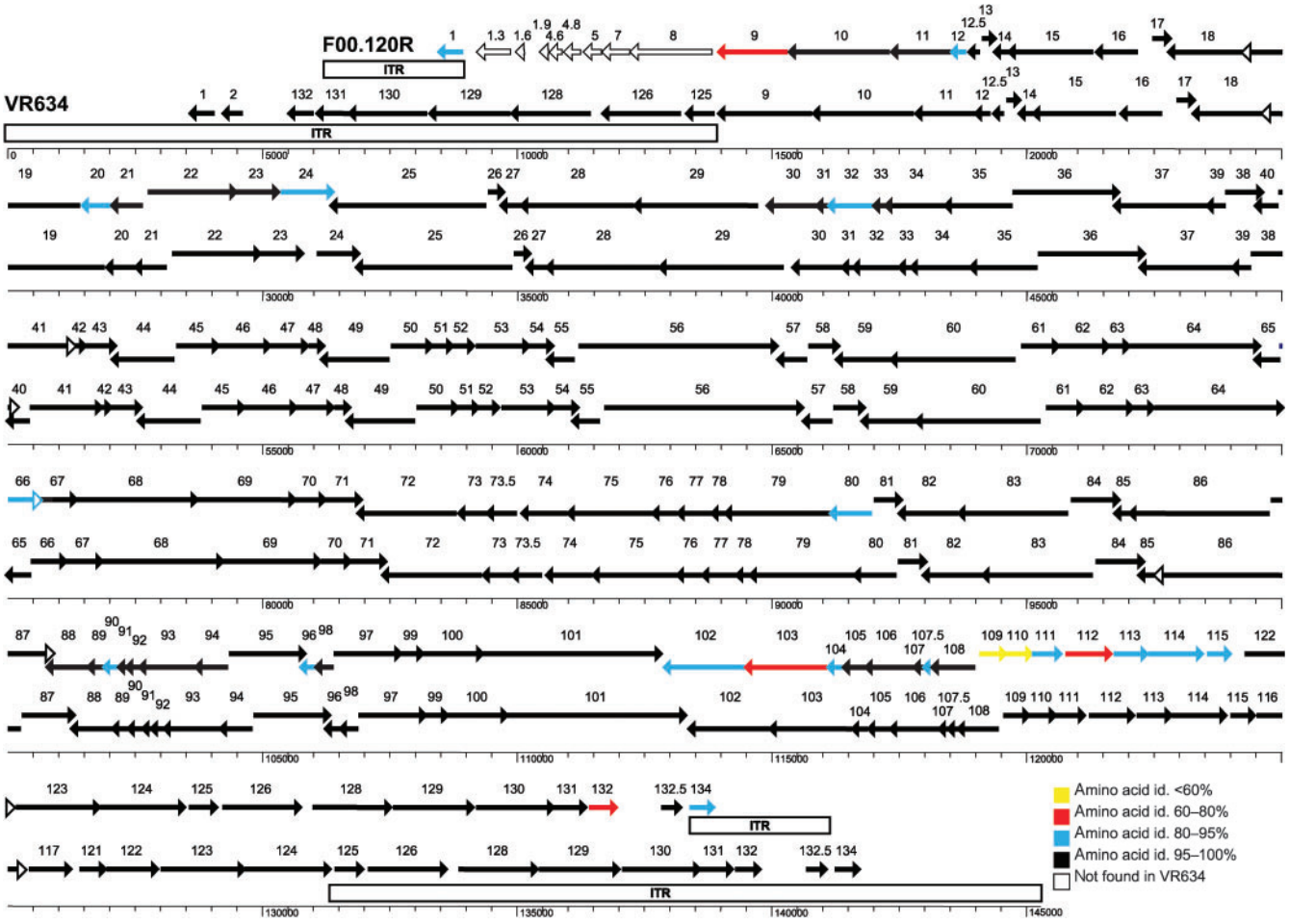

Fig. 2. Comparative map of F00.120R and VR634 genomes. Each ORF is represented by an arrow that indicates the size and direction of transcription; open arrowheads indicate that an ORF is split over two lines of the diagram. VR634 ORFs (bottom) are drawn to the scale based on the position of their start codons. F00.120R ORFs (top) are aligned with VR634 ORFs starting with ORF 009. Different colours of F00.120R arrows indicate amino acid identity of predicted proteins between the two viruses.

the site of PCPV gene 002. They may also suggest that, prior to recombination, PCPV 002 had been duplicated and translocated to the right genome terminus to create 132.5. The fragmented genes (001.6, 001.9, 004.6 and 004.8) are found in the region of the F00.120R genome corresponding to that region of the VR634 genome, which is likely to have been lost during *in vitro* culture of the virus and therefore we cannot predict if these genes are present in all PCPV isolates.

The other most obvious feature of the F00.120R and VR634 genomes is that both appear to have suffered a deletion of genes, in comparison to ORFV and BPSV, towards the right terminus of the genome. Specifically, in F00.120R genes 116–121, together with the 5′ end of gene 122, are missing, whereas VR634 is missing three genes, 118–120 and the 5′ end of gene 121 (Fig. 2, Table 2). These genes are specific to PPVs and their functions are unknown except for gene 117. The protein encoded by gene 117 of ORFV and PCPV (VR634) is known to bind and inhibit the ovine cytokines granulocyte-macrophage colony-stimulating factor and interleukin-2 (Deane *et al.*, 2000, 2009). Gene 122 is the equivalent of vaccinia virus (VACV) Copenhagen gene A51R, the biological function of which has still to be determined. The significance of the missing genes of F00.120R and VR634 is unknown. Future analyses of other isolates from cattle and reindeer will be needed to determine whether this is a common feature to PCPV or whether the deletion has arisen during laboratory culture of the viruses.

Comparison of predicted PPV protein sequences

Previous analyses have established that PPVs form a distinct group on the phylogenetic tree of mammalian poxviruses (Thomas *et al.*, 2003; Tikkanen *et al.*, 2004; Delhon *et al.*, 2004). The results of whole genome phylogenetic analyses and comparisons of the predicted protein sequences of F00.120R and VR634 with three

Table 2. ORFs of F00.120R and VR634 compared with the corresponding ORFs of ORFV strains NZ2, IA82 and SA00 and BPSV strain AR02

Italicized LT (left terminus) and RT (right terminus) indicate the existence of the orthologue of the ORF and its nucleotide position.

	PCPV					ORFV						BPSV		VACV		Predicted function
	F00.120R		VR634			NZ2		IA82		SA00		AR02		Copenhagen		
ORF	Nucleotide position	Length (aa)	Nucleotide position	Length (aa)	% Id*	Length (aa)	% Id*	Length (aa)	% Id*	Length (aa)	% Id*	Length (aa)	% Id*	ORF	Score†	
001	1859–1410	150	‡			149	75.2	149	74.5	149	81.9	147	57.8			Unknown
001.3	2785–2189	199				*RT (127)*	*75.5*	*RT (127)*	*76.6*	*RT (127)*	*76.5*	*RT (127)*	*87.6*			IL-10
001.6	3060–2956	35				Not present		Not present		Not present		Not present				Unknown
001.9	3538–3428	37				Not present		Not present		Not present		Not present				PPV ORF 002/124 hybrid like
002	*RT (132.5)*	*119*				117	84.6	117	84.6	115	80.9	Not present				Unknown
003	Not present					Not present		Not present		Not present		496				Ankyrin/F-box protein
004	Not present					Not present		Not present		Not present		519				Ankyrin/F-box protein
004.6	3804–3613	64				Not present		Not present		Not present		Not present				Similar to ORF 005
004.8	4168–3905	88				Not present		Not present		Not present		Not present				Similar to ORF 005
005	4573–4289	95				71	53.6	71	53.6	79	52.7	98	55.3			Unknown
006	*RT (132)*	*168*				*RT (132)*	*52.0*	*RT (132)*	*33.6*	*RT (132)*	*31.5*	152	34.2			VEGF
007	5133–4654	160				159	80.5	159	78.6	160	83.8	163	71.1	F2L	144	dUTPase
008	6746–5199	516				516	87.4	516	87.6	516	90.3	518	63.0	4 ORFs	54	Ankyrin/F-box protein
009	8233–6908	442	15782–13983	600	69.4	442	91.4	442	91.6	441	90.0	465	60.2	F11L	96	Unknown
010	10229–8295	645	17777–15843	645	98.3	641	92.0	643	92.2	643	91.8	643	75.1	F12L	160	EEV-maturation
011	11435–10302	378	18983–17850	378	99.7	378	96.0	378	95.0	378	95.2	378	84.4	F13L	308	EEV envelope phospholipase
012	11737–11468	90	19286–19017	90	85.6	89	70.8	89	70.8	89	69.7	85	42.7			Unknown
012.5	12000–11821	60	19549–19370	60	98.3	60	91.7	60	91.7	60	90.0	56	62.5			Unknown
013	12045–12278	78	19594–19830	79	98.7	79	83.3	79	82.1	79	83.3	73	42.7			Unknown
014	12600–12322	93	20152–19874	93	97.8	93	82.8	93	81.7	93	80.6	93	72.0			APC/C regulator
015	14222–12605	539	21774–20158	539	97.6	539	82.4	539	82.0	539	82.0	536	60.4			Unknown
016	15101–14322	260	22654–21875	260	95.4	258	75.7	259	76.2	259	77.4	249	56.0			Unknown
017	15384–15707	108	22937–23260	108	97.2	105	79.0	105	79.0	105	79.0	105	61.9	F17R	68	DNA-binding phosphoprotein
018	17156–15735	474	24710–23289	474	98.3	472	94.9	472	94.9	472	94.5	481	81.9	E1L	354	Poly(A)-polymerase subunit
019	19343–17169	725	26897–24723	725	98.1	725	91.2	725	91.2	725	90.8	725	73.9	E2L	286	Unknown
020	19961–19416	182	27508–26969	180	89.4	183	75.8	183	74.7	183	75.8	195	56.0	E3L	62	dsRNA-binding, interferon resistance
021	20572–19994	193	28119–27541	193	99.0	193	90.7	193	90.2	193	90.7	199	77.7	E4L	209	RNA-polymerase subunit RPO30
022	20666–22366	567	28213–29913	567	99.1	567	95.6	567	95.8	567	95.6	566	83.2	E6R	548	Unknown

Table 2. cont.

ORF	PCPV					ORFV						BPSV		VACV		Predicted function
	F00.120R		VR634			NZ2		IA82		SA00		AR02		Copenhagen		
	Nucleotide position	Length (aa)	Nucleotide position	Length (aa)	% Id*	Length (aa)	% Id*	Length (aa)	% Id*	Length (aa)	% Id*	Length (aa)	% Id*	ORF	Score†	
023	22390–23205	272	29937–30752	272	98.9	272	96.3	272	96.3	272	96.3	272	87.5	E8R	310	Membrane protein
024	23274–24278	335	31064–31861	266	94.3	288	73.2	288	73.9	292	72.1	227	60.7			Unknown
025	27314–24291	1008	34897–31874	1008	98.1	1008	94.5	1008	94.6	1008	94.5	1009	87.9	E9L	1131	DNA-polymerase
026	27344–27637	96	34927–35214	96	100	96	99.0	96	99.0	96	99.0	97	90.6	E10R	129	ERV/ALR homologue
027	28044–27634	137	35627–35217	137	97.8	137	95.6	137	96.4	137	95.6	137	83.2	E11L	93	Virion core protein
028	30214–28034	727	37788–35617	724	98.1	713	87.5	718	87.0	709	87.4	700	68.4	O1L	105	Unknown
029	32650–30260	797	40224–37834	797	96.9	797	84.1	797	84.3	797	83.8	807	67.4			Unknown
030	33824–32862	321	41397–40435	321	99.7	321	92.8	321	93.5	321	92.5	322	79.4	I1L	277	DNA-binding virion core protein
031	34046–33837	70	41619–41410	70	98.6	70	95.7	70	95.7	70	95.7	69	75.4	I2L	46	Unknown
032	34913–34059	285	42486–41632	285	84.9	285	81.7	285	82.4	285	82.0	288	66.9	I3L	196	DNA-binding phosphoprotein
033	35182–34945	78	42755–42522	78	98.7	78	92.3	78	92.3	78	92.3	86	71.8	I5L	41	IMV protein
034	36352–35186	389	43925–42759	389	99.5	389	94.9	389	95.1	389	94.9	389	81.5	I6L	189	Telomere-binding protein
035	37641–36352	430	45214–43925	430	99.1	430	96.7	430	97.0	430	96.5	430	83.7	I7L	479	Virion core protease
036	37647–39698	684	45220–47271	684	97.8	683	92.4	683	91.9	683	92.2	684	77.2	I8R	590	RNA helicase (NPH-II)
037	41484–39679	602	49057–47252	602	98.3	603	94.4	603	94.2	603	94.4	602	75.6	G1L	536	Zn-protease, virion morphogenesis
038	41821–42519	233	49394–50092	233	97.9	231	93.1	231	92.6	231	93.1	233	73.4	G2R	107	Late transcription elongation factor
039	41827–41498	110	49400–49071	110	100	110	90.0	110	90.0	110	90.0	111	71.8	G3L	44	Unknown
040	42860–42444	139	50433–50017	139	96.4	137	91.2	137	91.2	137	90.5	138	80.4	G4L	82	Glutaredoxin-like protein
041	42863–44224	454	50436–51803	456	98.9	452	89.3	452	89.5	452	87.3	441	69.4	G5R	177	FEN-1-like endonuclease
042	44229–44417	63	51808–51996	63	96.8	63	90.5	63	90.5	63	90.5	63	84.1	G5.5R	91	RNA-polymerase subunit RPO7
043	44449–45006	186	52028–52585	186	97.8	185	91.9	185	91.9	185	90.3	192	70.7	G6R	103	Unknown
044	46196–45009	396	53775–52588	396	98.5	397	87.4	398	87.6	398	87.4	391	67.4	G7L	168	Virion core protein
045	46229–47026	266	53808–54605	266	98.9	266	97.0	266	97.0	266	97.0	266	94.7	G8R	352	Late transcription factor
046	47044–48045	334	54623–55624	334	98.5	334	86.2	334	86.2	334	90.4	334	77.8	G9R	209	Myristylprotein
047	48049–48780	244	55628–56359	244	99.2	244	90.6	244	91.4	244	91.8	244	87.7	L1R	274	IMV protein
048	48829–49098	90	56408–56677	90	95.6	90	93.3	90	93.3	90	92.2	89	62.2	L2R	42	Unknown

Table 2. cont.

	PCPV					ORFV						BPSV		VACV		Predicted function
	F00.120R		VR634			NZ2		IA82		SA00		AR02		Copenhagen		
ORF	Nucleotide position	Length (aa)	Nucleotide position	Length (aa)	% Id*	Length (aa)	% Id*	Length (aa)	% Id*	Length (aa)	% Id*	Length (aa)	% Id*	ORF	Score†	
049	50417–49113	435	57990–56689	234	98.2	418	85.1	418	85.4	417	85.1	376	70.4	L3L	195	Unknown
050	50447–51223	259	58020–58796	259	99.2	259	92.3	259	91.9	259	91.9	256	81.6	L4R	236	Virion core protein
051	51248–51628	127	58821–59201	127	96.9	128	89.0	128	90.6	128	91.3	129	71.7	L5R	76	Membrane protein
052	51585–52037	151	59158–59610	151	99.3	151	85.4	151	85.4	151	86.1	151	78.1	J1R	63	Virion protein
053	52110–53117	336	59683–60690	336	99.1	336	96.7	336	96.4	336	96.1	337	82.7	J3R	367	Poly(A)-polymerase subunit
054	53035–53592	186	60608–61165	186	98.9	186	95.2	186	94.6	186	95.7	186	86.0	J4R	213	RNA-polymerase subunit RPO22
055	54049–53549	167	61622–61122	167	99.4	167	94.6	167	95.2	167	95.2	167	85.0	J5L	143	Late membrane protein
056	54132–57995	1288	61705–65568	1288	99.1	1289	96.4	1289	96.4	1289	96.4	1289	91.4	J6R	1723	RNA-polymerase subunit RPO147
057	58615–58073	181	66187–65645	181	98.9	181	86.7	181	86.7	181	86.7	179	69.3	H1L	125	Tyrosine phosphatase, virus assembly
058	58635–59207	191	66207–66779	191	100	191	94.8	191	94.8	191	94.8	194	83.8	H2R	183	IMV, viral entry
059	60290–59223	356	67856–66795	354	98.9	334	90.1	338	89.9	342	89.5	340	65.7	H3L	144	Immunodominant envelope protein
060	62705–60294	804	70271–67860	804	98.6	804	96.4	804	96.4	804	96.0	803	85.9	H4L	812	RNA-polymerase associated RAP94
061	62814–63518	235	70380–71075	232	95.3	228	71.9	228	70.6	227	72.2	242	54.6	H5R	45	Late transcription factor VLTF4
062	63546–64499	318	71103–72056	318	98.1	318	96.2	318	95.9	318	96.2	320	89.0	H6R	359	Topoisomerase I
063	64495–64908	138	72052–72465	138	100	138	92.0	138	91.3	138	91.3	139	65.2	H7R	34	Unknown
064	64946–67468	841	72503–75025	841	99.4	841	96.3	841	96.2	841	96.2	842	86.1	D1R	915	mRNA capping enzyme subunit
065	67900–67436	155	75463–74993	157	97.4	156	92.9	156	91.6	156	91.6	158	71.0	D2L	50	Virion protein
066	67887–68549	221	75450–76112	221	94.1	221	86.4	221	86.4	221	85.9	225	62.7	D3R	30	Virion protein
067	68552–69241	230	76121–76816	232	99.1	231	95.2	231	94.8	231	94.8	247	85.7	D4R	304	Uracil-DNA glycosylase
068	69258–71618	787	76833–79193	787	98.7	787	95.8	787	95.9	787	95.3	788	88.7	D5R	918	NTPase
069	71628–73532	635	79203–81107	635	99.2	635	96.9	635	97.0	635	97.2	650	92.6	D6R	905	Early transcription factor
070	73572–74129	186	81147–81692	182	95.6	190	93	190	91.9	190	93.5	176	83.0	D7R	187	RNA-polymerase subunit RPO18
071	74162–74833	224	81725–82396	224	99.6	224	96.9	224	96.9	224	96.9	223	80.7	D10R	114	NTP pyrophosphohydrolase

Table 2. cont.

	PCPV					ORFV						BPSV		VACV		Predicted function
	F00.120R		VR634			NZ2		IA82		SA00		AR02		Copenhagen		
ORF	Nucleotide position	Length (aa)	Nucleotide position	Length (aa)	% Id*	Length (aa)	% Id*	Length (aa)	% Id*	Length (aa)	% Id*	Length (aa)	% Id*	ORF	Score†	
072	76742–74829	638	84305–82392	638	98.7	638	98.3	638	98.3	638	98.3	638	88.4	D11L	726	NPH-1
073	77386–76811	192	84947–84372	192	97.4	188	85.1	188	86.7	188	85.6	194	62.5			Unknown
073.5	77924–77376	183	85485–84937	183	98.4	Not present		Not present		Not present		Not present				Similar to ORF 073
074	78916–78050	289	86475–85609	289	99.0	289	95.5	289	95.5	289	95.8	290	87.9	D12L	340	mRNA capping enzyme
075	80594–78960	545	88153–86519	545	98.5	545	95.6	545	95.6	545	96.0	545	84.8	D13L	637	Rifampicin resistance, membrane protein
076	81067–80618	150	88626–88177	150	98.0	150	96.7	150	96.7	150	96.7	150	86.7	A1L	112	Late transcription factor VLTF2
077	81782–81111	224	89340–88669	224	100	224	99.6	224	99.6	224	99.6	224	92.0	A2L	345	Late transcription factor VLTF3
078	82030–81782	83	89588–89340	83	96.4	82	85.4	82	86.6	83	89.2	80	70.9	A2.5L	62	Thioredoxin-like protein
079	84082–82046	679	91639–89603	679	97.6	676	89.6	675	89.6	675	90.0	683	74.4	A3L	477	Virion core, P4b precursor
080	84889–84101	263	92443–91658	262	89.3	334	71.5	324	72.2	328	70.7	244	60.8	A4L	27	Virion core protein
081	84928–85443	172	92482–92997	172	98.8	172	92.4	172	93.0	173	93.0	171	85.9	A5R	102	RNA-polymerase subunit RPO19
082	86583–85450	378	94137–93004	378	99.2	378	89.4	378	89.9	378	89.2	384	74.6	A6L	334	Unknown
083	88741–86624	706	96295–94178	706	98.0	706	95.6	706	95.8	706	95.6	706	89.4	A7L	853	Early transcription factor
084	88803–89711	303	96357–97265	303	99.3	303	95.7	303	95.7	303	95.4	307	83.8	A8R	265	Intermediate transcription factor
085	89967–89686	94	97521–97240	94	100	93	89.2	93	89.2	93	88.2	96	89.2	A9L	99	(Late) Virion membrane protein
086	92702–89985	906	100259–97539	907	98.2	905	90.8	905	90.6	905	90.8	908	76.7	A10L	663	Virion core protein P4a precursor
087	92717–93724	336	100274–101281	336	99.1	336	98.2	336	97.9	336	97.9	345	89.0	A11R	172	Virion formation
088	94516–93731	262	102073–101288	262	95.4	262	78.7	261	78.0	260	78.8	223	62.8	A12L	69	Virion core protein
089	94811–94536	92	102368–102093	92	100	92	92.4	92	91.3	92	91.3	78	62.8	A13L	17	Virion membrane protein
090	95112–94837	92	102669–102394	92	92.4	91	84.6	90	85.6	91	84.6	90	67.8	A14L	54	IMV membrane protein
091	95290–95132	53	102847–102689	53	100	53	86.8	53	86.8	53	86.8	53	84.9	A14.5L	65	Putative virulence factor, IMV
092	95560–95294	89	103117–102851	89	95.5	89	88.8	89	88.8	89	87.6	92	65.2	A15L	24	Unknown
093	96623–95550	358	104180–103107	358	98.6	358	91.6	358	91.9	358	92.2	359	83.0	A16L	274	Myristylated protein
094	97253–96657	199	104810–104214	199	98.5	196	90.3	196	90.3	196	90.3	201	81.4	A17L	98	Phosphorylated IMV membrane protein
095	97268–98731	488	104825–106288	488	98.0	488	95.3	488	95.1	488	95.1	489	89.8	A18R	419	DNA helicase

Table 2. cont.

	PCPV					ORFV						BPSV		VACV		Predicted function
	F00.120R		VR634			NZ2		IA82		SA00		AR02		Copenhagen		
ORF	Nucleotide position	Length (aa)	Nucleotide position	Length (aa)	% Id*	Length (aa)	% Id*	Length (aa)	% Id*	Length (aa)	% Id*	Length (aa)	% Id*	ORF	Score†	
096	98993–98709	95	106550–106266	95	88.4	91	80.2	91	80.2	90	80.0	83	66.3	A19L	47	Zn-finger protein
097	99329–100615	429	106886–108172	429	98.4	429	91.4	429	91.1	429	92.1	426	71.3	A20R	186	DNA-polymerase processivity factor
098	99330–99007	108	106887–106564	108	96.3	108	90.7	108	90.7	108	91.7	164	75.0	A21L	82	Unknown
099	100615–101052	146	108172–108609	146	100	146	98.6	146	98.6	146	98.6	146	93.8	A22R	167	Resolvase
100	101079–102218	380	108636–109775	380	97.1	380	96.8	380	96.8	380	96.1	381	84.2	A23R	357	Intermediate transcription factor VITF3
101	102246–105728	1161	109803–113285	1161	98.8	1161	97.7	1161	97.6	1161	97.3	1161	93.5	A24R	1699	RNA-polymerase RPO132
102	107403–105847	519	114957–113404	518	81.7	520	68.3	518	78.6	520	68.1	520	67.0	A26L/ A27L	77/38	A-type inclusion protein/fusion peptide
103	109012–107444	523	116572–114998	525	72.4	516	50	522	69.7	516	50.4	519	57.6	A26L	75	A-type inclusion protein
104	109323–109054	90	116886–116614	91	83.3	89	80.9	90	81.1	90	78.9	89	75.0	A27L	43	Viral fusion peptide
105	109786–109367	140	117349–116930	140	97.9	140	95.7	140	96.4	140	95.7	140	87.1	A28L	110	IMV surface protein
106	110752–109805	316	118315–117368	316	97.8	314	94.9	314	95.2	314	95.2	319	77.8	A29L	211	RNA-polymerase subunit RPO35
107	110931–110755	59	118494–118318	59	100	60	93.2	60	93.2	60	91.5	62	78.0	A30L	28	Virion morphogenesis
107.5	111081–110935	49	118668–118498	57	87.8	49	81.6	49	83.7	49	85.7	58	40.8			Unknown
108	111918–111103	272	119460–118690	257	98.8	266	95.8	266	95.8	274	97.4	259	90.3	A32L	263	ATPase, DNA packaging
109	112003–112485	161	119545–120021	159	58.4	161	56.1	159	58.4	164	70.6	162	48.1	A33R	36	EEV glycoprotein
110	112505–113005	167	120040–120534	165	49.1	165	46.7	165	49.1	167	89.2	167	52.4	A34R	54	EEV glycoprotein
111	113037–113573	179	120572–121108	179	93.9	179	84.4	179	84.4	179	84.9	184	66.5	A35R	54	Unknown
112	113692–114558	289	121227–122075	283	68.0	286	57.8	286	58.0	288	56.3	297	42.2	C23L, A41L	24	Chemokine-binding protein
113	114637–115266	210	122170–122811	214	80.5	209	69.6	211	67.8	200	74.1	199	46.6			Unknown
114	115313–116344	344	122858–123889	344	94.8	346	90.4	346	91.3	344	92.2	331	62.2			Unknown
115	116464–116902	146	124008–124445	146	84.0	145	74.8	143	75.5	149	80.8	132	36.4			Unknown
116	Not present		124517–125236	240		231		234		206		258				Unknown
117	Not present		125417–126211	265		265		265		265		264		A41L	29	GM-CSF/IL-2 inhibition factor
118	Not present		Not present													Unknown
119	Not present		Not present													Unknown

Table 2. cont.

	PCPV					ORFV						BPSV		VACV		Predicted function
	F00.120R		VR634			NZ2		IA82		SA00		AR02		Copenhagen		
ORF	Nucleotide position	Length (aa)	Nucleotide position	Length (aa)	% Id*	Length (aa)	% Id*	Length (aa)	% Id*	Length (aa)	% Id*	Length (aa)	% Id*	ORF	Score†	
120	Not present		Not present													Unknown
121	Not present		126417–126890	158		306		300		302		269				Unknown
122	117204–117995	264	126948–127913	322	97.3	323	87.1	323	87.5	323	86.4	322	57.3	A51R	30	Unknown
123	118084–119703	540	128005–129621	539	95.1	525	80.6	525	80.4	525	80.4	517	58.7	6 ORFs	62	Ankyrin/F-box protein
124	119746–121371	542	129664–131307	548	96.9	532	81.3	532	82.1	532	81.9	506	59.4			Unknown
125	121487–122005	173	131423–131941 *(13867–13349)*	173 *(173)*	97.1	173	79.2	173	82.1	173	80.3	177	63.4			Apoptosis inhibitor
126	122145–123650	502	132079–133584 *(13211–11706)*	502 *(502)*	98.6	497	87.5	497	87.7	497	87.9	506	60.6	4 ORFs	36	Ankyrin/F-box protein
127	*LT (001.3)*	*199*	Not present			186	75.5	186	76.6	184	76.5	185	87.6			IL-10
128	123915–125423	503	133858–135366 *(11432–9924)*	503 *(503)*	96.0	500	84.0	508	72.5	501	84.0	517	57.9	4 ORFs	66	Ankyrin/F-box protein
129	125487–127037	517	135429–136979 *(9861–8311)*	517 *(517)*	96.7	520	75.8	520	76.0	516	75.6	515	62.0	3 ORFs	61	Ankyrin/F-box protein
130	127126–128610	495	137065–138558 *(8225–6732)*	498 *(498)*	99.0	498	94.9	498	94.9	498	95.2	479	86.4	F10L	465	Protein kinase
131	128573–129250	226	138512–139198 *(6778–6092)*	229 *(229)*	98.7	225	92.5	225	88.9	226	90.7	224	76.1	F9L	47	Membrane protein
132	129343–129846	168	139289–139744 *(6001–5546)*	152 *(152)*	68.9	133	52.0	137	33.6	149	31.5	*LT (006)*	*34.2*			VEGF
132.5	130759–131115	119	140683–141039 *(4607–4251)*	119 *(119)*	99.2	*LT (002)*	*84.6*	*LT (002)*	*84.6*	*LT (002)*	*80.9*	Not present				Unknown
133	Not present		Not present			Not present		Not present		Not present		149				Unknown
134	131311–131760	150	141238–141687 *(4052–3603)*	150 *(150)*	89.3	149	75.2	149	74.5	149	81.9	147	57.8			Unknown

*The per cent amino acid identity with corresponding F00.120R ORF.

†The BLAST bit score between indicated VACV Copenhagen (GenBank accession number: M35027) and the corresponding F00.120R ORFs. Only bit score values >30 are shown except for ORFs 080, 089, 092, 107, 112 and 117, which are accepted orthologues of the indicated VACV ORFs based on their localization in the genome or known protein characteristics.

‡ITR. 001–008 are absent. Region contains eight genes duplicated from the right terminus (134–125).

strains of ORFV and one strain of BPSV revealed evidence of the close relationship between F00.120R and VR634 as well as that between these viruses and the other PPVs. Individual protein comparisons revealed highest amino acid identity between F00.120R and VR634 (~95 %), a result also inferred from a phylogenetic tree of the combined data from 132 PPV proteins (Fig. 3), indicating that F00.120R and VR634 should be classified as the same species of virus, namely PCPV.

This first analysis of the full genome sequence of PCPV confirms its classification as a distinct species of the genus *Parapoxvirus* (Fig. 3). Our analysis also reveals PCPV's relatively close genetic relationship to ORFV, in comparison to BPSV. For example, the average amino acid identity between the predicted proteins encoded by ORFV and F00.120R/VR634 was approximately 88 % in contrast to 73 % identity between F00.120R/VR634 and BPSV (Table 2). Similarly, phylogenetic analyses of amino acid alignments from both variable regions (ORFs 001–008 and 112–132) and the conserved region (ORFs 009–111) exhibited high bootstrap support for the tree presented in Fig. 3 (data not shown). This confirms the proposition from previous studies of partial gene sequences that PCPV and ORFV are genetically more closely related to each other than either of them is to BPSV (Inoshima *et al.*, 2001; Thomas *et al.*, 2003; Tikkanen *et al.*, 2004; Hosamani *et al.*, 2006). This is despite BPSV and PCPV sharing the same bovine host, whereas the natural hosts of ORFV are sheep and goats.

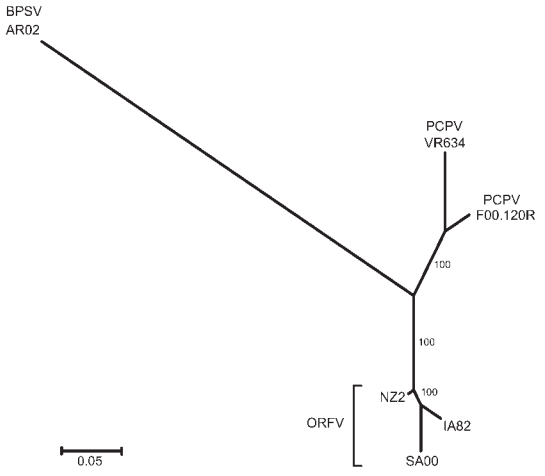

Fig. 3. Phylogenetic analysis of PPVs analysed in this study. The tree of concatenated data of proteins 001–134 of PCPV, ORFV and BPSV was generated with the maximum-likelihood method in the program RAxML-VI-HPC version 2.2.3 using the JTT model for amino acid substitution with four gamma rates. The reliability of the results was tested with bootstrap analysis of 100 replicates using the search parameters of the original analysis and the consensus tree was calculated with the program Consensus of the PHYLIP package version 3.66. The numbers on the branches indicate bootstrap support values inferred from 100 replicates. Bar, 0.05 substitutions per site.

All of the PPV genomes sequenced to date have been shown to possess 12 genes (ORFs 001/134, 005, 012, 012.5, 013, 024, 073, 113, 115, 124, 125, 006/132; Table 2) that are not present in other chordopoxviruses. The genomic locations of these unique PPV genes, as for most of the other genes, are conserved between different PPV species as is common with species belonging to the same genus (Tamames, 2001).

The five genes found to vary most between F00.120R and VR634 (ORFs 103, 109, 110, 112 and 132; Table 3) are also the most variable genes found between ORFV strains, with the exception of ORF 103 (Delhon *et al.*, 2004; Mercer *et al.*, 2006). Of these genes ORFs 109 and 110 are orthologues of VACV A33R and A34R, respectively, and encode envelope glycoproteins important to virus egress, dissemination and extracellular virus infectivity (Duncan & Smith, 1992; Roper *et al.*, 1996; McIntosh & Smith, 1996; Tan *et al.*, 2009). In addition, A33R has been shown to be one target of neutralizing antibodies that protected mice during experimental VACV infection (Fogg *et al.*, 2004). A33R and A34R are conserved in all orthopoxviruses sequenced to date, with amino acid identities ranging from 90 to 100 % between species. In A33R, the level of conservation is found to be higher in the N-terminal (99 %) than in the extracellular C-terminal (85 %; Roper *et al.*, 1996). It was suggested that this variability in the extracellular part may confer selective advantages to the virus when interacting with specific hosts *in vivo*. Although the corresponding proteins from the PPVs are much more variable between isolates (45–89 %) it was observed that the sequences from ORFVs isolated from sheep were more similar to each other than they were to the corresponding sequences in a virus isolated from a goat. It was thus suggested that these proteins could be important in host-range selection of the PPVs (Delhon *et al.*, 2004) and that they may also be responsible for the ability of different virus isolates to reinfect its host (Mercer *et al.*, 2005). The ORF 109 and 110 proteins from F00.120R are more similar to those from SA00 (Delhon *et al.*, 2004), the ORFV isolate from a goat (70.6 % ORF 109 and 89.2 % ORF 110) than they are to those from VR634 (58.4 % ORF 109 and 49.1 % ORF 110). In contrast, those sequences from VR634 show highest amino acid identity to sheep isolates of ORFV, namely NZ2 and IA82 (Table 3). Further analyses will be needed to clarify whether the results presented here are common to PCPV and if they have an impact either on the selection of, or the ability to reinfect, the host animal.

Immunomodulatory/virulence genes

Both the F00.120R and VR634 genomes are predicted to encode the majority of known or putative virulence factors thus far identified in the ORFV genome. These include the ankyrin-like repeat (ANK)/F-box proteins (ORFs 008, 123, 126, 128 and 129), the interferon-resistance protein (ORF 020), the chemokine-binding protein (ORF 112), viral vascular endothelial growth factor (vVEGF; ORF 132), viral

Table 3. Five most variable genes between PCPV strains and their relatedness to the corresponding genes of ORFV, BPSV and VACV strain Copenhagen (GenBank accession no. M35027) (Goebel *et al.*, 1990)

PCPV			ORFV			BPSV	VACV Copenhagen	Amino acid identity (%)
Amino acid identity (%) with F00.120R								
ORF	F00.120R	VR634	NZ2	IA82	SA00	AR02	ORF	
103		72.4	50.0	69.7	50.4	57.6	A26L	20.0
109		58.4	56.1	58.4	70.6	48.1	A33R	18.5
110		49.1	46.7	49.1	89.2	52.4	A34R	22.4
112		68.0	57.8	58.0	56.3	42.2	C23L/A41L	11.0/18.8
132		68.9	52.0	33.6	31.5	34.2	–	–
Amino acid identity (%) with VR634								
ORF							ORF	
103			49.8	83.7	50.0	58.4	A26L	19.4
109			72.8	69.4	54.1	53.2	A33R	22.2
110			80.0	80.6	49.1	61.8	A34R	22.5
112			55.1	52.5	54.0	43.0	C23L/A41L	10.0/18.8
132			36.9	40.5	57.6	37.9	–	–

interleukin-10 (vIL-10; ORF 001.3, present only in F00.120R), the GM-CSF inhibitory factor (ORF 117, present only in VR634) and an inhibitor of apoptosis (ORF 125). Neither is predicted to encode any additional virulence factors to those found in ORFV or BPSV, with the possible exception of the fragmented genes near the left end of F00.120R (ORFs 001.6, 001.9, 004.6 and 004.8). In contrast, neither F00.120R nor VR634 encodes the predicted 149 aa protein (ORF 133) or the two additional ANK proteins (ORFs 003 and 004) found in the BPSV genome.

The significance of F00.120R/VR634 lacking the two additional ANK proteins present in BPSV is currently not known, but almost all chordopoxviruses have several genes encoding either ANK or ANK/F-box proteins, which implies they are of importance to poxviruses (Mercer *et al.*, 2005). The ORFV ANK/F-box proteins (ORFs 008, 123, 126, 128 and 129) have been shown to be functionally active, interacting with components of the cellular SCF ubiquitin ligase complex via their F-box-like domains (Sonnberg *et al.*, 2008). Cellular F-box proteins facilitate protein degradation via the ubiquitin-proteasome pathway (Ho *et al.*, 2008; Zhang *et al.*, 2009), raising the possibility that poxvirus ANK/F-box proteins direct the removal of unwanted host cell proteins in order to favour viral replication. Inhibitory roles in ubiquitination pathways are also possible (Mercer *et al.*, 2005; Sonnberg *et al.*, 2008; Zhang *et al.*, 2009). Other poxvirus ANK/F-box proteins have been shown to inhibit virus-induced apoptosis (Ink *et al.*, 1995; Mossman *et al.*, 1996) and to influence virus virulence (Mossman *et al.*, 1996; Wang *et al.*, 2006), host-range and tissue tropism (Shchelkunov *et al.*, 1993; Ink *et al.*, 1995; Johnston *et al.*, 2005). Whether the PPV ANK/F-box proteins have any of these functions is yet to be determined. The F00.120R and VR634 ANK/F-box proteins have on average 96 % aa identity to each other and approximately 80 % aa identity with the corresponding proteins from ORFV, but only approximately 60 % aa identity with those from BPSV. Whether or not this has any significance for the host range of these viruses is not known.

The vIL-10 gene is found near the left end of the F00.120R genome (ORF 001.3) in contrast to the ORFV and BPSV genomes where it is situated near the right end of the genome (ORF 127). VR634 does not possess a vIL-10 gene, but this is thought to have been lost during cell culture passaging. Indeed, the partial sequence of the vIL-10 gene has been reported from five cattle isolates of PCPV in Norway (Klein & Tryland, 2005). The primary function of vIL-10 appears to be the suppression of early host responses to virus infection (Haig *et al.*, 2002). Both cellular IL-10 and vIL-10 have been shown to inhibit the production of IL-8 and tumour necrosis factor (TNF)-α by macrophages as well as the production of gamma interferon (IFN-γ) by lymphocytes (Moore *et al.*, 2001; Haig *et al.*, 2002).

The functional similarity, high amino acid identity and phylogenetic analyses of vIL-10 orthologues from ORFV, BPSV, Epstein–Barr virus, equid herpesvirus 2 with the corresponding sequences from their vertebrate hosts have suggested that these viruses have acquired the IL-10 gene from their hosts independently at different times during evolution (Hughes, 2002; Rziha *et al.*, 2003; Delhon *et al.*, 2004; Fleming *et al.*, 2007). The carboxy two-thirds of PPV and cellular IL-10s are highly conserved, but with certain amino acid differences that are helpful in predicting the genetic origin of the sequence. Although F00.120R IL-10 has a 12 aa insertion in this region (residues 116–127) in comparison to other vIL-10 molecules and the bovine, ovine and caprine IL-10 proteins, it also has 6 aa that are found in bovine IL-10, but not in ovine IL-10 (Rziha *et al.*, 2003). The IL-10 sequence from reindeer is not published,

but only 2 of these 6 aa are also shared with the IL-10 of *Cervus elaphus* (GenBank accession no. AAA85434.1), another species of deer belonging to the same family Cervidae as reindeer (Fig. 4). These results could support the view that F00.120R is a cattle virus that has been transmitted to reindeer. Further supporting evidence for this might come from the fact that F00.120R and the BPSV strains AR02, B177 and V660 share 14 aa in the highly variable N-terminal domain of the protein that are not found in any of the ORFV, ovine, *C. elaphus*, caprine or bovine IL-10s (Fig. 4). This is also evidence for the recombination event, discussed previously, that may have occurred between the ancestor of F00.120R and BPSV, in which a region of the BPSV genome that included the vIL-10 may have recombined into the PCPV genome.

A second viral gene thought to have been acquired from the host is the vVEGF gene, but because of the hypervariability of the sequence it is more difficult to predict its genetic origin (Ueda *et al.*, 2007). The vVEGF genes of PPVs are orthologues of mammalian VEGF gene family members, which stimulate proliferation and permeability of vascular endothelial cells and induce angiogenesis by binding to a set of mammalian tyrosine kinase receptors (Ferrara, 2004). It was demonstrated that all VEGFs of established PPV species (including PCPV VR634; Ueda *et al.*, 2003) also possess the ability to induce endothelial proliferation and angiogenesis and are likely to be responsible for the highly vascularized and proliferative nature of PPV lesions (Savory *et al.*, 2000; Wise *et al.*, 2003; Inder *et al.*, 2007; Ueda *et al.*, 2003, 2007). This is in spite of the fact that sequence variation of vVEGF proteins is remarkably high, varying from 35 to 63 % aa identity between species, whilst isolates of the same species show as little as 38 % aa sequence identity (Lyttle *et al.*, 1994; Mercer *et al.*, 2002, 2006; Wise *et al.*, 2003, 2007; Delhon

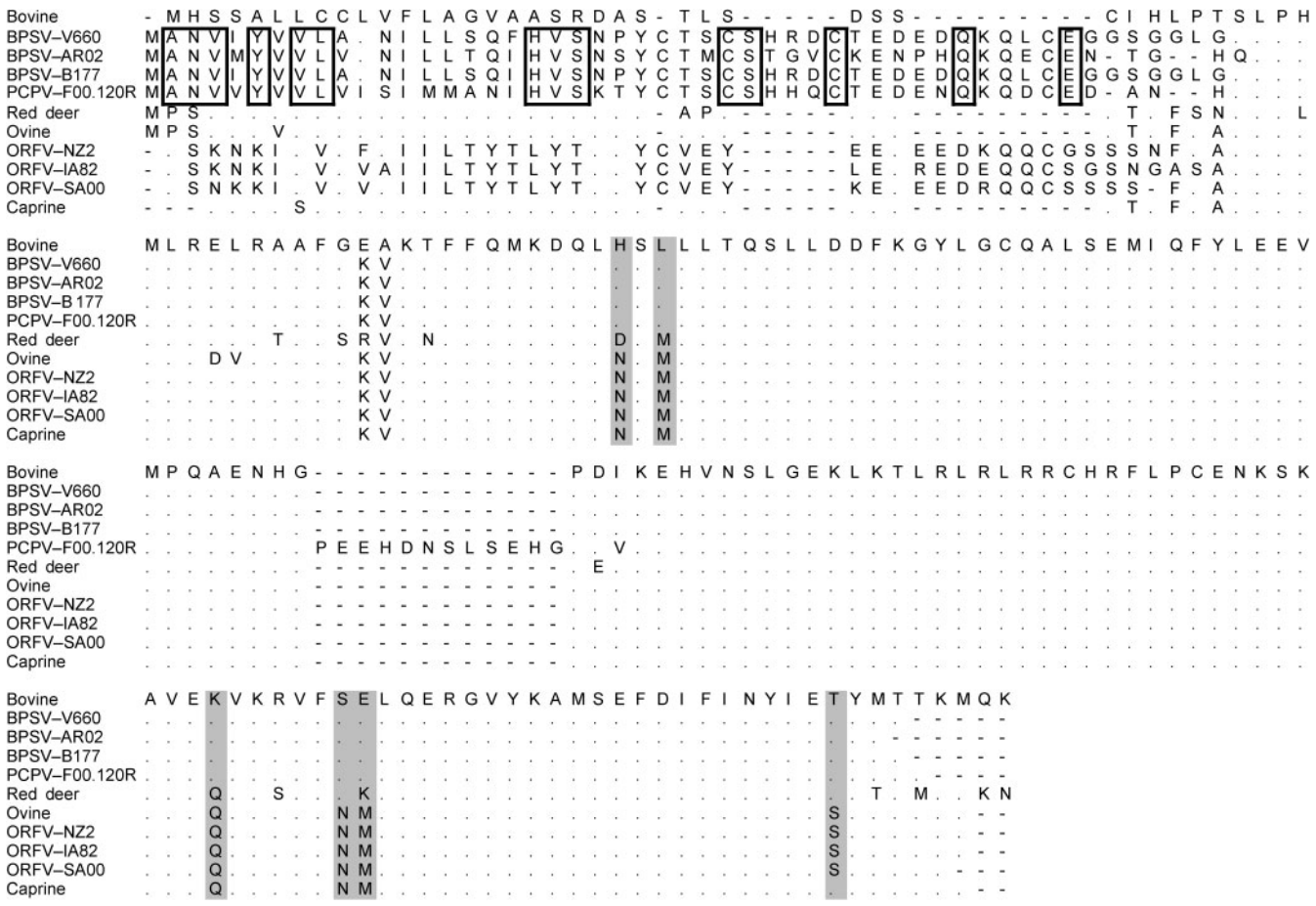

Fig. 4. Amino acid alignment of viral and host IL-10 sequences. Alignment was performed with CLUSTAL W 1.83 (Thompson *et al.*, 1994) and edited in GeneDoc v2.6.002 (Nicholas & Nicholas, 1997). Amino acids specific to PCPV and BPSV in variable N-terminal region are boxed and amino acids common for bovine and BPSV/PCPV IL-10 or ovine and ORFV IL-10 are shaded. Dots and lines indicate identical and missing amino acids in comparison to bovine sequence, respectively. GenBank accession numbers for IL-10 sequences not described in Methods are: AAA19011.1 (bovine), AAC13768.1 (ovine), AAA85434.1 (red deer), ABI20513.1 (caprine) and AAO31705.1 (BPSVB177). BPSV-V660 IL-10 is unpublished (N. Ueda).

et al., 2004; Ueda *et al.*, 2003, 2007; Inder *et al.*, 2007). The F00.120R and VR634 VEGF sequences share 69 % aa identity (Table 2). This is slightly less than the 75 % identity found between two BPSV isolates (Inder *et al.*, 2007), but considerably more than the 38 % identity found between two ORFV isolates, namely IA82 and SA00 (Delhon *et al.*, 2004). Therefore, it is not possible to use the VEGF sequences to infer whether or not F00.120R and VR634 are two distinct species of virus or two isolates of the same species.

The IFN resistance gene (ORF 020) is an orthologue of VACV E3L, which is essential for the broad host-range of VACV *in vitro* and affects virulence *in vivo* (Brandt & Jacobs, 2001; Vijaysri *et al.*, 2003, 2008). As a result of the variation seen between the N-terminal domains of the ORFV and BPSV proteins, Delhon *et al.* (2004) suggested that this domain might have a role to play in host range and pathogenesis. Our analysis of the variation between different PPV species does not clearly support a role in host range determination as there was no greater identity between BPSV and PCPV 020 proteins than between them and the corresponding ORFV proteins.

The orthologue of ORFV ORF 112 is found in both F00.120R and VR634. The ORFV ORF 112 gene encodes a protein that shares some amino acid and functional identity with the poxvirus type II CC-chemokine-binding (CBP-II) proteins of the genera *Orthopoxvirus* and *Leporipoxvirus* (Lalani *et al.*, 1998; Seet *et al.*, 2003a) and the VACV A41L chemokine-binding virulence factor (Clark *et al.*, 2006). Although most poxviruses have been shown to encode proteins that modulate host chemokine pathways in some way, there is not a single class of chemokine-modulator that is present in all poxviruses. This has been suggested to reflect differences in host and tissue tropism and, as a consequence, helps to explain clinical differences in diverse poxviral diseases (Seet *et al.*, 2003a, b). There is considerably more variation between the F00.120R and VR634 proteins (68 % aa identity) than is found between different ORFV isolates (85 % aa identity). Whether or not these differences have an influence on the function of the protein and/or reflect adaptation of F00.120R to reindeer remains to be determined.

Conclusions

This work presents a complete genomic sequence of an established strain of PCPV (VR634) together with genomic sequence of a PPV isolated from reindeer (F00.120R) exhibiting contagious stomatitis. The genomes of F00.120R and VR634 consist of a central core of conserved genes, flanked by more variable terminal regions as is found in other poxviruses. The G+C profile of the genomes, as well as the gene order and predicted protein homologies, indicate that F00.120R is an isolate of PCPV and that PCPV is correctly classified as a member of the genus *Parapoxvirus*. Furthermore, the analysis reveals that PCPV is more closely related to the ovine PPV, ORFV, than to its fellow bovine PPV, BPSV. This close relatedness may explain why both these viruses but not BPSV have been observed to cause infection of reindeer. Differences in immunomodulatory and virulence genes of PCPV and ORFV may be linked to the different disease phenotype observed in the papular stomatitis outbreak in reindeer during winter 1999–2000 (mostly mild inflammatory spots and ulcers in the mouth) compared with the more severe wart-like lesions seen in earlier outbreaks caused by ORFV-like viruses. This is the first indication that PCPV is able to infect a species other than bovine or human and thus expands the host range of this virus. Whether or not it then undergoes adaptation to allow more efficient transmission within its new host species is unknown, but a greater understanding of PPV biology and transmission between different animal species will provide insights into control measures that might reduce morbidity during epidemics.

METHODS

DNA purification and cloning of viral genomic DNA. The origin and history of reindeer parapoxvirus (F00.120R) and PCPV strain VR634 used in this study are presented in Table 1. Viral DNA was purified as previously described (Esposito *et al.*, 1981; Guo *et al.*, 2003 for F00.120R and Ueda *et al.*, 2003 for VR634). Purified F00.120R DNA was partially digested with *Sau*3AI (Roche) and cloned into the SuperCos 1 cosmid vector (Stratagene) to obtain a library containing viral fragments of 30–42 kb in length. In addition, for purposes of preparing probes for screening the cosmid library, and later for sequencing the genome ends, the viral DNA was digested with *Hin*dIII, *Kpn*I (Promega) and *Not*I (Roche) and the resulting fragments were cloned into the pBluescript SK− plasmid vector (Stratagene).

DNA sequencing strategies. The genomic sequence of F00.120R was derived by shotgun sequencing of plasmid sub-libraries of three cosmids covering the central region of the genome, and by sequencing transposon random insertion libraries of two plasmids derived from each terminus of the genome. Sub-libraries were prepared by using the TOPO Shotgun Subcloning kit (Invitrogen) and the transposon insertion libraries were constructed using the EZ::TN <KAN-2> Insertion kit (Epicentre) according to the manufacturer's instructions. Briefly, fragmented cosmids were cloned into the pCR4Blunt-TOPO plasmid vector and transformed into One Shot TOP10 *Escherichia coli* cells (Invitrogen). Colonies containing inserts were grown in TB medium with 50 μg kanamycin ml^{-1} for 18–22 h prior to plasmid purification. Transposon insertion libraries were produced by mixing an equimolar amount of <KAN-2 >transposon with the target plasmid. Transposon insertion clones were selected on agar plates containing 50 μg kanamycin ml^{-1} and cultured in 96-deep well plates for plasmid purification. Both shotgun and transposon insertion clones were purified using the Wizard SV96 Plasmid DNA Purification System (Promega) either manually using the Vac-Man 96 Vacuum manifold (Promega) or with a Biorobot 8000 (Qiagen).

Shotgun library clones were sequenced by using universal primers flanking the cloning site, and transposon insertion clones were sequenced by using transposon-specific forward and reverse primers. Sequencing reactions were performed by using Big Dye v.3.1 chemistry (Applied Biosystems) and the reactions were run on ABI 3730xl or ABI 3100 Avant genetic analysers. For each cosmid and plasmid, the individual sequences were edited and assembled into contigs by using the program SeqMan Pro of the Lasergene version

7.1 (DNASTAR). Gaps were closed by primer walking. The final consensus sequence represented on average sevenfold redundancy at each base position.

The genomic sequence of VR634 was determined with a 454/Roche GS FLX pyrosequencing system using protocols and reagents of the manufacturer (Roche Diagnostics). Five micrograms of fragmented VR634 DNA was converted to an single-stranded template DNA library using the GS FLX DNA library preparation kit and emulsion (em) PCRs were performed using the GS emPCR kit I. Sequencing was conducted by using a PicoTiterPlate and a GS LR70 sequencing kit. Four independent runs yielded 3858, 5037, 4043 and 6052 reads, which were assembled into contigs by using the software package of the GS FLX System (version 1.1.02). The gaps were closed by PCR and sequencing of the products. The final consensus sequence represented on average 25-fold redundancy at each base position.

Sequence analysis. Identification of ORFs putatively encoding proteins was performed by using the programs GATU (Tcherepanov *et al.*, 2006) and SeqBuilder (DNASTAR). The validity of the predicted protein sequences was confirmed by using BLASTP (Altschul *et al.*, 1990) available through the National Center for Biotechnology Information (NCBI) website (http://blast.ncbi.nlm.nih.gov/Blast.cgi). BLASTALL (NCBI BLAST version 2.2.15) was used to perform BLASTP searches of predicted F00.120R proteins against a BLAST database created by formatdb from vaccinia virus (VACV) Copenhagen predicted proteins. FASTA-formatted files of poxviruses and poxvirus proteins used in comparison were obtained from GenBank (http://www.ncbi.nlm.nih.gov/Genbank) or the Viral Bioinformatics Resource Center (http://athena.bioc.uvic.ca). ORFV IA82 and SA00 proteins were edited to correspond with the revisions suggested by Mercer *et al.* (2006).

Phylogenetic analyses. Phylogenetic analyses were performed on the predicted protein sequences. Individual amino acid sequences were aligned by using CLUSTAL W 1.83 (Thompson *et al.*, 1994) and the alignments concatenated into three separate blocks of data. Phylogenetic analysis was then performed separately on the concatenated genomic ends, proteins 001–008 and 112–134, the central core of the genome, proteins 009–111, and the entire genome, proteins 001–134. If a protein was missing from a particular virus it was coded as missing information for the purposes of the analyses.

Phylogenetic relationships were assessed by the maximum-likelihood method. Analyses were performed by using the fast-hill climbing method in the program RAxML-VI-HPC version 2.2.3 (Stamatakis *et al.*, 2005). To find the best topology of the tree for each alignment, ten random-addition-sequences (RAS), using the JTT evolutionary model for substitutions and four gamma classes for modelling the rate heterogeneity between sequence positions, were run. Since all ten RAS consistently gave the same topology and the same likelihood, further replication was not necessary.

The validity of the results was assessed by using bootstrap analysis. For each alignment, and the corresponding tree, 100 bootstrap replicates were performed using the same search parameters in RAxML-VI-HPC as for the original analysis. The consensus tree was calculated using the majority-rule method in the program Consensus from the PHYLIP package version 3.66 (Felsenstein, 2005).

ACKNOWLEDGEMENTS

M. H. had grants from Wihuri Foundation, Finland, N.U. and A. A. M. from the New Zealand Foundation for Research, Science and Technology, the Health Research Council of New Zealand and the University of Otago Research Grants. C. J. McI. was supported by the Scottish Government. The authors thank Dr J. Stanton, University of Otago for sequencing services and useful discussions, Ann Wood, Moredun Research Institute Edinburgh, for the help with cloning the F00.120R genome, Dr Mikael Björklund and Pekka Ellonen, Biomedicum Helsinki, for the help with automated plasmid purification and for sequencing services, respectively.

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