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RAPID COMMUNICATION

The Genome of Camelpox Virus

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Camelpox virus (CMLV), a member of the *Orthopoxvirus* genus in the *Poxviridae*, is the etiologic agent of a disease of camels. Here we report the CMLV genomic sequence with analysis. The 205,719-bp CMLV genome contains 211 putative genes and consists of a central region bound by identical inverted terminal repeats of approximately 7 kb. A high degree of similarity in gene order, gene content, and amino acid composition in the region located between CMLV017 and CMLV184 (average 96% amino acid identity to vaccinia virus (VACV)) indicates a close structural and functional relationship between CMLV and other known orthopoxviruses (OPVs). Notably, CMLV contains a unique region of approximately 3 kb, which encodes three ORFs (CMLV185, CMLV186, CMLV187) absent in other OPVs. These ORFs are most similar to B22R homologues found in other chordopoxvirus genera. Among OPVs, CMLV is the most closely related to variola virus (VARV), sharing all genes involved in basic replicative functions and the majority of genes involved in other host-related functions. Differences between CMLV and VARV include deletion and disruption of a large number of genes. Twenty-seven CMLV ORFs are absent in VARV, including seven full-length homologues of NMDA-like receptor, phospholipase D, Schlafen, MT-4 virulence, kelch, VACV C8L, and cowpox (CPXV) B21R proteins. Thirty-eight CMLV ORFs, some of which are fragments of larger genes, differ in size from corresponding VARV ORFs by more than 10% (amino acids). Genome structure and phylogenetic analysis of DNA sequences for all ORFs indicate that CMLV is clearly distinct from VARV and VACV and, as it has been suggested for VARV, it may have originated from a CPXV virus-like ancestor.

Introduction. Camelpox virus (CMLV) is the etiologic agent of camelpox, a disease of camels and dromedaries (*7*, *18*). CMLV is a member of the *orthopoxvirus* genus, one of eight genera within the *Chordopoxvirinae* subfamily of the *Poxviridae*. CMLV, along with variola (VARV), vaccinia (VACV), cowpox virus (CPXV), monkeypox virus, ectromelia virus, and taterapox virus, comprise the African–Eurasian group of orthopoxviruses (OPVs). Camelpox is widespread in the arid zones of Africa, the middle-east, and central Asia. The disease in nature causes significant economic impact attributable to high morbidity (up to 100%) and mortality (up to 25%), which results in a reduction in milk production and body weight (*18*). Disease presentation varies from localized lesions of the mouth, nose, muzzle, head, or neck to generalized infection involving the skin and respiratory system (*18*). Vaccination with attenuated strains of CMLV or VACV leads to long lasting immunity (*18, 34*). Clear evidence of human infection with CMLV virus has not been reported.

CMLV is one of the least studied OPVs and limited DNA sequence information is available (Table 1). Restriction endonuclease analysis only allows differentiation of CMLV isolates by different geographic regions (*8, 15*). Given the interest in understanding the genetic basis of viral host range and virulence and the origin and evolution of OPVs related to VARV, we have sequenced and analyzed the genome of a pathogenic field isolate of CMLV.

Results and Discussion. Organization of the CMLV genome. CMLV M-96 genome sequences were assembled into a contiguous sequence of 205,719 bp, which is slightly larger than a previous restriction enzyme-based size estimate of 196 kb (*8*). Predicted *Hin*dIII restriction fragments match previously published patterns (*8*). Because the hairpin loops were not sequenced, the leftmost nucleotide was arbitrarily designated base 1. The nucleotide composition is 66.8% A+T and is uniformly distributed. As with other poxviruses, the CMLV genome contains a central coding region bound by two identical inverted terminal repeat (ITR) regions (Fig. 1). Assembled CMLV ITR are 7736 bp and contain direct repeats and coding regions. Twenty-seven copies of a perfect 71-bp direct repeat, one 70-bp imperfect repeat, and one partial repeat extend from position 1 to 2039 and from 197,984 to 205,719. Six complete ORFs (CMLV001 to CMLV003 and

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TABLE 1

Features and Homologies of CMLV ORFs

ORF	Position (length in codons)	Best match BLASP2 Score	% ID[a]	Accession[b]	Species[c]	Putative function or similarity[d]	VARV[e] ORF	length codons	% ID[a]	VACV[f] ORF	length codons	% ID[a]	CPXV[g] ORF	length codons	% ID[a]
CMLV001	3100-2336 (255)	1262	96	P19063	VACV Lister	Chemokine binding, TM				C23L	244	95	D1L	255	96
CMLV002	4276-3230 (349)	1910	99	U87837	CMLV	TNF receptor II (CrmB), SP				C22L	122	89	D2L	351	94
CMLV003	6139-4367 (591)	3032	95	L22579	VARV	Ankyrin repeat				C21L	113	93	D3L	586	92
										C20L	103	66			
										C19L	259	81			
CMLV004	8367-6352 (672)	3246	92	Y11842	CPXV	Ankyrin repeat	D1L	57	90	C18L	150	93	D4L	672	92
										C17L	386	86			
CMLV005	8988-8530 (153)	793	97	Y11842	CPXV		D2L	143	97	C16L	181	94	D5L	153	97
CMLV006	9940-9230 (237)	972	94	Y15035	CPXV	NMDA receptor-like, SP, TM							S1R	210	94
CMLV007	10618-10412 (69)	382	100	AJ272009	CMLV	TNF receptor II (CrmE) fragment, TM									
CMLV008	11595-11251 (115)	198	58	X94355	CPXV	TM							D3L	833	58
CMLV009	12101-11598 (168)	826	92	X94355	CPXV		D3L	128	92				C4L	170	92
CMLV010	12238-12654 (139)	700	92	P20494	VACV	Vaccinia growth factor, SP, TM	D4R	140	92	C11R	142	92	C5R	138	91
CMLV011	13085-12810 (92)	470	96	P21043	VACV		D5L	330	95	C10L	331	96	C6L	331	95
CMLV012	13567-13232 (112)	552	97	P33861	VARV India-1967		D5L	330	94	C10L	331	95	C6L	331	96
CMLV013	14296-15021 (242)	1276	97	L22579	VARV	N1R/p28-like host range RING finger	D6R	242	97				C7R	242	95
CMLV014	15811-15626 (62)	329	96	X94355	CPXV								C10L	62	96
CMLV015	16367-16122 (82)	400	89	X94355	CPXV	Ankyrin repeat host range fragment	D9L	91	91	C9L	634	81	C11L	614	89
CMLV016	17121-16669 (151)	728	91	X94355	CPXV	Ankyrin repeat host range fragment				C9L	634	60	C11L	614	91
CMLV017	17738-17403 (112)	513	91	U18338	VARV Garcia-1966	Ankyrin repeat host range fragment	D10L	152	94	C9L	634	79	C11L	614	97
CMLV018	18405-17860 (182)	927	94	P21041	VACV					C8L	184	94	C12L	182	93
CMLV019	18929-18480 (150)	792	98	P33860	VARV India-1967	Host range	D11L	150	98	C7L	150	98	C13L	150	98
CMLV020	19629-19162 (156)	789	98	P34014	VARV India-1967		D12L	156	98	C6L	151	97	C14L	156	95
CMLV021	20387-19785 (201)	963	93	X94355	CPXV		D13L	134	95	C5L	204	94	C15L	205	93
CMLV022	21407-20463 (315)	1629	96	U18338	VARV Garcia-1966		D14L	316	96	C4L	316	96	C16L	315	96
CMLV023	22271-21477 (265)	1415	96	P10998	VACV	Complement binding, SP	D15L	263	93	C3L	263	96	C17L	259	93
CMLV024	23877-22342 (512)	2671	97	X94355	CPXV	TM	D16L	221	92	C2L	512	97	C18L	512	97
							D17L	79	90						
CMLV025	24578-24372 (69)	350	97	P33859	VARV India-1967		D18L	214	97	C1L	224	95	C19L	231	97
CMLV026	24999-24649 (117)	591	97	L22579	VARV	Virulence	P1L	117	97	N1L	117	94	P1L	117	97
CMLV027	25635-25354 (94)	432	93	P34019	VARV India-1967		P2L	177	93	N2L	175	88	P2L	175	93
CMLV028	27004-25667 (446)	2299	97	L22579	VARV	Ankyrin repeat	O1L	446	97	M1L	472	96	O1L	474	95
CMLV029	27711-27052 (220)	1158	97	P34017	VARV India-1967	SP	O2L	220	97	M2L	220	96	O2L	163	95
CMLV030	28367-28167 (67)	354	98	P20632	VACV	Host range fragment				K1L	284	98	M1L	284	92
CMLV031	30001-28883 (373)	1870	96	X94355	CPXV	Serpin, SPI-3	C2L	373	95	K2L	369	94	M2L	373	96
CMLV032	30318-30055 (88)	448	92	X69198	VARV India-1967	eIF2α-like PKR inhibitor	C3L	87	91	K3L	88	87	M3L	88	88
CMLV033	31652-30381 (424)	2169	96	X94355	CPXV	Phospholipase D-like				K4L	424	96	M4L	424	96
CMLV034	32187-31966 (74)	271	88	X94355	CPXV	Lysophospholipase-like fragment				K5L	136	86	M5L	276	88
CMLV035	32525-32962 (146)	743	97	L22579	VARV		C4R	149	97	K7R	149	94	M6R	161	97
CMLV036	33891-33040 (284)	1314	83	U18338	VARV Garcia-1966		C5L	237	79	F1L	226	93	G1L	238	88
CMLV037	34334-33894 (147)	755	97	M34368	VACV WR	dUTPase	C6L	147	98	F2L	147	96	G2L	147	97
CMLV038	35801-34362 (480)	2547	97	X94355	CPXV	Kelch-like, TM	C7L	161	94	F3L	480	96	G3L	485	97
CMLV039	36771-35815 (319)	1657	98	U18338	VARV Garcia-1966	Ribonucleotide reductase, small subunit, TM	C8L	333	97	F4L	319	97	G4L	319	97
CMLV040	37851-36805 (349)	1705	94	L22579	VARV	TM	C9L	348	94	F5L	321	90	G5L	323	90
CMLV041	38116-37811 (102)	352	97	U18338	VARV Garcia-1966		C10L	72	95	F6L	74	90	G6L	74	90
CMLV042	38285-38073 (71)	289	84	AF095689	VACV Tian Tan		C11L	79	87	F7L	92	71	G7L	80	83
CMLV043	38634-38440 (65)	351	98	P33868	VARV India-1967		C12L	65	96	F8L	65	95	G8L	65	95
CMLV044	39332-38697 (212)	1071	98	P21018	VACV	TM	C13L	212	96	F9L	212	98			
CMLV045	40638-39322 (439)	2311	99	U32589	VACV WR	Ser/Thr kinase, virus assembly	C14L	439	98	F10L	439	99			
CMLV046	41725-40664 (354)	1761	97	P21052	VACV		C15L	354	94	F11L	354	97			
CMLV047	43676-41772 (635)	3259	97	P33871	VARV India-1967	EEV maturation, TM	C16L	635	97	F12L	635	96			
CMLV048	44836-43721 (372)	1955	98	L22579	VARV	EEV envelope lipase, virus assembly	C17L	372	98	F13L	372	97			
CMLV049	45074-44856 (73)	344	93	Y16780	VARV minor		C18L	73	91	F14L	73	80			
CMLV050	45819-45346 (158)	827	99	P21020	VACV		C19L	161	99	F15L	159	99			
CMLV051	46520-45822 (233)	1166	96	U94848	VACV MVA		C20L	231	97	F16L	231	96			
CMLV052	46582-46884 (101)	529	99	U94848	VACV MVA	DNA-binding virion core	C21R	101	97	F17R	101	98			
CMLV053	48323-46887 (479)	2433	98	L22579	VARV	Poly(A) polymerase PAP_L	E1L	479	98	E1L	479	98			
CMLV054	50533-48323 (737)	3782	98	U94848	VACV MVA	TM	E2L	737	98	E2L	737	98			
CMLV055	51226-50657 (190)	957	96	P21081	VACV	dsRNA-binding PKR inhibitor, host range	E3L	192	96	E3L	190	96	NN	190	95
CMLV056	52058-51282 (259)	1368	99	P21082	VACV	RNA polymerase subunit RPO30	E4L	259	97	E4L	259	99			
CMLV057	52135-53121 (329)	1705	100	U32630	CMLV CMG		E5R	341	94	E5R	331	94	NN	319	87
CMLV058	53268-54968 (567)	2881	98	AF095689	VACV Tian Tan	TM	E6R	567	97	E6R	567	97			
CMLV059	55054-55548 (165)	793	90	P21048	VACV		NA	60	93	E7R	166	90			
CMLV060	55677-56495 (273)	1414	97	P33820	VARV India-1967	ER localized, TM	E8R	273	97	E8R	273	97			
CMLV061	59524-56507 (1006)	5212	98	L22579	VARV	DNA polymerase	E9L	1005	98	E9L	1006	98			
CMLV062	59556-59840 (95)	494	96	P33821	VARV	Potential redox, virus assembly	E10R	95	96	E10R	95	95			
CMLV063	60227-59841 (129)	661	98	P21051	VACV	Virion protein	E11L	129	98	E11L	129	98			
CMLV064	60932-61174 (81)	337	91	AF095689	VACV Tian Tan	TM	NA	115	93	EorfF	115	90			
CMLV065	61047-60217 (277)	1388	97	Y16780	VARV minor	TM	Q1L	666	97	O1L	666	92			
CMLV066	61900-61139 (254)	1233	97	Y16780	VARV minor		Q1L	666	97	O1L	666	95			
CMLV067	62560-62237 (108)	555	98	P20818	VACV	Glutaredoxin	Q2L	108	96	O2L	108	98			
CMLV068	63644-62709 (312)	1537	98	P20498	VACV	DNA-binding virion core, virus assembly	K1L	312	97	I1L	312	98			
CMLV069	63872-63657 (72)	369	98	P12922	VACV	TM	K2L	73	98	I2L	73	98			
CMLV070	64688-63876 (271)	1303	97	P12923	VACV WR	DNA-binding	K3L	269	97	I3L	269	97			
CMLV071	67084-64772 (771)	4022	99	P20503	VACV	Ribonucleotide reductase, large subunit	K4L	771	98	I4L	771	99			
CMLV072	67350-67114 (79)	381	98	P33001	VARV India-1967	IMV membrane, TM	K5L	79	98	I5L	79	97			
CMLV073	68517-67372 (382)	1945	98	P12925	VACV	TM	K6L	382	98	I6L	382	98			
CMLV074	69781-68513 (423)	2233	99	P20501	VACV	Virion core	K7L	423	99	I7L	423	99			
CMLV075	69787-71814 (676)	3478	98	P20502	VACV	NPH-II, RNA helicase	K8R	676	97	I8R	676	98			
CMLV076	73596-71824 (591)	3008	99	U94848	VACV MVA	Metalloprotease, virion morphogenesis	H1L	591	97	G1L	591	99			
CMLV077	73922-74581 (220)	1139	98	P32992	VARV India-1967	Putative transcriptional elongation factor	H2R	220	98	G2R	220	98			
CMLV078	73928-73596 (111)	563	100	P21024	VACV	SP	H3L	111	97	G3L	111	100			
CMLV079	74928-74557 (124)	638	100	P32994	VARV India-1967	Glutaredoxin 2, virion morphogenesis	H4L	124	100	G4L	124	98			
CMLV080	74931-76232 (434)	2223	98	U94848	VACV MVA		H5R	434	97	G5R	434	97			
CMLV081	76243-76431 (63)	323	100	J03399	VACV WR	RNA polymerase subunit RPO7	H5.5R	63	96	G5.5R	63	100			
CMLV082	76436-76930 (165)	867	99	U94848	VACV MVA		H6R	165	98	G6R	165	97			
CMLV083	78013-76901 (371)	1886	99	J03399	VACV WR	Virion core	H7L	371	99	G7L	371	99			
CMLV084	78044-78823 (260)	1332	99	P21029	VACV	Late transcription factor VLTF-1	H8R	260	98	G8R	260	99			
CMLV085	78846-79865 (340)	1808	98	P21030	VACV	Myristylated,TM	H9R	340	97	G9R	340	98			
CMLV086	79869-80618 (250)	1252	98	P20540	VACV	Myristylated IMV envelope, TM	M1R	250	98	L1R	250	98			

TABLE 1—*Continued*

CMLV087	80653-80913 (87)	424	97	P20843	VACV	TM	M2R	87	95	L2R	87	97					
CMLV088	81955-80909 (349)	1806	97	X76267	VARV Garcia-1966	TM	M3L	349	97	L3L	350	95					
CMLV089	81980-82732 (251)	1270	99	P20981	VACV	DNA-binding virion core VP8	M4R	251	98	L4R	251	99					
CMLV090	82745-83128 (128)	660	99	P07615	VACV	TM	M5R	128	97	L5R	128	99					
CMLV091	83088-83546 (153)	774	98	P21032	VACV		L1R	159	99	J1R	153	98					
CMLV092	83565-84095 (177)	927	100	S51129	CMLV	Thymidine kinase	L2R	177	97	J2R	177	97	NN	176	95		
CMLV093	84164-85162 (333)	1721	98	AF095689	VACV Tian Tan	Poly(A) polymerase PAP_S	L3R	333	98	J3R	333	96					
CMLV094	85080-85634 (185)	934	98	P07391	VACV	RNA polymerase subunit RPO22	L4R	185	98	J4R	185	98					
CMLV095	86102-85704 (133)	693	97	P33055	VARV India-1967	TM	L5L	133	96	J5L	133	96					
CMLV096	86207-90064 (1286)	6601	99	P20504	VACV	RNA polymerase subunit RPO147	L6R	1286	99	J6R	1286	99					
CMLV097	90579-90067 (171)	879	98	P20495	VACV	Virus assembly, Stat-1 inhibition	I1L	171	98	H1L	171	98					
CMLV098	90593-91159 (189)	985	99	P33061	VARV India-1967	TM	I2R	189	99	H2R	189	98					
CMLV099	92142-91168 (325)	1663	97	X76264	VARV Garcia-1966	IMV envelope p35, TM	I3L	325	97	H3L	324	96					
CMLV100	94530-92146 (795)	4042	98	P07241	VACV	RNA polymerase-associated RAP94, TM	I4L	795	98	H4L	795	98					
CMLV101	94716-95345 (210)	976	91	L22579	VARV	Late transcription factor VLTF-4	I5R	220	91	H5R	203	92					
CMLV102	95349-96290 (314)	1622	99	P08585	VACV	DNA topoisomerase	I6R	314	99	H6R	314	99					
CMLV103	96330-96767 (146)	733	97	U94848	VACV MVA		I7R	146	96	H7R	146	96					
CMLV104	96814-99345 (844)	4310	98	L22579	VARV	mRNA capping enzyme, large subunit	F1R	844	98	D1R	844	98					
CMLV105	99740-100450 (237)	1233	97	P33068	VARV India-1967	Virion protein	F2R	237	97	D3R	237	96					
CMLV106	99747-99310 (146)	737	98	P21008	VACV	Virion protein	F3L	146	97	D2L	146	98					
CMLV107	100453-101106 (218)	1167	99	L22579	VARV	Uracil DNA glycosylase, DNA replication	F4R	218	99	D4R	218	98					
CMLV108	101141-103495 (785)	4106	99	P04305	VACV WR	NTPase; DNA replication	F5R	785	98	D5R	785	99					
CMLV109	103539-105449 (637)	3271	99	P04308	VACV WR	Early transcription factor $VETF_S$, TM	F6R	637	98	D6R	637	98					
CMLV110	105479-105961 (161)	818	98	P04310	VACV WR	RNA polymerase subunit RPO18	F7R	161	98	D7R	161	98					
CMLV111	106841-105930 (304)	1593	99	X97857	CMLV CaP-2	IMV membrane, cell surface binding, TM	F8L	304	95	D8L	304	96	NN	304	92		
CMLV112	106883-107521 (213)	1084	99	P04311	VACV WR		F9R	213	98	D9R	213	99					
CMLV113	107521-108264 (248)	1273	99	L22579	VARV	*mut* T motif, gene expression regulator	F10R	248	99	D10R	248	97					
CMLV114	110163-108271 (631)	3225	99	L22579	VARV	NPH-I, transcription termination factor	N1L	631	99	D11L	631	98					
CMLV115	111061-110201 (287)	1470	99	P20980	VACV	mRNA capping enzyme, small subunit	N2L	287	99	D12L	287	99					
CMLV116	112747-111095 (551)	2839	99	P04321	VACV WR	Rifampicin resistance, IMV assembly	N3L	551	99	D13L	551	99					
CMLV117	113223-112774 (150)	769	99	U94848	VACV MVA	Late transcription factor VLTF-2	A1L	150	98	A1L	150	98					
CMLV118	113917-113246 (224)	1163	99	P07609	VACV WR	Late transcription factor VLTF-3	A2L	224	99	A2L	224	99					
CMLV119	114144-113917 (76)	392	98	L22579	VARV		A3L	76	98								
CMLV120	116093-114162 (644)	3337	99	P33818	VARV India-1967	Virion core P4b	A4L	644	99	A3L	644	98					
CMLV121	116973-116149 (275)	1329	93	P20983	VACV	Virion core, virion morphogenesis	A5L	271	93	A4L	281	93					
CMLV122	117011-117502 (164)	839	99	P20984	VACV	RNA polymerase subunit RPO19	A6R	164	98	A5R	164	99					
CMLV123	118620-117505 (372)	1823	98	P20985	VACV		A7L	372	97	A6L	372	98					
CMLV124	120776-118647 (710)	3672	99	C43497	VACV WR	Early transcription factor $VETF_L$	A8L	710	98	A7L	710	99					
CMLV125	120830-121693 (288)	1440	98	P20986	VACV	Intermediate transcription factor VITF-3	A9R	288	98	A8R	288	98					
CMLV126	122149-121733 (139)	483	87	M27634	VACV WR	IMV membrane, morphogenesis, SP, TM	A10L	95	90	A9L	99	88					
CMLV127	124828-122153 (892)	4577	99	Y16780	VARV minor	Virion core P4a	A11L	892	98	A10L	891	97					
CMLV128	124843-125799 (319)	1580	99	X76268	VARV Garcia-1966	TM	A12R	319	98	A11R	318	98					
CMLV129	126379-125807 (191)	952	96	P20989	VACV	Virion core	A13L	189	97	A12L	192	96					
CMLV130	126615-126406 (70)	347	97	AJ314916	CPXV Catpox5	IMV membrane, TM	A14L	68	92	A13L	70	92	NN	70	97		
CMLV131	126995-126726 (90)	462	98	P20991	VACV	IMV membrane, TM	A15L	90	98	A14L	90	98					
CMLV132	127174-127015 (53)	272	100	X76268		Virulence	NA	53	100	A14.5	53	100					
CMLV133	127447-127166 (94)	471	96	P20992	VACV		A16L	94	96	A15L	94	96					
CMLV134	128564-127434 (377)	2067	99	U94848	VACV MVA	Myristylated membrane, TM	A17L	377	97	A16L	378	99					
CMLV135	129178-128570 (203)	1042	99	P16711	VACV	Phosphorylated IMV membrane, TM	A18L	203	99	A17L	203	99					
CMLV136	129193-130671 (493)	2460	96	P16712	VARV	DNA helicase, transcriptional elongation	A19R	493	96	A18R	493	95					
CMLV137	130885-130658 (76)	379	97	P20994	VACV		A20L	76	93	A19L	77	97					
CMLV138	131235-132512 (426)	2162	98	P20995	VACV	DNA polymerase processivity factor	A22L	426	97	A20R	426	98					
CMLV139	131236-130889 (116)	603	99	P20996	VACV	TM	A21R	117	97	A21L	117	99					
CMLV140	132445-133008 (188)	958	97	U94848	VACV MVA	DNA processing	A23R	187	96	A22R	176	100					
CMLV141	133028-134173 (382)	1953	98	P20998	VACV	Intermediate transcription factor VITF-3	A24R	382	98	A23R	382	98					
CMLV142	134173-137664 (1164)	6014	99	P17474	CPXV Brighton Red	RNA polymerase subunit RPO132	A25R	1164	99	A24R	1164	99	NN	1164	99		
CMLV143	138446-138258 (63)	183	92	AF095689	VACV Tian Tan	ATI fragment	A28L	192	84				ATI	1284	89		
CMLV144	140677-138500 (726)	3677	99	Q05482	CMLV CP-1	ATI fragment, TMS	A29L	702	94				ATI	1284	91		
CMLV145	142215-140725 (497)	2529	94	X76266	VARV Garcia-1966	ATI-flanking protein, TM	A30L	498	94	A26L	322	97	NN	214	80		
CMLV146	142597-142247 (117)	598	100	X75156	CMLV CP-1	IMV membrane, fusion, virus assembly	A31L	110	97	A27L	110	96	NN	110	98		
CMLV147	143038-142601 (146)	760	99	P33847	VARV India-1967	TM	A31.5L	146	99	A28L	146	95					
CMLV148	143956-143042 (305)	1558	98	U94848	VACV MVA	RNA polymerase subunit RPO35	A32L	305	97	A29L	305	98					
CMLV149	144152-143922 (77)	389	100	P21088	VACV	Virion protein, maturation	A33L	77	100	A30L	77	100					
CMLV150	144312-144764 (151)	731	94	X76266	VARV Garcia-1966		A34R	140	90	A31R	124	98					
CMLV151	145546-144737 (270)	1384	98	P21055	VACV	DNA packaging, virus assembly	A35L	270	98	A32L	300	98					
CMLV152	145664-146215 (184)	928	96	P33850	VARV India-1967	EEV, TM	A36R	184	96	A33R	185	95					
CMLV153	146242-146745 (168)	888	100	L22579	VARV	EEV, TM	A37R	168	100	A34R	168	98					
CMLV154	146790-147317 (176)	913	98	P21058	VACV		NA	60	93	A35R	176	98					
CMLV155	147387-148040 (218)	1064	94	P21059	VACV	EEV, TM	A38R	216	93	A36R	221	94					
CMLV156	148113-148901 (263)	1345	98	P24762	VACV WR	TM	A40R	68	91	A37R	263	98					
CMLV157	148994-149173 (60)	305	95	X69198	VARV India-1967		NA	62	95	NA	64	92					
CMLV158	150006-149176 (277)	1415	97	P33853	VARV India-1967	CD47-like membrane glycoprotein, TM	A41L	277	97	A38L	277	94					
CMLV159	150798-151028 (77)	365	97	D11079	VACV WR	Semaphorin-like fragment	A43R	139	65	A39R	403	95					
CMLV160	152047-151391 (219)	1130	95	P24766	VACV WR	TM	A44L	218	94	A41L	219	94					
CMLV161	152219-152617 (133)	684	98	P33828	VARV India-1967	Profilin-like	A45R	133	98	A42R	133	96					
CMLV162	152658-153242 (195)	998	96	P33855	VARV India-1967	TM	A46R	195	96	A43R	194	96					
CMLV163	153248-153481 (78)	371	98	M72474	VACV WR												
CMLV164	154618-153581 (346)	1816	98	P26670	VACV WR	Hydroxysteroid dehydrogenase-like	A47L	210	95	A44L	346	97					
CMLV165	154665-155039 (125)	649	100	AF349014	CMLV	Superoxide dismutase-like	A48R	125	98	A45R	125	96					
CMLV166	155032-155751 (240)	1206	97	L22579	VARV	Toll-like receptor inhibitor	A49R	240	97	A46R	214	89					
CMLV167	156286-155921 (122)	580	95	P21067	VACV		J1L	244	95	A47L	244	95					
CMLV168	156501-156286 (72)	349	97	Y16780	VARV minor		J1L	244	95	A47L	244	91					
CMLV169	156596-157276 (227)	1109	96	A21996	VACV	Thymidylate kinase	J2R	205	97	A48R	204	98					
CMLV170	157328-157570 (81)	276	87	P21068	VACV		J3R	162	85	A49R	162	87					
CMLV171	157845-159500 (552)	2801	97	P33798	VARV India-1967	DNA ligase	J4R	552	97	A50R	552	96					
CMLV172	159556-160557 (334)	1735	97	Y15035	CPXV	TM	J5R	334	96	A51R	334	94	A51R	334	97		
CMLV173	161027-160758 (90)					SP											
CMLV174	161596-162453 (286)	1343	94	Y15035	CPXV	Kelch-like fragment				A55R	564	94	A54R	564	94		
CMLV175	162497-163288 (264)	1388	95	Y15035	CPXV	Kelch-like fragment, TM	J6R	172	83	A55R	564	94	A54R	564	95		
CMLV176	163341-164297 (319)	1399	87	Y16780	VARV minor	EEV hemagglutinin, SP, TM	J7R	313	85	A56R	315	86	A55R	314	85		
CMLV177	164611-164901 (97)	461	93	Y15035	CPXV	Guanylate kinase fragment	J8R	151	90	A57R	193	91	A56R	197	93		
CMLV178	165062-165961 (300)	1556	98	Y16780	VARV minor	Ser/Thr kinase, DNA replication	B1R	300	97	B1R	300	97	B1R	300	96		
CMLV179	166055-167560 (502)	2478	91	Y15035	CPXV	Schlafen-like				B2R	219	97	B2R	503	91		
										B3R	124						
CMLV180	167763-169454 (564)	2833	96	U18339	VARV Garcia-1966	Ankyrin repeat, TM	B5R	558	96	B4R	558	94	B3R	558	95		

TABLE 1—*Continued*

CMLV181	169561-170511 (317)	1658	96	X65519	VARV Harvey	EEV host-range, SP, TM	B6R	317	96	B5R	317	92	B4R	317	92
CMLV182	170596-171135 (180)	804	87	Y15035	CPXV		B7R	65	96	B6R	173	85	B5R	183	87
CMLV183	171442-171720 (93)	457	90	AF012825	ECTV Moscow	Virulence fragment				B7R	182	88	B6R	182	90
CMLV184	171775-172572 (266)	1404	98	L22579	VARV	IFN-γ receptor, SP	B8R	266	98	B8R	272	92	B7R	271	92
CMLV185	172669-173154 (162)	145	27	AF325528	LSDV Neethling 2490	B22R-like									
CMLV186	173330-174094 (255)	582	47	AB025319	YMTV	B22R-like									
CMLV187	174230-174898 (223)	518	46	AF170726	MYXV Lausanne	B22R-like									
CMLV188	175522-176196 (225)	750	64	Y15035	CPXV	M-T4-like virulence, SP				B9R	77	78	B8R	221	64
CMLV189	176345-177847 (501)	2512	95	Y15035	CPXV	Kelch-like				B10R	166	90	B9R	501	95
CMLV190	178201-179058 (286)	1443	94	P21098	VACV	Ser/Thr kinase	B11R	104	83	B12R	283	94	B11R	283	93
CMLV191	179027-180184 (386)	1754	97	Y15035	CPXV	Serpin, SPI-2	B12R	344	96	B13R	116	93	B12R	345	97
										B14R	222				
CMLV192	180249-180740 (164)	842	94	AF012825	ECTV Moscow	TM	B13R	149	95	B15R	149	94	B13R	149	95
CMLV193	181013-181216 (68)	197	79	X69198	VARV India-1967	IL-1 receptor fragment				B16R	326	76	B14R	326	76
CMLV194	181140-181418 (93)	453	95	P25212	VACV WR	IL-1 receptor fragment				B16R	326	94	B14R	326	95
CMLV195	181430-181182 (83)	313	75	U18339	VARV Garcia-1966	TM	B14L	69	88						
CMLV196	181488-181697 (70)	321	90	Q04523	CPXV Brighton Red	IL-1 receptor fragment				B16R	326	90	B14R	326	92
CMLV197	182337-182125 (71)	359	92	P21075	VACV		B15L	340	90	B17L	340	92	B15L	340	92
CMLV198	182866-182459 (136)	714	98	P21075	VACV		B15L	340	98	B17L	340	98	B15L	340	97
CMLV199	183200-183934 (245)	1210	96	U18339	VARV Garcia-1966	Ankyrin repeat fragment	B16R	574	96	B18R	574	94	B16R	574	96
CMLV200	184119-184412 (98)	468	87	P21076	VACV	Ankyrin repeat fragment	B16R	574	88	B18R	574	87	B16R	574	87
CMLV201	184551-185615 (355)	1795	94	L22579	VARV	IFN-α/β binding, SP	B17R	354	94	B19R	353	89	B17R	351	91
CMLV202	185719-188067 (783)	4019	96	L22579	VARV	Ankyrin-repeat	B18R	787	96	B20R	127	94	B18R	795	89
CMLV203	188149-188916 (256)	1234	91	Y15035	CPXV	Kelch-like	B19R	70	90				B19R	557	91
CMLV204	189161-189583 (141)	663	92	Y15035	CPXV	Kelch-like	B20R	88	91				B19R	557	92
CMLV205	190059-191174 (372)	1930	97	L22579	VARV	Serpin, SPI-1	B21R	372	97	C12L	353	92	B20R	375	92
CMLV206	191360-191926 (189)	858	89	Y15035	CPXV	SP				C13L	65	85	B21R	190	89
										C14L	82	89			
CMLV207	192188-197794 (1869)	9302	94	L22579	VARV	B22R-like, TM	B22R	1897	94				B22R	1933	85
CMLV208	198241-199368 (376)	1857	93	Y11842	CPXV	Ankyrin-repeat				B23R	386	86	H2R	672	93
										B24R	150	93			
CMLV209	199581-201353 (591)	3032	95	L22579	VARV	Ankyrin-repeat	G1R	585	95	B25R	259	81	H3R	586	92
										B26R	103	66			
										B27R	113	93			
CMLV210	201444-202490 (349)	1910	99	U87837	CMLV	TNF receptor II (CrmB), SP	G2R	348	91	B28R	122	89	H4R	351	94
CMLV211	202620-203384 (255)	1262	96	P19063	VACV Lister	Chemokine binding, TM	G3R	253	94	B29R	244	95	H5R	255	96

Note. Shaded areas indicate length differences with CMLV (>10%). Thick line boxes indicate OPV ORFs fragmented in CMLV. Thin line boxes indicate CPXV sequences not available. NA indicates nonannotated and NN no name available.

[a] % ID refers to the percentage of amino acid identity in the BLASP2 analysis.

[b] Accession numbers are from the GenBank or SwissProt database.

[c] Virus abbreviations are as described in the text and as follows: ECTV, ectromelia virus; LSDV, lumpy skin disease virus; YMTV, yaba monkey tumor virus; and MYXV, myxoma virus.

[d] Function was deduced either from the degree of similarity to known genes or from the presence of Prosite signatures. SP, signal peptide prediction; TM transmembrane segment as predicted by Psort (http://psort.nibb.ac.jp) (*31*, *33*).

[e] Homologous ORF from the variola virus Bangladesh genome (Accession No. L22579).

[f] Homologous ORF from the vaccinia virus Copenhagen genome (Accession No. M35027).

[g] Homologous ORF from cowpox virus GRI-90 (Accession Nos. Y11842 and Y15035).

CMLV209 to CMLV211) are the present ITR regions. ITRs also include 1385 bases of CMLV004 and CMLV208. CMLV exhibits compact gene arrangement with few overlapping ORFs and contains no apparent introns or large regions of noncoding DNA. CMLV contains 211 putative gene-encoding proteins of 53–1869 amino acids, which are similar to previously described poxvirus genes (Fig. 1; Table 1). A 2.9-kb region contains CMLV185 to CMLV187, ORFs absent from other OPVs and similar to B22R-like genes from other chordopoxvirus genera (Table 1). The conserved central genomic region (ORFs CMLV017 to CMLV184) is collinear with VACV C9L to B8R and contains 172 genes oriented in both directions. In terminal genomic regions, genes are oriented largely toward the termini.

Nucleic acid biogenesis, virion structure, and virion assembly. CMLV contains all the conserved poxviral genes involved in basic replicative functions, including genes encoding the DNA polymerase, RNA polymerase subunits, mRNA transcription initiation, elongation and termination factors, and enzymes which direct posttranscriptional processing of viral mRNA (*19*) (Table 1). CMLV contains a complement of nucleotide metabolism genes similar to those found in other OPVs. These CMLV proteins potentially involved in nucleotide metabolism include homologues of thymidine kinase, thymidylate kinase, dUTP pyrophosphatase, and the large and small subunit of ribonucleotide reductase (Table 1).

CMLV encodes homologues of most OPV proteins known to be structural or involved in virion morphogenesis (Table 1). These include proteins present in the virion core, proteins present in the intracellular mature virus (IMV) and associated membranes, potential enzymes involved in protein modification, DNA packaging and redox activity, and proteins associated with the release of extracellular enveloped virions (EEV) (Table 1).

Host-related functions. CMLV contains genes which likely function in modulation or evasion of host immune responses, modulation or inhibition of host cell apoptosis, and other aspects of cell or tissue tropism. CMLV proteins potentially involved in immune evasion include homologues of a 35-kDa chemokine-binding protein (CMLV001 and CMLV211), tumor necrosis factor receptor II crmB (CMLV002 and CMLV210), complement binding protein (CMLV023), double-stranded RNA-dependent protein kinase inhibitors (CMLV032 and CMLV055),

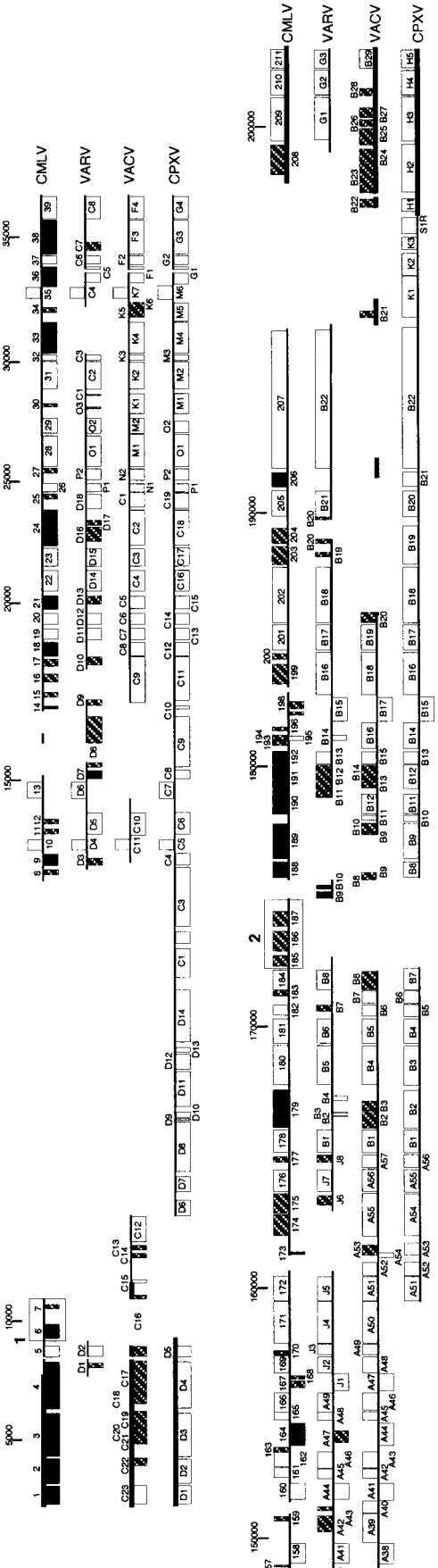

FIG. 1. Graphical representation of the left (A) and right (B) genomic coding regions of CMLV aligned with those of VARV, VACV, and CPXV. ORFs transcribed to the right are located above horizontal lines; ORFs transcribed to the left are below. Nucleotide positions are indicated above ORFs. Red color indicates differences between CMLV and VARV, including ORFs with significant length differences (>10%) and ORFs present in only one virus. Hatching indicates ORFs truncated in comparison with the longest OPV homologue (>10%). Thick lines at the ends of the figure indicate ITR. Box 1 marks a CMLV region translocated in comparison to CPXV. Box 2 marks a region with CMLV-specific B22R-like ORFs.

Stat1-inhibitor (CMLV097) (*22*), serine proteinase inhibitors (CMLV031, CMLV191, CMLV205), CD47-like protein (CMLV 158), IL-1/Toll-like receptor inhibitor (CMLV166) (*5*), interferon-γ receptor (CMLV184), and interferon-α/β binding protein (CMLV201) (*20*).

CMLV encodes homologues of poxviral proteins known to affect viral virulence or host range (Table 1). These include homologues of VACV C7L host range (CMLV019) (*25*), N1L virulence (CMLV026) (*16*), and A14.5L virulence (CMLV132) (*4*) proteins, myxoma (MYXV) M-T4 virulence protein (CMLV188) (*3*), and a homologue of rabbit fibroma virus (RFV) N1R/ectromelia virus p28 host range factor (CMLV013) (*28*) (Table 1). CMLV encodes a unique complement of 12 ankyrin repeat-containing proteins (Table 1), which are believed to be associated with functions involving viral host range and prevention of infection-induced apoptosis (*21*). It has been suggested that the specific complement of ankyrin genes found in a poxvirus significantly affects viral virulence or host range, and this may be the case for CMLV (*2, 29*).

Notably, CMLV006 is a homologue of CPXV S1R and human CGI-119 (Accession No. AF151877) (71% amino acid identity). The function of these viral and cellular S1R-like genes is unknown; however, they resemble the glutamate-binding subunit of the cellular *N*-methyl-D-apartate receptor (NMDA) and contain putative signal peptide and transmembrane domains. The glutamate-binding subunit is a component of a receptor complex capable of forming L-glutamate-activated ion channels (*17*). CMLV and CPXV are the only known poxviruses containing this gene.

Several CMLV ORFs with potential host range and virulence functions are truncated, or in some cases fragmented, compared to viral and cellular homologues. These include ORFs representing regions of the crmE TNF-R homologue (CMLV007), VACV K1L host range protein (CMLV030) (*25*), the semaphorin-like protein (CMLV159), guanylate kinase (CMLV177), VACV B7R virulence protein (CMLV183), and multiple regions of VACV B16R IL-1 binding protein (CMLV193, CMLV194, and CMLV196) (Table 1). These smaller ORFs which have been annotated here may or may not encode functional proteins.

Comparison of CMLV to other orthopoxviruses. CMLV is similar to other OPVs in overall genome structure and composition. Gene order, ORF length, and amino acid identity are most conserved in the region located between CMLV017 and CMLV184 (Table 1), which is collinear with VARV and VACV (average 96% amino acid identity to VACV) (Table 1). Exceptions include the lack in CMLV of homologues of VARV C1L, E7L, A26L, A27L, A39L, A42R, B2L, B3L, and B4L, and of homologues of VACV K6L, A25L, A40R, A52R, and A53R.

Genomic differences between CMLV and other OPVs are greater in terminal regions (CMLV001 to CMLV017

and CMLV184 to CMLV211) where ORF collinearity and average amino acid identity decreases (82% to VACV) due to small and large nucleotide insertions, deletions, and translocations. Notable differences are the deletion from the left end of the CMLV genome of a 14.5-kb region which is present in CPXV (Fig. 1) and the insertion of a 2.9-kb region (position 172,582 to 175,508), which is absent in VACV and CPXV (Fig. 1, Box 2). This region contains three small ORFs (CMLV185 to CMLV187) with limited similarity to regions of CMLV207 and its OPV homologues, VARV and CPXV B22R (up to 31% amino acid identity), but more closely resembles regions of B22R homologues found in other poxviral genera including capripox, yatapox, and leporipoxviruses (Table 1). These CMLV ORFs likely represent fragmented remains of a second B22R gene. This region also includes 510 bp, which are unique to CMLV (position 172,582 to 173,090) and VARV and corresponds to a region of CMLV185; however, VARV does not contain an ORF within this region (Table 1, Fig. 1).

CMLV is distinct but closely related to VARV and VACV. BLAST analysis of CMLV ORFs indicates that they are most similar to VACV and VARV, and in some cases to available CPXV ORFs (Table 1). Phylogenetic analysis of all CMLV proteins using maximum-likelihood and neighbor-joining distance methods indicates that CMLV is clearly separated (high bootstrap support) and nearly equidistant between VARV and VACV (data not shown). Nucleotide-based maximum-likelihood analysis of all genomic regions coding for CMLV ORFs also clearly separates CMLV from VARV and VACV (Fig. 2 and data not shown). This analysis on nucleotide sequences suggested that 59% of the 211 CMLV coding sequences were most similar to VARV, with the remainder being closer to VACV or CPXV. The phylogenetic distance between VARV and CMLV at the nucleotide level was consistently smaller than the distance between VARV and VACV, suggesting that CMLV is the closest known relative of VARV (Fig. 2).

Genomic differences, which include nucleotide insertions and deletions (1–4.8 kb), affect a number of CMLV and VARV genes involved in virulence and host range. CMLV contains 27 ORFs absent in VARV, including homologues of NMDA receptor-like protein (CMLV006), TNFR II crmE fragment (CMLV007), ankyrin repeat proteins (CMLV016, CMLV208), phospholipase D-like protein (CMLV033), lysophospholipase-like protein fragment (CMLV034), Schlafen-like protein (CMLV179), M-T4-like virulence protein (CMLV188), kelch-like proteins (CMLV174 and CMLV189), interleukin 1 receptor (IL-R) fragments (CMLV193, CMLV194, CMLV196), VACV K1L-like host range fragment (CLMV030), additional copies of CMLV001–CMLV003 found in the left ITR of CMLV, and 17 ORFs of unknown function (Table 1). Conversely, CMLV lacks homologues of VARV interleukin-18 binding protein (D7L), ankyrin repeat protein (D8L), ATI-like proteins (A26L and A27L), and ORFs of unknown function (A39L, A42L, B2L, B3L, B4L, B9R, and B10R). Thirty-eight CMLV ORFs are significantly different in length from corresponding ORFs in VARV (>10% difference) (Table 1). CMLV ORFs significantly shorter than those in VARV (19 ORFs) include homologues of VACV C10L (CMLV011 and CMLV012), semaphorin-like protein (CMLV159), ankyrin repeat host range protein (CMLV015 and CMLV017), and guanylate kinase (CMLV177). CMLV ORFs significantly longer (19 ORFs) than those in VARV include homologues of ankyrin repeat proteins (CMLV004), kelch-like proteins (CMLV038 and CMLV175), hydroxysteroid dehydrogenase (CMLV164), IMV membrane protein (CMLV126), serine-threonine kinase (CMLV190), SPI-2 serpin (CMLV191), and ORFs of unknown function (Table 1). Although some of these length differences may have no functional significance, others are likely to affect aspects of host range and may functionally differentiate CMLV from VARV.

CMLV ORF arrangement, content, and length suggest that it may have originated from a CPXV-like ancestor (Table 1 and Fig. 1) (*2, 29*). Similar to VARV and VACV, CMLV ORF arrangement is collinear with CPXV in the variable terminal regions except for the absence in CMLV of large genomic regions found on the left (D6L to C4L and C7R to C10) and right (K1R to K2R) ends of the CPXV genome (Fig. 1). A genomic translocation (CMLV006–CMLV007) (Fig. 1, Box 1) and insertion of B22R-like ORFs (CMLV185–CMLV187) also differentiate CMLV from CPXV. CMLV contains CPXV-like genes and genomic regions that are significantly different or absent in VARV or VACV, supporting the notion that CPXV contains the most complete OPV genome (Fig. 1; Table 1). Twenty-eight CMLV ORFs are significantly shorter (<10%) than the 19 homologous CPXV ORFs (Fig. 1, hatched boxes; Table 1) and thus may represent nonfunctional truncated proteins or gene fragments. These features suggest an overall reduction in coding capacity during adaptation of poxviruses to a more specific host range and are consistent with similar events in other organisms (*23*).

Conclusions. CMLV gene content and organization indicate a close structural and functional relationship to other OPVs, and phylogenetically CMLV may be the closest relative of VARV. Genes involved in basic replicative mechanisms including mRNA biogenesis, DNA replication, and virion structure and assembly are highly conserved. Major genomic differences between CMLV and other OPVs occur in terminal genomic regions affecting a large number of genes with likely functions involving virulence or host range. The differences clearly distinguish CMLV from other OPVs. CMLV genomic sequence provides a basis from which comparisons with other OPVs may be made, thus contributing to our understanding of the genetic basis of OPVs virulence and host range.

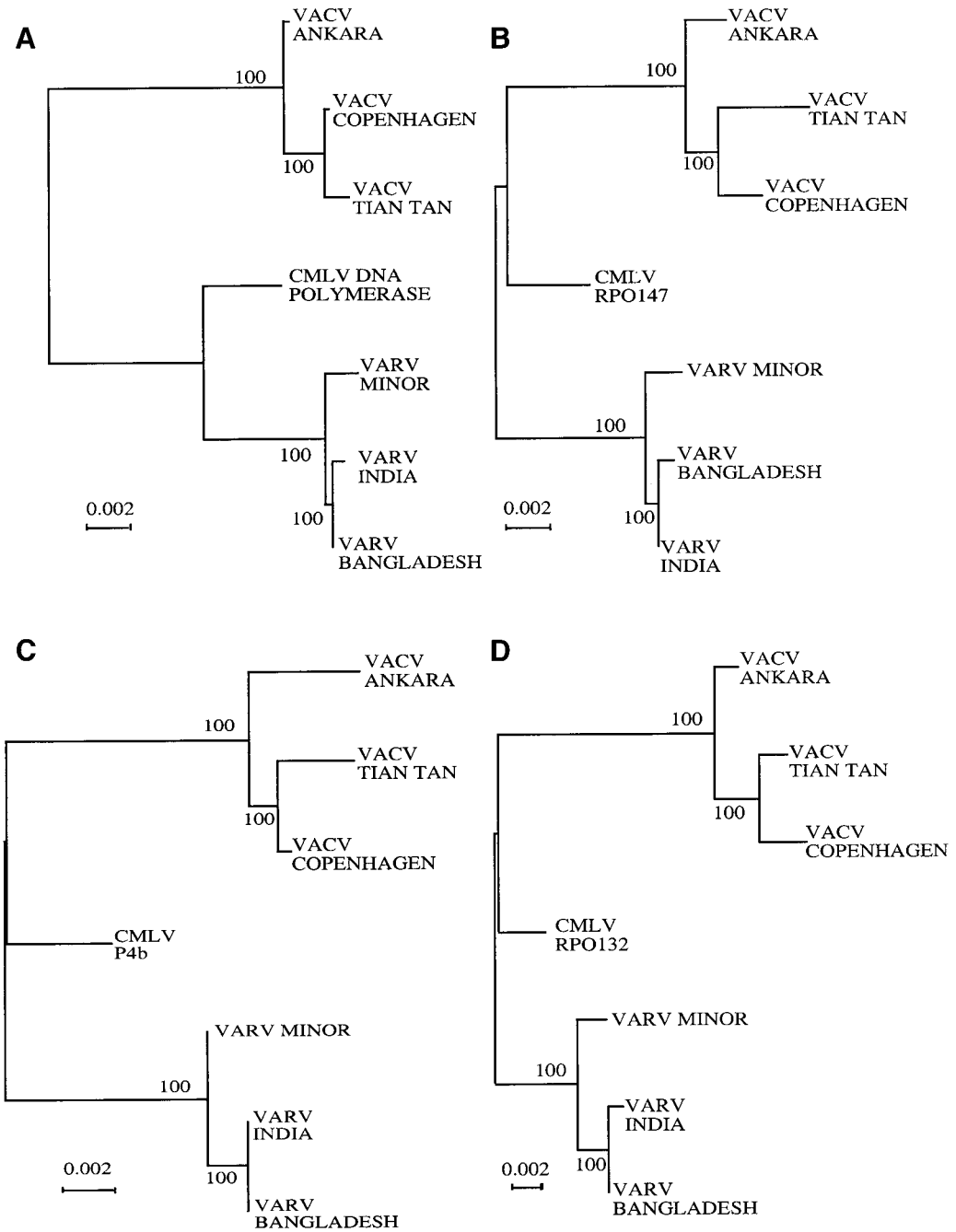

FIG. 2. Phylogenetic analysis of (A) DNA polymerase; (B) RPO147; (C) major coat protein P4b; and (D) RPO132. DNA sequences from complete genes were aligned with ClustalW. Unrooted trees were generated using maximum likelihood with the HKY model of substitution and 1000 bootstraps (*12*). Bootstrap values greater than 90% are in bold. Bar indicates nucleotide distances. Similar results were obtaining using neighbor joining analysis (data not shown).

Materials and Methods. CMLV DNA isolation, cloning, sequencing, and sequence analysis. CMLV genomic DNA was extracted from purified virions obtained from sheep kidney cells infected with the pathogenic M-96 strain (Mangistauskiy), which was isolated from sick camels in Mangistausskaya oblast, Kazakhstan. Virus was passaged once in 11-day-old chicken embryos and twice in sheep kidney cells. Viral DNA was extracted using standard isolation procedures (*35*). Random DNA fragments were obtained by incomplete enzymatic digestion with *Tsp*509 I endonuclease (New England Biolabs, Beverly, MA). DNA fragments of 1.0–6.0 kb were cloned into the dephosphorylated *Eco*RI site of pUC19 plasmids and grown in *Escherichia coli* DH10B cells (Gibco BRL, Gaithersburg, MD). Double-stranded pUC19 plasmids were purified using alkaline lysis according to

the manufacturer's instruction (Eppendorf 5 Prime, Boulder, CO). DNA templates were sequenced from both ends with M13 forward and reverse primers using dideoxy chain-terminator sequencing chemistries (*27*) and the Applied Biosystem PRISM 3700 automated DNA sequencer (PE Biosystems, Foster City, CA). Chromatogram traces were base-called with Phred (*10*), which also produced a quality file containing a predicted probability of error at each base position. The sequences were assembled with Phrap (*9*) and CAP3 (*14*) using quality files and default settings to produce a consensus sequence with some subsequent manual editing using the Consed sequence editor (*13*). Gap closure was achieved by primer walking of gap-spanning clones and sequencing of PCR products. The final DNA consensus sequence represented on average sevenfold redundancy at each base position and the estimated error rate was 0.13 base per 10 kb. ORFs longer than 30 amino acids with a methionine start codon were evaluated for coding potential using the Glimmer (*26*) computer program. Genomic comparisons were done with Sim2, Blast, and Smith–Waterman alignments (*1, 6, 24*). Multiple alignments and phylogenetic analysis of annotated ORFs were done with Phylo_Win (*11, 12*) and Tree-Puzzle (*32*). Due to the high percentage amino acid identity in ORFs contained within the conserved central region (>97%) and to maximize the number of informative changes, phylogenetic comparisons among OPV ORFs were also done at the DNA level.

CMLV was compared to variola major virus strain Bangladesh-1975 (GenBank Accession No. L22579) and vaccinia virus strain Copenhagen (GenBank Accession No. M35027) and to available cowpox virus sequences from strain GRI-90 (GenBank Accession Nos. Y11842 and X94355). All references to VARV, VACV, and CPXV refer to these strains unless indicated otherwise.

Nucleotide sequence accession number. The CMLV genome sequence was assigned GenBank Accession No. AF438165.

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