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Completion of full length genome sequence of novel avian paramyxovirus strain APMV/Shimane67 isolated from migratory wild geese in Japan

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ABSTRACT. The nucleotide sequences of nucleocapsid protein (N); phosphoprotein (P); matrix protein (M); hemagglutinin-neuraminidase (HN); and large polymerase protein (L) genes, 3′-end leader, 5′-end trailer and intergenic regions of the avian paramyxovirus (APMV) strain goose/Shimane/67/2000 (APMV/Shimane67) were determined. Together with previously reported data on fusion protein (F) gene sequence [46], the determination of the genome sequence of APMV/Shimane67 has been completed in this study. The genome of APMV/Shimane67 comprised 16,146 nucleotides in length and contains six genes in the order of 3′-N-P-M-F-HN-L-5′. The features of the APMV/Shimane67 genome (*e.g.*, nucleotide length of whole genome and each of the six genes, and predicted amino acid length of each of the six genes) were distinct from those of other APMV serotypes. Phylogenetic analysis indicated that although APMV/Shimane67 was grouped with APMV-1, -9 and -12, the evolutionary distance between APMV/Shimane67 and these viruses was longer than that observed between intra-serotype viruses. These results show that the genome sequence of APMV/Shimane67 contains specific characteristics and is distinguishable from other types of APMV.

KEY WORDS: APMV, avian paramyxovirus, avulavirus, complete genome sequence, phylogenetic tree

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Avian paramyxovirus (APMV) is a member of the genus *Avulavirus* in the subfamily *Paramyxovirinae* of the family *Paramyxoviridae*. APMV comprises nine known serotypes (APMV-1–APMV-9), based on hemagglutination inhibition and neuraminidase inhibition assay [23]. APMV-1, also called Newcastle disease virus (NDV), is one of the most important pathogens for poultry, because the infection of the virulent type of APMV-1 (velogenic) is highly lethal. Thus, APMV-1 is the most characterized virus among all other APMV serotypes. In addition to APMV-1, the association of APMV-2, -3, -6 and -7 with poultry disease was reported [23]. APMV-2 causes respiratory disease in chickens and turkeys, whereas APMV-3, -6 and -7 cause respiratory disease or disorder in egg production of turkeys. Alternatively, APMV-4, -5, -8 and -9 have not been reported to infect poultry; APMV-4, -8 and -9 were mainly isolated from waterfowl, such as ducks and geese, whereas APMV-5 was isolated from budgerigar and was associated with diarrhea and high mortality. Recently, new types of APMV have been isolated from rockhopper penguins (APVM-10), common snipes (APVM-11) and wigeon (APVM-12) [3, 22, 41]. Intracerebral pathogenicity index test using one-day-old chicks suggested that APMV-10 and -12 revealed little or no virulence in chickens, resembling the low or non-virulent (lentogenic) NDV.

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APMVs are pleomorphic, enveloped viruses containing a negative-sense, single-stranded RNA genome. The genome size of APMV ranged from approximately 14,900 to 17,260 nucleotides (nt) long [3, 22, 35, 41]. The exact value for genome length is divisible by six (rule of six), which is the basic feature for efficient replication of viral genome among members of the subfamily *Paramyxovirinae* [5]. APMV genome contains nucleocapsid protein (N); phosphoprotein (P); matrix protein (M); fusion protein (F); hemagglutinin-neuraminidase (HN); and large polymerase protein (L) genes, similar to other members of the family Paramyxoviridae [35]. APMV-6 contains an additional gene that encodes the small hydrophobic protein (SH) [6]. Each gene encodes a single viral structural protein with the exception of P gene. mRNA transcribed from P gene has a potential to translate an additional nonstructural protein, termed V protein [38]. V proteins are translated from mRNA containing one guanine residue insertions, respectively, at the RNA editing site. The 3′- and 5′-ends of each gene possess the non-coding sequences, known as gene-start (GS) and gene-end (GE), which are conserved among similar types of APMVs and function as transcriptional promoters and terminators. The non-coding region boundaries between GE and GS are termed as intergenic sequence (IGS) that comprised various nucleotides. At the 3′- and 5′-ends of the APMV genome, non-coding leader (Le) and trailer (Tr) sequences exist and act as promoters for replication of genomic and antigenomic RNAs [21].

In the past, most of the available complete genome sequence of APMVs had been from APMV-1. However, recently, complete genome sequences from prototype of other APMV serotypes have been published [17, 29, 30, 36, 37, 40, 44, 45]. Moreover, the number of reports on complete

genome sequences from APMVs other than APMV-1 has been increasing in recent years [1, 3, 4, 6, 15, 18, 22, 33, 39, 41, 42]. In these studies, the phylogenetic trees that were constructed using whole genome or individual genes revealed the correlation between genetic classification and serotyping.

During our continuous surveillance for the presence of avian influenza A viruses and APMVs in wild birds, we isolated APMV/Shimane67 from the feces of geese collected in 2000 [46]. The F gene of APMV/Shimane67 shared 42.9–62.7% and 28.9–67.3% identities at nt and deduced aa levels, respectively, with those of other APMVs. The deduced aa sequence at the F protein cleavage site of APMV/Shimane67 was QVRENR/LVG, which resembles the motif of lentogenic NDV. Phylogenetic analysis revealed that APMV/Shimane67 had relationship with NDV, APMV-9 and APMV-12, but was distinct from those APMVs. These results and serological analysis demonstrated the possibility that APMV/Shimane67 was distinct from the already existing APMVs. In this study, we completed the determination of whole genome sequences of APMV/Shimane67 to further understand its molecular characteristics and compare with other APMVs genome.

MATERIALS AND METHODS

Extraction of viral RNA, RT-PCR and nucleotide sequencing: The isolation and characterization of APMV/Shimane/67 have been previously described [46]. Viral genomic RNA was extracted from infected allantoic fluid using QIAamp Viral RNA Mini Kit (QIAGEN, Tokyo, Japan), according to the manufacturer's instructions manual. The cDNA of the APMV/Shimane67 genome was synthesized using Primescript Reverse Transcriptase (TaKaRa Bio, Otsu, Japan) and amplified using SapphireAmp Fast PCR Master Mix (TaKaRa Bio) and pairs of oligonucleotide primers. The sequences of oligonucleotide primers used in this study are available upon request. After purification from agarose gel using QIAquick Gel Extraction Kit (QIAGEN), PCR products were sequenced using BigDye Terminater v3.1 Sequencing Kit (Applied Biosystems, Foster City, CA, U.S.A.) and analyzed using the 3130 xl Genetic Analyzer (Applied Biosystems).

Determination of 3′- and 5′-ends of the viral genome sequence: The 3′-end of the viral genome (Le region) sequence of APMV/Shimane67 was determined by a method previously described [43]. To determine the 5′-end of the viral genome (Tr region), cDNA from the 5′-end of the genome was amplified using SMART PCR cDNA Synthesis Kit (Clontech, Palo Alto, CA, U.S.A.) and virus-specific primers, according to the manufacturer's instructions manual. The amplified cDNA was used for determining the nucleotide sequence as described above.

Analysis of nucleotide and deduced amino acid sequences: The molecular weight (MW) and isoelectric point (pI) of protein were calculated by Compute pI/Mw tool (http://web.expasy.org/compute_pi/) [11]. The transmembrane region of HN protein was predicted by the SOSUI system (http://harrier.nagahama-i-bio.ac.jp/sosui/sosui_submit.html) [13]. The alignment of nt and deduced amino acid (aa) sequences and calculation of evolutionary distance in nt substitutions per site were conducted using the Clustal X program [19]. Phylogenetic trees were generated by Neighbor-Joining method [34] with 1,000 bootstraps using Clustal X program and then visualized with NJPlot [31]. The nucleotide sequence data reported in this study have been deposited in the DDBJ database under the accession number LC041132. Whole genome sequences of other APMVs for comparison with APMV/Shimane67 were from the following sources (abbreviation and accession number): APMV-1/goose/Alaska/415/91 (APMV-1/415, AB524405); APMV-1 strain LaSota (APMV-1/LaSota, AF077761); APMV-1 strain SF02 (APMV-1/SF02, AF473851); APMV-2/chicken/California/Yucaipa/56 (APMV-2/Yukaipa, EU338414); APMV-2/finch/Northern Ireland/Bangor/73 (APMV-2/Bangor, HM159995); APMV-3/parakeet/Netherland/449/75 (APMV-3/NLD, EU403085); APMV-3/turkey/Wisconsin/68 (APMV-3/WI, EU782025); APMV-4/duck/Hong Kong/D3/75 (APMV-4/HK/D3, FJ177514); APMV-4/KR/YJ/06 (APMV-4/KR/YJ, EU877976); APMV-5/budgerigar/Kunitachi/74 (APMV-5/Kunitachi, GU206351); APMV-6/duck/Hong Kong/18/199/77 (APMV-6/HK/D199, EU622637); APMV-6/duck/Italy/4524-2/07 (APMV-6/ITA/4524-2, GQ406232); APMV-7/dove/Tennessee/4/75 (APMV-7/TN, FJ231524); APMV-8/goose/Delaware/1053/76 (APMV-8/DE, FJ215863); APMV-8/pintail/Wakuya/20/78 (APMV-8/Wakuya, FJ215864); APMV-9/duck/New York/22/78 (APMV-9/NY, EU910942); APMV-10/penguin/Falkland islands/324/2007 (APMV-10/FLK, HM147142); APMV-11/common snipe/France/100212/2010 (APMV-11/FRA, JQ886184); and APMV-12/wigeon/Italy/3920-1/2005 (APMV-12/ITA/3920-1, KC333050).

RESULTS

Genomic features of APMV/Shimane67: The genome characteristics of APMV/Shimane67 and some other APMVs are summarized in Table 1. The genome of APMV/Shimane67 comprised 16,146 nt that was slightly AU-rich (A 25.9%, C 22.1%, G 20.5% and U 31.5%). The genome of APMV/Shimane67 contained six viral protein genes in the order 3′-N-P-M-F-HN-L-5′, which was identical to other APMVs, except for APMV-6. The SH protein gene existing in the APMV-6 genome was not present in the APMV/Shimane67 genome. The full genome sequence of APMV/Shimane67 had the highest nt identity with APMV-12/ITA/3920-1 (62.2%), intermediate nt identities with APMV-1 and -9 (53.7%–55.0%), and lower nt identities with APMV-2, -3, -4, -5, -6, -7, -8, -10 and -11 (41.9%–44.8%) (Table 2).

The Le sequence of APMV/Shimane67 was 55 nt in length, which was the same as that of other APMVs. In contrast, the Tr sequence of APMV/Shimane67 was 776 nt in length, which was the longest in the Avulavirus genus. Marcos *et al*. [21] reported that the first 18 nt of the Le and Tr regions and thrice-repeated motif (3′-NNNNGC-5′) at 73–90 nt of the APMV-1 genomic and antigenomic RNA func-

Table 1. Comparison of nucleotide and amino acid length between APMV/Shimane67, APMV-1, -9 and-12

Gene		APMV/Shimane67	APMV-1/LaSota	APMV-1/415	APMV-1/SF02	APMV-9/NY	APMV-12/ITA/3920-1
Full genome (nt)		16,146	15,186	15,198	15,192	15,438	15,132
Leader (nt)		55	55	55	55	55	55
Trailer (nt)		776	114	114	114	47	60
N	3'-noncoding (nt)	60	66	66	66	66	66
	ORF (nt)	1,482	1,470	1,470	1,470	1,470	1,482
	5'-noncoding (nt)	179	210	210	217	192	162
	Total (nt)	1,721	1,746	1,746	1,753	1,728	1,710
	Amino acid	493	489	489	489	489	493
N-P intergenic (nt)		14	2	2	1	19	7
P	3'-noncoding (nt)	95	83	83	83	113	95
	ORF (nt)	1,194	1,188	1,200	1,188	1,260	1,218
	5'-noncoding (nt)	223	180	180	180	248	190
	Total (nt)	1,512	1,451	1,463	1,451	1,621	1,503
	Amino acid	397	395	399	395	419	405
P-M intergenic (nt)		1	1	1	1	6	3
M	3'-noncoding (nt)	34	34	34	34	34	34
	ORF (nt)	1,101	1,095	1,095	1,095	1,095	1,095
	5'-noncoding (nt)	200	112	112	112	161	151
	Total (nt)	1,335	1,241	1,241	1,241	1,290	1,280
	Amino acid	366	364	364	364	364	364
M-F intergenic (nt)		2	1	1	1	30	11
F [a)]	3'-noncoding (nt)	45	46	46	46	55	52
	ORF (nt)	1,638	1,662	1,662	1,662	1,656	1,641
	5'-noncoding (nt)	175	84	84	84	67	88
	Total (nt)	1,858	1,792	1,792	1,792	1,778	1,781
	Amino acid	545	553	553	553	551	546
F-HN intergenic (nt)		14	31	31	31	22	61
HN	3'-noncoding (nt)	92	91	91	91	97	91
	ORF (nt)	1,833	1,734	1,851	1,716	1,740	1,845
	5'-noncoding (nt)	145	177	59	195	293	136
	Total (nt)	2,070	2,002	2,001	2,002	2,130	2,072
	Amino acid	610	577	616	571	579	614
HN-L intergenic (nt)		25	47	48	47	0	42
L	3'-noncoding (nt)	13	11	11	11	11	11
	ORF (nt)	6,600	6,615	6,615	6,615	6,633	6,609
	5'-noncoding (nt)	150	77	77	77	69	106
	Total (nt)	6,763	6,703	6,703	6,703	6,713	6,727
	Amino acid	2,201	2,204	2,204	2,204	2,210	2,202

a) The nucleotide sequence of APMV/Shimane67 F gene was reported by Yamamoto *et al*. [46].

tioned as promoters of viral genome replication. Moreover, similar sequence motifs were reported in APMV-2 genome [40]. Fourteen nt of the Le and Tr sequences, with the exception of the 9th nt from the 3′- and 5′-terminal of the genome, were complementary in the APMV/Shimane67 genome (Fig. 1a). Twelve nt of the 3′ Le and 11 nt of the 5′ Tr of the APMV/Shimane67 genome were relatively conserved with those of other APMVs and were completely matched with APMV-1 (Fig. 1b and 1c). The three times repeated motifs, 3′-GGUGGC-5′, 3′-ACAAGC-5′ and 3′-UCAGGC-5′, and 3′-AUUUCC-5′, 3′-UCCAGC-5′ and 3′-UUCAGC-5′ were found in 73–90 nt from the 3′-terminus of the genome and antigenome of APMV/Shimane67, respectively. The GS signal of APMV/Shimane67 was well preserved, and its consensus sequence was "ACGGGCAGAA" (Fig. 2a). In contrast, the preservation of GE signal sequences of APMV/Shimane67 was relatively low. The consensus sequence of the APMV/Shimane67 GE signal was $TTAAGA_{5–6}$, whereas that of the M gene diverged at positions 1 (A), 2 (A), 3 (T) and 5 (T). The HN gene had one nt difference at the fifth position (T); whereas the L gene also contained two nt differences at the second (A) and third (G) positions. APMV/Shimane67 GS and GE sequences had similarities with those of APMV-1, -9 and -12 (Fig. 2b). The IGS of APMV/Shimane67 varied in nucleotide sequence and length (Fig. 2a). The IGS length of NP-P, P-M, M-F, F-HN and HN-L junctions was 14 nt, one nt, two nt, 14 nt and 25 nt, respectively. The last nt at IGS of APMV/Shimane67 was T at all times, and this could act with the GS sequences to initiate mRNA transcription.

N gene and N protein: The N gene of APMV/Shimane67 was 1,721 nt in length and contained an open reading frame (ORF) that encoded 493 aa, with MW of 54,045 Da and

Table 2. Nucleotide (nt) and deduced amino acid (aa) sequence identities between APMV/Shimane67 and other APMVs (%)

Virus	Full genome	N		P		V	M		HN		L	
		nt	aa	nt	aa	aa	nt	aa	nt	aa	nt	aa
APMV-1/LaSota	55.0	58.6	57.9	53.8	42.3	38.5	56.7	53.3	56.9	55.1	56.6	54.4
APMV-1/415	55.0	59.0	58.3	53.6	40.5	34.9	56.5	53.9	56.1	55.3	56.7	55.6
APMV-1/SF02	54.6	58.4	56.4	52.3	42.8	37.6	56.3	52.8	56.1	55.1	57.0	55.5
APMV-2/Yucaipa	44.8	47.9	39.8	39.6	24.2	25.2	43.1	27.8	44.4	34.1	46.3	37.5
APMV-2/Bangor	44.6	47.0	39.3	40.1	22.9	24.4	43.8	27.8	43.3	34.5	46.4	32.0
APMV-3/NLD	41.9	48.5	38.7	41.0	23.7	20.5	40.2	25.2	43.8	37.2	43.6	33.2
APMV-3/WI	41.9	47.7	38.6	40.0	24.2	19.3	41.1	25.5	45.4	36.9	43.8	33.4
APMV-4/HK/D3	42.3	48.8	37.0	40.5	21.7	24.1	39.4	24.5	44.2	36.5	43.4	32.5
APMV-4/KR/YJ	42.3	48.4	37.0	39.7	22.5	25.1	40.8	24.4	44.0	36.3	43.1	32.6
APMV-5/Kunitachi	43.5	48.7	36.9	40.9	22.9	25.2	43.4	29.9	43.4	33.8	46.4	37.5
APMV-6/HK/D199	42.9	49.1	40.6	41.3	22.4	26.7	43.6	29.6	44.3	31.9	47.5	38.4
APMV-6/ITA/4524-2	43.4	49.0	40.2	42.9	22.7	26.1	43.6	30.4	42.9	30.7	47.6	38.5
APMV-7/TN	44.8	49.1	39.1	38.7	20.0	26.7	44.2	30.3	45.2	37.9	47.5	40.3
APMV-8/DE	44.8	47.8	39.2	40.6	24.9	27.2	42.4	29.5	43.8	34.2	47.1	39.1
APMV-8/Wakuya	44.7	47.7	39.4	40.6	25.2	26.7	42.0	28.7	43.4	33.8	47.3	39.1
APMV-9/NY	53.7	58.6	56.0	51.2	40.4	32.1	55.3	50.8	54.5	54.7	56.0	53.4
APMV-10/FLK	44.6	47.9	40.4	39.0	23.3	23.3	42.0	28.1	43.4	35.2	46.4	38.0
APMV-11/FRA	43.0	48.1	40.2	40.1	23.7	26.6	38.9	25.8	45.5	37.4	47.0	40.1
APMV/12/ ITA/3920-1	62.2	67.6	74.4	61.4	51.3	45.2	65.1	73.6	61.3	60.5	63.1	65.5

a

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Leader   3'- UGGUUUGUCUCUAAGACAUUCCAUGGAAUUUUAUGCUUAAUUAUUA
                ||||||||  |||||   |   |    |   | | |         | |||
Trailer  5'- ACCAAACAAAGAUUUGGAGAACAAAAUAAUACCGACUUGUCAUAUA
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b

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                          3'-end
APMV/Shimane67            UGGUUUGUCUCUAAGACAUUCCAUGGAAUUUUAUGCUUAAUUAUUAACUUGAUCG
NDV/LaSota                ............U..G..C..A...CU.....CC....CC.CG.......C.G..
APMV-2/Yucaipa            ........UC..U.UC.....GU..C.U..AG.AU..AUU..GG..U...AGG.A
APMV-3/NLD                ..A.......U.U.AUU..GAACAAUC..CAGCG...A.U.AGGCGAU.AU..AA
APMV-4/HK/D3              ..C...U...UCUUAUUU.C.GUCUUCGGAAA..UU.CCU.GGGACC.GAC.G.A
APMV-5/Kunitachi          ........UC..U.CG.G...GUA...U...A.AUU.AUCGC.C..U.ACU..A.
APMV-6/HK/D199            ........UC..UU.GU..A.G.ACCCC.GAA.....CUCGCGAAC.U..UGG.A
APMV-7/TN                 ........UC..G.CGU....GUCAC.U...G.AAA...U..U...UUGAA.G.A
APMV-8/DE                 ........UC..U.CGUUC.GGU..CCC..GA.AUU.AUU..G...G...UG.AA
APMV-9/NY                 ........U...UUA......U...C.....CUG....C..CG..G...GA.G.A
APMV-11/FRA               .......GUC...GUGUGAC.GC..C.C.C.ACAU...UU...A..G...C...A
APMV-12/ITA/3920-1        ...AA.....UAG.....AA.....C...A..UCU..ACU....AU....U.U.U
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c

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                          5'-end
APMV/Shimane67            ACCAAACAAAGAUUUGGAGAACAAAAUAAUACCGACUUGUCAUAUAUCGACAUAU
NDV/LaSota                ..................U...UG.CGAG.CUA.ACUCAAGA...AU.GUG.GC.A
APMV-2/Yucaipa            ....G...UG..A.A.C.A.G...CG...CU.A.UGA.UAUGC.GUG.UUAUAU.
APMV-3/NLD                ..UAA.....A.G..AU.AUGAUUUU...AU.A.UC..CAACCU.U.GUU.U.UG
APMV-4/HK/D3              ..UAA.U...AGACAUA-------------------------------------
APMV-5/Kunitachi          .........G....AC....GUU..G.U.AUUU..A.CA.AU.U.UGUUUACACC
APMV-6/HK/D199            .........G..AA.CAUA.G.UUUU.G.CUAU..UCCU.GUCGAC.UUC..GG.
APMV-7/TN                 .........G..C.GCAUA.G..GUG....A..UUUUAAUAA.A.AUAACUUUCG
APMV-8/DE                 ....G.A.UG..A.ACAG..AAU.CGGU.AUUUUCAC.AAU.C.AU.UUUU..UA
APMV-9/NY                 ............AA.U.UA.GAU.CG.U.A.GACCGAA..AGC.AC.G-------
APMV-11/FRA               .......CAG....A.A.U.GG.GU.CU.AUAA.UUGA..A....UGU.UUUGUC
APMV-12/ITA/3920-1        ..................UGG.UU.CG.CU.UAGU.G..UCTTCUCGGGUUAC.U.
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Fig. 1. Nucleotide sequences of the 3′-leader and 5′-trailer regions of the APMV/Shimane67 genome. (a) Complementary nucleotide pairs are indicated by the vertical bars. Alignment of the (b) 3′-leader and (c) 5′-trailer regions of the sequences from APMV/Shimane67 and other APMVs. Identical nucleotides with APMV/Shimane67 and gaps are shown by dots and dashes, respectively. Nucleotide sequences are shown in genomic sense.

a

	Gene start	Gene stop	Intergenic
NP	UGCCCGUCUU	AAUUCUUUUUU	GUUUGACUGUCUUA
P	UGCCCGUCUU	AAUUCUUUUUU	A
M	UGCCCGUCUU	UUAUAUUUUUU	CA
F	UGCCCGUCUU	AAUUCUUUUU	CUAGGCCUAAGUUA
HN	UGCCCGUCUU	AAUUAUUUUUU	ACUGUUACAAUCACUGUUUUUCGGA
L	UCCCCGUCUU	AUCUCUUUUUU	
Consensus	UGCCCGUCUU	AAUUCU$_{5-6}$	

b

	Gene start	Gene stop
APMV/Shimane67	UGCCCGUCUU	AAUUCU$_{5-6}$
APMV-1/LaSota	UGCCCAUCUU	AAUUCU$_{6}$
APMV-2/Yucaipa	CCCCCGCU(G/U)U	AAUUAU$_{6}$
APMV-3/NLD	UCCUCGCCUU	AAUUAU$_{5-7}$
APMV-4/HK/D3	CACCCCUUCC	AAUUAAU$_{5}$
APMV-5/Kunitachi	CCCCCUUNNN	AAU(A/U)NU$_{5-7}$
APMV-6/HK/D199	CUC$_{5-6}$UUC	AAUN$_{1-2}$AU$_{4-6}$
APMV-7/TN	CUCCCNCUNN	AAUNNUUUNU$_{1-3}$
APMV-8/DE	CCCCCGC(G/U)N	AAUUNU$_{6}$
APMV-9/NY	UGCCCAUCUU	AAUNA$_{6}$
APMV-10/FLK	CCCCGCUGGG	AAUUCU$_{6}$
APMV-11/FRA	CGCCCGCUUC	AAUUAU$_{6-7}$
APMV-12/ITA/3920-1	UGCCCGUCUU	AAUUCU$_{6-8}$

Fig. 2. (a) The gene-start, gene-end and intergenic sequences of APMV/Shimane67. (b) Alignment of the consensus gene-start and gene-end sequences from APMV/Shimane67 and other APMVs. Nucleotide sequence is shown in genomic sense.

pI of 5.29. The nt sequence of APMV/Shimane67 N gene ORF was 67.6% identical with APMV-12/ITA/3920-1, 58.4%–59.0% identical with APMV-1 and -9, and 47.0%–49.1% identical with the rest of the APMVs. The predicted aa sequence of APMV/Shimane67 N gene demonstrated 74.4%, 56.0%–58.3% and 36.9%–40.6% identities with APMV-12/ITA/3920-1, APMV-1 and -9 and the rest of the APMVs, respectively (Table 2). There are three highly conserved regions [region 1, QXW(I,V)XXXK(A,C)XT; region 2, FXXT(I,L)(R,K)Φ(G,A)(L,I,V)XT; and region 3, FXXXXYPXXΦSΦAMG] (where X represents any amino acid and Φ represents an aromatic amino acid and, either of the residues in parentheses can be present at that position) in the central domain of the N protein of the subfamily *Paramyxovirinae* [25]. Among these regions, region 3 is particularly important, because this region is thought to be involved in the N–N protein self-assembly [26]. The N protein of APMV/Shimane67 contained aa sequences similar to these motifs: ^{171}QIWVTLAKAMT^{181}, ^{266}FFLTLKYGINT^{277} and ^{322}FAPAEYSLMYSFSMG^{336} (Fig. 3). The 7th (P) and 13th (A) aa of region 3 motif were replaced with ^{328}S and ^{334}S, respectively. The aa sequence of region 2 was completely identical among APMV/Shimane67, APMV-1 and APMV-12.

P gene and P/V proteins: The P gene of APMV/Shimane67 genome was 1,512 nt long. The P gene of avulaviruses encodes two proteins, P and V [35, 38]. The P protein is expressed from mRNA that is directly transcribed from the genomic RNA. The nt identities of APMV/Shimane67 P protein ORF were 61.4% with APMV-12/ITA/3920-1, 51.2%–53.8% with APMV-1 and -9, and 38.7%–42.9% with the other remaining APMVs (Table 2). The V proteins is produced through mRNA that contains insertion of one non-template G residues at the RNA editing site. The nt at positions 436–444 of the P gene ORF, 5′-AAAAAAGGG-3′ (mRNA sense), was predicted as the RNA editing site of APMV/Shimane67 (Fig. 4a). This nt sequence was completely identical with that of APMV-5/Kunitachi, APMV-6/HK/D199, APMV-7/TN, APMV-9/NY and APMV-12/ITA/3920-1. The deduced aa lengths of P and V proteins were 397 aa (MW 41,992 Da, pI 6.22) and 241 aa (MW 25,929 Da, pI 4.80), respectively. These two proteins shared the N-terminal 148 aa. The aa identities in P and V proteins between APMV/Shimane67 and other APMVs were comparatively low, ranging from 20.0% (APMV-7/TN) to 51.3% (APMV-12/ITA/3920-1) and 19.3% (APMV-3/WI) to 45.2% (APMV-12/ITA/3920-1), respectively (Table 2). Seven cysteine residues (^{198}C, ^{202}C, ^{214}C, ^{216}C, ^{219}C, ^{223}C and ^{226}C) and the ^{179}HRRE^{182} and ^{197}WCNP^{200} motifs were conserved at the C-terminus of APMV/Shimane67 V protein as well as in other APMVs (Fig. 4b).

M gene and M protein: The M gene of APMV/Shimane67 contained 1,335 nt and encoded a protein with 366 aa with MW of 39,902 Da and pI of 9.63. The ORF and predicted aa sequence of APMV/Shimane67 M gene had identities in 65.1% and 73.6% with APMV-12/ITA/3920-1, 55.3%–56.7% and 50.8%–53.9% with APMV-1 and -9, and 38.9%–44.2% and 24.4%–30.4% with the remaining APMVs, respectively (Table 2). There are two functional aa sequences one is nuclear localization signal (NLS), and the other is the late domain in the M protein of APMV-1

```
                        Regin 1      Region 2       Region 3
                                           V
                         V    C        LK AI
Motif                 QXWIXXXKAXT  FXXTIRΦGLXT  FXXXXYPXXΦSΦAMG
                      171     181  267     277  322          336
APMV/Shimane67        QIWVTLAKAMT  FFLTLKYGINT  FAPAEYSLMYSFSMG
APMV-1/LaSota         .V...V.....  ...........  ......AQL...A..
APMV-2/Yucaipa        ...IAAI.S..  .....RF..G.  ....NF.TL..YA..
APMV-3/NLD            ...ILV.....  .LT..R...G.  ...GN...L..YA..
APMV-4/HK/D3          ...ILV.....  .LT..R...G.  ...GNFPH...YA..
APMV-5/Kunitachi      ....SAM.S..  .....RF..G.  ...GN.P.I..YA..
APMV-6/HK/D199        ....AAM.S..  .....RF..G.  ...GN.P....YA..
APMV-7/TN             .A..AAI.S..  .....RF.VG.  ....N.T.L..YA..
APMV-8/DE             ....SAI.S..  ......F.VG.  ....N..T...YA..
APMV-9/NY             .V..TV..S..  ......F.VG.  ....E.AQL..YA..
APMV-10/FLK           ...IAAI.C..  .....RF..G.  ....N..TL..YA.D
APMV-11/FRA           ....AVI.S..  .....RF.LG.  ...SN...L..YA..
APMV-12/ITA/3920-1    .L.........  ...........  .....FA.....A..
```

Fig. 3. Alignment of the conserved amino acid motif regions of APMV N proteins. The motifs are shown at the upper lines (X and Φ represent any amino acid and aromatic amino acid, respectively). Identical amino acids between APMV/Shimane67 and other APMVs are shown by dots. Numbers indicate the amino acid positions of the APMV/Shimane67 N protein.

a

```
                      2331      2334
APMV/Shimane67        AAAAAAGGGCAGAT
APMV-1/LaSota         T.........CC..
APMV-2/Yucaipa        T........G.AGG
APMV-3/NLD            TTT......GGCCC
APMV-4/HK/D3          TTT......GG.CC
APMV-5/Kunitachi      ..........CC.G
APMV-6/HK/D199        .........G.A.G
APMV-7/TN             .........TTAG.
APMV-8/DE             T.........CCTC
APMV-9/NY             ..........TC.A
APMV-10/FLK           T..........CTG
APMV-11/FRA           .G..GG...TC...
APMV-12/ITA/3920-1    .........ATC..
```

b

```
                      177                    +   +           + +  +   +  + 230
APMV/Shimane67        PGHRREHSISWSTE--GIITTSWCNPDCAPITSTPQQFACRCGKCPKFCRLCSRDV
APMV-1/LaSota         ..........TMG--.VT.I.....S.S..KAE.R.YP.I..S..AT....AS.D
APMV-2/Yucaipa        ......Y.FISRDG--RLEV.....V.S..R E.RREK.T..T..ES.I..RQPN
APMV-3/NLD            S.....Y.....PGSGTFQ.ET....A.S.V.AF.K.YK.A RQ..R..D..FNPA
APMV-4/HK/D3          FP....MAIVTDKATGD.ELVE....G.TAVRIE.TRLD.V..H..TI.S..MY.D
APMV-5/Kunitachi      ......Y.LFF.DG--RCSI.E....T.R...AI.SVQR.T..E..RR.SM.WN.S
APMV-6/HK/D199        RR....F....RHG--OC.LAE....V...V.PE.RT.K.I..R..RV.IN.RN.S
APMV-7/TN             ......Y.FA..AGTNKF.VS.....T....RPY.TVER....N.....PG.QSAL
APMV-8/DE             ......Y.FTTFHG--TTRVI.....Q.T..RAR.IYDE....E..TT.IM.RD.K
APMV-9/NY             G.......MV.NAN--..V.I.....V.S.V.YE.RE.T.S..S..TE....AGSH
APMV-10/FLK           ......YS.INEHG--STLVE.....N.T..RAY.RREK.I.RR..TT.I..RD.N
APMV-11/FRA           V.....YILRSEG--NQY.VE.....K....RAN.IREQ....Y..RA.TM.Y*
APMV-12/ITA/3920-1    ...........EK.--...SLG....I...V.AE.RK.Q....E..PT....ARDA
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Fig. 4. Alignment of (a) nucleotide sequences of RNA editing sites (mRNA sense) and (b) C-terminal regions of V protein. Identical nucleotides or amino acids with APMV/Shimane67 are shown by dots. Conserved cysteine residues of V protein are shown by cross. Conserved HRRE and WCNP motifs are shaded gray. Numbers indicate the nucleotide or amino acid positions of the (a) P gene ORF and (b) V protein of APMV/Shimane67, respectively.

[7, 10]. NLS comprised a bipartite clustering of basic amino acids (*e.g.*, RKGKKVTFDKLEKKIRS of APMV-1/LaSota M protein). In the APMV/Shimane67 M protein, the putative bipartite NLS motif (^{248}KGNKISVDKLELKIRR^{263}) was found at positions 248–263 aa (Fig. 5a). The FPIV late domain that contributes to efficient viral release and replication was at positions 23–26 aa of the APMV-1 M protein [10]. Furthermore, a comparable sequence motif in other APMV M proteins was reported [37, 44, 45]. A similar aa sequence motif (FPVV) was found at positions 23–26 aa in the M protein of APMV/Shimane67 (Fig. 5b).

HN gene and HN protein: The HN gene for APMV/Shimane67 comprised 2,070 nt with one ORF that encoded a protein with 610 aa with MW of 67,492 Da and pI of 5.42. The nt and deduced aa of APMV/Shimane67 HN gene had the highest identities with APMV-12/ITA/3920-1 at 61.3% and 60.5%, followed by that with APMV-1 and -9 at 54.5%–56.9% and 54.7%–55.3%, and other APMVs at 42.9%–45.5% and 30.7%–37.9%, respectively (Table 2). The HN protein of paramyxoviruses is a type II transmembrane protein. The transmembrane region of APMV/Shimane67 HN protein was predicted at positions 25–47 by the SOSUI system [13]. Five N-glycosylation motifs (N-X-S/T) were found at positions 119, 341, 392, 481 and 604 aa of APMV/Shimane67 HN protein (Table 3). Two of these, at positions 119 and 392 aa, were relatively conserved among avulaviruses, because the HN proteins of APMV-1, -2, -5, -6, -7, -8, -9, -10 and -12, and APMV-2, -4, -5, -6, -7, -8 and -11 also contained the N-glycosylation motif at positions corresponding to the 119 and 392 aa, respectively. The sialic acid-binding motif NRKSCS preserved among HN proteins of paramyxoviruses [24] was identified at positions 234–239 of the HN protein of APMV/Shimane67 (Table 3). Twelve aa (^{174}R, ^{175}I, ^{198}D, ^{236}K, ^{258}E, ^{299}Y, ^{317}Y, ^{401}E, ^{416}R, ^{498}R, ^{526}Y and ^{547}E) engaged in sialic acid-binding and neuraminidase

a

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                          243                 267
APMV/Shimane67            SCLDSKGNKISVDKLELKIRRMSIS
APMV-1/LaSota             TTV.R..K.VTF...K...SLDL.
APMV-2/Yucaipa            KKTNA..ESRTISN..G.V.A.G.K
APMV-3/NLD                LKRTA.QRRRTPSEIMQ.V.K.GFR
APMV-4/HK/D3              LKK.R...MRTLSQAAD.V...N.L
APMV-5/Kunitachi          KKTNR..ADR..LQIKE.V.K.GLK
APMV-6/HK/D199            KRT.RR.VDR..ENIRN.V.A.GLK
APMV-7/TN                 KKTNAS.KPR.LEDMRK.V.D.G.K
APMV-8/DE                 KKTSS..KPRTL.E.KT.VKN.GLK
APMV-9/NY                 .NI.KR.K.VTFE...K.....ELT
APMV-10/FLK               KKTNA..EARTLVN.QE.V.A.G.K
APMV-11/FRA               AKKNM..TLR.IQDVTE.VK..ALA
APMV-12/ITA/3920-1        .AI.KH...LAMER..N.....DM.
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b

```
                          18          31
APMV/Shimane67            NTLLAFPVVLQEID
APMV-1/LaSota             SN.....I...GTG
APMV-2/Yucaipa            IE..SY.LIMKDTG
APMV-3/NLD                TQ.....LISEKTE
APMV-4/HK/D3              KE..S..LIPVTGP
APMV-5/Kunitachi          NR.....I.MK.SA
APMV-6/HK/D199            VR.....IIMKPKE
APMV-7/TN                 CS.....IIM.TTS
APMV-8/DE                 SS..S..L..KET.
APMV-9/NY                 SSI....I.MEATG
APMV-10/FLK               LE..S..LIFRQQ.
APMV-11/FRA               LE.....IIVERGQ
APMV-12/ITA/3920-1        SA.........D.G
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Fig. 5. Alignment of (a) putative bipartite nuclear localization signal and (b) late domain of APMV M protein. The arginine and lysine residues are underlined in (a). The putative late domains are shaded gray. Identical amino acids with APMV/Shimane67 are shown by dots. Numbers indicate the amino acid positions of the APMV/Shimane67 M protein.

Table 3. Comparison of amino acid position and sequences of functional domains of the HN protein between APMV/Shimane67 and other APMVs

Virus	Amino acid positions of potential N-linked glycosylation site	Sialic acid-binding motif	Sialic acid-binding and neuraminidase activity
APMV/Shimane67	119, 341, 392, 481, 604	^{234}NRKSCS^{239}	^{174}R, ^{175}I, ^{198}D, ^{236}K, ^{258}E, ^{299}Y, ^{317}Y, ^{401}E, ^{416}R, ^{498}R, ^{526}Y, ^{547}E
APMV-1/LaSota	119, 341, 433, 481, 538[a)]	.....[b)]	.....
APMV-2/Yucaipa	119, 278, 345, 392, 481	.....	.....
APMV-3/NLD	33, 53, 58, 115, 309, 322, 380, 493, 494	.....	.....
APMV-4/HK/D3	11, 57, 142, 322, 380, 392, 443	.....	.....
APMV-5/Kunitachi	60, 119, 148, 278, 346, 392	.....	.....
APMV-6/HK/D199	119, 278, 346, 377, 392, 438, 483	.....	.....
APMV-7/TN	60, 119, 145, 278, 343, 377, 392, 484, 513, 564	.....	.....
APMV-8/DE	119, 278, 392, 507	.....	.....
APMV-9/NY	119, 147, 228, 341, 348, 433, 481	.....	.....
APMV-10/FLK	119, 147, 278, 352, 432,	.....	.....
APMV-11/FRA	147, 150, 266, 278, 345, 392, 431, 479, 484	.....	.....
APMV-12/ITA/3920-1	119, 147, 341, 348, 594	.....	.....

a) Numbering of amino acid residues corresponds to APMV/Shimane67 HN protein sequnece. b) The identical amino acids between APMV/Shimane67 and other APMVs are shown by dot.

(NA) activity of APMV-1 [8, 9, 14] were also completely conserved in the HN protein of APMV/Shimane67 (Table 3). Moreover, the HN protein of other APMVs perfectly possessed these aa. The aa sequence alignment of HN protein from APMVs demonstrated that the HN protein of APMV/Shimane67, APMV-9/mallard/Italy/5709/2007 and APMV-12/ITA/3920-1 contained aa sequences corresponding to the 45 aa extension, which was found in the C-terminal end of HN protein of lentogenic APMV-1, such as strains D26, Ulster and 415 (Fig. 6). The removal of C-terminal 42 aa extension by proteolytic cleavage converts into the biologically active form of lentogenic APMV-1 HN protein [27, 28]. Recently, Yuan *et al.* [47] demonstrated that the C-terminal 45 aa extension of lentogenic APMV-1 HN protein autoinhibited receptor binding and catalytic activities by blocking the NA-active and second sialic binding sites. In addition, the intermolecular disulfide bond formed by the cysteine residue at 596 aa in the C-terminal extension was critical for the expression of autoinhibition of HN activities. Although there was no cysteine residue and aa identity in the C-terminal extension region of APMV/Shimane67, the three extreme C-terminal residues (SWP), which masked the NA active site in the APMV-1 HN protein, were identical to APMV-1.

L gene and L protein: The APMV/Shimane67 L gene was 6,763 nt long and encoded a single ORF, giving a deduced protein of 2,199 aa (MW 248,209 Da and pI 6.86). The ORF and aa sequences of APMV/Shimane67 L gene were 63.1% and 65.5% identical with APMV-12/ITA/3920-1, and 56.0%–57.0% and 53.4%–55.6% identical with APMV-1 and -9, respectively. In contrast, fewer identities (43.1%–47.6% in nt and 32.0%–40.3% in aa sequences) were observed between APMV/Shimane67 and APMV-2 to -8, APMV-10 and APMV-11 (Table 2). The L protein of non-segmented negative-strand RNA viruses contains six conserved aa domains (I-VI) [32]. Among these domains, domain III corresponding to at positions 635–826 aa of APMV/Shimane67 was relatively highly conserved among avulaviruses (Fig. 7a). The QGDNQ sequence, identified in the motif C of domain III as the putative active site for nucleotide polymerization [20], was found on the APMV/Shimane67 L protein at positions 747–751 aa. The putative ATP-binding site comprising K-$X_{18–21}$-G-X-G-X-G was found at positions 1756–1782 aa of domain IV of APMV/Shimane67 L protein (Fig. 7b) [12, 32].

Phylogenetic analysis: To understand genetic relationships, phylogenetic trees were constructed based on the nt sequences of full length genome and the N, P, M, HN and L genes of APMV/Shimane67 and other viruses from the five members of the family *Paramyxovirinae* (*Avulavirus*, *Henipavirus*, *Morbillivirus*, *Respirovirus* and *Rubulavirus*) (Fig. 8 and Supplementary Figs. 1–5). Essentially, all trees demonstrated similar grouping patterns, except for APMV-7 and -11, and clearly divided according to the five classifications of paramyxoviruses. Furthermore, a similar phylogenetic tree was constructed by the F gene sequences [46]. APMV/Shimane67 was classified as a member of the genus Avulavirus, but distinct from other APMVs. The APMV/Shimane67 fell into the group that comprised APMV-1, -9 and -12 and had the closest relationship with APMV-12/ITA/3920-1. This branching pattern was supported by high bootstrap values.

DISCUSSION

In the present study, we determined the nt sequences of N, P, M, HN and L genes, Le, Tr and IGS of APMV/Shimane67. Together with our previous study of the F gene sequencing [46], whole genome sequencing of APMV/Shimane67 was completed.

The genome of APMV/Shimane67 basically had following common features with other APMVs: agreement with the "rule of six"; gene order (3′-N-P-M-F-HN-L-5′) and existence of GS and GE signal, and IGS; complementation of 3′- and 5′-terminus sequence; existence of three times repeated motif; existence of the putative RNA editing site of P gene. In addition, the coding proteins of APMV/Shimane67 genome contained following conserved aa sequence motifs with other APMVs: N protein self-assembly motif; cysteine-rich region and HRRE and WCNP motifs of V protein; putative bipartite NLS motif and the late domain of M protein; amino acids constituting the sialic acid binding site of HN protein; putative active site for nucleotide polymerization and ATP-binding site of L protein. These nt and aa characteristics of APMV/Shimane67 can contribute to the efficient replication and transcription of viral genome, and viral growth.

By contrast, the genome of APMV/Shimane67 had differences with other APMVs in terms of some details, such as the nt length of whole genome, six genes (N, P, M, F, HN and L) and IGS, and predicted aa length of six genes, which were almost unique in APMV/Shimane67 (Table 1). The genome size of APMV/Shimane67 was 16,146 nt long. This genome size is different from any other known APMVs genome and was the fifth longest among APMVs. With the exception of APMV/Shimane67, there are four APMVs, which have genome longer than 16,000 nt: APMV-3 with 16,182–16,272 nt; APMV-5 with 17,262 nt; APMV-6 with 16,174–16,236 nt; and APMV-11 with 17,412 nt [3, 6, 17, 18, 37, 42, 45]. The relatively large genome size of APMV/Shimane67 is attributable to its long Tr sequence (776 nt). The Tr sequence longer than 700 nt was also found in APMV-3/NLD genome [17]. However, the significance of these long Tr sequence is unclear, and a further study is needed to clarify this issue. The phylogenetic trees indicated that APMV/Shimane67 had the closest relationship with APMV-12/ITA/3920-1. When comparing full genome sequences, the evolutionary distance between APMV/Shimane67 and APMV-12/ITA/3920-1 was 0.357 nt substitutions per site. This distance was longer than those observed within serotypes, such as the distance between APMV-2/Yucaipa and APMV-2/Bangor at 0.291, APMV-3/NLD and APMV-3/WI at 0.292, and APMV-6/TWN/Y1 and APMV-6/ITA/4524-2 at 0.265. These strains were antigenically and genetically divided into subgroups in each serotype [2, 18, 39, 45]. Thus, the APMV/Shimane67 and APMV-12/ITA/3920-1 had greater degrees of genetic diversity than that found within the subgroups of APMV-2,

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                    547                                                             610
APMV/Shimane67      EIGNTLFGEFRIVPLLLEVYSE-----KGKSLKSSFD-GWEDISINNPLRPLDN----HRVDPILISNYTSSWP*
APMV-1/415          ..S.............V.ILRDEGRSEARSA.TTQGHP..N.EVVDPIFCAVT.----QTDHRQKLEE.AQ...*
APMV-1/D26          ..S.............V.ILKDDGVREARSGRL.QLQE..K.DIVSPIFCDAK.----QTEYRRELES.AA...*
APMV-1/Ulster       ..S.............V.ILKDDGVREARAGRL.QLRE..K.DIVSPIFCDAK.----QTEYRRELES.AA...*
APMV-1/LaSota       ..S.............V.ILKDDGVREARSG*
APMV-9/NY           ..E.........T...S.IIFDPNLEPSDT.RN*
APMV-9/ITA/5709     ............T...S.IILDPSLETSD..HNRVKSAPEDGRWIDPASIA.TAGNASSLHSNATSAGPVD.Q.*
APMV-12/ITA/3920-1  ................I..QKTP--LTRRSE.RQQMPQPPI.L.ID..FCAPSG----NLSRKNA.DE.AN...*
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Fig. 6. Alignment of C-terminal 45 extension of APMV-1 HN protein and the corresponding regions of APMV/Shimane67, APMV-9 and −12. Identical amino acids with APMV/Shimane67 are shown by dots. Gaps and the stop codons are shown by dashes and asterisks, respectively. Numbers indicate the amino acids positions of the APMV/Shimane67 HN protein. The GenBank accession of APMV-1/D26, Ulster and APMV-9/ITA/5709 is M19432, M19478 and GU068587, respectively.

```
a
                       Motif A                 Motif B                  Motif C        Motif D
                    635         647   706                      731   744      753   814         826
APMV/Shimane67      FITTDLSKYCLNW     IFIVSARGGIEGLCQKLWSMISIAAI     CMVQGDNQVI     KDGKILSQMLKNA
APMV-1/LaSota       ......Q......     .Y................T.......     ..........     ...A....V...S
APMV-2/Yucaipa      .L....Q......     ..................T....SI.     A.........     WE.R....A....
APMV-3/NLD          ......Q......     .....P..A.......M.TI...S..     S.........     Y..RV.P.L...I
APMV-4/HK/D3        YM....K......     ...I.........Q....TG....IA     ATL......L     FN.S....C...F
APMV-5/Kunitachi    .L....Q......     .....P..........M.T....S..     S.......A.     FE.R....G...A
APMV-6/HK/D199      .L....Q......     .....P..........M.T.......     S.......A.     FE......L....
APMV-7/TN           YL....Q......     .....P..........M.TL....L.     S.........     FE.RV...I....
APMV-8/DE           .L....E....Q.     .....P..........M.T....S..     A.........     FE.RL...V....
APMV-9/NY           Y.....Q......     .....G............TT...S..     ..........     ........A....
APMV-10/FLK         .L....Q......     .....P..........M.T....SI.     A.........     WE.R....L....
APMV-11/FRA         .L....Q......     .....P..........M.T....S..     S.........     FE......S....
APMV-12/ITA/3920-1  .L...........     .......................S..     ..........     ...R....L....
```

```
b
                    1756                       1782
APMV/Shimane67      KVGQIFSIPEVRQTKGGVSLFLGEGSG
APMV-1/LaSota       .ASHLL.V....CARH.N..Y.A....
APMV-2/Yucaipa      .YAVVGASV.KV.PTRST..YI.....
APMV-3/NLD          RQYRYVLSQLGHDQL..AT.Y......
APMV-4/HK/D3        RSAALLASGALSGLPE.S..Y....Y.
APMV-5/Kunitachi    .ISGLLAKGLCKGLPH.HG.YIA....
APMV-6/HK/D199      .AAG.LTT.GFLNLPK.NG.Y.A.S..
APMV-7/TN           .ASSLIASDILKGGPL.D..Y.C....
APMV-8/DE           .YAIAYAVS.KRSARL.G..YI.....
APMV-9/NY           .AANLL.L..VR.ARF.N..Y.A....
APMV-10/FLK         .YAVLYASERKTAKSS.D..YI.....
APMV-11/FRA         .YAGLY.SEFFRGMPQ.DA.Y......
APMV/12/ITA/3920-1  .I..L........SR.TNA.Y......
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Fig. 7. Alignment of (a) the conserved domain III of L protein of non-segmented negative strand RNA viruses and (b) the putative ATP-binding site of the L protein. The QGDNQ and K-$X_{18\text{-}21}$–G-X-G-X-G motifs are underscored. Identical amino acids with APMV/Shimane67 are shown by dots. Numbers indicate the amino acid positions of the APMV/Shimane67 L protein.

-3 or -6. Although there are no defined genetic criteria to differentiate the typing of APMVs, recent studies attempted and demonstrated that classification based on genetic analysis was correlated with conventional serotyping [3, 22, 35, 41]. Our previous serological analysis demonstrated that APMV/Shimane67 was distinct from APMV-1, -2, -3, -4, -6 and -7 [46]. Moreover, sequence analysis of F gene of APMV/Shimane67 indicated that APMV/Shimane67 was genetically diverse from other APMV serotypes. Thus, the results obtained in this study emphasized the possibility that APMV/Shimane67 would be a novel APMV type. Quite recently, Karamendin *et al.* [16] reported the whole genome sequence of a novel APMV isolated from a white fronted goose in northern Kazakhstan (GenBank accession number KU64513). The genome length of APMV/Shimane67 is 150 nt longer than that of Kazakhstan APMV, while genome nt identity of these strains was very high (approximately 96%). The deduced aa sequence identities of the N, P, M, F, HN and L proteins between APMV/Shimane67 and Kazakhstan APMV are 99.6%, 99.2%, 98.9%, 99.6%, 99.1% and 99.6%, respectively. Although there is no serological evidence for the close relationship between APMV/Shimane67 and

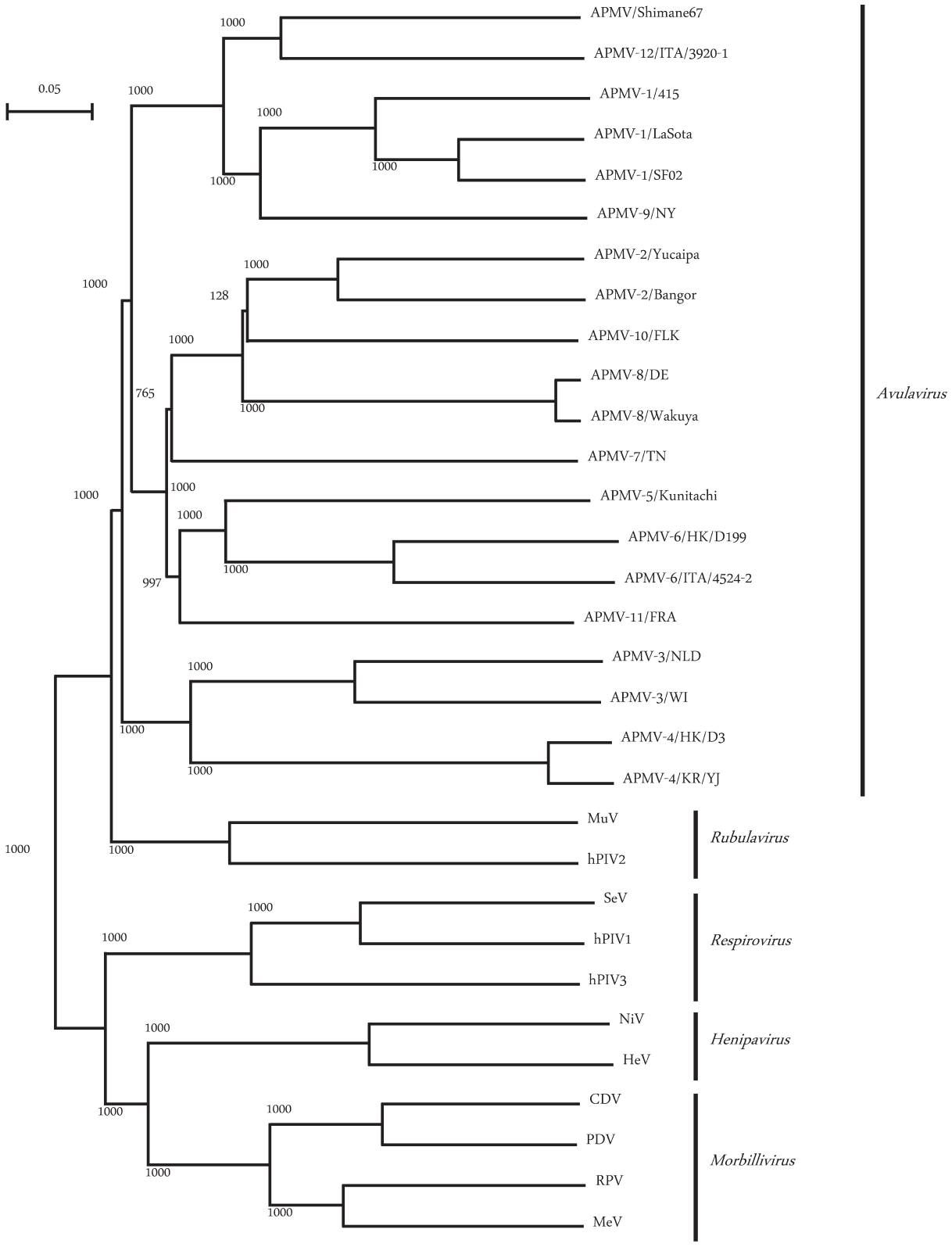

Fig. 8. Phylogenetic analyses of the complete genome from members of the subfamily *Paramyxovirinae*. Phylogenetic trees are generated with the program ClustalX [19] and viewed using NJplot [31]. The numbers at the branches represent bootstrap values from 1,000 replicates. The number of nucleotide substitutions per site (scale bar) is shown.

Kazakhstan APMV, the genomic similarities of APMV/Shimane67 and Kazakhstan APMV suggest that these two strains would be classified as the same serotype, APMV-13.

REFERENCES

1. Abolnik, C., de Castro, M. and Rees, J. 2012. Full genomic sequence of an African avian paramyxovirus type 4 strain isolated from a wild duck. *Virus Genes* **45**: 537–541. [Medline]
2. Anderson, C., Kearsley, R., Alexander, D. J. and Russell, P. H. 1987. Antigenic variation in avian paramyxovirus type 3 isolates detected by mouse monoclonal antibodies. *Avian Pathol.* **16**: 691–698. [Medline] [CrossRef]
3. Briand, F. X., Henry, A., Massin, P. and Jestin, V. 2012. Complete genome sequence of a novel avian paramyxovirus. *J. Virol.* **86**: 7710. [Medline] [CrossRef]
4. Bui, V. N., Mizutani, T., Nguyen, T. H., Trinh, D. Q., Awad, S. S. A., Minoungou, G. L., Yamamoto, Y., Nakamura, K., Saito, K., Watanabe, Y., Runstadler, J., Huettmann, F., Ogawa, H. and Imai, K. 2014. Characterization of a genetic and antigenic variant of avian paramyxovirus 6 isolated from a migratory wild bird, the red-necked stint (Calidris ruficollis). *Arch. Virol.* **159**: 3101–3105. [Medline] [CrossRef]
5. Calain, P. and Roux, L. 1993. The rule of six, a basic feature for efficient replication of Sendai virus defective interfering RNA. *J. Virol.* **67**: 4822–4830. [Medline]
6. Chang, P. C., Hsieh, M. L., Shien, J. H., Graham, D. A., Lee, M. S. and Shieh, H. K. 2001. Complete nucleotide sequence of avian paramyxovirus type 6 isolated from ducks. *J. Gen. Virol.* **82**: 2157–2168. [Medline] [CrossRef]
7. Coleman, N. A. and Peeples, M. E. 1993. The matrix protein of Newcastle disease virus localizes to the nucleus via a bipartite nuclear localization signal. *Virology* **195**: 596–607. [Medline] [CrossRef]
8. Connaris, H., Takimoto, T., Russell, R., Crennell, S., Moustafa, I., Portner, A. and Taylor, G. 2002. Probing the sialic acid binding site of the hemagglutinin-neuraminidase of Newcastle disease virus: identification of key amino acids involved in cell binding, catalysis, and fusion. *J. Virol.* **76**: 1816–1824. [Medline] [CrossRef]
9. Crennell, S., Takimoto, T., Portner, A. and Taylor, G. 2000. Crystal structure of the multifunctional paramyxovirus hemagglutinin-neuraminidase. *Nat. Struct. Biol.* **7**: 1068–1074. [Medline] [CrossRef]
10. Duan, Z., Hu, Z., Zhu, J., Xu, H., Chen, J., Liu, H., Hu, S. and Liu, X. 2014. Mutations in the FPIV motif of Newcastle disease virus matrix protein attenuate virus replication and reduce virus budding. *Arch. Virol.* **159**: 1813–1819. [Medline] [CrossRef]
11. Gasteiger, E., Hoogland, C., Gattiker, A., Duvaud, S., Wilkins, M. R., Appel, R. D. and Bairoch, A. 2005. Protein identification and analysis tools on the ExPASy server. pp. 571–607. *In*: The Proteomics Protocols Handbook. (Walker, J. M. ed.), Humana Press, Totowa.
12. Harcourt, B. H., Tamin, A., Halpin, K., Ksiazek, T. G., Rollin, P. E., Bellini, W. J. and Rota, P. A. 2001. Molecular characterization of the polymerase gene and genomic termini of Nipah virus. *Virology* **287**: 192–201. [Medline] [CrossRef]
13. Hirokawa, T., Boon-Chieng, S. and Mitaku, S. 1998. SOSUI: classification and secondary structure prediction system for membrane proteins. *Bioinformatics* **14**: 378–379. [Medline] [CrossRef]
14. Iorio, R. M., Field, G. M., Sauvron, J. M., Mirza, A. M., Deng, R., Mahon, P. J. and Langedijk, J. P. 2001. Structural and functional relationship between the receptor recognition and neuraminidase activities of the Newcastle disease virus hemagglutinin-neuraminidase protein: receptor recognition is dependent on neuraminidase activity. *J. Virol.* **75**: 1918–1927. [Medline] [CrossRef]
15. Jeon, W. J., Lee, E. K., Kwon, J. H. and Choi, K. S. 2008. Full-length genome sequence of avain paramyxovirus type 4 isolated from a mallard duck. *Virus Genes* **37**: 342–350. [Medline] [CrossRef]
16. Karamendin, K., Kydyrmanov, A., Seidalina, A., Asanova, S., Sayatov, M., Kasymbekov, E., Khan, E., Daulbayeva, K., Harrison, S. M., Carr, I. M., Goodman, S. J. and Zhumatov, K. 2016. Complete genome sequence of a novel avian paramyxovirus (APMV-13) isolated from a wild bird in Kazakhstan. *Genome Announc.* **4**: e00167-16. [Medline] [CrossRef]
17. Kumar, S., Nayak, B., Collins, P. L. and Samal, S. K. 2008. Complete genome sequence of avian paramyxovirus type 3 reveals an unusually long trailer region. *Virus Res.* **137**: 189–197. [Medline] [CrossRef]
18. Kumar, S., Nayak, B., Samuel, A. S., Xiao, S., Collins, P. L. and Samal, S. K. 2010. Complete genome sequence of avian paramyxovirus-3 strain Wisconsin: evidence for the existence of subgroups within the serotype. *Virus Res.* **149**: 78–85. [Medline] [CrossRef]
19. Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., Valentin, F., Wallace, I. M., Wilm, A., Lopez, R., Thompson, J. D., Gibson, T. J. and Higgins, D. G. 2007. Clustal W and Clustal X version 2.0. *Bioinformatics* **23**: 2947–2948. [Medline] [CrossRef]
20. Malur, A. G., Gupta, N. K., De Bishnu, P. and Banerjee, A. K. 2002. Analysis of the mutations in the active site of the RNA-dependent RNA polymerase of human parainfluenza virus type 3 (HPIV3). *Gene Expr.* **10**: 93–100. [Medline]
21. Marcos, F., Ferreira, L., Cros, J., Park, M. S., Nakaya, T., García-Sastre, A. and Villar, E. 2005. Mapping of the RNA promoter of Newcastle disease virus. *Virology* **331**: 396–406. [Medline] [CrossRef]
22. Miller, P. J., Afonso, C. L., Spackman, E., Scott, M. A., Pedersen, J. C., Senne, D. A., Brown, J. D., Fuller, C. M., Uhart, M. M., Karesh, W. B., Brown, I. H., Alexander, D. J. and Swayne, D. E. 2010. Evidence for a new avian paramyxovirus serotype 10 detected in rockhopper penguins from the Falkland Islands. *J. Virol.* **84**: 11496–11504. [Medline] [CrossRef]
23. Miller, P. J. and Koch, G. 2013. Newcastle disease, other avian paramyxoviruses, and avian metapneumovirus infections. pp. 89–138. *In*: Diseases of Poultry, 13th ed. (Swayne, D. E., Glisson, J. R., McDougald. L. R., Nolan, L. K., Suarez, D. L. and Nair. V. eds.), Wiley-Blackwell, Hoboken.
24. Mirza, A. M., Deng, R. and Iorio, R. M. 1994. Site-directed mutagenesis of a conserved hexapeptide in the paramyxovirus hemagglutinin-neuraminidase glycoprotein: effects on antigenic structure and function. *J. Virol.* **68**: 5093–5099. [Medline]
25. Morgan, E. M. 1991. Evolutionary relationships of Paramyxovirus nucleocapsid-associated proteins. pp. 163–179. *In*: The Paramyxoviruses (Kingsbury, D. W. ed.), Plenum Press, New York.
26. Myers, T. M., Pieters, A. and Moyer, S. A. 1997. A highly conserved region of the Sendai virus nucleocapsid protein contributes to the NP-NP binding domain. *Virology* **229**: 322–335. [Medline] [CrossRef]
27. Nagai, Y. and Klenk, H. D. 1977. Activation of precursors to both glycoporteins of Newcastle disease virus by proteolytic cleavage. *Virology* **77**: 125–134. [Medline] [CrossRef]

28. Nagai, Y., Klenk, H. D. and Rott, R. 1976. Proteolytic cleavage of the viral glycoproteins and its significance for the virulence of Newcastle disease virus. *Virology* **72**: 494–508. [Medline] [CrossRef]
29. Nayak, B., Kumar, S., Collins, P. L. and Samal, S. K. 2008. Molecular characterization and complete genome sequence of avian paramyxovirus type 4 prototype strain duck/Hong Kong/D3/75. *Virol. J.* **5**: 124. [Medline] [CrossRef]
30. Paldurai, A., Subbiah, M., Kumar, S., Collins, P. L. and Samal, S. K. 2009. Complete genome sequences of avian paramyxovirus type 8 strains goose/Delaware/1053/76 and pintail/Wakuya/20/78. *Virus Res.* **142**: 144–153. [Medline] [CrossRef]
31. Perrière, G. and Gouy, M. 1996. WWW-query: an on-line retrieval system for biological sequence banks. *Biochimie* **78**: 364–369. [Medline] [CrossRef]
32. Poch, O., Blumberg, B. M., Bougueleret, L. and Tordo, N. 1990. Sequence comparison of five polymerases (L proteins) of unsegmented negative-strand RNA viruses: theoretical assignment of functional domains. *J. Gen. Virol.* **71**: 1153–1162. [Medline] [CrossRef]
33. Rosseel, T., Lambrecht, B., Vandenbussche, F., van den Berg, T. and Van Borm, S. 2011. Identification and complete genome sequencing of paramyxoviruses in mallard ducks (Anas platyrhynchos) using random access amplification and next generation sequencing technologies. *Virol. J.* **8**: 463. [Medline] [CrossRef]
34. Saitou, N. and Nei, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4**: 406–425. [Medline]
35. Samal, S. K. 2011. Newcastle disease and related avian paramyxoviruses. pp. 69–114. *In*: The Biology of Paramyxoviruses. (Samal, S. K. ed.), Caister Academic Press, Norfolk.
36. Samuel, A. S., Kumar, S., Madhuri, S., Collins, P. L. and Samal, S. K. 2009. Complete sequence of the genome of avian paramyxovirus type 9 and comparison with other paramyxoviruses. *Virus Res.* **142**: 10–18. [Medline] [CrossRef]
37. Samuel, A. S., Paldurai, A., Kumar, S., Collins, P. L. and Samal, S. K. 2010. Complete genome sequence of avian paramyxovirus (APMV) serotype 5 completes the analysis of nine APMV serotypes and reveals the longest APMV genome. *PLoS ONE* **5**: e9269. [Medline] [CrossRef]
38. Steward, M., Vipond, I. B., Millar, N. S. and Emmerson, P. T. 1993. RNA editing in Newcastle disease virus. *J. Gen. Virol.* **74**: 2539–2547. [Medline] [CrossRef]
39. Subbiah, M., Nayak, S., Collins, P. L. and Samal, S. K. 2010. Complete genome sequences of avian paramyxovirus serotype 2 (APMV-2) strains Bangor, England and Kenya: evidence for the existence of subgroups within serotype 2. *Virus Res.* **152**: 85–95. [Medline] [CrossRef]
40. Subbiah, M., Xiao, S., Collins, P. L. and Samal, S. K. 2008. Complete sequence of the genome of avian paramyxovirus type 2 (strain Yucaipa) and comparison with other paramyxoviruses. *Virus Res.* **137**: 40–48. [Medline] [CrossRef]
41. Terregino, C., Aldous, E. W., Heidari, A., Fuller, C. M., De Nardi, R., Manvell, R. J., Beato, M. S., Shell, W. M., Monne, I., Brown, I. H., Alexander, D. J. and Capua, I. 2013. Antigenic and genetic analyses of isolate APMV/wigeon/Italy/3920-1/2005 indicate that it represents a new avian paramyxovirus (APMV-12). *Arch. Virol.* **158**: 2233–2243. [Medline] [CrossRef]
42. Tian, Z., Chai, H., Li, F., Sun, J., Chen, G., Hu, X., Hua, Y. and Xiang, W. 2012. Complete nucleotide sequence of avian paramyxovirus type 6 strain JL isolated from mallard ducks in China. *J. Virol.* **86**: 13112. [Medline] [CrossRef]
43. Tsunekuni, R., Ito, H., Otsuki, K., Kida, H. and Ito, T. 2010. Genetic comparisons between lentogenic Newcastle disease virus isolated from waterfowl and velogenic variants. *Virus Genes* **40**: 252–255. [Medline] [CrossRef]
44. Xiao, S., Paldurai, A., Nayak, B., Subbiah, M., Collins, P. L. and Samal, S. K. 2009. Complete genome sequence of avian paramyxovirus type 7 (strain Tennessee) and comparison with other paramyxoviruses. *Virus Res.* **145**: 80–91. [Medline] [CrossRef]
45. Xiao, S., Subbiah, M., Kumar, S., De Nardi, R., Terregino, C., Collins, P. L. and Samal, S. K. 2010. Complete genome sequences of avian paramyxovirus serotype 6 prototype strain Hong Kong and a recent novel strain from Italy: evidence for the existence of subgroups within the serotype. *Virus Res.* **150**: 61–72. [Medline] [CrossRef]
46. Yamamoto, E., Ito, H., Tomioka, Y. and Ito, T. 2015. Characterization of novel avian paramyxovirus strain APMV/Shimane67 isolated from migratory wild geese in Japan. *J. Vet. Med. Sci.* **77**: 1079–1085. [Medline] [CrossRef]
47. Yuan, P., Paterson, R. G., Leser, G. P., Lamb, R. A. and Jardetzky, T. S. 2012. Structure of the ulster strain newcastle disease virus hemagglutinin-neuraminidase reveals auto-inhibitory interactions associated with low virulence. *PLoS Pathog.* **8**: e1002855. [Medline] [CrossRef]