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Genetic Diversity of Artybash Virus in the Laxmann's Shrew (*Sorex caecutiens*)

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Abstract

Although based on very limited M and L segment sequences, Artybash virus (ARTV) was proposed previously as a unique hantavirus harbored by the Laxmann's shrew (*Sorex caecutiens*). To verify this conjecture, lung tissues from 68 Laxmann's shrews, captured during 2006 to 2014 in eastern Siberia, Russia, and Hokkaido, Japan, were analyzed for ARTV RNA using reverse transcription polymerase chain reaction (RT-PCR). ARTV RNA was detected in six Laxmann's shrews. Pairwise alignment and comparison of partial- and full-length S, M, and L segment sequences from these Laxmann's shrews, as well as phylogenetic analyses, using maximum likelihood and Bayesian methods indicated that ARTV was distinct from other soricine shrew-borne hantaviruses and representative hantaviruses harbored by rodents, moles, and bats. Taxonomic identity of the ARTV-infected Laxmann's shrews was confirmed by full-length cytochrome *b* mitochondrial DNA sequence analysis. Our data indicate that the hantavirus previously known as Amga virus (MGAV) represents genetic variants of ARTV. Thus, the previously proposed designation of ARTV/MGAV should be replaced by ARTV.

Key Words: Hantavirus—Japan—RT-PCR—Russia—Shrew.

Introduction

Recent discovery of genetically distinct hantaviruses (family Bunyaviridae and genus *Hantavirus*) in multiple species of soricine and crocidurine shrews (order Eulipotyphla, family Soricidae, and subfamilies Soricinae and Crocidurinae), as well as in moles (family Talpidae and subfamilies Talpinae and Scalopinae) and insectivorous bats (order Chiroptera), challenges the conventional view that rodents (order Rodentia and families Muridae and Cricetidae) are the principal reservoir hosts. Broad taxonomic and geographic distribution of hosts indicates that small mammals other than rodents played a significant role in the evolutionary history of hantaviruses and points to the urgent need to understand the diversity, distribution, and phylogeography of hantaviruses worldwide (Bennett et al. 2014, Yanagihara et al. 2014).

Seewis virus (SWSV), originally detected in the Eurasian common shrew (*Sorex araneus*) in Switzerland (Song et al. 2007), has now been found across the vast distribution of its soricine reservoir host in the Czech Republic (Schlegel et al. 2012), Finland (Kang et al. 2009a, Ling et al. 2014), Germany (Schlegel et al. 2012), Hungary (Kang et al. 2009a), Poland (Gu et al. 2014), Russia (Yashina et al. 2010), Slovakia (Schlegel et al. 2012), and Slovenia (Korva et al. 2013; Resman et al. 2013).

Genetically distinct hantaviruses have also been detected in other *Sorex* species, including Ash River virus in the North American masked shrew (*Sorex cinereus*) and Jemez Springs virus in the dusky shrew (*Sorex monticolus*) (Arai et al. 2008a), Kenkeme virus in the Asian flat-skulled shrew (*Sorex roboratus*) (Kang et al. 2010), Asikkala virus in the Eurasian pygmy shrew (*Sorex minutus*) (Radosa et al. 2013), Yakeshi virus in the taiga shrew (*Sorex isodon*) (Guo et al. 2013), Qian

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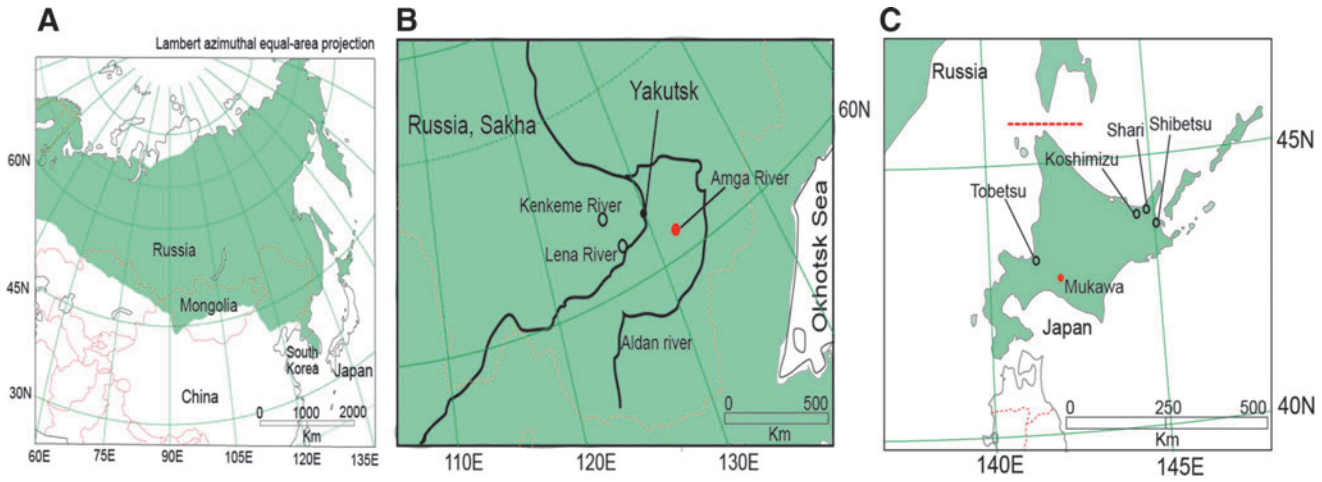

FIG. 1. **(A)** Map showing the geographic distribution of the Laxmann's shrew (*Sorex caecutiens*) across Eurasia, where it inhabits broad-leaved forests on lowland and mountainous areas. **(B)** Map showing trap sites of Laxmann's shrews along the Lena River (N61°45′14.399 E129°31′30.0), Amga River (N61°35′34.810 E132°56′24.053), and Kenkeme River (N62°04′11.996 E128°56′16.821) in the Sakha Republic of Russia. **(C)** Map showing trap sites of Laxmann's shrews in Koshimizu (N43°56′10.615 E144°26′31.750), Shibetsu (N43°37′43.589 E145°11′34.594), Mukawa (N42°50′30.073 E142°10′01.675), Shari (N43°55′23.998, E144°50′39.800), and Tobetsu (N43°12′11.084 E141°26′58.999) in Hokkaido, Japan. *Red circles* indicate trap sites of ARTV-positive shrews.

Hu Shan virus in the stripe-back shrew (*Sorex cylindricauda*) (Zuo et al. 2014), and Sarufutsu virus in the long-clawed shrew (*Sorex unguiculatus*) (Arai et al. unpublished data).

In addition, based on very limited M and L segment sequences detected in a Laxmann's shrew (*Sorex caecutiens*) captured near Teletskoye Lake in the Altai Republic of western Siberia, a new hantavirus, named Artybash virus (ARTV), has been proposed. In an attempt to validate its reservoir host species and explore the genetic diversity of ARTV, we analyzed lung tissues collected from Laxmann's shrews trapped in eastern Siberia, Russia, and Hokkaido, Japan. Genetic and phylogenetic analyses, based on partial- and full-length genomes of a hantavirus formerly called Amga virus (MGAV), show that it represents genetic variants of ARTV. Thus, instead of referring to this hantavirus as ARTV/MGAV, as previously proposed (Bennett et al. 2014), ARTV should be the preferred designation.

Materials and Methods

Trapping and sample collection

Laxmann's shrews, which are widely distributed from northern Fennoscandia through Siberia and across northern Japan (Fig. 1A), were captured at three sites in the Sakha Republic, Russia, during July and August 2006 (Fig. 1B and Table 1) and five sites in Hokkaido, Japan, between 2008 and 2014 (Fig. 1C and Table 1). Protocols for trapping, euthanasia, and tissue processing were performed according to well-established guidelines (Animal Care and Use Committee 1998).

RNA extraction and reverse transcription polymerase chain reaction

Total RNA was extracted from lung tissues using the PureLink Micro-to-Midi Total RNA Purification Kit (Invitrogen, San Diego, CA) or MagDEA® RNA 100 Kit (Precision System Science; PSS, Matstudo, Japan). cDNA was synthesized using SuperScript III First-Strand Synthesis System (Invitrogen) or PrimeScript™ II 1st strand cDNA Synthesis Kit (Takara Bio, Otsu, Japan) and an oligonucleotide primer (OSM55: 5′-TAGTAGTAGACTCC-3′) designed from the genus-specific conserved 3′-end of the S, M, and L segments of all hantaviruses. For initial screening by RT-PCR, primers were based on highly conserved regions of shrew-borne hantavirus genomes: S (outer: OSM55F, HTN-S6: 5′-AGCTCNGGATCCATN TCATC-3′; inner: Cro2F: 5′-AGYCCNGTNATGRGWGTN

Table 1. Capture Sites of Laxmann's Shrews Tested for Hantavirus RNA

				Total		*Positive*	
Country	*Republic/prefecture*	*Location*	*Year*	*Male*	*Female*	*Male*	*Female*
Russia	Sakha	Lena River	2006	4	1	0	0
		Amga River	2006	7	7	4	1
		Kenkeme River	2006	15	5	0	0
Japan	Hokkaido	Koshimizu	2008	1	2	0	0
		Shibetsu	2008	0	1	0	0
		Mukawa	2010	3	1	1	0
		Shari	2011	0	1	0	0
		Tobetsu	2013	2	2	0	0
			2014	9	7	0	0
Total				41	27	5	1

RTYGG-3′ and Cro2R: 5′-ANGAYTGRTARAANGANGAY TTYTT-3′) and L (outer: Han-L1880F: 5′-ATGAARNTN TGTGCNATNTTTGA-3′ and Han-L3000R: 5′-GCNGAR TTRTCNCCNGGNGACCA-3′; inner: Han-L2520F: 5′-AT NWGHYTDAARGGNATGTCNGG-3′ and Han-L2970R: 5′-CCNGGNGACCAYTTNGTDGCATC-3′). Oligonucleotide primers designed for amplification and sequencing of the full genome of ARTV are shown in Table 2. Nested PCR cycling conditions and methods for DNA sequencing have been previously described (Arai et al. 2008a, 2008b, Kang et al. 2009b).

Genetic and phylogenetic analysis

Pairwise alignment and comparison of partial- and full-length S, M, and L segment sequences of hantaviruses from Laxmann's shrews, as well as other representative rodent-, shrew-, mole-, and bat-borne hantaviruses, were performed using the ClustalW method (Thompson et al. 1994). Phylogenetic analyses were conducted using maximum likelihood and Bayesian methods, implemented in MrBayes 3.1 (Ronquist and Huelsenbeck 2003) and RAxML (Stamatakis et al. 2008), under the best-fit $GTR + I + \Gamma$ model of evolution using MrModeltest 2.3 (Posada 2008). Two replicate Bayesian Metropolis–Hastings Markov chain Monte Carlo runs, each consisting of six chains of 10 million generations sampled every 100 generations with a burn-in of 25,000 (25%), resulted in 150,000 trees overall. Topologies were evaluated by bootstrap analysis of 100 iterations, and posterior node probabilities were based on 10 million

Table 2. Oligonucleotide Primers for the Amplification of the S, M, and L Genomic Segments of Artybash Virus

Segment	*Primer*	*Sequence (5′→3′)*	*Length*	*Polarity*
S	OSM55	TAG TAG TAG ACT CC	14	+
	CAS-381F	CAN GTG GNC ARA CWG CWG AYT GG	23	+
	Han-S604F	GCH GAD GAR HTN ACA CCN GG	20	+
	Cro2F	AGY CCN GTN ATG RGW GTN RTY GG	24	+
	Han-S974R	TCN GGN GCH CHN GCA AAN AHC CA	23	−
	Cro2R	ANG AYT GRT ARA ANG ANG AYT TYT T	25	−
	HTN-S6	AGC TCN GGA TCC ATN TCA TC	20	−
	MKWS-1612R	AGA GTG TTT GAG GTA GTG GAG TG	23	−
	Han-S3R1	TAG TAG TAN NCT CCN	15	−
	PHS-3endR	TAG TAG TAT ACT CCT TGA AAA GC	23	−
M	OSM55	TAG TAG TAG ACT CC	14	+
	JAM-241F	GTT CAT GYA GYA TGG ATG TRC A	22	+
	MKWM-433R	TNC GCA TCT TGTA RGC CTG YTC	22	−
	HTM-1490F	TGT GTN CCW GGN TTY CAT GGN T	22	+
	CAM-1685F	ACN AAG GGY TCW ATG GTN TGT GA	23	+
	SHM-1706R	CAT ACA TCA CAN ACC ATW GAA CC	23	−
	SHM-2371F	TGY AAC CCN GTN GAT TGY CCW GG	23	+
	MKWM-2458R	ATA GGC AGT CCC GAC AGC CTT	21	−
	MKWM-2631R	CAT GAT RTC NCC AGG RTC NCC	21	−
	TM-2957R	GAA CCC CAD GCC CCNTCY AT	20	−
	AM-3280F	CCA TAT CTA TGA TGA TGG TGC	21	+
	PHM-3endR	TAG TAG TAG ACT CCG CAA GAA	21	−
L	OSM55	TAG TAG TAG ACT CC	14	+
	PHL-173F	GAT WAA GCA TGA YTG GTC TGA	21	+
	CAL-539F	TAT NTC MAC ACA RTG GCC WAG T	22	+
	HTL-577R	CMC CNK CAT THC KYC TAC TNG GC	23	−
	SL-761F	CCT ATT TNA GTA YTG TAA GGW NTG G	25	+
	Han-L1880F	CAR AAR ATG AAR NTN TGT GC	20	+
	CS-L-2319R	CTT CYT CAT TYA CAT TMC CAT G	22	−
	Han-L2520F	ATN WGH YTD AAR GGN ATG TCN GG	23	+
	Tal-L2855F	GAA AGG GCA TTN MGA TGG GCN TCA GG	26	+
	Tal-L2928F	GNA AAY TNA TGT ATG TNA GTG C	22	+
	HAN-L-F1	ATG TAY GTB AGT GCW GAT GC	20	+
	HAN-L-F2	TGC WGA TGC HAC NAA RTG GTC	21	+
	Han-L3000R	GCN GAR TTR TCN CCN GGN GAC CA	23	−
	HAN-L-R2	GCR TCR TCW GAR TGR TGD GCA A	22	−
	HAN-L-R1	AAC CAD TCW GTY CCR TCA TC	20	−
	MKWL-4020F	YAA YCA TGA RAG GTT GGG NGA G	22	+
	MKWL-4132F	GAG ACT TGG AGT GAN CAA CAY CC	23	+
	SL-4321R	AAYGTTACCCAYTCACCAYCCA	22	−
	PHL-5167R	CAT AYT GYT THC CTG AAT AWG C	22	−
	CAL-6375F	GKR TWG TKA ARG GYT GGG GWG A	22	−
	HNL-6388R	CTC WGT YAA RTC ATA WGG ATC	21	−
	HNL-3R	TAG TAG TAK GCT CCG	15	−

Mixed bases: B = G, T, C; D = G, A, T; H = A, T, C; K = G, T; M = A, C; *N* = A, T, G, C; R = A, G; W = A, T; and Y = C, T.

Table 3. Sequence Identities of the Full-Length S, M, and L Segments of ARTV Strain Mukawa AH301 from *Sorex caecutiens* in Japan to Other Representative Hantaviruses

	S		*M*		*L*	
Virus	*1290 nt*	*429 aa*	*3420 nt*	*1139 aa*	*6456 nt*	*2151 aa*
ARTV Amga MSB148558	1290 nt[a] 79.8%	429 aa 93.5%	1088 nt 78.1%	362 aa 95.0%	4598 nt 79.7%	1532 aa 96.0%
ARTV Amga MSB148559	—	—	—	—	1401 nt 79.8%	467 aa 97.4%
ARTV Amga MSB148436	—	—	727 nt 78.3%	242 aa 97.1%	2454 nt 80.2%	818 aa 97.3%
ARTV Amga MSB148457	—	—	683 nt 78.2%	227 aa 96.9%	2397 nt 80.0%	799 aa 97.4%
ARTV Amga MSB148347	582 nt 76.5%	194 aa 88.7%	729 nt 78.2%	243 aa 97.5%	1302 nt 80.2%	434 aa 97.2%
ARTV ART502	837 nt 76.9%	279 aa 92.8%	247 nt 79.8%	82 aa 96.3%	347 nt 80.1%	115 aa 96.5%
ARTV Galkino2712	559 nt 75.9%	186 aa 89.2%	—	—	—	—
ARTV Parnaya1205	767 nt 76.8%	255 aa 92.6%	—	—	347 nt 80.4%	115 aa 95.7%
SWSV mp70	1290 nt[a] 77.1%	429 aa 86.7%	250 nt 78.0%	83 aa 91.6%	3288 nt 77.9%	1096 aa 94.3%
KKMV MSB148794	1290 nt[a] 74.6%	429 aa 83.3%	1002 nt 79.1%	334 aa 91.6%	4295 nt 77.9%	1432 aa 91.5%
QHSV YN05-284	1290 nt[a] 72.7%	429 aa 83.5%	1430 nt 76.2%	476 aa 89.9%	450 nt 79.8%	149 aa 93.3%
YKSV Si-210	1290 nt[a] 74.3%	429 aa 84.0%	3420 nt[a] 76.8%	1139 aa 88.2%	339 nt 79.4%	113 aa 91.3%
ASIV Drahany	1290 nt[a] 73.2%	429 aa 84.7%	3420 nt[a] 76.8%	1139 aa 87.3%	1729 nt 79.3%	576 aa 93.8%
ARRV MSB73418	1059 nt 62.5%	353 aa 64.0%	—	—	347 nt 75.8%	115 aa 86.1%
JMSV MSB144475	1290 nt[a] 65.8%	429 aa 69.1%	1412 nt 72.6%	470 aa 79.8%	4928 nt 74.8%	1642 aa 85.7%
CBNV CBN-3	1287 nt[a] 64.7%	428 aa 69.0%	3420 nt[a] 68.9%	1139 aa 73.9%	412 nt 75.0%	137 aa 84.9%
TGNV Tan826	442 nt 62.4%	147 aa 61.2%	—	—	412 nt 71.8%	137 aa 77.4%
BOWV VN1512	1296 nt[a] 63.6%	431 aa 67.8%	3438 nt[a] 67.0%	1145 aa 66.8%	6477 nt[a] 70.9%	2158 aa 78.2%
AZGV KBM15	540 nt 60.7%	180 aa 60.6%	687 nt 71.0%	229 aa 73.8%	4556 nt 71.4%	1518 aa 77.7%
JJUV SH42	1287 nt[a] 60.8%	428 aa 61.8%	3408 nt 66.2%	1135 aa 65.8%	6474 nt[a] 71.3%	2157 aa 77.6%
MJNV Cl 05-11	1311 nt[a] 53.8%	436 aa 45.3%	3363 nt[a] 51.7%	1120 aa 42.5%	6450 nt[a] 62.3%	2149 aa 61.7%
TPMV VRC66412	1308 nt[a] 50.9%	435 aa 44.2%	3366 nt[a] 52.3%	1121 aa 43.1%	6450 nt[a] 61.6%	2149 aa 61.7%
ULUV FMNH158302	1187 nt 52.8%	395 aa 45.7%	1389 nt 52.8%	463 aa 42.8%	6459 nt[a] 61.9%	2152 aa 61.9%
KMJV FMNH174124	1269 nt[a] 53.7%	423 aa 47.6%	1240 nt 53.8%	413 aa 45.1%	6447 nt[a] 63.0%	2148 aa 62.7%
RKPV MSB57412	1287 nt[a] 60.1%	428 aa 60.3%	3411 nt 57.7%	1136 aa 51.8%	6462 nt[a] 65.3%	2153 aa 67.9%
OXBV Ng1453	1287 nt[a] 64.4%	428 aa 68.3%	3426 nt 66.9%	1141 aa 67.4%	4345 nt 72.4%	1448 aa 80.3%
ASAV N10	1302 nt[a] 65.1%	433 aa 70.0%	3423 nt[a] 71.6%	1140 aa 77.6%	6456 nt[a] 75.4%	2151 aa 85.0%
NVAV Te34	1287 nt[a] 56.1%	428 aa 49.7%	3384 nt[a] 53.5%	1127 aa 44.8%	6474 nt[a] 63.6%	2157 aa 62.1%

For virus names and hosts, refer to legend of Figure 2.
[a]Complete coding region.
—, sequences unavailable; aa, amino acids; ARTV, Artybash virus; nt, nucleotides; SWSV, Seewis virus.

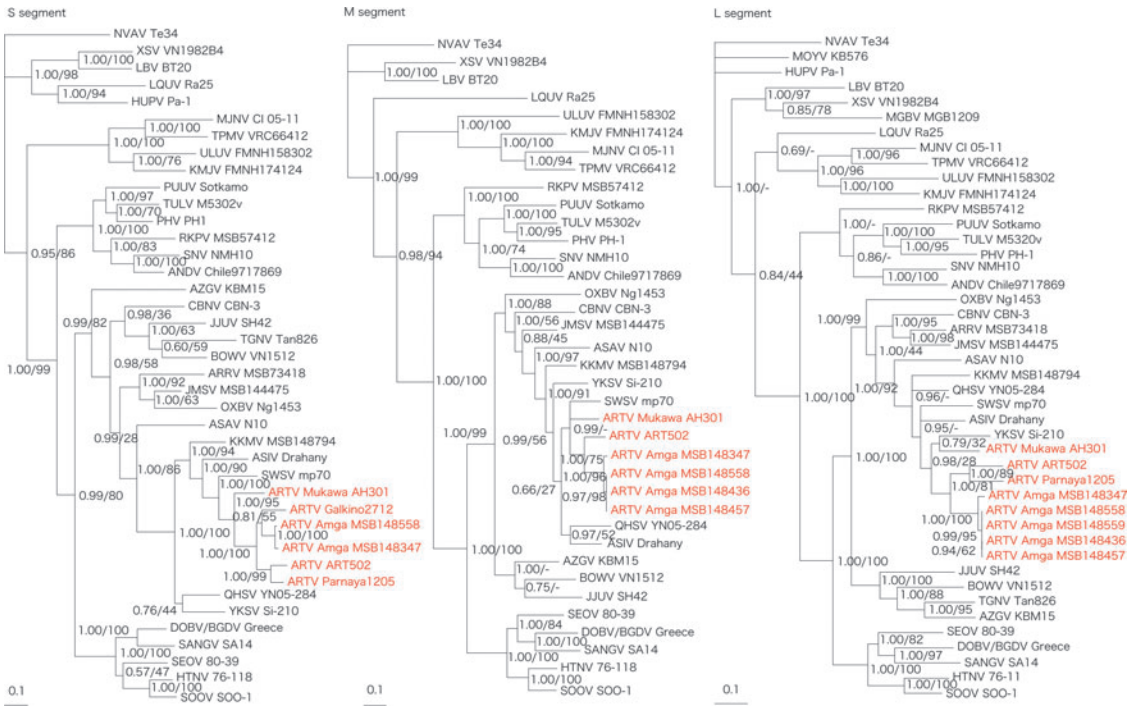

FIG. 2. Phylogenetic trees generated by Bayesian and maximum likelihood methods under the best-fit GTR + I + Γ model of evolution as estimated based on the entire coding regions of the full-length 1290-nucleotide S, 3420-nucleotide M, and 6456-nucleotide L genomic segments of Artybash virus (ARTV) strain Mukawa AH301. Phylogenetic trees show the positions of ARTV Mukawa AH301 (GenBank accession numbers: S: KF974360; M: KF974359; and L: KF974361), ARTV Amga MSB148558 (S: KM201411; M: KM201412; and L: KM201413), ARTV Amga MSB148559 (S: KM201414), ARTV Amga MSB148436 (M: KM201415 and L: KM201416), ARTV Amga MSB148457 (M: KM201417 and L: KM201418), ARTV Amga MSB148347 (S: KM201419; M: KM201420; and L: KM201421), ARTV ART502 (S: KM288698; M: EU424340; and L: EU424339), ARTV Galkino2712 (S: KM288699), and ARTV Parnaya1205 (S: KU253274 and L: KU253275) from *Sorex caecutiens*. Also shown are Ash River virus (ARRV MSB73418, S: EF650086 and L: EF619961) from *Sorex cinereus*, Jemez Springs virus (JMSV MSB144475, S: FJ593499; M: FJ593500; and L: FJ593501) from *Sorex monticolus*, Seewis virus (SWSV mp70, S: EF636024; M: EF636025; and L: EF636026) from *Sorex araneus*, Asikkala virus (ASIV Drahany, S: KC880342; M: KC880345; and L: KC880348) from *Sorex minutus*, Kenkeme virus (KKMV MSB148794, S: GQ306148; M: GQ306149; and L: GQ306150) from *Sorex roboratus*, Qian Hu Shan virus (QHSV YN05-284, S: GU566023; M: GU566022; and L: GU566021) from *Sorex cylindricauda*, Yakeshi virus (YKSV Si-210, S: JX465423; M: JX465403; and L: JX465389) from *Sorex isodon*, and Cao Bang virus (CBNV CBN-3, S: EF543524; M: EF543526; and L: EF543525) from *Anourosorex squamipes*, as well as Thottapalayam virus (TPMV VRC66412, S: AY526097 and L: EU001330) from *Suncus murinus*, Imjin virus (MJNV Cl 05-11, S: EF641804; M: EF641798; and L: EF641806) from *Crocidura lasiura*, Azagny virus (AZGV KBM15, S: JF276226; M: JF276227; and L: JF276228) from *Crocidura obscurior*, Tanganya virus (TGNV Tan826, S: EF050455 and L: EF050454) from *Crocidura theresea*, Bowé virus (BOWV VN1512, S: KC631782; M: KC631783; and L: KC631784) from *Crocidura douceti*, Jeju virus (JJUV SH42, S: HQ663933; M: HQ663934; and L: HQ663935) from *Crocidura shantungensis*, Uluguru virus (ULUV FMNH158302, S: JX193695; M: JX193696; and L: JX193697) from *Myosorex geata*, and Kilimanjaro virus (KMJV FMNH174124, S: JX193698; M: JX193699; and L: JX193700) from *Myosorex zinki*. Mole-borne hantaviruses include Asama virus (ASAV N10, S: EU929072; M: EU929075; and L: EU929078) from *Urotrichus talpoides*, Nova virus (NVAV Te34, S: KR072621; M: KR072622; and L: KR072623) from *Talpa europaea*, Oxbow virus (OXBV Ng1453, S: FJ5339166; M: FJ539167; and L: FJ593497) from *Neurotrichus gibbsii*, and Rockport virus (RKPV MSB57412, S: HM015223; M: HM015222; and L: HM015221) from *Scalopus aquaticus*. Bat-borne hantaviruses include Magboi virus (MGBV MGB1209, L: JN037851) from *Nycteris hispida*, Mouyassué virus (MOYV KB576, L: JQ287716) from *Neoromicia nanus*, Huangpi virus (HUPV Pa-1, S: JX473273 and L: JX465369) from *Pipistrellus abramus*, Longquan virus (LQUV Ra-25, S: JX465415; M: JX465397; and L: JX465381) from *Rhinolophus sinicus*, Laibin virus (LBV BT20, S: KM102247; M: KM102248; and L: KM102249) from *Taphozous melanopogon*, and Xuan Son virus (XSV VN1982B4, S: KC688335; M: KU976427; and L: JX912953) from *Hipposideros pomona*. Other taxa include Sin Nombre virus (SNV NMH10, S: NC_005216; M: NC_005215; and L: NC_005217), Andes virus (ANDV Chile9717869, S: AF291702; M: AF291703; and L: AF291704), Prospect Hill virus (PHV PH-1, S: Z49098; M: X55129; and L: EF646763), Tula virus (TULV M5302v, S: NC_005227; M: NC_005228; and L: NC_005226), Puumala virus (PUUV Sotkamo, S: NC_005224; M: NC_005223; and L: NC_005225), Dobrava virus/Belgrade virus (DOBV/BGDV Greece, S: NC_005233; M: NC_005234; and L: NC_005235), Hantaan virus (HTNV 76-118, S: NC_005218; M: NC_005219; and L: NC_005222), Soochong virus (SOOV SOO-1, S: AY675349; M: AY675353; and L: DQ056292), Sangassou virus (SANGV SA14, S: JQ082300; M: JQ082301; and L: JQ082302) and Seoul virus (SEOV 80-39, S: NC_005236; M: NC_005237; and L: NC_005238). The numbers at each node are posterior node probabilities (*left*) based on 150,000 trees: two replicate Markov chain Monte Carlo runs, consisting of six chains of 10 million generations each sampled every 100 generations with a burn-in of 25,000 (25%) and bootstrap values (*right*), based on 100 replicates executed on the RAxML BlackBox Web server.

generations and estimated sample sizes more than 100 (implemented in MrBayes).

Host species identification

Because shrews are inherently difficult to identify by morphological features alone, host verification of ARTV-infected shrews was confirmed by analyzing the entire 1140-base pair cytochrome *b* gene of mitochondrial DNA (mtDNA), amplified by PCR, using modified universal primers (Cy-14726F: 5′-GACYARTRRCATGAAAAAYCAYCGTTGT-3′ and Cy-15909R: 5′-CYYCWTYIYTGGTTTACAAGACYAG-3′) (Arai et al. 2008b). A Bayesian approach, as described above, with midpoint rooting was used to determine the phylogenetic relationship between Laxmann's shrews and other shrew and mole species known to harbor distinct hantaviruses.

Results and Discussion

ARTV RNA was detected by RT-PCR in five of 39 (12.8%) and one of 29 (3.4%) Laxmann's shrews captured in Russia and Japan, respectively (Table 1). All but one of the six ARTV-infected shrews were males.

Analysis of the full-length nucleotide and amino acid sequences of the S, M, and L segments (GenBank accession numbers: KF974360, KF974359, and KF974361, respectively) of ARTV strain Mukawa AH301 showed considerable divergence from representative hantaviruses harbored by shrews in the Soricinae subfamily in Eurasia (Table 3): S, 22.9–27.3% and 13.3–16.0%; M, 20.9–23.8% and 8.4–12.7%; and L, 20.2–22.1% and 5.7–8.7%, respectively. In contrast, alignment and comparison between ARTV strain Mukawa AH301 and the very limited S, M, and L segments of ARTV strains ART502, Galkino2712, and Parnaya1205 showed higher sequence similarity at the amino acid level (Table 3).

Phylogenetic analyses of ARTV strain Mukawa AH301, based on the full-length coding regions (comprising 1290-nucleotide S, 3420-nucleotide M, and 6456-nucleotide L segment sequences), showed similar topologies, with ARTV strains from the Sakha Republic and ARTV strains detected independently in Laxmann's shrews captured in the Altai Republic and Khabarovsk Krai of Russia forming a separate geographic-specific cluster (Fig. 2). Moreover, ARTV strains were distinct from recently detected hantaviruses harbored by other *Sorex* shrew species in Eurasia. In contrast, differences in the phylogenetic positions of some soricine shrew-borne hantaviruses may be attributed to the limited sequences, especially for the M and L segments.

The identities of the six ARTV-infected shrews were confirmed as Laxmann's shrews (GenBank no. KF974362 for strain AH301; GU562415, GU562416, GU562417, GU562418, and GU562422 for strains MSB148558, MSB148559, MSB148436, MSB148457, and MSB148347, respectively). The cytochrome *b* mtDNA sequences of the ARTV-infected Laxmann's shrews from Japan and Russia differed by 4.0–6.4%. The ARTV-negative shrews from Japan were also identified as Laxmann's shrews by analysis of the 1140-nucleotide cytochrome *b* mtDNA gene (GenBank nos. KU760858 to KU760884). However, the full-length cytochrome *b* mtDNA sequence variation was 0–1.1% and 0.5–1.1% among Laxmann's shrews in Japan and Russia, respectively.

Host phylogenies based on mtDNA cytochrome *b* sequences of shrew and mole species, which harbor genetically distinct hantaviruses, showed two separate lineages of Laxmann's shrews (Fig. 3). This was consistent with previous studies suggesting at least two genetic races of Laxmann's

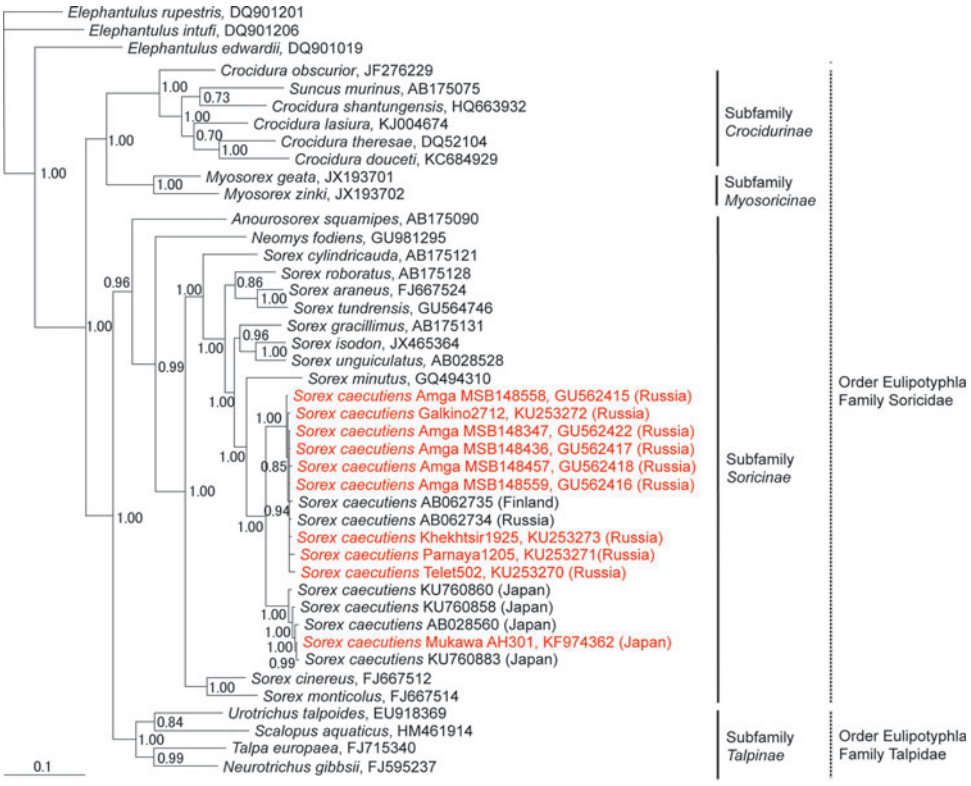

FIG. 3. Bayesian phylogenetic tree based on 1140 nucleotides of the cytochrome *b* mtDNA of shrews and moles (order Eulipotyphla and families Talpidae and Soricidae). The tree was rooted using *Elephantulus* (order Macroscelidea, GenBank nos. DQ901019, DQ901206, and DQ901201) as the outgroup. Numbers at nodes indicate posterior probability values based on 150,000 trees: two replicate Markov chain Monte Carlo runs, consisting of six chains of 10 million generations each sampled every 100 generations with a burn-in of 25,000 (25%). GenBank numbers for all taxa are provided in the tree.

shrews distributed in Eurasia and Japan (Ohdachi et al. 2003). That is, based on analysis of the full-length cytochrome *b* gene, Laxmann's shrews were separated into Hokkaido and Eurasian Continent–Sakhalin–Cheju clusters. Individuals in the latter cluster do not always reflect the geographical proximity of their capture locations, which is consistent with an ancestral isolation of the Hokkaido population, occurring ~13,000 years before present (Tanabe et al. 2015), and recent rapid range expansion of the modern Eurasian Continent–Sakhalin–Cheju population (Ohdachi et al. 2003).

The phylogeographic variation in ARTV reflects the distinct evolutionary histories of these variants. Similar patterns of geographic variation have also been reported for rodent-borne hantaviruses. For example, geographic-specific genetic variants have been reported for Puumala virus in the bank vole (*Myodes glareolus*) (Garanina et al. 2009), Tula virus in the European common vole (*Microtus arvalis*) (Song et al. 2004), and Andes virus in the colilargo (*Oligoryzomys longicaudus*) (Torres-Perez et al. 2011). Both Muju virus in the royal vole (*Myodes regulus*) (Lee et al. 2014) and Hokkaido virus in the grey red-backed vole (*Myodes rufocanus*) and northern red-backed vole (*Myodes rutilus*) (Yashina et al. 2015) may represent genotypes of Puumala virus. These examples point to the urgency of studying the phylogeographic variation of viruses and their hosts to provide an essential foundation for understanding their evolutionary histories and as a prelude to forecasting their future emergence under changing environmental conditions (Hope et al. 2013, Campbell et al. 2015).

Although ARTV was detected in a single Laxmann's shrew captured in the Mukawa area in Japan, the evidence is compelling that this *Sorex* shrew species harbors ARTV across its broad geographic range. Our findings suggest that Laxmann's shrews, resident on Hokkaido Island before the geologic separation from the Eurasian continent, might have already been infected with ARTV. Renewed attempts to isolate ARTV from Laxmann's shrews captured in Eurasian Continent–Sakhalin–Cheju and Japan are warranted to better understand its evolutionary origins and phylogeography, as well as its pathogenic potential in humans.

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Author Disclosure Statement

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