Lymphocytic Choriomeningitis Virus Lineage V in Wood Mice, Germany

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We identified a novel lineage of lymphocytic choriomeningitis virus, tentatively named lineage V, in wood mice (*Apodemus sylvaticus*) from Germany. Wood mouse–derived lymphocytic choriomeningitis virus can be found across a substantially greater range than previously thought. Increased surveillance is needed to determine its geographic range and zoonotic potential.

Lymphocytic choriomeningitis virus (LCMV; species *Mammarenavirus choriomeningitidis*) is a single-stranded RNA virus that has a bisegmented genome and ambisense coding strategy (*1*). LCMV is a zoonotic virus that causes encephalitis, meningitis, and sudden infant death syndrome in humans (*2*,*3*) and callitrichid hepatitis in New World primates (family Callitrichidae) (*4*). According to phylogenetic analysis, LCMV lineages I–IV are recognized. The most common, lineages I and II, are found worldwide (the house mouse, *Mus musculus*, is a reservoir host), whereas lineage III was found in 1 patient in Georgia, USA. Lineage IV was identified by sequencing small (S) RNA segments obtained from wood mice (*Apodemus sylvaticus*) found at 3 sites in southern Spain (*5*). That same study also reported the presence of LCMV antibodies in *M. musculus*, *M. spretus*, and *Rattus norvegicus* (Norway) rats in Spain (*5*). Similarly, LCMV-reactive antibodies have been found in wood mice from Austria (*6*) and in yellow-necked field mice (*Apodemus flavicollis*) and voles from Italy (*7*). LCMV reemerged in Germany in a captive golden lion tamarin (*Leontopithecus rosalia*) and sympatric wild *M. musculus domesticus* mice (*8*). We report the discovery of LCMV RNA in wood mice from Germany.

High-throughput sequencing of pooled brain tissue from *Apodemus* spp. captured in southern Germany revealed the presence of LCMV sequence reads (Appendix, https://wwwnc.cdc.gov/EID/article/30/2/23-0868-App1.pdf). We tested brain tissue samples from each of those animals (4 yellow-necked field mice and 13 wood mice) separately by reverse transcription PCR (*9*). We found LCMV amplification products of the expected length only in 2 wood mouse samples (KS20/3119 and KS20/3122). In addition, we tested 132 rodents and shrews collected during 2005–2018, representing 5 species from the same geographic region in Bavaria, Germany, as the 2 LCMV RNA-positive animals. Those 132 animals were negative for LCMV RNA by using conventional panarenavirus reverse transcription PCR (Appendix Table 1, Figure 1).

We captured all 134 animals (132 rodents and shrews plus 2 LCMV-positive wood mice) near natural forest or reforested areas at an altitude of 366–620 m by using line trapping. We placed traps 2 m apart within lines and 10 m between lines. We trapped animals 1 time per year for 2 consecutive nights during 2005–2018.

We assembled nearly complete sequences of LCMV large (L) and S RNA segments and host mitochondrial cytochrome b DNA from brain tissue of the 2 LCMV-positive wood mice and performed phylogenetic analyses. We deposited LCMV sequences obtained in this study in GenBank (accession nos. OR135709–12). The L (7,144 nt) and S (3,342 nt) sequences contained complete coding regions except for the first ≈55 nt and last ≈18 nt of the L segment and first ≈18 nt and last ≈24 nt of the S segment. For all 3 coding regions examined (L protein, glycoprotein, and nucleocapsid protein), virus sequences from the 2 mice formed a separate monophyletic clade (tentatively named lineage V) that is ancestral to all previously published LCMV sequences (Figure; Appendix Figures 2, 3) and highly divergent at the nucleotide and amino acid sequence levels (Appendix Table 2). Phylogenetic analysis of wood mouse mitochondrial cytochrome b sequences showed that both LCMV-positive animals clustered with *Apodemus sylvaticus* subclade 2b (Appendix Figure 4), the same subclade as the mice from Spain in which LCMV lineage IV was discovered (*5*).

In conclusion, we identified a new LCMV lineage in wood mice from southern Germany. Unlike Dandenong virus, an unclassified mammarenavirus that

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falls within lineage II (both L and S segments), sequences from lineage V constitute their own distinct clade that is basal to other known LCMV lineages. Host mitochondrial DNA sequences indicated the wood mice from Germany belonged to the same clade as those in which LCMV lineage IV was previously identified in Spain. The serologic evidence of LCMV in wood mice from Italy (*7*) and Austria (*6*) combined with LCMV RNA detection in wood mice from Spain (*5*) and this study suggest that wood mouse-derived

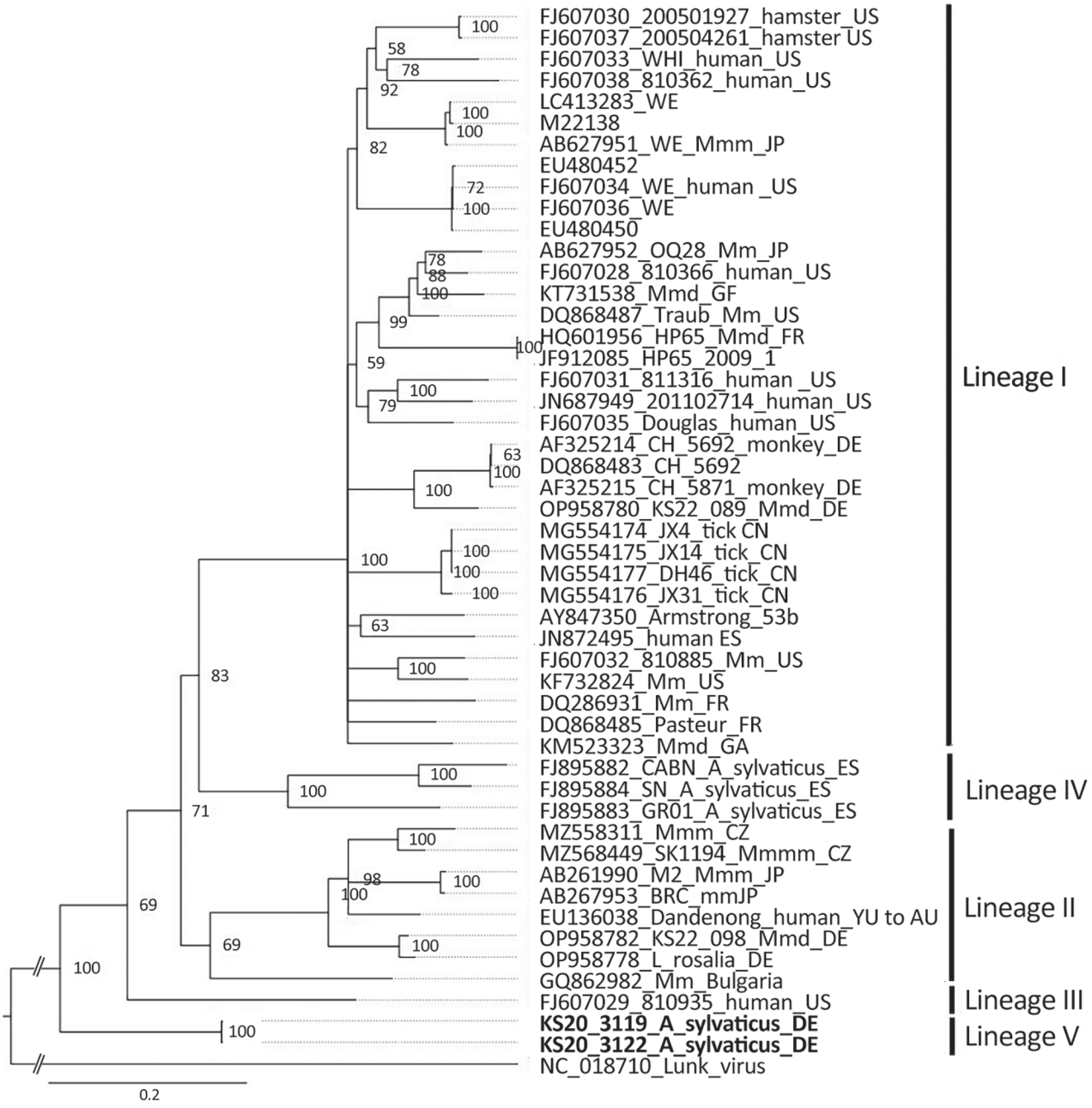

Figure. Phylogenetic analysis of the nucleocapsid protein encoding region of lymphocytic choriomeningitis virus lineage V identified in wood mice, Germany (boldface), and reference sequences. Bayesian inference method was used to analyze the 1,674-nt open reading frame corresponding to codons 1–558 without the stop codon. GenBank accession number, strain name, host species, and country of origin (if known) are shown. Roman numerals I–IV represent the different virus lineages as defined previously (*10*). Lunk virus from *Mus minutoides* mice was used as an outgroup. WE and Armstrong are laboratory strains of lymphocytic choriomeningitis virus. Scale bar indicates nucleotide substitutions per site. Asyl, *Apodemus sylvaticus*; AU, Australia; BG, Bulgaria; CN, China; CZ, Czech Republic; DE, Germany; ES, Spain; FR, France; GA, Gabon; GF, French Guiana; JP, Japan; Mm, *Mus musculus*; Mmm, *M. musculus musculus*; Mmd, *M. musculus domesticus*; SK, Slovakia; US, United States; YU, former Yugoslavia.

LCMV can be found across a substantially greater range than previously thought. Greater surveillance is needed to determine the geographic range and diversity of LCMV in small mammals and the potential infection risk to humans.

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About the Author

Mr. Mehl is a doctoral candidate at the Friedrich-Loeffler-Institut in Greifswald-Insel Riems, Germany. His research interests focus on how small mammal ecology, microbiome diversity, and ecotoxicology influence disease ecologies.

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Appendix

Additional Methods

Genetic screening of wood mice and other sympatric small mammals was performed by using a SuperScript III One-Step RT-PCR Kit (Thermo Fisher Scientific, https://www.thermofisher.com) and arenavirus primers (*1*). Brain tissues were homogenized and nucleic acids were isolated by using a NucleoMag VET kit (Macherey-Nagel, https://www.mn-net.com) and KingFisher Flex Purification System (Thermo Fisher Scientific). High-throughput sequencing was performed as previously described (*2*). Coding sequences of lymphocytic choriomeningitis virus large and small segments and host mitochondrial cytochrome b DNA were obtained through a de novo assembly with SPAdes version 3.13.0 (*3*). Sequences were aligned by using the ClustalW algorithm in BioEdit (*4*). The best-fit nucleotide substitution model was determined by using JModelTest2 (*5*,*6*). Thereafter, phylogenetic trees were constructed by using MrBayes v.3.2.7 (*7*). For all 3 coding regions, Bayesian tree reconstruction was performed by using a general time reversal substitution model with gamma distribution and a proportion of invariable sites. Twenty million generations were run; trees were sampled every 1000 generations, and the first 25% were discarded as burn-in. For maximum-likelihood tree construction of *Apodemus sylvaticus* cytochrome b, we used the general time reversal substitution model with gamma distribution and a proportion of invariable sites. Bootstraps were calculated from 1000 replications (*8*). LCMV sequences obtained in this study were deposited in GenBank and are available under the accession numbers OR135709–12.

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Appendix Table 1. Number of animals tested for lymphocytic choriomeningitis virus*

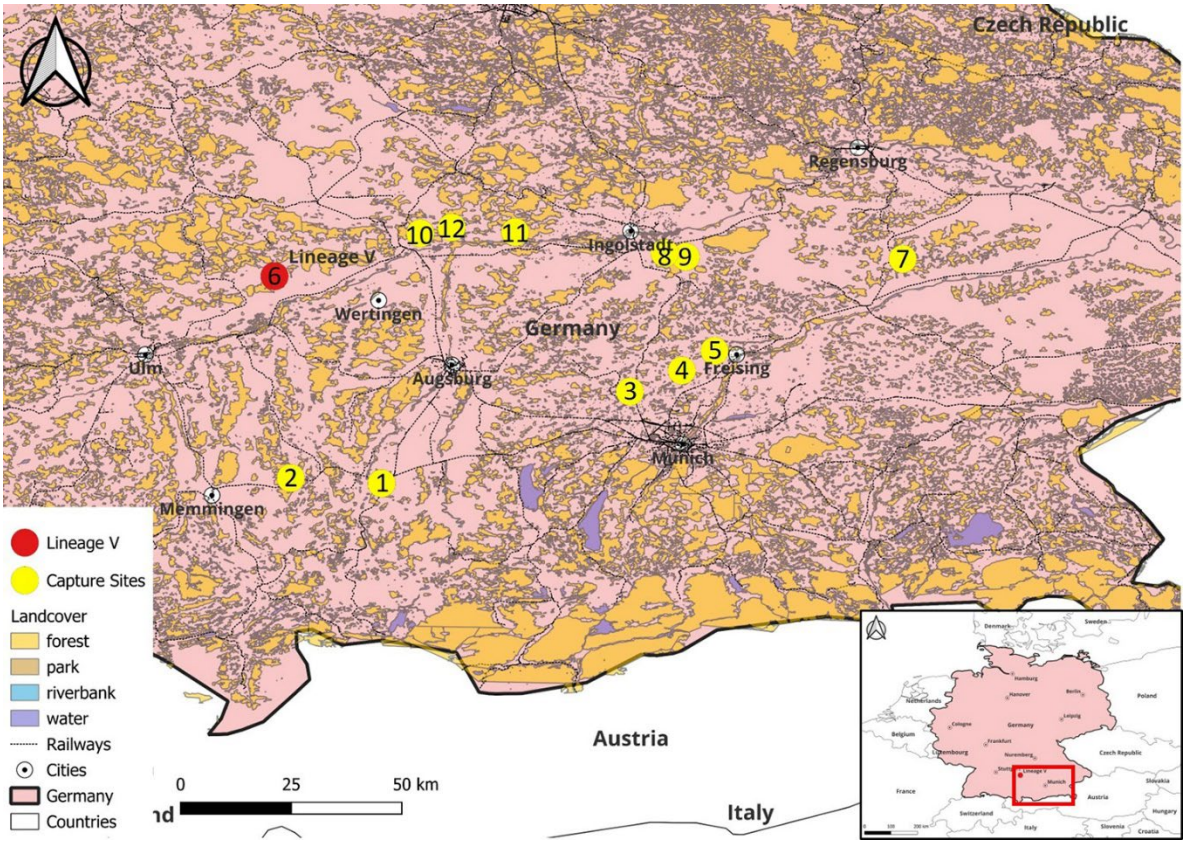
Site	Species	Common name	No. positive/total no. tested
1	*Apodemus sylvaticus*	Wood mouse	0/2
2	*Apodemus sylvaticus*	Wood mouse	0/5
3	*Sorex araneus*	Common shrew	0/2
4	*Myodes glareolus* (syn. *Clethrionomys glareolus)*	Bank vole	0/3
5	*Apodemus sylvaticus*	Wood mouse	0/3
6	*Apodemus sylvaticus*	Wood mouse	2/3
	Myodes glareolus (syn. *Clethrionomys glareolus)*	Bank vole	0/29
	Microtus agrestis	Field vole	0/6
7	*Myodes glareolus* (syn. *Clethrionomys glareolus)*	Bank vole	0/3
8	*Sorex araneus*	Common shrew	0/1
9	*Microtus agrestis*	Field vole	0/9
	Sorex araneus	Common shrew	0/1
10	*Myodes glareolus* (syn. *Clethrionomys glareolus)*	Bank vole	0/18
	Sorex araneus	Common shrew	0/2
11	*Apodemus flavicollis*	Yellow-necked field mouse	0/28
	Myodes glareolus (syn. *Clethrionomys glareolus)*	Bank vole	0/9
	Microtus agrestis	Field vole	0/2
	Sorex araneus	Common shrew	0/2
12	*Apodemus sylvaticus*	Wood mouse	0/1
	Myodes glareolus (syn. *Clethrionomys glareolus)*	Bank vole	0/2
	Microtus agrestis	Field vole	0/3
Totals	*Apodemus sylvaticus*	Wood mouse	2/14
	Apodemus flavicollis	Yellow-necked field mouse	0/28
	Myodes glareolus	Bank vole	0/64
	Microtus agrestis	Field vole	0/20
	Sorex araneus	Common shrew	0/8

*Locations of sites are presented in Appendix Figure 1. syn, synonym.

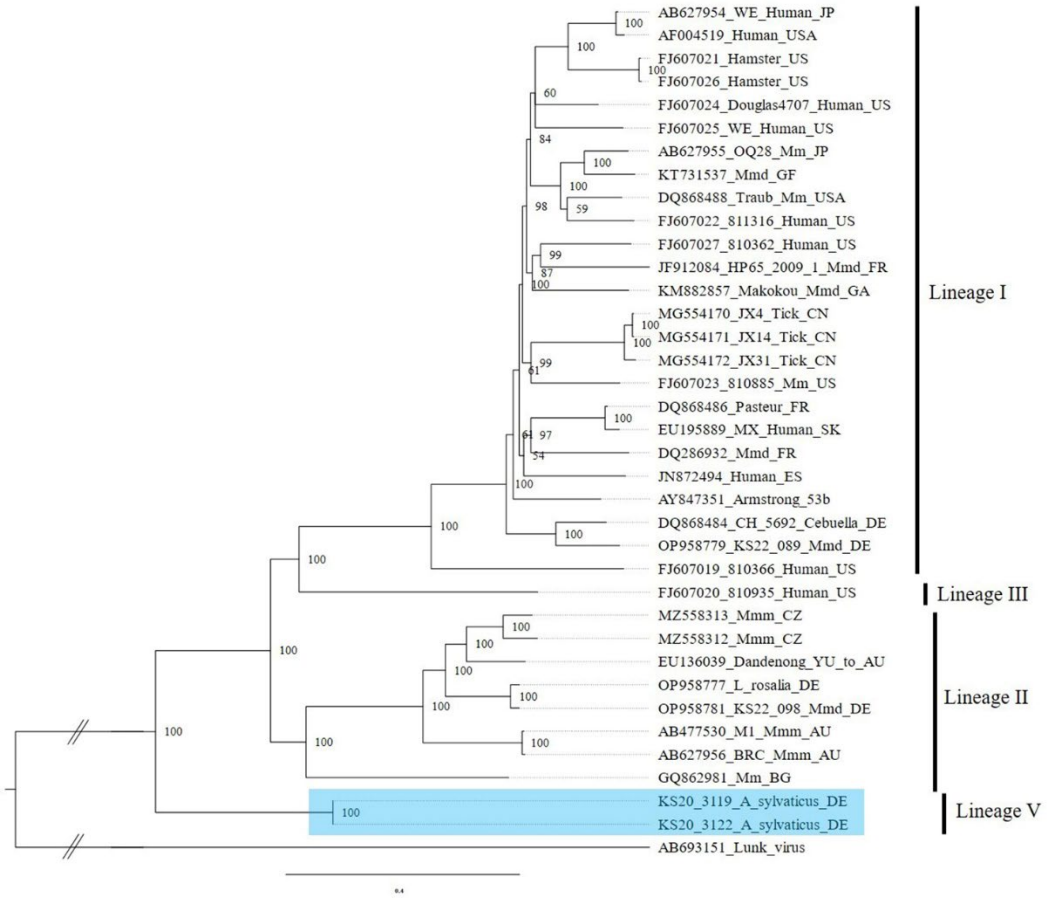
Appendix Table 2. Nucleotide and amino acid sequence differences between the newly identified lineage V and recognized lineages of lymphocytic choriomeningitis virus*

	Large protein		Glycoprotein		Nucleoprotein	
Clade	Nucleotide	Amino acid	Nucleotide	Amino acid	Nucleotide	Amino acid
Lineage I	28.4 (29.2)	22.4 (24.1)	24.4 (25.5)	16.1 (18.5)	21.3 (23.5)	7.8 (11.8)
Lineage II	27.8 (28.4)	21.7 (22.2)	23.5 (24.0)	14.3 (15.7)	20.7 (21.4)	7.0 (7.9)
Lineage III	28.3 (28.3)	21.8 (21.8)	23.6 (23.6)	16.7 (16.7)	20.0 (20.0)	6.3 (6.3)
Lineage IV	ND	ND	25.3 (26.0)	17.7 (19.1)	20.4 (21.4)	8.9 (9.5)
Lunk virus outgroup	36.4 (36.4)	35.8 (35.8)	29.7 (29.7)	22.1 (22.1)	27.6 (27.6)	17.7 (17.7)

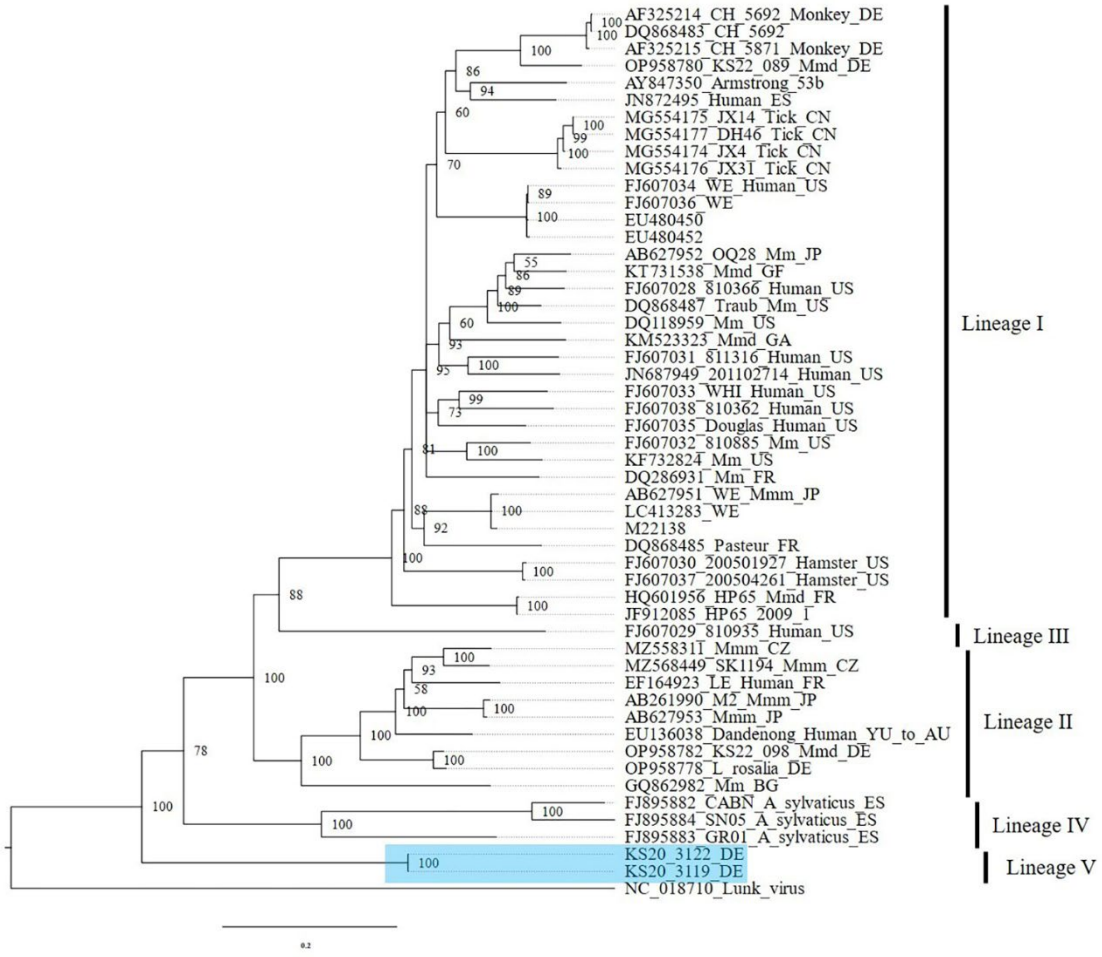
*Values are mean (maximum) % sequence differences. ND, no data available.



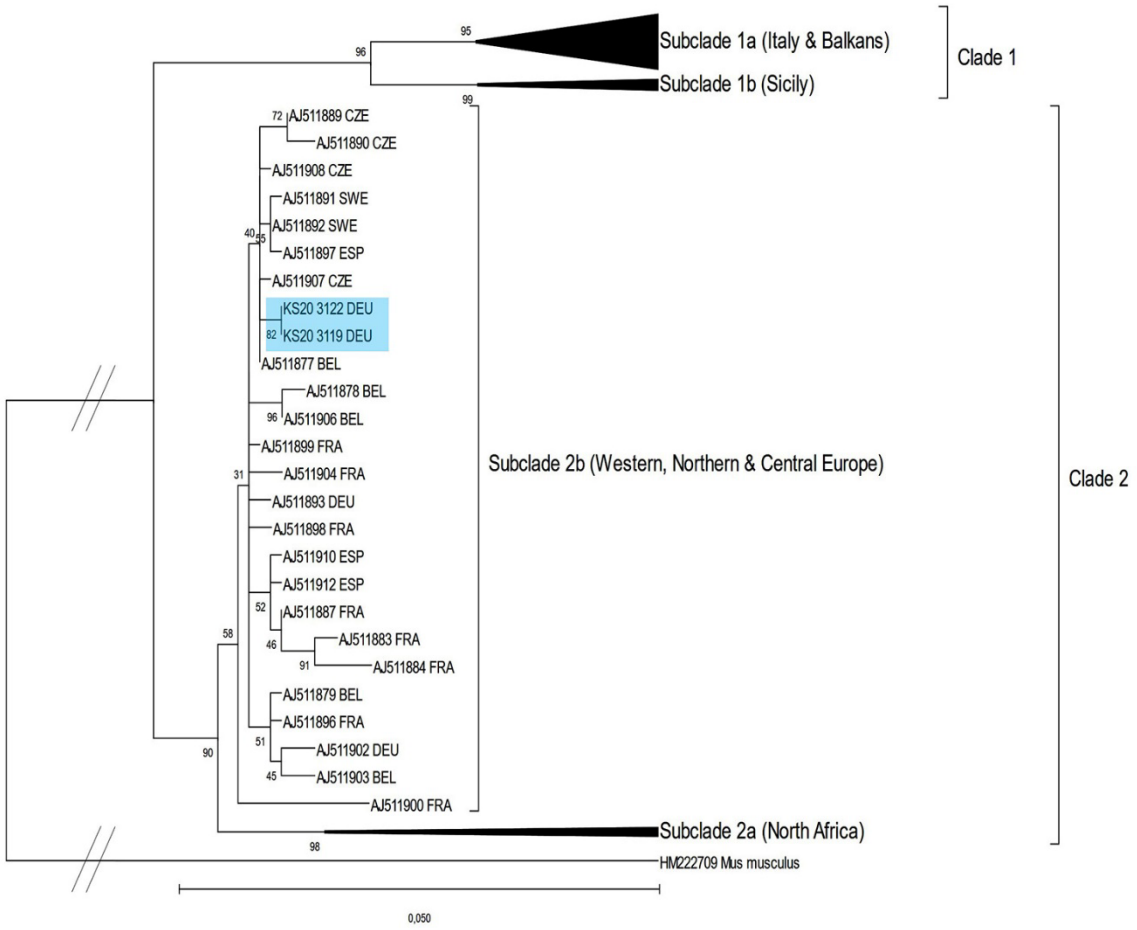
Appendix Figure 1. Map showing animal trapping site locations and land cover types in southern Germany. Red square in inset map indicates the area in southern Germany where trapping occurred. Yellow and red circles indicate capture sites in the main map. Red circle shows location where 2 *Apodemus sylvaticus* mice infected with lymphocytic choriomeningitis virus lineage V were found (site 6). Map was produced by using QGIS 3.32.0 (QGIS Geographic Information System, Open Source Geospatial Foundation Project, http://qgis.org). Data were obtained from OpenStreetMap (https://www.openstreetmap.org).



Appendix Figure 2. Phylogenetic analysis of the L protein encoding region of lymphocytic choriomeningitis virus lineage V in wood mice, Germany. Bayesian inference method was used to analyze 6,597 nt corresponding to codons 2–2210 without the start codon or the 10 codons (including the stop codon) at the 3′ end. Sequences from this study are highlighted in blue. Lunk virus from *Mus minutoides* mice was used as an outgroup. Sequence names are comprised of the GenBank accession number, strain name, host species, and country of origin (if known). Countries are represented by their international organization of standardization code. Roman numerals I–III represent the different virus lineages as defined previously (*9*). L-segment sequences for lineage IV were not available. WE and Armstrong are laboratory strains of lymphocytic choriomeningitis virus. Asyl, *Apodemus sylvaticus*; AU, Australia; BG, Bulgaria; CN, China; DE, Germany; ES, Spain; FR, France; GA, Gabon; GF, French Guiana; JP, Japan; Mm, *Mus musculus*; Mmm, *Mus musculus musculus*; Mmd, *Mus musculus domesticus*; SK, Slovakia; US, United States; YU, former Yugoslavia. Scale bar indicates nucleotide substitutions per site.



Appendix Figure 3. Phylogenetic analysis of the glycoprotein encoding region of lymphocytic choriomeningitis virus lineage V in wood mice, Germany. Bayesian inference method was used to analyze 1,494 nt corresponding to codons 1–498, excluding the stop codon. Sequences from this study are highlighted in blue. Lunk virus from *Mus minutoides* mice was used as an outgroup. Sequence names are comprised of the GenBank accession number, strain name, host species and country of origin (if known). Countries are represented by their international organization of standardization code. Roman numerals I–IV represent the different virus lineages as defined previously (*9*). WE and Armstrong are laboratory strains of lymphocytic choriomeningitis virus. Asyl, *Apodemus sylvaticus*; AU, Australia; BG, Bulgaria; CN, China; CZ, Czech Republic; DE, Germany; ES, Spain; FR, France; GA, Gabon; GF, French Guiana; JP, Japan; Mm, *Mus musculus*; Mmm, *Mus musculus musculus*; Mmd, *Mus musculus domesticus*; SK, Slovakia; US, United States; YU, former Yugoslavia. Scale bar indicates nucleotide substitutions per site.



Appendix Figure 4. Phylogenetic analysis of mitochondrial cytochrome b DNA from *Apodemus sylvaticus* mice. Maximum-likelihood method was used for partial cytochrome b nucleotide sequences (909 nt, corresponding to codons 4718–5020). Sequences from this study are highlighted in blue. Sequence from *Mus musculus* mice was used as an outgroup. Sequence names are comprised of the GenBank accession number and country of origin (if known). Countries are represented by their international organization of standardization code. Clades are defined as previously described (*10*). Wood mouse phylogeny can be divided into 2 major clades according to mitochondrial cytochrome b DNA phylogenetics: clade 1 comprises populations from Italy, Sicily, and the Balkans and clade 2 comprises populations from North Africa and western, northern, and central Europe (*10*). Those clades can be further subdivided into subclades: subclade 1a, Italy and Balkans; 1b, Sicily; 2a, North Africa, and 2b, central Europe (*10*). Sequences obtained from wood mice from this study clustered in subclade 2b. Scale bar indicates nucleotide substitutions per site.