Local maintenance and genomic diversity of lymphocytic choriomeningitis virus in natural populations of house mice in the Czech Republic over a 24-year period

Ivana Jezkova[1], Alena Fornůsková[1], Ľudovít Ďureje[1], Miloš Macholán[2], Jaroslav Piálek[1], and Joëlle Goüy de Bellocq[1]

[1]Ustav biologie obratlovcu Akademie ved Ceske republiky
[2]Institute of Animal Physiology and Genetics of the Czech Academy of Sciences

March 03, 2025

Abstract

Lymphocytic choriomeningitis virus (LCMV) is a neglected rodent-borne zoonotic virus primarily infecting house mice. The virus can be highly pathogenic, particularly in immunocompromised individuals and in congenital infections. LCMV is distributed worldwide but shows local clustering, probably due to the highly structured populations of its hosts and the vertical transmission of the pathogen. These factors should also promote long-term virus persistence in wild populations, yet this aspect remains largely unexplored. To investigate this, we resampled a transect in the western Czech Republic that was primarily studied more than a decade ago. Additionally, we analyzed a sample collection from Buškovice, a locality where LCMV was first detected in 2008, to trace virus presence back to the year 2000. Positive samples underwent whole-genome characterization to assess the virus's genetic structure over space and time. We detected intermittent presence over 24 years in a geographically limited area, where LCMV was already present in 2000 and remained detectable in 2023. Phylogenetic analysis showed no clear spatio-temporal clustering, suggesting that virus persistence in Buškovice is a dynamic process involving mouse dispersal between neighboring villages. Given LCMV's zoonotic potential and house mouse synanthropy, these findings highlight the need for continuous monitoring in the region.

Title

Local maintenance and genomic diversity of lymphocytic choriomeningitis virus in natural populations of house mice in the Czech Republic over a 24-year period

Authors

Ivana Jezkova[1], Alena Fornůsková[1], Ľudovít Ďureje[1], Miloš Macholán[2], Jaroslav Piálek[1], Joëlle Goüy de Bellocq[1]

Affiliations

[1] Institute of Vertebrate Biology of the Czech Academy of Sciences, Brno, Czech Republic

[2] Institute of Animal Physiology and Genetics of the Czech Academy of Sciences, Brno, Czech Republic

Corresponding author

Ivana Jezkova, jezkova@ivb.cz, Institute of Vertebrate Biology of the Czech Academy of Sciences, Květná 170/8, 603 65 Brno, Czech Republic, +420 560 590 607

Keywords

LCMV, Arenaviridae, Mammarenavirus, house mouse, *Mus musculus* , Rodent-borne viruses

Running Head

LCMV maintenance in mice over 24 years

ABSTRACT

Lymphocytic choriomeningitis virus (LCMV) is a neglected rodent-borne zoonotic virus primarily infecting house mice. The virus can be highly pathogenic, particularly in immunocompromised individuals and in congenital infections. LCMV is distributed worldwide but shows local clustering, probably due to the highly structured populations of its hosts and the vertical transmission of the pathogen. These factors should also promote long-term virus persistence in wild populations, yet this aspect remains largely unexplored. To investigate this, we resampled a transect in the western Czech Republic that was primarily studied more than a decade ago. Additionally, we analyzed a sample collection from Buškovice, a locality where LCMV was first detected in 2008, to trace virus presence back to the year 2000. Positive samples underwent whole-genome characterization to assess the virus's genetic structure over space and time. We detected intermittent presence over 24 years in a geographically limited area, where LCMV was already present in 2000 and remained detectable in 2023. Phylogenetic analysis showed no clear spatio-temporal clustering, suggesting that virus persistence in Buškovice is a dynamic process involving mouse dispersal between neighboring villages. Given LCMV's zoonotic potential and house mouse synanthropy, these findings highlight the need for continuous monitoring in the region.

1. INTRODUCTION

Lymphocytic choriomeningitis virus (LCMV) is a rodent-borne virus with cosmopolitan distribution due to its primary reservoir host, the house mouse (*Mus musculus*) (1). Other wild rodents (2,3) or domesticated and pet animals can also serve as reservoirs (4,5). Humans can become infected after contact with rodent secreta or excreta or by inhalation of aerosolized particles. Human-to-human transmission has been documented during transplantation or vertically during intrauterine development (4,6). In healthy people, the infection is usually asymptomatic or with mild symptoms. However, in immunocompromised individuals, the infection can develop into life-threatening conditions, e.g., aseptic meningitis, encephalopathy or encephalitis. Congenital infection can lead to miscarriage or severe defects in the fetus, often of neurodevelopmental manifestation (6).

LCMV is an enveloped RNA virus belonging to the genus*Mammarenavirus* , family *Arenaviridae* (1). The genome, approximately 10.6 kb long, consists of two segments: The large (L) segment carries the RNA-dependent RNA polymerase (L) gene and the RING finger protein (Z) gene whereas the small (S) segment carries the viral glycoprotein precursor (GPC) gene and the nucleoprotein (NP) gene.

In natural house mouse populations, LCMV can be transmitted both horizontally and vertically. When transmitted horizontally, infected individuals typically mount an immune response that clears the infection. However, when transmitted vertically, i.e., when mice are infected with LCMV during the intrauterine period or early in life, they fail to develop an effective immune response, resulting in a chronic, asymptomatic, lifelong infection and the agent again can be transmitted vertically to their offspring which in turn become chronically infected (5,7). LCMV infections in natural mouse populations have been shown to be patchy at local and regional scales (5). This pattern was observed in a recent study in Central Europe, where two subspecies of the house mouse, *Mus musculus musculus* and *M. m. domesticus,* come into contact and form a narrow hybrid zone ((8); see Figure 1A). After serological and molecular screening of more than 700 mice sampled between 2008 and 2014, LCMV was detected in only four localities within a 12 km^2 area in the *musculus* territory in the Czech Republic. In one locality, Buškovice, LCMV was detected in 2008 and 2014, suggesting that the virus could persist in this area for several years.

House mouse populations form small, stable and relatively isolated communities known as "demes" (9,10). This population structure combined with the vertical transmission of LCMV should favor the focal spread of the virus (5) but also its long-term persistence within affected areas. The maintenance of LCMV in natural

mouse populations has been understudied, although this is a key factor in understanding the dynamics of the virus in reservoir populations and the risk to humans. The aim of this study was therefore to investigate the focal and long-term maintenance of the virus in this region and to assess its genetic structure in space and time.

2. MATERIALS AND METHODS

2.1. Sample collection and LCMV detection

In 2022, 216 house mice from 54 localities in the region between north-eastern Bavaria (Germany) and western Bohemia (Czech Republic) were sampled as in (8) and tested for LCMV antibodies (Figure 1B, Table). As LCMV can also infect other rodent species (2,3,5), 89 small mammals other than house mice (see Table) but living in sympatry with them, were also tested for LCMV antibodies. Two additional mice were sampled in Buškovice in 2023 and included in the screening. The serum of these individuals was tested with the ELISA kit IM-698 C-EB (XpressBio, https://xpressbio.com). Of these 218 house mice, 98 individuals, either from Buškovice or from localities within a 21 km radius of Buškovice, were further analysed for the presence of LCMV RNA in lung tissue. RNA was extracted using the RNeasy kit (Qiagen) and subjected to one-step RT-PCR (Invitrogen SuperScript IV System, ThermoFisher Scientific) targeting a 340-nucleotide fragment of the L gene (12), following (8).

To go back in time and extend the period for assessing the long-term maintenance of LCMV in Buškovice, 60 individuals were tested from a -80 °C frozen collection of kidney/lung tissue from house mice sampled in 2000, 2003, 2004, 2008 and 2014 (Table). The molecular one-step RT-PCR screening was performed as described above.

2.2. Whole genome sequencing and phylogenetic analyses

Five LCMV-positive samples were selected for whole genome sequencing: JPC2844 from 2000, ST5763 and ST5769 from 2003, SK2670 from 2014 and JHZ22_002 from 2022. The xGen^{TM} Broad-range RNA Lib Prep Kit (Integrated DNA Technology) was used for library preparation and pooled libraries were sequenced with 150 paired-end reads on a DNBSEQ-G400 at BGI Genomics (Poland). Mouse reads were removed after mapping against the GRCm39 mouse reference genome using BWA. Unmapped reads were trimmed and *de novo* assembled with metaSPAdes (13) and scaffolds classified using BlobTools (14). The metaSPAdes scaffolds were then polished by mapping reads to the scaffolds with a consensus threshold of 60% using Geneious mapper (Geneious, https://www.geneious.com) to obtain complete LCMV L and S segments. The L, NP and GPC gene sequences of the newly characterized strains were aligned with the sequences of LCMV strains SK1194 and SK1042 originating from two localities eight and four kilometers from Buškovice (see (8) and Appendix Figure) and with the Dandenong strain originating from the former Yugoslavia (16). Bayesian phylogenetic analysis was performed in MrBayes 3.2.7 (15) using the GTR model with gamma rates for all three genes. Pasteur and Armstrong strains from Clade I sensu Albariño et al. (2010) (16) were used as outgroups.

3. RESULTS

3.1. Virus detection

The serological screening revealed that LCMV was present in only one of 216 house mice sampled in the region between north-eastern Bavaria and western Bohemia in 2022. The positive sample came from the subspecies*musculus* from the locality Buškovice. In addition, one of the two mice from the same locality sampled in 2023 was also positive. LCMV antibodies were not detected in any of the 89 rodent individuals found in sympatry with the house mice which included 48 individuals of the genus *Apodemus* (Table). *Apodemus sylvaticus* has been shown to be a reservoir for LCMV in Spain and Germany (2,3). RT-PCR screening of the 98 samples centered around Buškovice revealed one additional*musculus* individual positive for LCMV RNA and originating from Buškovice 2022 (Table, Appendix Table).

RT-PCR screening of older samples from our tissue collection from Buškovice revealed five positive samples

for LCMV RNA: 1 of 2 samples from 2000, 3 of 16 samples from 2003, and 1 of 7 samples from 2014 were positive (Table). An overview of the years in which LCMV-positive individuals were found in Buškovice, either by serological or molecular screening, and combining the data from Fornuskova et al. (8) and this study is shown in Figure 2. LCMV was detected in six of the eight years for which we had samples available (no LCMV was found in 2004 and 2019), in a total period of 24 years.

3.2. Sequence and phylogeny analyses

Whole genome sequencing of five LCMV strains was successful and allowed us to assemble their complete genomes. All strains had unique S and L sequences, but with limited diversity: the average pairwise nucleotide/amino acid identity was 96.5%/99.6% for the NP gene, 95.3%/97.9% for the GPC gene and 94.6%/96.7% for the L gene. The topologies of the phylogenetic trees were similar for all three genes (Figure 3), suggesting the absence of recombination within the S segment or reassortment between the S and L segments. The five newly characterized strains clustered with the two LCMV strains previously characterized in this region, . The tree topologies did not showed a clear temporal or spatial clustering of samples. The samples are not all descendant from a single viral lineage for the whole period of 24 years. Instead, the samples were divided into two temporal clusters, 2000 – 2003 and 2014 – 2022, suggesting that the LCMV lineage present in Buškovice in 2000 was replaced between 2003 and 2014 by a lineage also present in the nearby village of Nepomyšl in 2009.

4. DISCUSSIONS

We investigated the spatial clustering and the long-term maintenance of LCMV in wild house mouse populations in Central Europe, where the two subspecies known to be natural reservoirs of the virus meet. There, LCMV was found more than a decade ago in four localities, Nepomyšl, Kryry, Žihle, and Buškovice, within 12 km^2 (8). Our study, based on samples from 216 mice captured in 2022 (along with two additional samples from 2023), reinforces the previous finding, with the virus detected exclusively in the *musculus* territory. During this sampling, we did not catch any mice in Nepomyšl and Kryry so it was not possible to determine whether the virus is still present in these localities or not. However, samples from Žihle were all negative and we did not detect LCMV in other localities surrounding Buškovice, which confirms the LCMV presence is still very limited in space. This focal distribution has already been reported both locally and on a large scale. In Baltimore, United States, LCMV infection was shown to vary drastically between neighboring houses in the same block (18). At the regional scale, a serological study carried out in West Germany from 1960 to 1962 showed that 65/1795 positive mice were mainly localized in the regions of North Rhine-Westphalia and southern Lower Saxony (19). In other regions, no or only a few localities were found positive for LCMV, with no positive localities in Bavaria.

The detection of the virus in Buškovice in 2023, i.e., 15 years after the first detection (8), prompted us to investigate whether the virus was already present in this locality before 2008 by analyzing an old tissue collection. We detected LCMV in 2000, the earliest sample available in our collection. Overall, the virus was detected in six out of eight time points over a 24-year period, either by detection of viral RNA or using antibodies. Although LCMV was not detected in Buškovice in 2009, it was present in neighboring localities in the same year (Nepomyšl and Kryry) and in 2010 (Žihle) (8). The maintenance of the virus from generation to generation in captive house mice has been known for a long time due to the vertical transmission of the virus (5). However, data on the long-term maintenance of LCMV in wild mice are rare. To our knowledge, until our study, the longest LCMV presence in nature was evidenced in a serological study of wild rodents in northern Italy, which showed that the prevalence of LCMV in *Apodemus flavicollis* fluctuated considerably over a 7-year period (20). The discontinuous detection in Buškovice is to be expected due to the very low LCMV prevalence in this region - 2.4% and 0.8% in serological and molecular assays, respectively (8) - and the limited number of mice captured in each locality.

The patchy occurrence of LCMV and its long-term maintenance may be due to the combination of the house mouse population structure and the vertical transmission of the virus (5). House mice form relatively stable demes with low dispersal ability (10, 17). Therefore, a deme that initially acquires an LCMV through

horizontal transmission could easily maintain the virus over many generations by vertical transmission within the deme. The turn-over of the viral lineage observed between 2003 and 2022 suggests that, despite the strong genetic structure, dispersal of individuals between adjacent populations occurs and is sufficient to exchange the virus between neighboring villages.

5. CONCLUSIONS

This study confirms the focal occurrence of LCMV in the sampled region and reports its persistence in wild mouse populations over a period of 24 years. Thus, human exposure to this zoonotic virus is geographically limited but persistent over time. This has important implications for public health and pest control strategies. Targeted measures in a few localities could potentially eradicate the mouse demes that sustain LCMV, and thus reduce the risk of transmission to humans. The ability of the virus to persist in mouse populations over time emphasizes the need for continuous surveillance to protect public health and prevent spillover.

Statements

Funding statement

This work was funded through the Czech Science Foundation grant 22-32394S. Computational resources were provided by the e-INFRA CZ project (ID:90254), supported by the Ministry of Education, Youth and Sports of the Czech Republic. Institutional support from the Czech Academy of Sciences RVO:68081766.

Conflict of interest disclosure:

The authors declare no conflicts of interest.

Data availability statement

Data provided in supplementary materials. The complete genomes of the five new strains were deposited in GenBank (AN: PQ726963-PQ726972).

Acknowledgments: We would like to acknowledge the help of our colleagues and the kindness of local farmers during the sample collection.

Permissions

Research permits and approval (no. 27/2007) by the Institutional Committee and Czech Academy of Sciences Committee for animal welfare according to Czech law and UBO-834/Sekr-e/2021 from the Czech Ministry of Agriculture.

Author Contributions

Study design JGB, AF, data collection AF, LD, MM, JP, JGB, data analysis IJ, AF, JGB, data interpretation IJ, JGB, draft manuscript preparation IJ, manuscript revision IJ, JBG. All the authors approved the final version of the manuscript.

Emails and ORCiD numbers Ivana Jezkova: jezkova@ivb.cz, 0009-0000-4707-8401

Alena Fornůsková: a.fornuskova@seznam.cz, 0000-0003-3319-8394

Ľudovít Ďureje: dureje@gmail.com, 0009-0000-9406-7377

Miloš Macholán: macholan@iach.cz, 0000-0001-5663-6831

Jaroslav Piálek: jpialek@ivb.cz, 0000-0002-0829-7481

Joëlle Goüy de Bellocq: joellegouy@gmail.com, 0000-0001-5831-6284

References

1. Radoshitzky SR, Buchmeier MJ, Charrel RN, Gonzalez JPJ, Günther S, Hepojoki J, et al. ICTV Virus Taxonomy Profile: Arenaviridae 2023. J Gen Virol. 2023;104:001891.

2. Mehl C, Adeyemi OA, Wylezich C, Höper D, Beer M, Triebenbacher C, et al. Lymphocytic Choriomeningitis Virus Lineage V in Wood Mice, Germany. Emerg Infect Dis. 2024;30:399–401.

3. Ledesma J, Fedele CG, Carro F, Lledó L, Sánchez-Seco MP, Tenorio A, et al. Independent Lineage of Lymphocytic Choriomeningitis Virus in Wood Mice (*Apodemus sylvaticus*), Spain. Emerg Infect Dis. 2009;15:1677–80.

4. Amman BR, Pavlin BI, Albariño CG, Comer JA, Erickson BR, Oliver JB, et al. Pet rodents and fatal lymphocytic choriomeningitis in transplant patients. Emerg Infect Dis. 2007;13:719–25.

5. Childs JE, Klein SL, Glass GE. A Case Study of Two Rodent-Borne Viruses: Not Always the Same Old Suspects. Front Ecol Evol. 2019;7:35.

6. Olivieri NR, Othman L, Flannery DD, Gordon SM. Transmission, seroprevalence, and maternal-fetal impact of lymphocytic choriomeningitis virus. Pediatr Res. 2024;95:456–63.

7. Lehmann-Grube F. Persistent infection of the mouse with the virus of lymphocytic choriomeningitis. J Clin Pathol Suppl (R Coll Pathol). 1972;6:8–21.

8. Fornůsková A, Hiadlovská Z, Macholán M, Piálek J, Gouy de Bellocq J. New Perspective on the Geographic Distribution and Evolution of Lymphocytic Choriomeningitis Virus, Central Europe. Emerg Infect Dis. 2021;27:2638–47.

9. Reimer JD, Petras ML. Breeding Structure of The House Mouse,*Mus Musculus* , in A Population Cage. Journal of Mammalogy. 1967;48:88–99.

10. Mikula O, Macholán M, Ďureje Ľ, Hiadlovská Z, Daniszová K, Janotová K, et al. House mouse subspecies do differ in their social structure. Ecol Evol. 2022;12:e9683.

11. Baird SJE, Macholán M. What can the *Mus musculus musculus* /*M. m. domesticus* hybrid zone tell us about speciation? In: Macholán M, Baird SJE, Munclinger P, Piálek J, eds. Evolution of the House Mouse. Cambridge University Press; 2012:334-372.

12. Vieth S, Drosten C, Lenz O, Vincent M, Omilabu S, Hass M, et al. RT-PCR assay for detection of Lassa virus and related Old World arenaviruses targeting the L gene. Trans R Soc Trop Med Hyg. 2007;101:1253–64.

13. Nurk S, Meleshko D, Korobeynikov A, Pevzner PA. metaSPAdes: a new versatile metagenomic assembler. Genome Res. 2017;27:824–34.

14. Laetsch DR, Blaxter ML. BlobTools: Interrogation of genome assemblies. F1000Research; 2017;6:1287.

15. Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, et al. MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice Across a Large Model Space. Systematic Biology. 20121;61:539–42.

16. Albariño CG, Palacios G, Khristova ML, Erickson BR, Carroll SA, Comer JA, et al. High Diversity and Ancient Common Ancestry of Lymphocytic Choriomeningitis Virus. Emerg Infect Dis. 2010;16:1093–1100.

17. Pocock MJO, Hauffe HC, Searle JB, Dispersal in house mice. Biol J Linn Soc. 2005; 84: 565–583.

18. Childs JE, Glass GE, Korch GW, Ksiazek TG, and LeDuc JW. Lymphocytic choriomeningitis virus infection and house mouse (*Mus musculus*) distribution in urban Baltimore. *Am. J. Trop. Med. Hyg. 1992;* 47:27–34.

19. Ackermann R, Bloedhorn H, Kupper B, Winkens I, Scheid W. Uber die Verbreitung des Virus der lymphocytaren Choriomeningitis unter den Mausen in Westdeutschland. Zentralbl Bakt Parasitol Infekt Hyg. 1992;194:407–430.

20. Tagliapietra V, Rosà R, Hauffe HC, et al. Spatial and temporal dynamics of lymphocytic choriomeningitis virus in wild rodents, northern Italy. Emerg Infect Dis. 2009;15(7):1019-1025.

Table. Overview of the sampling year, geographic origin, tested individuals and screening assays.

Figure 1. Legend.

Localities sampled in Bavaria-Bohemia in Fornůsková et al. (8), panel A) and the current study, panel B). The red dashed line is the center of the house mouse hybrid zone between *Mus musculus musculus* and *M. m. domesticus* .

Figure 2. Legend.

Long-term maintenance of LCMV in the Buškovice locality is indicated by the number of positive/tested samples using (A) ELISA and (B) RT-PCR. The current study is represented in black, while data from Fornůsková et al. (8) is shown in blue. A single entry from 2014 (1*) indicated the presence of LCMV in a viral metagenomic dataset.

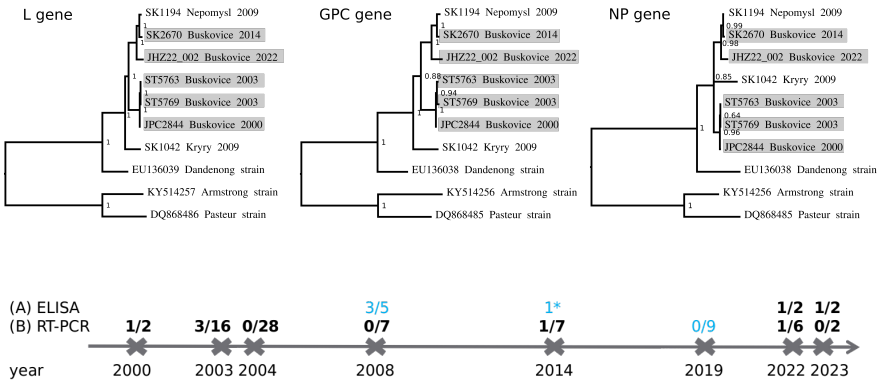
Figure 3. Legend.

Phylogeny inference based on nucleic acid sequences of the LCMV L, GPC and NP genes separately, using the Bayesian approach. Posterior probabilities are shown at the nodes. Armstrong and Pasteur LCMV strains from clade I (sensu 16) were used as outgroups. Samples characterized in this study are highlighted in grey.

Appendix Table. Samples of mice and other rodent species, together with their localities, used in the study.

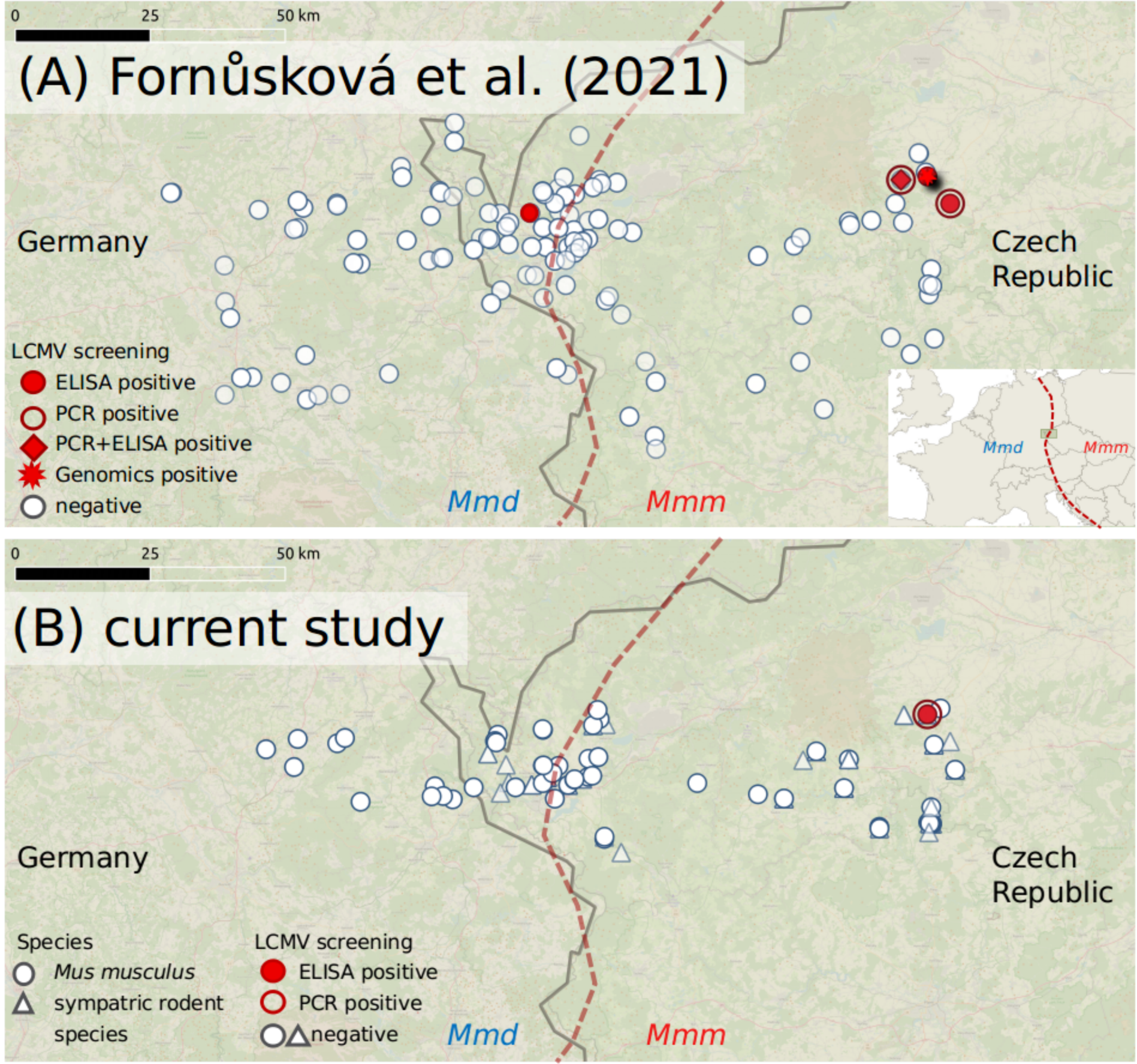
Appendix Figure. Legend.

Location of Buškovice and adjacent villages where LCMV was previously detected by Fornůsková et al. (8).



Hosted file

`Table.docx` available at https://authorea.com/users/897853/articles/1273824-local-maintenance-and-genomic-diversity-of-lymphocytic-choriomeningitis-virus-in-natural-populations-of-house-mice-in-the-czech-republic-over-a-24-year-period


0
25
50 km
(A) Fornůsková et al. (2021)
Germany
Czech Republic
LCMV screening
ELISA positive
PCR positive
PCR+ELISA positive
Genomics positive
negative
Mmd
Mmm
(B) current study
Species
Mus musculus
sympatric rodent species
LCMV screening
ELISA positive
PCR positive
negative