



Hantavirus Brno loanvirus is highly specific to the common noctule bat (*Nyctalus noctula*) and widespread in Central Europe

Maysaa Dafalla¹ · Anna Orłowska² · Sinan Julian Keleş³ · Petra Straková⁴ · Kore Schlottau⁵ · Kathrin Jeske¹ · Bernd Hoffmann⁵ · Gudrun Wibbelt⁶ · Marcin Smreczak² · Thomas Müller⁷ · Conrad Martin Freuling⁷ · Xuejing Wang⁸ · Jerzy Rola² · Stephan Drewes¹ · Sasan Fereidouni³ · Gerald Heckel^{8,9} · Rainer G. Ulrich^{1,10}

Received: 10 March 2022 / Accepted: 29 October 2022
© The Author(s) 2022

Abstract

Bat-associated hantaviruses have been detected in Asia, Africa and Europe. Recently, a novel hantavirus (Brno loanvirus, BRNV) was identified in common noctule bats (*Nyctalus noctula*) in the Czech Republic, but nothing is known about its geographical range and prevalence. The objective of this study was to evaluate the distribution and host specificity of BRNV by testing bats from neighbouring countries Germany, Austria and Poland. One thousand forty-seven bats representing 21 species from Germany, 464 bats representing 18 species from Austria and 77 bats representing 12 species from Poland were screened by L segment broad-spectrum nested reverse transcription-polymerase chain reaction (RT-PCR) or by BRNV-specific real-time RT-PCR. Three common noctules from Germany, one common noctule from Austria and three common noctules from Poland were positive in the hantavirus RNA screening. Conventional RT-PCR and primer walking resulted in the amplification of partial L segment and (almost) complete S and M segment coding sequences for samples from Germany and partial L segment sequences for samples from Poland. Phylogenetic analysis of these nucleotide sequences showed highest similarity to BRNV from Czech Republic. The exclusive detection of BRNV in common noctules from different countries suggests high host specificity. The RNA detection rate in common noctules ranged between 1 of 207 (0.5%; Austria), 3 of 245 (1.2%; Germany) and 3 of 20 (15%; Poland). In conclusion, this study demonstrates a broader distribution of BRNV in common noctules in Central Europe, but at low to moderate prevalence. Additional studies are needed to prove the zoonotic potential of this hantavirus and evaluate its transmission within bat populations.

Keywords Hantavirus · Bats · RT-qPCR · Host · Europe · Host specificity

Edited by Juergen Richt.

✉ Rainer G. Ulrich
rainer.ulrich@fli.de

- ¹ Institute of Novel and Emerging Infectious Diseases, Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Südufer 10, 17493 Greifswald-Insel Riems, Germany
- ² Department of Virology, National Veterinary Research Institute, 57 Partyzantów Avenue, 24-100 Pulawy, Poland
- ³ Research Institute of Wildlife Ecology, University of Veterinary Medicine Vienna, Savoyenstraße 1a, 1160 Vienna, Austria
- ⁴ Veterinary Research Institute, Hudcova 296/70, 621 00 Brno, Czech Republic
- ⁵ Institute of Diagnostic Virology, Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Südufer 10, 17493 Greifswald-Insel Riems, Germany

- ⁶ Leibniz Institute for Zoo and Wildlife Research, Alfred-Kowalke-Straße 17, 10315 Berlin, Germany
- ⁷ Institute of Molecular Virology and Cell Biology, Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Südufer 10, 17493 Greifswald-Insel Riems, Germany
- ⁸ Institute of Ecology and Evolution, University of Bern, Baltzerstrasse 6, 3012 Bern, Switzerland
- ⁹ Quartier Sorge - Batiment Amphipole, Swiss Institute of Bioinformatics, 1015 Lausanne, Switzerland
- ¹⁰ German Center for Infection Research (DZIF), Partner Site Hamburg-Lübeck-Borstel-Riems, Südufer 10, 17493 Greifswald-Insel Riems, Germany

Hantaviruses, family *Hantaviridae*, order *Bunyavirales*, are currently classified into four genera: *Orthohantavirus*, *Thottimvirus*, *Mobatvirus* and *Loanvirus* [1, 2]. The hantavirus genome consists of three RNA segments of negative polarity. The small (S) segment encodes the nucleocapsid (N) protein and the large (L) segment encodes the RNA-dependent RNA polymerase (RdRp). The glycoprotein precursor (GPC) is encoded by the medium (M) segment and cotranslationally cleaved at a conserved sequence motif into N-terminal Gn and C-terminal Gc [3].

Hantavirus disease in humans is currently thought to be associated with certain rodent-borne hantaviruses. Haemorrhagic fever with renal syndrome is caused by different hantaviruses in the Old World and by Seoul orthohantavirus, detected in both the Old and New World. In the New World, hantavirus cardiopulmonary syndrome is caused by Andes orthohantavirus, Sin nombre orthohantavirus [4], and related viruses.

Most rodent-borne hantaviruses exhibit strong host specificity, explained by virus–host coevolution [5, 6]. The host association of hantaviruses is usually evaluated by field studies of sympatric small mammals. Here, the reservoir is defined by the most frequent molecular detection of a given hantavirus in a single species or closely related species [6]. The geographical distribution of a given hantavirus follows the range of its reservoir; however, phylogeographic processes and ecological factors influencing reservoir populations may result in the absence of a hantavirus in certain areas [7–9].

Hantaviruses were initially thought to be exclusively rodent-borne, despite the first hantavirus being isolated from the Asian house shrew, *Suncus murinus* [10]. Moreover, the use of broad-spectrum L segment reverse transcription-polymerase chain reaction (RT-PCR) assays resulted in the identification of novel hantaviruses in shrews and moles [11–22]. Furthermore, novel hantaviruses have also recently been detected and characterized in bat species from Africa (e.g. Mouyassue virus (MOYV) in Cote d'Ivoire; Magboi virus (MGBV) in Sierra Leone; Makokou virus (MAKV) in Gabon) and Asia (e.g. Xuan Son virus (XSV) in Vietnam; Huangpi virus (HUPV), Longquan loanvirus (LQUV); Laibin mobatvirus (LBV) in China; Quezon mobatvirus (QZNV) in the Philippines; Đakrông virus (DKGV) in Vietnam) [23–30]. For most of these viruses, the host specificity is not well documented. The recent finding of Brno loanvirus (BRNV) in common noctule bats (*Nyctalus noctula*) shows the presence of bat-borne hantaviruses in Europe [31].

Here we aimed to evaluate the presence, prevalence and host specificity of BRNV through a field survey of bats from Germany, Austria and Poland.

Bats and/or bat swab samples were collected from Poland, Austria and Germany [32] (Fig. 1a). Animal carcasses were dissected, and tissue samples and body cavity fluid were

collected following standard protocols. Bat species were determined by morphological characters or molecular methods (for details see Supplementary Information). Tissue as well as oropharyngeal and rectal swab samples were subjected to RNA isolation and conventional RT-PCR and a novel BRNV-specific real-time RT-qPCR both targeting the L segment (for primer sequences and binding site positions see Table S1). Phylogenetic and sliding window analyses of sequence data and prediction of functional domains followed standard procedures (see Supplementary Information).

Screening of organ pool samples, from 1047 bats from 21 species from Germany [33], by RT-qPCR resulted in the identification of three positive samples (Table 1). Of the 618 oropharyngeal and rectal swab samples and 155 pooled tissue samples collected from 464 bats, representing 18 species, from Austria one tissue sample was positive (Table 1). Additional screening of the 1047 bats from Germany by conventional RT-PCR tested the same three samples positive and therefore confirmed the results of the RT-qPCR analysis (see Table 1). Subsequent analyses of common noctules ($N = 245$) from Germany by RT-PCR assays targeting partial M and S segment revealed two positive samples each; in contrast, re-amplification of the RT-qPCR-positive sample from Austria by conventional RT-PCR failed, most likely due to a lower sensitivity of the conventional assay. Of the 77 bats belonging to 12 species from Poland, three common noctules were positive by conventional nested RT-PCR of lung, liver and kidney samples (Table 1). All positive samples originated from common noctules with an estimated prevalence of 1.2% (3 of 245; 95% confidence interval, CI, 0.25–3.5%) for Germany, 0.5% (1 of 207; CI 0.01–2.7%) for Austria and 15% (3 of 20; CI 3.2–37.9%) for Poland. The here observed prevalence for common noctules in Poland might be biased by the low number of samples. The positive common noctules originated from two sites in central Germany, one site in north-western Germany, one site in Lower Austria and two locations in Southern Poland (Fig. 1a).

Phylogenetic analysis confirmed high similarity between common noctule-derived partial L segment sequences from this study and two recently discovered sequences from the Czech Republic [31], but showed a clear divergence to other bat-, insectivore- and rodent-borne hantaviruses (Fig. 1b). The nucleotide and amino acid sequence identity of the partial L segment and RdRp sequences of BRNV strains ranged between 94.3–100% and 95.2–100%, respectively (Table S2).

A primer-walking-based approach (for details see Supplementary Information) resulted in the determination of the almost complete coding sequences (CDS) of the S and M segments of BRNV from two common noctules from Germany (sample BH 08/16–276 / sample BH 08/16–23). Phylogenetic analyses and pairwise sequence comparisons of the CDS confirmed the high similarity to the BRNV prototype

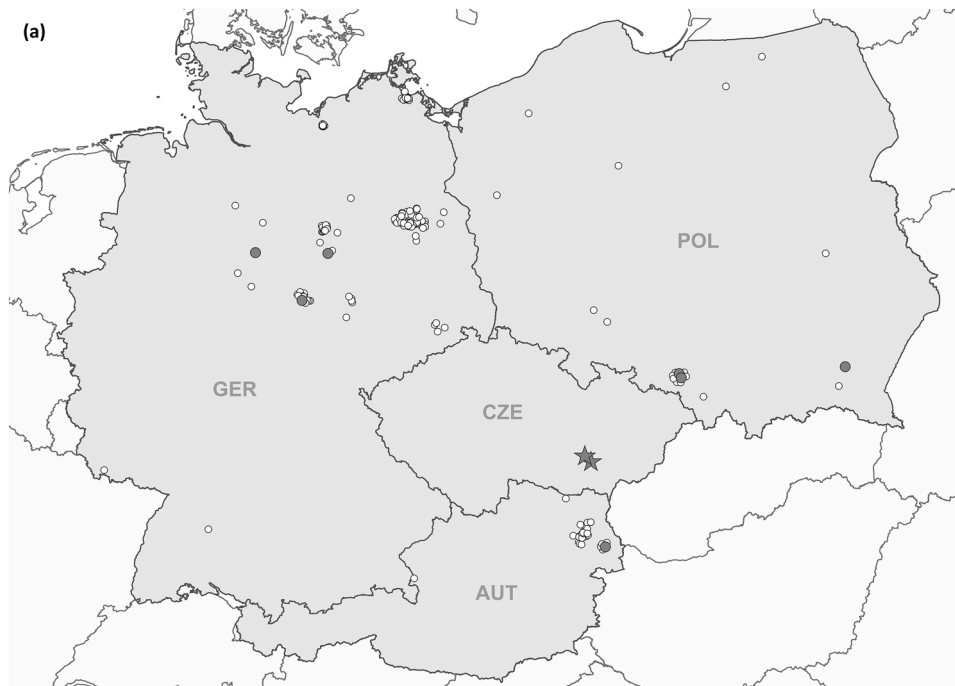


Fig. 1 Geographical origin of bat samples and evolutionary relationships of Brno loanvirus (BRNV) sequences. **a** Geographical origin of common noctule bat (*Nyctalus noctula*) samples from Germany ($n=245$, GER), Austria ($n=207$, AUT) and Poland ($n=20$, POL) tested for BRNV. The geographical origin of the two noctule bats from the Czech Republic (city of Brno, CZE) where BRNV was discovered is indicated. The origin of BRNV RNA positive and negative common noctules is indicated in the map by black dots and grey dots, respectively. For clarity, the sampling locations of BRNV-negative bats ($N=1133$) from 20 other species are not shown. **b–d** Phylogenetic consensus trees of partial L segment (**b**), (almost) complete S segment (**c**) and M segment (**d**) sequences of the novel BRNV strains, prototype BRNV strain from the Czech Republic, Longquan loanvirus and reference sequences of the other *Mammantavirinae* genera.

sequences from the Czech Republic (Fig. 1c, d; Table S3). The BRNV sequences of both segments were clearly separated from sequences of other bat-borne hantaviruses and the rodent- and insectivore-borne hantaviruses.

The N protein of BRNV prototype strain CZE 7_2012 of 423 amino acid residues was predicted to contain a non-cytoplasmic domain, a transmembrane domain, a cytoplasmic domain and three coiled coil structures (Fig. S1a). Amino acid sequence diversity of the novel BRNV sequences was highest within the transmembrane domain and at the N terminus of the non-cytoplasmic domain/coiled coil structures.

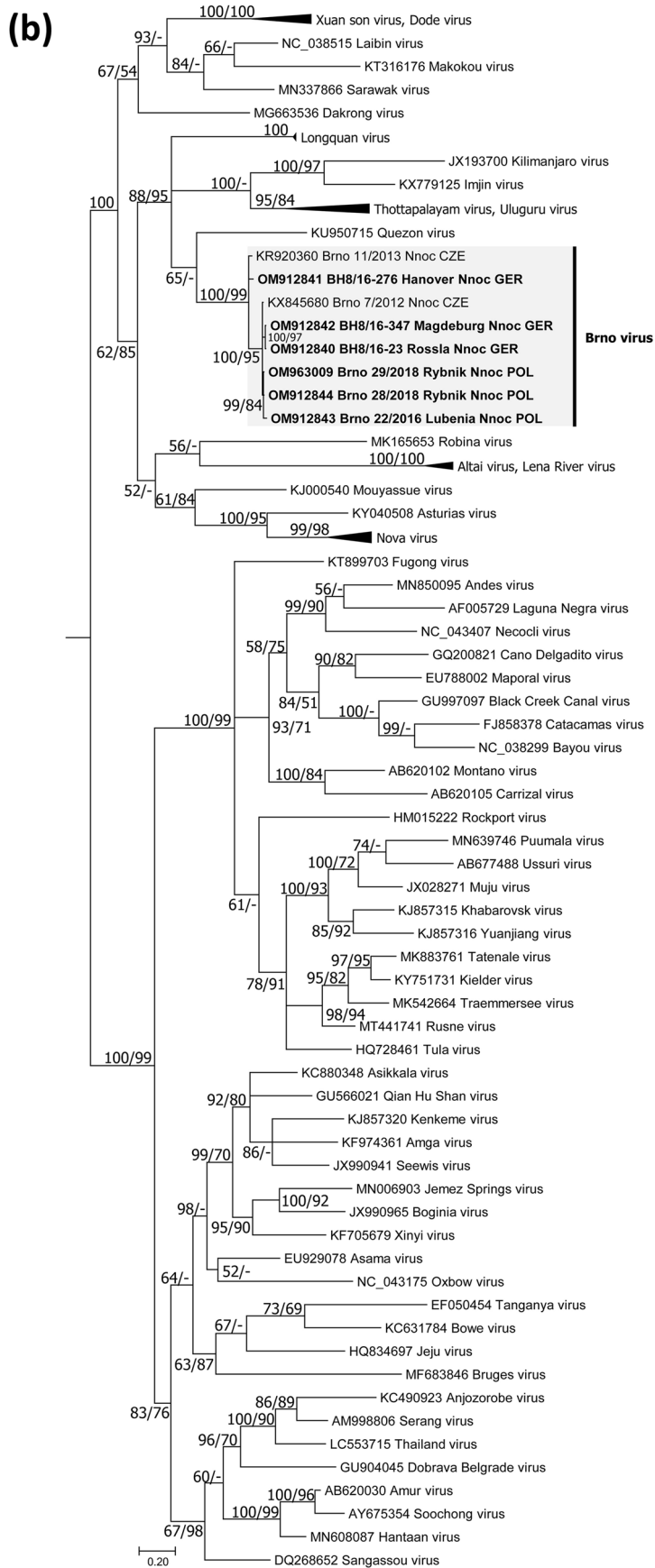
The prototype strain GPC (1136 residues) was predicted to consist of an 18 amino acid signal peptide at the N terminus of Gn, followed by a non-cytoplasmic domain, a transmembrane domain and a cytosolic tail, including an immunoreceptor tyrosine-based activation (ITAM) motif, followed by the putative Gn/Gc pentapeptide cleavage site (residues 649–653; Fig. S1b). This pentapeptide in the prototype and

Alignments were constructed using the Clustal W Multiple Alignment algorithm implemented in Bioedit (V7.2.3.) [38]. The most suitable substitution model was determined by jModelTest v2.1.6 [39]. The consensus trees are based on Bayesian analyses with 6×10^6 to 1×10^7 generations and a burn-in phase of 25% using MrBayes v3.2.6 [40] and Maximum-Likelihood analyses with 1000 bootstrap replicates performed with the aid of FasttreeMP v2.1.10 [41] and 50% cut-off using the General Time Reversible (GTR) substitution model with invariant sites and a gamma-distributed shape parameter for both algorithms. The consensus tree was established by transferring the bootstrap values to the Bayesian tree only if branches were supported by both trees. All tree reconstructions were done on CIPRES Science Gateway [42]. *Nnoc* *Nyctalus noctula*

the novel BRNV strains (“WGSSA”) differ from the conserved motif “WAASA” found in most orthohantaviruses [34, 35], mobatviruses and all thottimviruses, but also from the putative cleavage site “WASSA” in Longquan loanvirus and “WAVSA” in Asama, Bruges and Fugong orthohantaviruses (Table S4). The predicted Gc protein includes a non-cytoplasmic domain and ends after a transmembrane domain in a short cytoplasmic tail. The highest sequence divergence was seen within the Gn part of GPC, with peaks at the N terminus and the cytoplasmic domain (see Fig. S1b).

The tissue distribution of BRNV RNA was investigated for the three common noctules from Germany by RT-qPCR analyses. The highest viral RNA loads were observed in liver, lung and kidney samples (Table S5). In one animal, the brain and spleen samples were positive, whilst in another, the intestine sample was positive. The conventional RT-PCR analyses of different tissues of the three bats from Poland

Fig. 1 (continued)



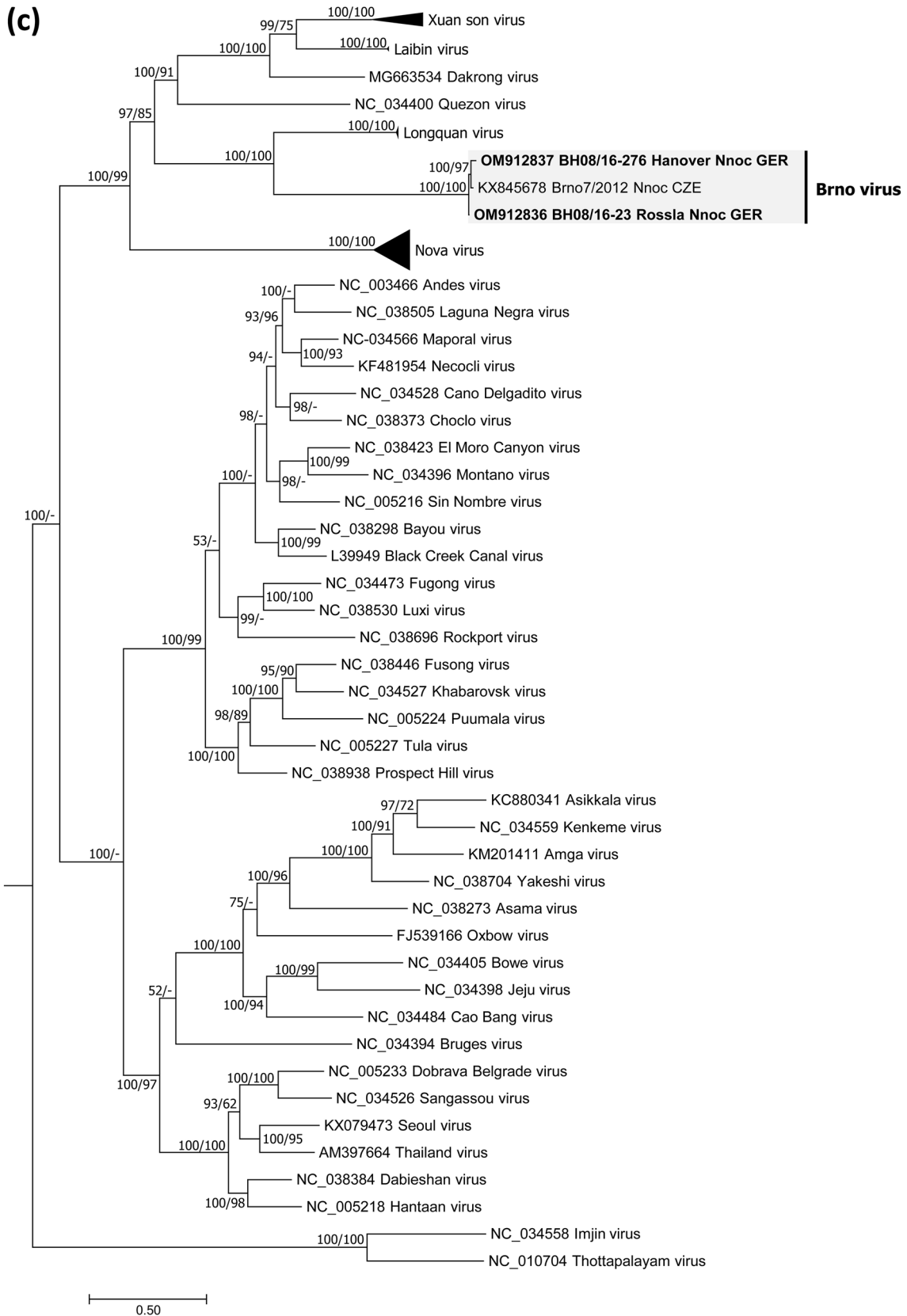


Fig. 1 (continued)

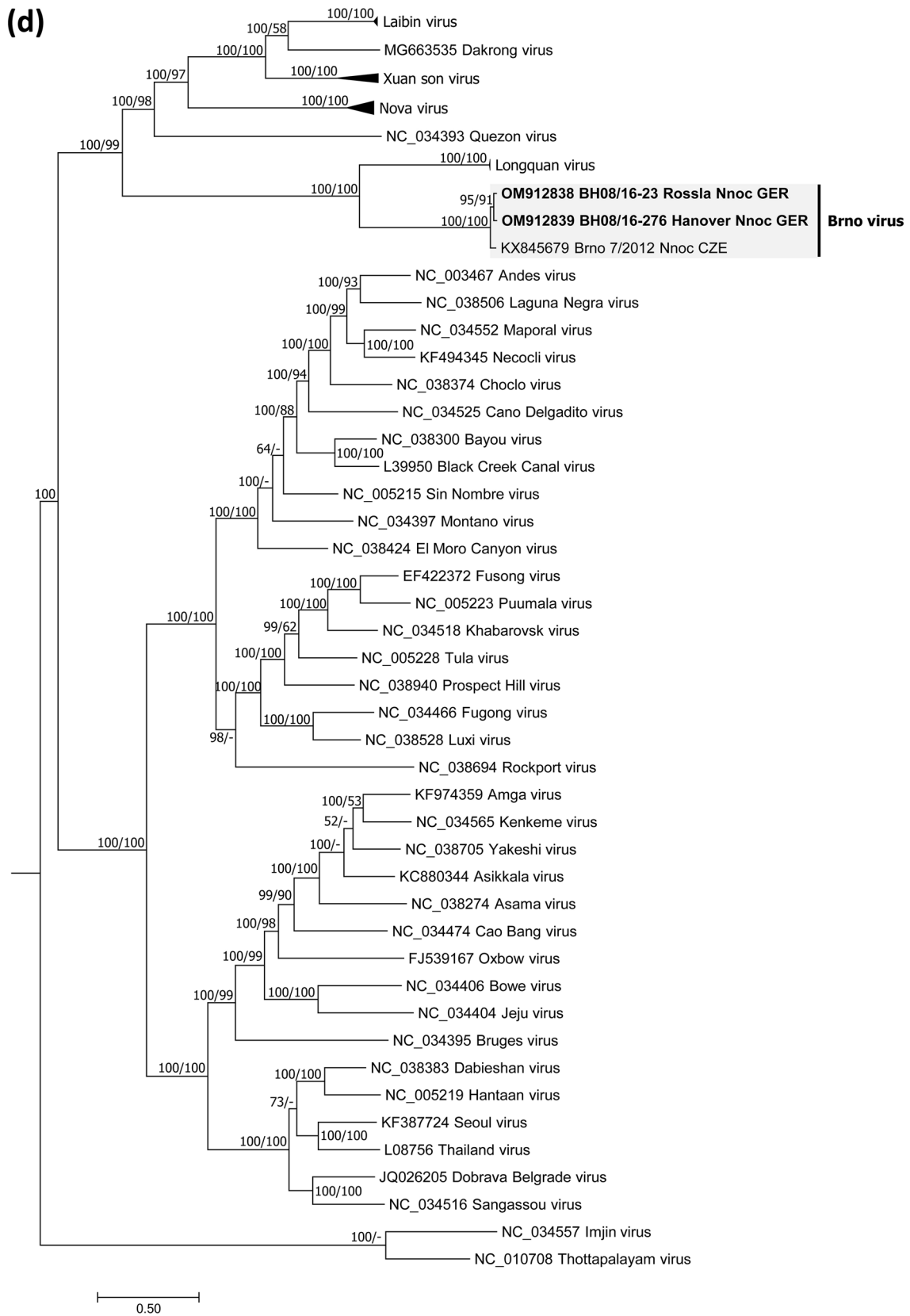


Fig. 1 (continued)

Table 1 Results of Brno loanvirus-specific RT-qPCR screening of bats from Germany and Austria and of nested RT-PCR for samples from Poland

Family	Subfamily	Genus	Common name	Scientific name	No. of positive animals/total no. of animals				
					Germany*	Austria	Poland		
Vespertilionidae	Vespertilioninae	<i>Nyctalus</i>	Common noctule	<i>Nyctalus noctula</i>	3/245	1/207	3/20		
			Lesser noctule	<i>Nyctalus leisleri</i>	0/19	0/1	–		
		<i>Pipistrellus</i>	Common pipistrelle	<i>Pipistrellus pipistrellus</i>	0/387	0/30	0/5		
			Soprano pipistrelle	<i>Pipistrellus pygmaeus</i>	0/6	0/3	0/4		
			Nathusius' pipistrelle	<i>Pipistrellus nathusii</i>	0/51	0/33	0/3		
			Kuhl's pipistrelle	<i>Pipistrellus kuhlii</i>	0/3	0/45	–		
			Savi's pipistrelle	<i>Hypsugo/Pipistrellus savii</i>	–	0/51	–		
			<i>Vespertilio</i>	Parti-coloured bat	<i>Vespertilio murinus</i>	0/23	0/43	0/5	
		<i>Plecotus</i>	Brown long-eared bat	<i>Plecotus auritus</i>	0/43	–	0/4		
			Grey long-eared bat	<i>Plecotus austriacus</i>	0/16	0/11	–		
		<i>Eptesicus</i>	Northern bat	<i>Eptesicus nilssonii</i>	0/3	–	0/2		
			Serotine bat	<i>Eptesicus serotinus</i>	0/56	0/7	0/17		
		<i>Barbastella</i>	Western barbastellus	<i>Barbastella barbastellus</i>	0/4	0/11	–		
		Myotinae	<i>Myotis</i>	Bechstein's bat	<i>Myotis bechsteinii</i>	0/6	–	–	
				Brandt's bat	<i>Myotis brandti</i>	0/16	–	–	
				Pond bat	<i>Myotis dasycneme</i>	0/3	–	0/1	
	Daubenton's bat			<i>Myotis daubentonii</i>	0/46	0/2	0/3		
	Greater mouse-eared bat			<i>Myotis myotis</i>	0/18	0/1	–		
	Whiskered bat			<i>Myotis mystacinus</i>	0/48	0/6	0/2		
	Natterer's bat			<i>Myotis nattereri</i>	0/47	–	–		
Alcathoe Whiskered bat	<i>Myotis alcathoe</i>			0/2	–	0/1			
Geoffroy's bat	<i>Myotis emarginatus</i>			–	0/2	–			
Pteropodidae	Pteropodinae			<i>Rousettus</i>	Egyptian fruit bat	<i>Rousettus aegyptiacus</i>	0/2	–	–
Miniopteridae	Miniopterinae			<i>Miniopterus</i>	Schreiber's bent-winged bat	<i>Miniopterus schreibersii</i>	–	0/5	–
Rhinolophidae	Rhinolophinae			<i>Rhinolophus</i>	Greater horseshoe bat	<i>Rhinolophus ferrumequinum</i>	–	0/3	–
					Lesser horseshoe bat	<i>Rhinolophus hipposideros</i>	–	0/2	–
Species not identified**					0/3	0/1	0/10		
Total					3/1047	1/464	3/77		

*Results of the RT-qPCR were confirmed by conventional RT-PCR, detecting the same three samples as positive

**for three samples from Germany, one sample from Austria and 10 samples from Poland the bat species could not be identified

revealed the detection of viral RNA in the livers of two bats and in one kidney and one lung sample (Table S5).

This study detected BRNV in common noctules from Germany, Poland and Austria, suggesting a broader geographical distribution of this hantavirus in Central Europe. The multiple detection of this hantavirus in the same bat species from different regions, together with the absence of BRNV-specific RNA in other sympatric bat species, indicated its clear host specificity to the common noctule.

It should be noted that the prevalence estimates in common noctules from Germany, Austria (this study) and the Czech Republic [31] might be affected by inherent biases in sampling methods. For the prevalence in common noctules from Poland, it is also noteworthy that two BRNV-positive bats were found dead at the same place and time in Silesia.

The overall intra-species sequence divergence was found to be rather low, both at the nucleotide sequence level, for S, M and L segments (max. 2.3%, 4.4%, and 6.4%), and amino acid levels, for the encoded proteins (0%, 0.7%, 2.9%), in comparison to the intra-species sequence divergence in rodent-borne hantaviruses.

The common noctule (*Nyctalus noctula*) is one of the largest bats in Western and Central Europe and common throughout Europe, Asia, and North Africa [36]. Common noctules typically live in forests, but populations can also be found in human settlements [37]. The occurrence of this bat species in urban regions may raise a public health concern due to a potential risk of transmission of this bat-borne hantavirus to humans. The characterization of BRNV, its host association and geographical distribution may ultimately

assist in the prevention of its emergence in humans and other animals. Future investigations in other regions where common noctules are endemic will profit from our novel RT-qPCR assay, which shows here an (almost) identical sensitivity to the nested RT-PCR assay for BRNV detection, at least in Germany. The performance of this assay should be validated in the future based on synthetic RNA molecules, as long as a cell culture isolate of BRNV is lacking. Further studies will be dedicated to analyse the zoonotic potential of this bat-borne hantavirus and evaluate its transmission within bat populations.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11262-022-01952-2>.

Acknowledgements The provisioning of bats by regional veterinary laboratories in Poland is kindly acknowledged. We would like to thank Kim Lea Molle and Patrycja Potyrała for their excellent technical assistance, René Ryll for support of the phylogenetic analysis and Calvin Mehl for critical reading of the manuscript.

Author contributions AO, KS, BH, TM, SF and RGU designed the study. AO, MS, SJK, GW and CMF collected all bats. MD, AO, SJK, PS, KS, KJ and XW performed all molecular investigations, including sequence determination and analyses. BH, SD, SF and GH supervised the sequence analyses. MD, PS, KS, KJ, SD, SJK, TM, CMF, SF, GH and RGU wrote the manuscript draft. All authors contributed to the final version of the manuscript and approved it.

Funding Open Access funding enabled and organized by Projekt DEAL. Maysaa Dafalla acknowledges support by the German Academic Exchange Service (DAAD) [Personal ref. no.: 91529281]. GH was supported by the Swiss National Science Foundation (31003A_176209). The investigations in the laboratory of RGU were supported by the Helmholtz Association within the Initiative and Networking Fund for Infection Research Greifswald (project HAN-Tadapt-022021). Petra Straková was supported by project NU21-05-00143 of the Ministry of Health of the Czech Republic.

Data availability The entire information on the bats investigated here is given within the manuscript and the Supplementary Information. All new Brno loanvirus sequences are deposited at GenBank under the accession numbers OM912836- OM912844.

Declarations

Conflict of interest The authors declare that they have no competing interests.

Ethical approval The collection of dead bats in Germany, Poland and Austria was performed in the frame of passive rabies surveillance and virus infection studies in animals. As for rabies testing, the testing of dead found bats is recommended and laid down in the EUROBATS agreement, resolution 5.2, Bats and rabies. The bats in Austria were handled and cared for in accordance with the Animal Protection guidelines and legal approval of the sampling had been granted (Ethical committee approval ETK-08/02/2018).

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes

were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Walker PJ, Siddell SG, Lefkowitz EJ, Mushegian AR, Adriaenssens EM, Alfenas-Zerbini P, Davison AJ, Dempsey DM, Dutilleul BE, Garcia ML, Harrach B, Harrison RL, Hendrickson RC, Junglen S, Knowles NJ, Krupovic M, Kuhn JH, Lambert AJ, Lobočka M, Nibert ML, Oksanen HM, Orton RJ, Robertson DL, Rubino L, Sabanadzovic S, Simmonds P, Smith DB, Suzuki N, Van Doornslaer K, Vandamme AM, Varsani A, Zerbini FM (2021) Changes to virus taxonomy and to the international code of virus classification and nomenclature ratified by the international committee on taxonomy of viruses (2021). *Arch Virol* 166:2633–2648. <https://doi.org/10.1007/s00705-021-05156-1>
- Laenen L, Vergote V, Calisher CH, Klempa B, Klingstrom J, Kuhn JH, Maes P (2019) *Hantaviridae*: current classification and future perspectives. *Viruses* 11:788. <https://doi.org/10.3390/v11090788>
- Schmaljohn CS, Dalrymple JM (1983) Analysis of Hantaan virus RNA: evidence for a new genus of Bunyaviridae. *Virology* 131:482–491. [https://doi.org/10.1016/0042-6822\(83\)90514-7](https://doi.org/10.1016/0042-6822(83)90514-7)
- Childs JE, Ksiazek TG, Spiropoulou CF, Krebs JW, Morzunov S, Maupin GO, Gage KL, Rollin PE, Sarisky J, Enscore RE, Frey JK, Peters CJ, Nichol ST (1994) Serologic and genetic identification of *Peromyscus maniculatus* as the primary rodent reservoir for a new hantavirus in the southwestern United States. *J Infect Dis* 169:1271–1280. <https://doi.org/10.1093/infdis/169.6.1271>
- Plyusnin A, Morzunov SP (2001) Virus evolution and genetic diversity of hantaviruses and their rodent hosts. *Curr Top Microbiol Immunol* 256:47–75. https://doi.org/10.1007/978-3-642-56753-7_4
- Plyusnin A, Vapalahti O, Vaheri A (1996) Hantaviruses: genome structure, expression and evolution. *J Gen Virol* 77(Pt 11):2677–2687. <https://doi.org/10.1099/0022-1317-77-11-2677>
- Schatz J, Ohlendorf B, Busse P, Pelz G, Dolch D, Teubner J, Encarnação JA, Mühle RU, Fischer M, Hoffmann B, Kwasnitschka L, Balkema-Buschmann A, Mettenleiter TC, Müller T, Freuling CM (2014) Twenty years of active bat rabies surveillance in Germany: a detailed analysis and future perspectives. *Epidemiol Infect* 142:1155–1166. <https://doi.org/10.1017/S0950268813002185>
- Schatz J, Fooks AR, McElhinney L, Horton D, Echevarria J, Vázquez-Moron S, Kooi EA, Rasmussen TB, Müller T, Freuling CM (2013) Bat rabies surveillance in Europe. *Zoonoses Public Health* 60:22–34. <https://doi.org/10.1111/zph.12002>
- Saxenhofer M, Weber de Melo V, Ulrich RG, Heckel G (2017) Revised time scales of RNA virus evolution based on spatial information. *Proc Biol Sci* 284. <https://doi.org/10.1098/rspb.2017.0857>
- Carey DE, Reuben R, Panicker KN, Shope RE, Myers RM (1971) Thottapalayam virus: a presumptive arbovirus isolated from a shrew in India. *Indian J Med Res* 59:1758–1760
- Gu SH, Dormion J, Hugot JP, Yanagihara R (2014) High prevalence of Nova hantavirus infection in the European mole (*Talpa europaea*) in France. *Epidemiol Infect* 142:1167–1171. <https://doi.org/10.1017/S0950268813002197>
- Gu SH, Hejduk J, Markowski J, Kang HJ, Markowski M, Polatyńska M, Sikorska B, Liberski PP, Yanagihara R (2014) Co-circulation of soricid- and talpid-borne hantaviruses in Poland.

- Infect Genet Evol 28:296–303. <https://doi.org/10.1016/j.meegid.2014.10.017>
13. Gu SH, Kumar M, Sikorska B, Hejduk J, Markowski J, Markowski M, Liberski PP, Yanagihara R (2016) Isolation and partial characterization of a highly divergent lineage of hantavirus from the European mole (*Talpa europaea*). *Sci Rep* 6:21119. <https://doi.org/10.1038/srep21119>
 14. Gu SH, Markowski J, Kang HJ, Hejduk J, Sikorska B, Liberski PP, Yanagihara R (2013) Boginia virus, a newfound hantavirus harbored by the Eurasian water shrew (*Neomys fodiens*) in Poland. *Virol J* 10:160. <https://doi.org/10.1186/1743-422X-10-160>
 15. Kang HJ, Arai S, Hope AG, Song JW, Cook JA, Yanagihara R (2009) Genetic diversity and phylogeography of Seewis virus in the Eurasian common shrew in Finland and Hungary. *Virol J* 6:208. <https://doi.org/10.1186/1743-422X-6-208>
 16. Kang HJ, Bennett SN, Sumibcay L, Arai S, Hope AG, Mocz G, Song JW, Cook JA, Yanagihara R (2009) Evolutionary insights from a genetically divergent hantavirus harbored by the European common mole (*Talpa europaea*). *PLoS One* 4:e6149. <https://doi.org/10.1371/journal.pone.0006149>
 17. Klempa B, Fichet-Calvet E, Lecompte E, Auste B, Aniskin V, Meisel H, Denys C, Koivogui L, ter Meulen J, Krüger DH (2006) Hantavirus in African wood mouse, Guinea. *Emerg Infect Dis* 12:838–840. <https://doi.org/10.3201/eid1205.051487>
 18. Laenen L, Vergote V, Kafetzopoulou LE, Wawina TB, Vassou D, Cook JA, Hugot JP, Deboutte W, Kang HJ, Witkowski PT, Köppen-Rung P, Krüger DH, Ličková M, Stang A, Striešková L, Szemeš T, Markowski J, Hejduk J, Kafetzopoulos D, Van Ranst M, Yanagihara R, Klempa B, Maes P (2018) A novel hantavirus of the European Mole, Bruges virus, is involved in frequent Nova virus coinfections. *Genome Biol Evol* 10:45–55. <https://doi.org/10.1093/gbe/evx268>
 19. Laenen L, Vergote V, Nauwelaers I, Verbeeck I, Kafetzopoulou LE, Van Ranst M, Maes P (2015) Complete Genome Sequence of Nova virus, a Hantavirus circulating in the European Mole in Belgium. *Genome Announc* 3:e00770–00715. <https://doi.org/10.1128/genomeA.00770-15>
 20. Radosa L, Schlegel M, Gebauer P, Ansorge H, Heroldová M, Jánová E, Stanko M, Mošanský L, Fričová J, Pejčoch M, Suchomel J, Purchart L, Groschup MH, Krüger DH, Ulrich RG, Klempa B (2013) Detection of shrew-borne hantavirus in Eurasian pygmy shrew (*Sorex minutus*) in Central Europe. *Infect Genet Evol* 19:403–410. <https://doi.org/10.1016/j.meegid.2013.04.008>
 21. Schlegel M, Radosa L, Rosenfeld UM, Schmidt S, Triebenbacher C, Löhr PW, Fuchs D, Heroldová M, Jánová E, Stanko M, Mošanský L, Fričová J, Pejčoch M, Suchomel J, Purchart L, Groschup MH, Krüger DH, Klempa B, Ulrich RG (2012) Broad geographical distribution and high genetic diversity of shrew-borne Seewis hantavirus in Central Europe. *Virus Genes* 45:48–55. <https://doi.org/10.1007/s11262-012-0736-7>
 22. Song JW, Gu SH, Bennett SN, Arai S, Puorger M, Hilbe M, Yanagihara R (2007) Seewis virus, a genetically distinct hantavirus in the Eurasian common shrew (*Sorex araneus*). *Virol J* 4:114. <https://doi.org/10.1186/1743-422X-4-114>
 23. Xu L, Wu J, He B, Qin S, Xia L, Qin M, Li N, Tu C (2015) Novel hantavirus identified in black-bearded tomb bats, China. *Infect Genet Evol* 31:158–160. <https://doi.org/10.1016/j.meegid.2015.01.018>
 24. Weiss S, Witkowski PT, Auste B, Nowak K, Weber N, Fahr J, Mombouli JV, Wolfe ND, Drexler JF, Drosten C, Klempa B, Leendertz FH, Krüger DH (2012) Hantavirus in bat, Sierra Leone. *Emerg Infect Dis* 18:159–161. <https://doi.org/10.3201/eid1801.111026>
 25. Sumibcay L, Kadjo B, Gu SH, Kang HJ, Lim BK, Cook JA, Song JW, Yanagihara R (2012) Divergent lineage of a novel hantavirus in the banana pipistrelle (*Neoromicia nanus*) in Côte d'Ivoire. *Virol J* 9:34. <https://doi.org/10.1186/1743-422X-9-34>
 26. Arai S, Nguyen ST, Boldgiov B, Fukui D, Araki K, Dang CN, Ohdachi SD, Nguyen NX, Pham TD, Boldbaatar B, Satoh H, Yoshikawa Y, Morikawa S, Tanaka-Taya K, Yanagihara R, Oishi K (2013) Novel bat-borne hantavirus, Vietnam. *Emerg Infect Dis* 19:1159–1161. <https://doi.org/10.3201/eid1907.121549>
 27. Guo WP, Lin XD, Wang W, Tian JH, Cong ML, Zhang HL, Wang MR, Zhou RH, Wang JB, Li MH, Xu J, Holmes EC, Zhang YZ (2013) Phylogeny and origins of hantaviruses harbored by bats, insectivores, and rodents. *PLoS Pathog* 9:e1003159. <https://doi.org/10.1371/journal.ppat.1003159>
 28. Tesikova J, Bryjova A, Bryja J, Lavrenchenko LA, Gouy de Bellocq J (2017) Hantavirus strains in East Africa related to western African hantaviruses. *Vector Borne Zoonotic Dis* 17:278–280. <https://doi.org/10.1089/vbz.2016.2022>
 29. Arai S, Taniguchi S, Aoki K, Yoshikawa Y, Kyuwa S, Tanaka-Taya K, Masangkay JS, Omatsu T, Puentespina R Jr, Watanabe S, Alviola P, Alvarez J, Eres E, Cosico E, Quibod M, Morikawa S, Yanagihara R, Oishi K (2016) Molecular phylogeny of a genetically divergent hantavirus harbored by the Geoffroy's rousette (*Rousettus amplexicaudatus*), a frugivorous bat species in the Philippines. *Infect Genet Evol* 45:26–32. <https://doi.org/10.1016/j.meegid.2016.08.008>
 30. Witkowski PT, Drexler JF, Kallies R, Ličková M, Bokorová S, Mananga GD, Szemes T, Leroy EM, Krüger DH, Drosten C, Klempa B (2016) Phylogenetic analysis of a newfound bat-borne hantavirus supports a laurasiatherian host association for ancestral mammalian hantaviruses. *Infect Genet Evol* 41:113–119. <https://doi.org/10.1016/j.meegid.2016.03.036>
 31. Straková P, Dufkova L, Širmarová J, Salát J, Bartonička T, Klempa B, Pfaff F, Höper D, Hoffmann B, Ulrich RG, Růžek D (2017) Novel hantavirus identified in European bat species *Nyctalus noctula*. *Infect Genet Evol* 48:127–130. <https://doi.org/10.1016/j.meegid.2016.12.025>
 32. Schatz J, Freuling CM, Auer E, Goharriz H, Harbusch C, Johnson N, Kaipf I, Mettenleiter TC, Mühlendorfer K, Mühle RU, Ohlendorf B, Pott-Dörfer B, Prüger J, Ali HS, Stiefel D, Teubner J, Ulrich RG, Wibbelt G, Müller T (2014) Enhanced passive bat rabies surveillance in indigenous bat species from Germany - a retrospective study. *PLoS Negl Trop Dis* 8:e2835. <https://doi.org/10.1371/journal.pntd.0002835>
 33. Schlottau K, Eggerbauer E, Freuling CM, Beer M, Müller T, Hoffmann B (2020) Rapid molecular species identification of indigenous bats from Germany for surveillance purposes. *Infect Genet Evol* 78:104140. <https://doi.org/10.1016/j.meegid.2019.104140>
 34. Löber C, Anheier B, Lindow S, Klenk HD, Feldmann H (2001) The Hantaan virus glycoprotein precursor is cleaved at the conserved pentapeptide WAASA. *Virology* 289:224–229. <https://doi.org/10.1006/viro.2001.1171>
 35. Schmaljohn CS, Schmaljohn AL, Dalrymple JM (1987) Hantaan virus M RNA: coding strategy, nucleotide sequence, and gene order. *Virology* 157:31–39. [https://doi.org/10.1016/0042-6822\(87\)90310-2](https://doi.org/10.1016/0042-6822(87)90310-2)
 36. Csorba G, Smeenk C, Lee BP (2016) The identity of *Vespertilio oreias* Temminck, 1840-solving a taxonomic puzzle. *Zootaxa* 4205:564–570. <https://doi.org/10.11646/zootaxa.4205.6.4>
 37. Voigt CC, Kingston T (2016) Bats in the Anthropocene. In: Voigt CC, and Kingston T (eds). *Bats in the Anthropocene: conservation of bats in a changing world*. Springer International Publishing AG, Switzerland, pp. 1–9. <https://doi.org/10.1007/978-3-319-25220-9>
 38. Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl Acids Symp Ser* 41:95–98.

39. Darrriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nat Methods* 9:772. <https://doi.org/10.1038/nmeth.2109>
40. Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst Biol* 61:539–542. <https://doi.org/10.1093/sysbio/sys029>
41. Price MN, Dehal PS, Arkin AP (2010) FastTree 2 - approximately maximum-likelihood trees for large alignments. *PLoS One* 5:e9490. <https://doi.org/10.1371/journal.pone.0009490>
42. Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES science gateway for inference of large phylogenetic trees. In: 2010 gateway computing environments workshop (GCE). IEEE, New Orleans, LA, USA, pp 1–8. <https://doi.org/10.1109/GCE.2010.5676129>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.