

Review

Phylogeographic aspects of bat lyssaviruses in Europe: A Review

Heliana Dundarova^{1*}, Nadya Ivanova-Aleksandrova², Sarka Bednarikova³, Irina Georgieva², Krasimir Kirov⁴, Kalina Miteva¹, Boyko Neov¹, Peter Ostoich¹, Jiri Pikula³, Jan Zukal⁵, and Peter Hristov¹

¹Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 1 Tsar Osvoboditel Blvd., 1000 Sofia, Bulgaria, <https://orcid.org/0000-0002-3136-6457>

²National Centre of Infectious and Parasitic Diseases, 26 Yanko Sakazov Blvd., 1504 Sofia, Bulgaria

³Department of Ecology and Diseases of Zoo Animals, Game, Fish and Bees, University of Veterinary Sciences Brno, Palackého tř. 1946/1, 612 42 Brno, Czech Republic

⁴Faculty of Biology, University of Plovdiv "Paisii Hilendarski", 24 Tzar Assen" str., 4000 Plovdiv, Bulgaria

⁵Institute of Vertebrate Biology of the Czech Academy of Sciences, Květná 8, 603 65 Brno, Czech Republic

* Corresponding author: heliana.dundarova@iber.bas.bg ORCID: <https://orcid.org/0000-0002-3136-6457>

Simple Summary: Bats (Order Chiroptera) are the only mammals capable of active flight and widely distributed across the European continent. The order is increasingly recognized as a reservoir of viruses that can cross species barriers (i.e., "spillover") to infect humans and other domestic and wild mammals. Rabies is a zoonotic disease with an important public health hazard associated with the lyssavirus distribution. This review is focused on the evolution, geographic distribution and transmission routes of bat lyssaviruses. In addition, we discussed potential scenario for zoonotic transfer of the non-specific Phylogroup II lyssavirus in Europe from the contact zone of the Palearctic and Ethiopian realms.

Abstract: During the last few decades bat lyssaviruses have become the topic of intensive molecular and epidemiological investigations. Since ancient times rhabdoviruses have caused fatal encephalitis in humans which has led to research into effective strategies for their eradication. Modelling of potential future cross-species virus transmissions forms a substantial component of the recent infection biology of rabies. In this article we summarise the available data on the phylogeography of both bats and lyssaviruses in Europe and the adjacent regions, especially in the contact zone between the Palearctic and Ethiopian realms. Within these zones three bat families are present with high potential for cross-species transmission and the spread of lyssaviruses in Phylogroup II to Europe (part of the western Palearctic). The lack of effective therapies for rabies viruses in Phylogroups II and III generates impetus for additional phylogenetic and virological research within this geographical region.

Keywords: lyssavirus phylogroups, Chiroptera, evolution, transition routes, health

1. Introduction

The Order Chiroptera has a Laurasiatherian origin ("laurasian beasts"), evolved between 50 and 70 million years ago (MYA), and has undergone rapid diversification ([1, 2]. Due to their capabilities of self-powered flight and echolocation, bats [3] comprise over 20%, or more than 1400 species, of all modern mammals and are globally distributed, with the exception of the extreme polar regions [4]. They have many characteristics that differentiate them from other mammalian species, such as their unique physiology [5, 6], metabolism [7], and immune system [2, 8, 9]. These features make them a suitable reservoir for viral zoonoses [4,10, 11] and more than 200 viruses have been isolated from or detected in bats [see 4, 12, 13,14]. The order comprises 45 species in Europe [15] from two superfamilies the Rhinolophoidea and Vespertilionoidea [16], representing a natural reservoir of RNA-viruses (Table 1). Viruses from 11 families have been isolated on the continent

[17], and bat lyssaviruses in Europe (family Rhabdoviridae) have been the subject of detailed reviews by [18, 19, 20, 21].

Lyssaviruses are a genus of negative-sense single-strand RNA viruses in the family Rhabdoviridae, subfamily Alpharhabdovirinae. Notably, they are members of the order Mononegavirales, which includes other prominent zoonotic pathogens such as filoviruses (Ebola, Marburg, etc.) and the neurotropic Bornaviridae [22]. Based on genetic divergence lyssaviruses are classified into 17 different viral species according to the latest ICTV Taxonomy release [23]. Recently, two putative new lyssaviruses were published [24, 25, 26]. Apart from the Mokola virus (MOKV) and Ikoma lyssavirus (IKOV), which have rodents and African civets as a reservoir, respectively [25, 27, 28], the rest of the lyssaviruses are transmitted by Chiroptera [27, 29].

Table 1. European bat species, their IUCN conservation status and lyssavirus presence.

Species		IUCN	Bat lyssaviruses in Europe					
Rhinolophidae		status	EBLV-1	EBLV-	BBLV	KBLV	LLEBV	WCBV
2								
<i>Rhinolophus blasii</i>	Blasius's horseshoe bat	VU						
	Mediterranean							
<i>Rhinolophus euryale</i>	horseshoe bat	VU						
<i>Rhinolophus fer-rumequinum</i>	Greater horseshoe bat	NT	+					
<i>Rhinolophus hippo-sideros</i>	Lesser horseshoe bat	NT						
	Mehely's horseshoe bat							
<i>Rhinolophus mehelyi</i>	bat	VU						
Vespertilionidae								
	Western Barbastelle bat							
<i>Barbastella barbastellus</i>	bat	VU						
<i>Eptesicus bottae</i>	Botta’s Serotine	N/A						
<i>Eptesicus nilssonii</i>	Northern bat	LC						
	Isabelline Serotine bat		+					
<i>Eptesicus isabellinus</i>	bat	N/A						
<i>Eptesicus serotinus</i>	Common Serotine	LC	+					
<i>Hypsugo savii</i>	Savi's pipistrelle	LC						
	Alcathoe whiskered bat							
<i>Myotis alcathoe</i>	bat	DD						
	Steppe whiskered bat							
<i>Myotis aurascens</i>	bat	LC						
<i>Myotis bechsteinii</i>	Myotis bechsteinii	VU						
	Lesser mouse-eared bat		+					
<i>Myotis blythii</i>	bat	NT						
<i>Myotis brandtii</i>	Brandt's bat	LC				+		
<i>Myotis capaccinii</i>	Long-fingered bat	VU						
<i>Myotis dasycneme</i>	Pond bat	NT		+				

<i>Myotis daubentonii</i>	Daubenton's bat	LC		+		
<i>Myotis escaleraei</i>	Escaleraei bat	N/A				
<i>Myotis emarginatus</i>	Geoffroy's bat	LC				
	Greater mouse-eared bat		+			
<i>Myotis myotis</i>		LC				
<i>Myotis mystacinus</i>	Whiskered bat	LC				
<i>Myotis nattereri</i>	Natterer's bat	LC	+		+	
	Maghreb mouse-eared bat					
<i>Myotis punicus</i>		NT				
<i>Nyctalus azoreum</i>	Azorean bat	EN				
<i>Nyctalus lasiopterus</i>	Greater noctule bat	DD				
<i>Nyctalus leisleri</i>	Leisler's bat	LC				
<i>Nyctalus noctula</i>	Common noctule	LC	+			
<i>Pipistrellus kuhlii</i>	Kuhl's pipistrelle	LC				
<i>Pipistrellus hanaki</i>	Hanaki's Dwarf Bat	N/A				
<i>Pipistrellus maderensis</i>	Madeira pipistrelle	EN				
	Nathusius's pipistrelle		+			
<i>Pipistrellus nathusii</i>		LC				
<i>Pipistrellus pipistrellus</i>	Common pipistrelle	LC	+			
<i>Pipistrellus pygmaeus</i>	Pygmy pipistrelle	LC				
	Brown long-eared bat		+			
<i>Plecotus auritus</i>		LC				
<i>Plecotus austriacus</i>	Grey long-eared bat	LC				
	Kolombatovic's Long-eared bat					
<i>Plecotus kolombatovici</i>		NT				
<i>Plecotus macrobullaris</i>	Mountain long-eared bat					
		NT				
	Sardinian long-eared bat					
<i>Plecotus sardus</i>		VU				
	Tenerife long-eared bat					
<i>Plecotus teneriffae</i>		EN				
<i>Vespertilio murinus</i>	Parti-coloured bat	LC	+			
Miniopteridae						
<i>Miniopterus schreibersii</i>	Schreibers's long-fingered bat		+		+	+
		NT				
Molossidae						
<i>Tadarida teniotis</i>	European free-tailed bat		+			
		LC				
Pteropodidae						
<i>Rousettus aegyptiacus</i>		N/A	+			
	Egyptian fruit bat	(EN?)				

Abbreviations: IUCN red list categories: EN: Endangered – Very high risk of extinction in the wild; VU: Vulnerable – High risk of extinction in the wild; NT: Near Threatened – Likely to become threatened in the near future; LC: Least

Concern – Does not qualify for a more at-risk category. Widespread and abundant taxa are included in this category; DD: Data Deficient – Inadequate information to make a direct, or indirect, assessment of its risk of extinction based on its distribution and/or population status; N/A: not assessed

The genus includes the classical rabies virus (RABV), Lyssavirus Aravan (ARAV), Lyssavirus australis (ABLV), Lyssavirus Bokeloh (BBLV), Lyssavirus Duvenhage (DUVV), Lyssavirus Hamburg (EBLV-1), Lyssavirus Helsinki (EBLV-2), Lyssavirus Gan-noruwa (GBLV), Lyssavirus Ikoma (IKOV), Lyssavirus Irkut (IRKV), Lyssavirus Khujand (KHUV), Lyssavirus Lagos (LBV), Lyssavirus Lleida (LLEBV), Lyssavirus Mokola (MOKV), Lyssavirus Shimoni (SHIBV), Kotalahti bat lyssavirus (KBVL), Lyssavirus Cau-casicus (WCBV) and Lyssavirus Formosa, which includes Taiwan bat lyssavirus -1 (TWBLV-1) and Taiwan bat lyssavirus-2 (TWBLV-2) [21, 24, 30, 31, 32, 33]. The current study aims to review the evolution, phylogeography and transmission routes of bat lyssaviruses in Europe.

2. Origin, Evolution and Geographic Distribution of Bat Lyssaviruses

Despite the greater diversity of African lyssaviruses [34], Hayman et al [35] assumed that they have a Palearctic origin and highlighted “Out of Africa” hypothesis. Lyssa-viruses most recent common ancestor (MRCA) evolved from an insect rhabdovirus before 7,000 - 11,000 years ago [30,36] which was transmitted to representatives of the order Chi-roptera and spread globally [36, 37]. According to Rupprecht et al. [30] Africa is the most likely home to the ancestors of taxa within the Genus Lyssavirus, Family Rhabdoviridae. According to this review a large number of different lyssaviruses co-evolved with bats, as ultimate reservoirs, over millions of years. It is assumed that all mammals be susceptible to infection with the rabies virus. Apart from bats as reservoirs of infection the so-called mesocarnivores (canids, raccoons, skunks etc.) also play a significant role in the spread and transmission of the rabies virus. The fact that the biological localization of the virus is the central nervous system poses a real challenge for its eradication.

Lyssaviruses have undergone purifying selection followed by neutral evolution of the viral genomes, [38]. The low rate of nonsynonymous evolution of lyssaviruses is prob-ably the result of constraints imposed by the need to replicate in multiple cell types (mus-cle, peripheral and central nervous systems, and salivary glands) within the host, which in turn boosts cross-species transmission (e. g. different groups of mammals), or because viral proteins are not subject to immune selection, which means existing lyssaviruses are well adapted to their reservoir [39, 40]. The host switching of the classic rabies lyssavirus – RABV from bats to other mammals is estimated to have occurred 800 to 1,400 years ago, which does not explain the timing of the oldest putative human rabies cases, estimated to have circulated 4,000 years ago in ancient Mesopotamia [41]. A possible explanation is that the Mesopotamian RABV lineage disappeared as a consequence of genetic drift (loss of polymorphism) or its high fatality rates [41]. According to Rupprecht et al and Badrane et al [42, 43], bats are the primary evolutionary host of rabies viruses as a reservoir of all existing lyssaviruses except MOKV and IKOV, whereas other mammals and humans only maintain several lineages of RABV, including the extinct Mesopotamian strain [30, 41, 43].

In Europe bat lyssaviruses (Fig. 1) were detected in the United Kingdom, the Neth-erlands, Finland, Denmark, Poland, Czech Republic, Germany, Switzerland, France, Spain, Hungary, Italy, Croatia, Bulgaria, Ukraine and Russia [19, 21, 44, 45, 46, 47, 48, 49]. During the last two decades, previously unknown lyssaviruses were isolated as follows: WCBV in 2002 on the European side of the Caucasus Mts. [50], BBLV in 2010 from Ger-many [51], LLEBV in 2011 from Spain [52], and KBLV in 2017 from Finland [24].

The most frequent lineages are EBLV-1, first reported in 1955 from Germany, and EBLV-2, isolated in 1985 in Switzerland [44, 45]. EBLV-1 is exclusively detected in Serotine bats (*Eptesicus serotinus*) while EBLV-2 is mainly found in Daubenton’s bats (*Myotis daubentonii*). EBLV-1 is present in two forms: EBLV-1a and EBLV-1b. EBLV-1a displays a

wide geographical distribution between France and Russia with phylogenetic homogeneity – an indication of extensive dispersal by bats [20, 53]. Recent research has shown that EBLV-1 is associated with the bat *E. serotinus* of the mountainous parts of Southern Europe, such as the French Alps or the Iberian Peninsula [54]. EBLV-1 showed the risk of spillover because of its close phylogenetic relation with a different bat, the *E. isabellinus*. The phylogenetic analysis of nine EBLV-1 strains of *E. serotinus* distributed in south of the Pyrenees revealed that two of them closely related with EBLV-1a sequences from Southern France i.e. this group expanded to Northern Spain. The results of the conducted research give the authors reason to assume the expansion of EBLV-1a subtype across southern France, with very recent arrival to the Iberian Peninsula i.e. current southwards dissemination [54]. In contrast, EBLV-1b is distributed between Spain and Poland with a well-defined geographic structure, indicating restricted contact between bat populations [20, 53]. Therefore EBLV-1b had the potential to spread southwards according to the *E. isabellinus* distribution. The lineage of EBLV-1 is presumed to have arisen 500 to 750 years ago and has a relatively recent origin [53]. Conversely, the lineage of EBLV-2 is dated to more than 8000 years ago, with current establishment in Europe within the last 2000 years. [55]. EBLV-2 has been reported in Western Europe and is also represented by two forms: EBLV-2a and EBLV-2b [46, 56]. The first occurs in the United Kingdom, Netherlands, Germany, Switzerland and Denmark, while the second includes the Finnish EBLV-2 strains, and a strain from Switzerland [55], where the divergence of the Finnish strains from the Swiss strain occurred within the last 200 years [55].

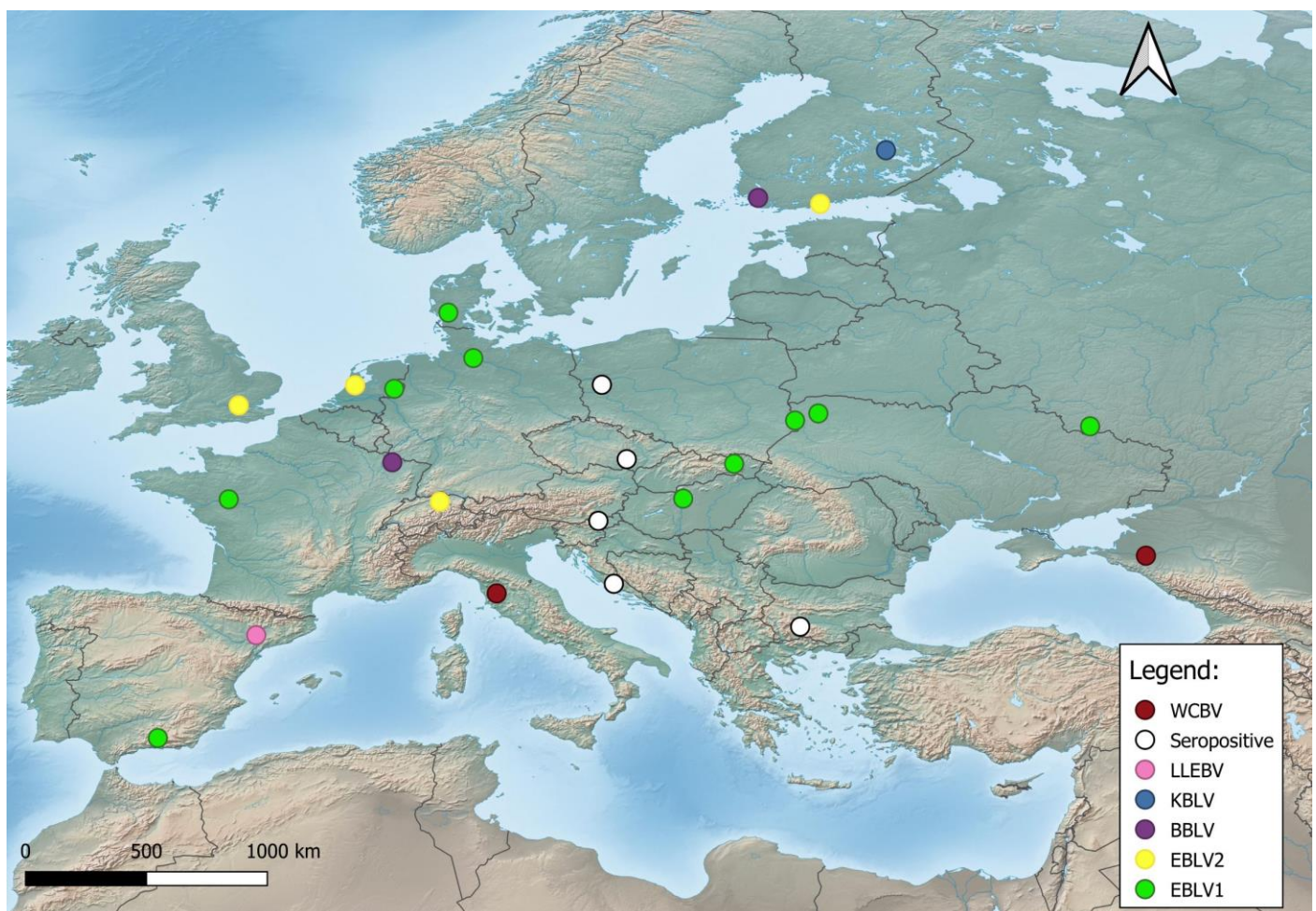


Figure 1. Distribution of bat lyssaviruses in Europe. Abbreviations used: **EBLV1** - European bat-1 lyssavirus; **EBLV2** - European bat-2 lyssavirus; **BBLV** - Bokeloh bat lyssavirus; **KBLV** - Kotalahti bat lyssavirus; **WCBV** - West Caucasian bat lyssavirus; **LLEBV** - Lleida bat lyssavirus; **Seropositive** - Seropositive Blood samples.

3. Virion structure and genome

Rhabdoviruses (family Rhabdoviridae) have a characteristic bullet-shaped virion morphology, with an envelope derived from the plasma membrane of the infected host cell and approximate dimensions of ~75 nm and a length ~180 nm, which distinguishes them from other taxa in the order Mononegavirales: Bornaviridae, Filoviridae, and Paramyxoviridae. They include an approximately 12 kb long non-segmented, linear, single-strand RNA genome. The basic genome includes five genes that encode (from 3' to 5') the nucleoprotein (or nucleocapsid protein, N), phosphoproteins (P), matrix protein (M), glycoprotein (G) and large protein (L, RNA - dependent RNA polymerase) [57, 58].

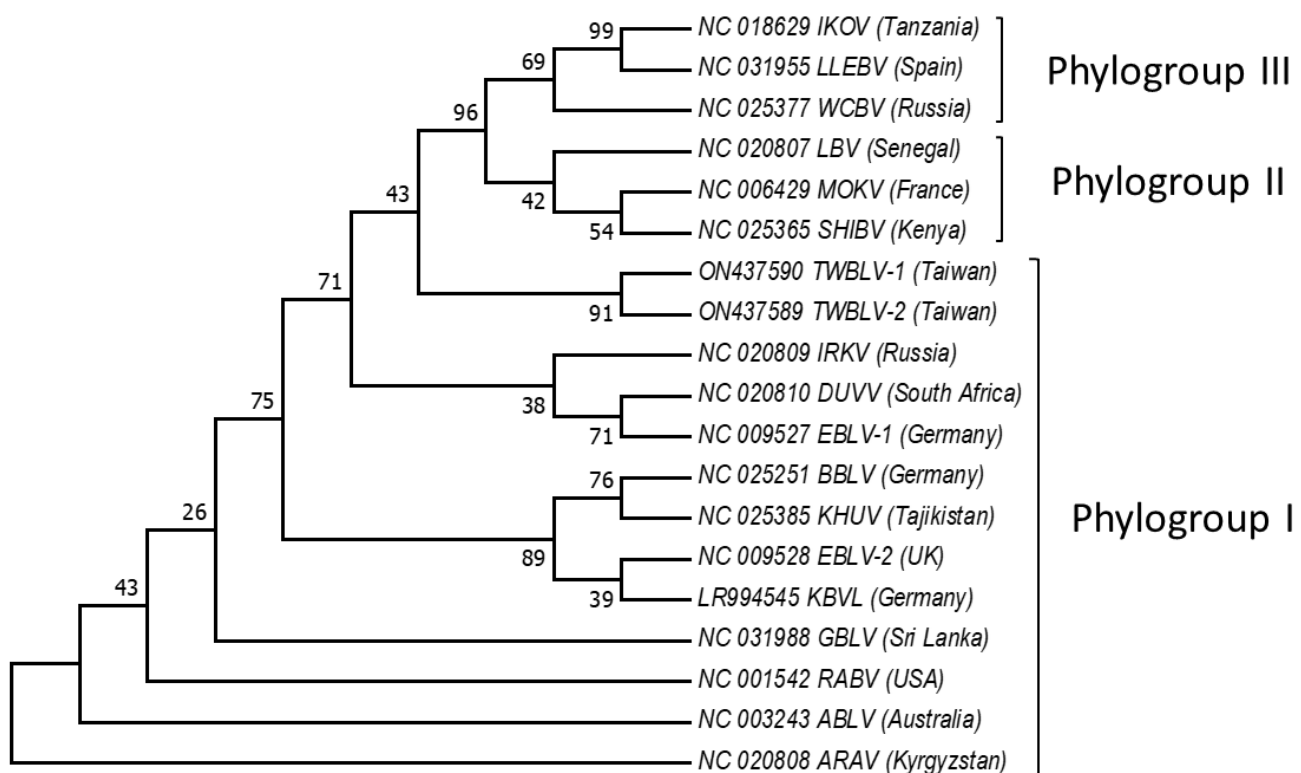
The lyssaviruses archetypes are design by variations of long non-coding region in the G gene and the small alternative open reading frames known as ORFs in the P gene [59]. ORFs present an ancestral pseudogene which are used for studying virus–host interactions in WCBV [37] due to the outstanding size which is 40% larger than in other bat lyssaviruses [60]. It has been found that in some rhabdoviruses very long non-coding regions (up to 749 nt) were present either within or between transcriptional units [37]. This region seems to serve as a resource to *de novo* emergence of genes which may be related to elucidating the taxonomy, phylogeny and evolution of lyssaviruses. This is most likely to occur when ORFs are present in transcribed non-coding regions (UTRs) such as the in the so-called 'pseudogene ψ region' of WCBV which is unique to an ORF of 180 nt has been identified lyssaviruses. The *de novo* creation of genes in non-transcribed intergenic regions (IGRs), as well as those present in the G-L gene junctions of various hapavirus, is associated with prior or simultaneous evolution of new or modified transcriptional control sequences. In the trend towards increasing genome size and complexity in rhabdoviruses, the loss of a gene and/or genes is also likely to have occurred periodically, which may also be evolutionarily determined in family Rhabdoviridae [37].

4. Phylogeny of bat lyssaviruses

Based on the sequence analysis of lyssavirus N gene, serologic cross-reactivity and pathogenicity bat lyssaviruses are divided into three phylogroups [35, 61, 62, 63] (Fig. 2, S1). For simplicity, we used 19 reference available in GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>) for our phylogenetic analysis. European viruses are included in Phylogroups I and III. Due to this fact, Phylogroup II is discussed only as a potential scenario for cross-species bat transmission.

Figure 2. Phylogeny of bat lyssaviruses. The sequences of the N gene of representative reference sequences of lyssaviruses used in the analysis were derived from Genbank. The evolutionary history was inferred by using the Maximum Likelihood method and General Time Reversible model [64]. There was a total of 568 positions in the final dataset. Evolutionary analyses were conducted in MEGA X [65]. Virus names are: classical rabies virus (RABV), Lyssavirus Aravan (ARAV), Lyssavirus australis (ABLV), Lyssavirus Bokeloh (BBLV), Lyssavirus Duvenhage (DUVV), Lyssavirus Hamburg (EBLV-1), Lyssavirus Helsinki (EBLV-2), Lyssavirus Gannoruwa (GBLV), Lyssavirus Ikoma (IKOV), Lyssavirus Irkut (IRKV), Lyssavirus Khujand (KHUV), Lyssavirus Lagos (LBV), Lyssavirus Lleida (LLEBV), Lyssavirus Mokol (MOKV), Lyssavirus Shimoni (SHIBV), Kotalahti bat lyssavirus (KBVL), Lyssavirus Caucasicus (WCBV) and Lyssavirus Formosa, which includes Taiwan bat lyssavirus -1 (TWBLV-1) and Taiwan bat lyssavirus-2 (TWBLV-2).

Phylogroup I includes species RABV, ARAV, ABLV, BBLV, DUVV, EBLV-1, EBLV-2, GBLV, IRKV, KBLV, KHUV, TWBLV -1 and TWBLV -2 whereas LBV, MOKV, and SHIBV form Phylogroup II [24, 31, 43, 44, 62, 66, 67]. Phylogenetically, species LLEBV, IKOV and WCBV appear related and represent phylogroup III [27, 50, 68, 69]. Phylogroup I is divided into two major groups: the first includes the Palearctic species IRKV, EBLV-1, TWBLV-1, TWBLV-2 and African DUVV lyssaviruses and the second ARAV, BBLV, KHUV and EBLV-2 which are also rabies viruses with Palearctic distribution, as well as Australian - ABLV, Oriental - GBLV and American - RABV lyssaviruses [35]. Interestingly,



EBLV-1 is most closely related to DUVV, and IRKV, while EBLV-2 to KBLV, KHUV and BBLV [30, 32]. Based on the close phylogenetic relation between EBLV-1 and DUVV species [70] hypothesized that EBLV-1 originated in North Africa and spread to Europe (Iberian Peninsula) via the Strait of Gibraltar. However, [35] present phylogenetic evidence, based on the rabies N gene sequences, that EBLV-1 and DUVV share a common ancestor with IRKV (isolate from Russia), and both have been transferred to Africa from the Palearctic region, and Europe in particular. Phylogenetic relationships in Phylogroup III demonstrate close phylogenetic relatedness between the LLEBV virus from Spain with the Eurasian WCBV and the African non-bat related IKOV lyssavirus [35, 71]. Genetically LLEBV is more closely related to IKOV than to WCBV.

For a better understanding of lyssavirus phylogeny and their current distributions, a closer look at their bat species reservoirs is required. Genomic and mitochondrial analyses have placed bats into two suborders: Yinpterochiroptera – including the five families in the superfamily Rhinolophoidea plus the flying foxes - Pteropodidae, and Yangochiroptera – including the three superfamilies: Emballonuroidea, Vespertilionoidea, and Noctilionoidea, comprising a total of 13 families. Two superfamilies (Rhinolophoidea and Vespertilionoidea) are of particular interest in Europe because their representatives are the main reservoir of lyssaviruses. The greater horseshoe bat (*Rhinolophus ferrumequinum*) (Rhinolophidae, Rhinolophoidea), and the Vespertilionoidea species Greater mouse-eared bat (*Myotis myotis*), Lesser mouse-eared bat (*M. blythii*), Natterer's bat (*M. nattereri*), Serotine bat (*Eptesicus serotinus*), Meridional serotine (*E. isabellinus*), Common pipistrelle (*Pipistrellus pipistrellus*), Nathusius's pipistrelle (*P. nathusii*), Brown long-eared bat (*Plecotus auritus*), Common noctule (*Nyctalus noctula*), Parti-coloured bat (*Vesperilio murinus*) (Vespertilionidae), Common bent-wing bat (*Miniopterus schreibersii*) (Miniopteroidea), European free-tailed bat (*Tadarida teniotis*) (Molossidae) have all been documented as being infected by EBLV-1 [47, 48, 72, 73, 74]. Regardless of the high number of bat hosts recorded for EBLV-1, EBVL-2 is restricted to *Myotis daubentonii* and *M. dasycneme* [44, 46]. KBLV was found only in *Myotis brandtii* [24] and BBLV only in *M. nattereri* [74, 75]. However, the phylogeny of the Natterer's bat group is more complex. In Europe, *M. nattereri* is composed of *M. escalerae* (Iberia), *M. species A* (Italy and parts of the Pyrenees), *M. species B* (Northwest Africa), *M. species C* (Corsica), and the nominal form, *M. nattereri* present across the rest of Europe [76, 77, 78]. According to [79] BBLV-positive bats in Germany and France were of the nominal form. [80] provided a detailed phylogenetic analysis and reported signs of repeated hybridization between the Natterer's bat lineages [80], with southern France a probable contact zone between *M. species* and *M. nattereri*. Additional research could reveal the potential of BBLV to spread to other Natterer's bat lineages.

The Common bent-wing bat (*Miniopterus schreibersii*) has been proven to be a host to IRKV and DUVV from Phylogroup I, and WCBV and LLEBV from Phylogroup III. The species seems to be a universal reservoir for both phylogroups not only in Europe but also in Asia and Africa [35, 81], due to the fact that *M. schreibersii* is a strictly cave-dwelling species [76–82] capable of long-distance migration [83]. The most abundant European lyssavirus group - EBLV-1 has still not been detected in the Common bent-wing bat despite its close phylogenetic relationship to DUVV.

5. Transmission routes of bat lyssaviruses

5.1. Bat intra- and cross-species transmission

The main transmission route of rabies viruses is via a bite from the host and the virions released into the saliva during the clinical period of rabies or/and during the end of the incubation period [84, 85, 86]. All lineages from Phylogroup I are transmitted by bats whereas the classical rabies virus – RABV has adapted to spread via carnivores and through bat species restricted to the Americas [21]. In Europe, EBLV-1 is the most prevalent lyssavirus among the Chiroptera, due to bat colony sizes, species richness, and the presence of migratory species. [87], suggested that in the roost the main factor for virus spillover is the large number of species and their individual mobility. However, roost sizes less than approximately 200 individuals are not enough for efficient maintenance of the lyssavirus infection [88]. On the other hand, migrant species such as *Pipistrellus nathusii*, *Nyctalus noctula*, *N. leisleri* and *Miniopterus schreibersi* can play a key role for the dispersal of EBLV-1 in Europe, and *M. schreibersi* also for WCBV and LLEBV [83, 89, 90, 91]. These species are capable of flying long distances between summer and winter roosts and increasing the lyssavirus geographical range via cross-species transition is an expected scenario. Consequently, seasonal bat movements between hibernacula, breeding and mating sites are an important prerequisite for successful virus cross-species spillover. For instance, hibernation is characterized by decreased activity in the bat immune system and metabolism [92, 93], thereby extending incubation periods and allowing virus persistence

between transmission periods. Based on the transcriptomic responses of bat cells to EBLV-1 [94], concluded that the lack of bat cell reaction to infection in conditions simulating hibernation may contribute to the virus tolerance or persistence in bats. In addition, long hibernation roosts could facilitate transmission of EBLV-1 between geographically separated breeding populations [19]. This statement is also supported by a study focused on RABV in the Americas, where *Myotis* species have been found to harbor a diverse range of RABV variants, suggesting that increased contact between species increases viral transmission [68]. Breeding colonies are a good site to understand intraspecies transmission of antibodies via intra-uterine transfer, or viral transmissions as a result of biting or daily communal grooming via antibodies intra-uterine transfer [95] or biting during daily grooming [19, 96]. Nevertheless, characterizing EBLV-1 dynamics in juvenile bats is difficult due to unequal equilibration between them and adults and the chance that mothers can transfer antibodies to them via the placenta or during lactation [90, 97]. The most efficient intra- and interspecific lyssavirus transmission route is during the mating period, due to aggressive male behavior when defending territory against other males and during mating with females [19]. In support of this assertion most cases of bat rabies in Europe peak in August and September [19, 85, 98].

Bats naturally infected with RABV have the virus in their nasal mucosa, leading to the proposition that airborne transmission of RABV between bats living in enclosed areas is possible [99, 100, 101]. Laboratory experiments showed that aerosols of the RABV virus were successfully overcome by bats but were fatal for the majority of experimental mice [102]. Johnson et al. [103] conducted a similar experiment with EBLV-2, where mice were intranasally inoculated and two of them developed the disease between 16- and 19-days post-infection. In addition, four cases of human rabies infection via aerosols were reported between 1956 and 1977 in a USA cave [102]. Li et al. [104] discovered that within caves bat guano exhibited a great diversity of viral species.

5.2. Other vertebrates cross-species transmission

Seidlova et al. [48] reported that lyssavirus seroprevalence in Palearctic bats is 3.2%, with 2.0% in Europe. Due to the adequately taken measures for terrestrial mammals rabies eradication in Europe [105], bats remain potential lyssavirus reservoir for humans in Europe [75]. Even so transmission is rare, the most recent case of bat-human spillover was in southwest central France in 2019 when a human male died from EBLV-1 (<https://www.zmescience.com/ecology/animals-ecology/extremely-rare-case-of-death-from-bat-rabies-in-france/>), one human died from EBLV-2 in Finland in 1985, and a third in the UK in 2002 [46]. Due to growing urbanization, pets present an expected intermediate host for bat-to-human lyssavirus transmission, and both EBLV-1 and WCBV have been found in cats in France [106] and Italy [49, 107]. Experimental data suggests that the infectious dose for lyssaviruses is low and certainly some human infections, especially those involving transmission from bats, support this finding [108].

5.3. Within the contact zone of the Palearctic and Ethiopian realms – potential scenarios

Heretofore, Phylogroup II was only known from the African continent in two bat families: Pteropodidae and Hipposideridae [28, 109, 110]. LBV was isolated from the Straw-coloured fruit bat (*Eidolon helvum*) and Egyptian fruit bat (*Rousettus aegyptiacus*), and SHIBV from the Striped leaf-nosed bat (*Macronycteris vittatus*).

Compared with other Pteropodids, *Rousettus aegyptiacus* has the northernmost distribution, reaching Cyprus and southern Turkey [111], and roosts in a variety of underground sites [112] with other Palearctic species [113]. As a rule, cave-dwelling bats form large summer and winter colonies [82], where cross-species pathogen transmission is common [114]. A recent study [115] has shown that the families Rhinolophidae and Pteropodidae were a common factor in cross-species transmission of β -coronaviruses between continents due to their close phylogenetic relationship. This might also be applicable across the contact zones of different zoogeographic realms, e. g., Africa

(Ethiopian realm) and Europe (Palearctic realm). Considering that the distributions of *Rousettus aegyptiacus* and *Rhinolophus ferrumequinum* overlap in the Southeastern zone of the Palearctic, on the border with the Ethiopian realm (fig. 3), the successful transfer of lyssaviruses from Phylogroup II via the Egyptian fruit bat to other cave-dwelling species, e. g., *R. ferrumequinum*, could be a plausible scenario due to their close phylogenetic relationship within the suborder Yinpterochiroptera [16]. Furthermore, phylogeny can act as a biotic factor driving the occurrence of RNA virus cross-species transmission between closely related host species [116]. For example, *R. aegyptiacus* and various species in the genus *Rhinolophus* are known to be successful virus reservoirs of β -coronavirus, Marburg virus, Henipavirus, Orthorubulavirus, Pararubulavirus and Lyssaviruses [14, 28, 97].

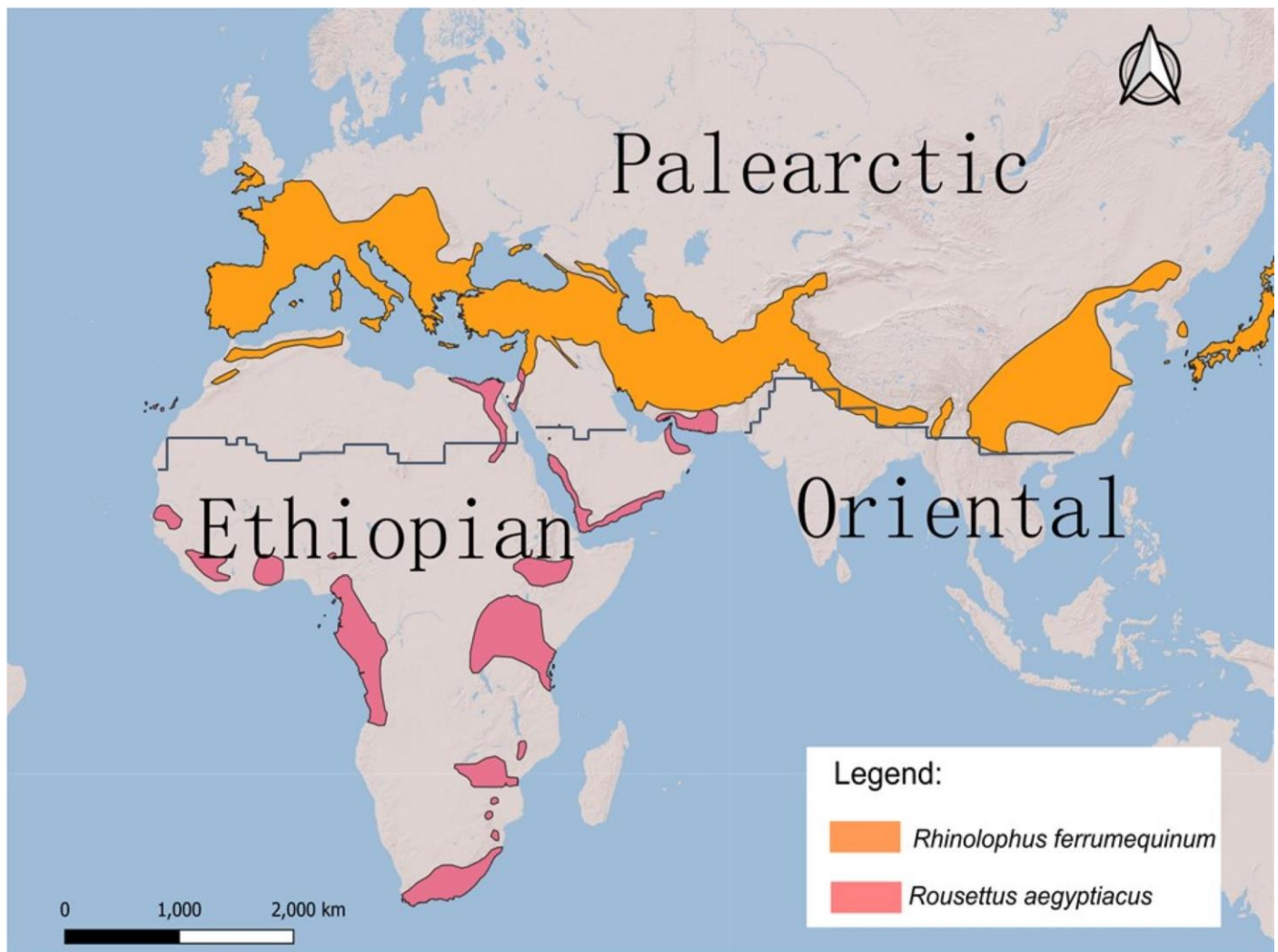


Figure 3. Zoogeographical distributions of *Rousettus aegyptiacus* and *Rhinolophus ferrumequinum* according to IUCN and their contact zone between the Ethiopian and Palearctic realms.

Additional molecular investigations are needed to solve the relationship between Phylogroup II and the other phylogroups. This is an important consideration since Phylogroup II is spread within the families Pteropodidae and Hipposideridae, both of which are within the suborder Yinpterochiroptera and closely related to the family Rhinolophidae, which includes cave-dwelling species widely distributed across Europe. Taking into account the close phylogenetic relationship between these bat families, the spread of the lyssavirus Phylogroup II to the Palearctic realm is a possible scenario within the contact zone with the Ethiopian realm. From the epidemiological point of view, these

bat families and the contact zone are significant for cross-species bat transmission routes of β -coronaviruses.

6. Conclusions

Bat lyssaviruses in Europe belong to two phylogroups: I and III, based on the molecular analyses of rabies G and N genes. The phylogeny of the lyssaviruses is closely related to the phylogeography of Palearctic bat species. EBLV-1 has been detected in species in the superfamilies Yangochiroptera and Yinpterochiroptera, which demonstrates the virus plasticity between highly divergent bat lineages. In contrast, EBLV-2, KBLV and BBLV have limited distributions in Europe, with their reservoirs restricted to *Myotis* spp. Phylogenetic relationships within Phylogroup I demonstrate that EBLV-1 and DUVV are closely related and spillover between different bat families, at a large distance from each other, is possible (Europe – Africa), while the second closest group, EBLV-2, KBLV and BBLV has a restricted distribution in just one bat genus. The existing rabies vaccines developed for RABV eradication are effective against lyssaviruses from Phylogroup I but offer little to no protection against phylogroup II and III viruses.

Living under the shadow of the recent COVID-19 pandemic, the detection and prevention of future pathogens is of crucial importance. The newly discovered bat lyssaviruses (BBLV, LLEBV and KBVL) and vesiculoviruses from the USA (SDRV1 and SDRV2) and China (YSBV, TYBV, and QZBV) warn us that the Rhabdoviridae represent an unexplored pathogen pool with new and yet to be described viruses potentially adverse to human health.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

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