

Novel and emerging pathogens detected in ticks from wolves in the Iberian Peninsula, Western Europe

Manena Fayos

Centro de Recuperación de Fauna Silvestre de Cantabria

Aránzazu Portillo

Center of Rickettsiosis and Arthropod-Borne Diseases (CRETAV), Infectious Diseases Department, San Pedro University Hospital-Center for Biomedical Research from La Rioja (CIBIR)

Carlos Sacristán

Centro de Investigación en Sanidad Animal (CISA-INIA), CSIC

Cristina Cervera-Acedo

Center of Rickettsiosis and Arthropod-Borne Diseases (CRETAV), Infectious Diseases Department, San Pedro University Hospital-Center for Biomedical Research from La Rioja (CIBIR)

Irene Sacristán

Centro de Investigación en Sanidad Animal (CISA-INIA), CSIC

Roser Velarde

Wildlife Ecology & Health group (WE&H) and Servei d'Ecopatologia de Fauna Salvatge (SEFaS), Departament de Medicina i Cirurgia Animals, Universitat Autònoma de Barcelona (UAB)

Ana Carolina Ewbank

Centro de Investigación en Sanidad Animal (CISA-INIA), CSIC

Natalia Sastre

Servei Veterinari de Genètica Molecular (SVGM), Departament de Ciència Animal i dels Aliments, Universitat Autònoma de Barcelona (UAB)

Paula Santibáñez

Center of Rickettsiosis and Arthropod-Borne Diseases (CRETAV), Infectious Diseases Department, San Pedro University Hospital-Center for Biomedical Research from La Rioja (CIBIR)

José A. Oteo

Center of Rickettsiosis and Arthropod-Borne Diseases (CRETAV), Infectious Diseases Department, San Pedro University Hospital-Center for Biomedical Research from La Rioja (CIBIR)

Ana M. Palomar

ampalomar@riojasalud.es

Center of Rickettsiosis and Arthropod-Borne Diseases (CRETAV), Infectious Diseases Department, San Pedro University Hospital-Center for Biomedical Research from La Rioja (CIBIR)

Article

Keywords: Canis lupus signatus, tick-borne viruses, tick-borne bacteria, tick-borne protozoan, Spain

Posted Date: September 2nd, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10699711/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Ticks pose an increasing public and veterinary health concern. Despite the importance of wildlife in the enzootic cycle of tick-borne diseases, the knowledge about ticks from wolves is scarce. The Iberian wolf is a highly valuable model species in this regard due to its unique combination of ecological and epidemiological traits. In this study, we aimed to investigate the ticks and associated microorganisms collected from Iberian wolves (*Canis lupus signatus*) in the north of Spain. Over a seven-year period, we collected 392 ticks from 72 wolves and identified five species: *Ixodes ricinus* (66.58%), *Dermacentor reticulatus* (21.68%), *Haemaphysalis concinna* (5.87%), *Ixodes hexagonus* (4.08%) and *Rhipicephalus bursa* (1.79%). The ticks were pooled and screened for viral, bacterial and protozoan microorganisms using molecular approaches. Among the microorganisms found, we describe three novel tick phenuiviruses (*Ixovirus*, *Hudivirus* and *Uukuvirus* spp.) and the first circulation of the human pathogen *Uukuvirus tachengense*, the species *Mivirus boleense*, *Ixovirus norvergiae* and *Rickettsia kotlanii*, and the genus *Allocryptoplasma* in the Iberian Peninsula, Western Europe. Our work also expands the known geographical distribution of tick-borne pathogens and underscores the role of the wolf as a sentinel species for ecosystem health.

INTRODUCTION

Ticks are considered the most important vectors of pathogenic microorganisms in wild and domestic animals and the second most important in humans after mosquitoes¹. Thus, the investigation of ticks and tick-borne diseases (TBD) deserves special attention, especially in areas where bioclimatic features favor the abundance of wildlife species and ectoparasites, and where there is a close interface between wildlife, domestic animals and humans². TBD can affect wildlife species by decreasing their fitness, reproduction and survival³. In turn, wildlife can act as reservoirs of tick-borne microorganisms and as amplifiers of ticks^{4,5}. Beyond their impact on wildlife, TBD can affect domestic animals causing disease and important economic losses and may be associated with zoonotic events⁶. A wide range of viral, bacterial and protozoan microorganisms are zoonotic and capable of causing severe and potentially life-threatening diseases in humans^{1,7}. Known tick-borne diseases are on the rise, while novel tick-borne pathogens are identified continuously and the pathogenic potential of newly identified microorganisms should not be underestimated^{8–10}.

Wolves (*Canis lupus*) are apex predators with a long lifespan that interact with numerous prey species, which makes them valuable disease sentinels and potential reservoirs of infectious agents¹¹. In addition, these vertebrates have a wide home range, which enables them to interact with ticks across distant environments, contributing to their dispersal and promoting the emergence of TBD outbreaks⁴. Furthermore, in some regions of northern Spain, wolves coexist in human dominated landscapes, with interfaces of continuous contact between wildlife, domestic animals and humans¹². Given the ecological, socio-environmental and epidemiological characteristics of wolves, the investigation of the ticks they carry and their associated microorganisms constitutes an interesting and valuable study

model. Although there are several studies that report the detection and prevalence of tick-borne microorganisms in wolves^{11,13}, information of the microorganisms harbored by their ticks is still scarce and limited to studies that collect ticks from different wildlife species and include a few wolves^{5,14}. To characterize the microorganisms directly from the ticks provides complementary information to the host-based studies, because this approach helps visualize more broadly the diversity of the microorganisms that circulate within the ecosystems and facilitates the early detection of emerging infectious agents^{7,10}. Herein, our goal was to investigate the diversity and abundance of the tick species parasitizing Iberian wolves (*Canis lupus signatus*) and to characterize the viral, bacterial and protozoan microorganisms associated with these parasites.

RESULTS

Tick species and life stage

A total of 392 ticks were collected from 72 Iberian wolves over a seven-year period (2017–2023). Five hard tick species were identified based on their morphological features: *Ixodes ricinus* (66.58%), *Dermacentor reticulatus* (21.68%), *Haemaphysalis concinna* (5.87%), *Ixodes hexagonus* (4.08%), and *Rhipicephalus bursa* (1.79%) (Table 1). The genetic identification of one *H. concinna* immature specimen was also carried out based on the obtained mitochondrial 16S rRNA sequence (Supplementary Tables 1 and 2). The geographic distribution and abundance of collected tick species studied is represented in the Fig. 1.

Table 1
Species, number of specimens, number of pools and life stage of ticks retrieved from Iberian wolves

Tick species	No. of ticks/No. of pools				
	Larvae	Nymphs	Adult males	Adult females	Total
<i>Dermacentor reticulatus</i>			43/14	42/18	85/32
<i>Haemaphysalis concinna</i>		6/2	9/2	8/2	23/6
<i>Ixodes hexagonus</i>		11/1		5/3	16/4
<i>Ixodes ricinus</i>	1/1	2/2	61/6	197/38	261/47
<i>Rhipicephalus bursa</i>			4/2	3/2	7/4

Microorganism detection

The collected ticks were grouped into 93 pools (Table 1). After confirming successful nucleic acid extraction by amplification of a fragment of the tick 16S rRNA gene, all pools were screened for the selected viral, bacterial and protozoan microorganisms. Several viruses of the families *Chuviridae* and

Phenuviridae, bacteria belonging to the genera *Anaplasma*, *Ehrlichia*, *Alloccryptoplasma*, *Wolbachia*, *Midichloria*, *Borrelia*, *Rickettsia* and *Francisella*; and protozoa species of the genera *Babesia* and *Hepatozoon* were detected. All the pools tested PCR-negative for the detection of flaviviruses, nairoviruses and *Neoehrlichia* spp. Table 2 summarizes the microorganisms detected in this study, together with their minimum infectious rate (MIR) and their estimated pooled prevalence (EPP) percentages. Sequence identities are provided in the Supplementary Tables 2 and 3.

Table 2
Microorganisms detected and pooled prevalence in ticks from Iberian wolves.

Microorganism	Tick species. No. of positive pools (tick stage); MIR% (CI)-EPP% (CI)				
	<i>Dermacentor reticulatus</i>	<i>Haemaphysalis concinna</i>	<i>Ixodes hexagonus</i>	<i>Ixodes ricinus</i>	<i>Rhipicephalus bursa</i>
Mivirus boleense					3 (2M,1F); 42.9 (6.2–79.5)-0.8 (0.2–2.0)
Hudivirus sp.				1 (1F) 0.4 (0.1–2.1)-0.3 (0–1.1)	
Ixovirus norvergiae				3 (3M); 1.2 (0.2–3.3)-0.8 (0.2–2.0)	
Ixovirus spp.			2 (1N,1F); 12.5 (1.5–38.3)-0.8 (0.2–2.0)		
Uukuvirus tachengense	23 (13M,10F); 27.1 (17.6–36.5)-6.2 (4.0–9.0)				
Uukuvirus sp.					3 (2M,1F); 42.9 (6.2–79.5)-0.8 (0.2–2.0)
Anaplasma phagocytophilum				7 (2M,5F); 2.7(0.7–4.6) -1.9 (0.8–3.6)	
Ehrlichia canis					1 (1M);
MIR: Minimum infectious rate; EPP: Maximum-likelihood Estimate of Pooled Prevalence; CI: 95% Confidence Interval; F: Female; M: Male; N: Nymph; L: Larvae; ¹ : Detected only with a single molecular marker; therefore, co-feeding transmission cannot be excluded					

Microorganism	Tick species. No. of positive pools (tick stage); MIR% (CI)-EPP% (CI)				
	<i>Dermacentor reticulatus</i>	<i>Haemaphysalis concinna</i>	<i>Ixodes hexagonus</i>	<i>Ixodes ricinus</i>	<i>Rhipicephalus bursa</i>
					14.3 (0.3–57.9)-0.3 (0–1.1)
Allocryptoplasma spp.				1 (1M); 0.4 (0.07–2.1)-0.3 (0–1.1)	
Wolbachia sp.			1 (1F); 6.3(0–28.0)-0.3 (0–1.1)		
Midichloria mitochondrii				11 (11F); 4.2 (1.8–6.6)-3.0 (1.6–5.0)	
Borrelia lusitaniae				1 (1M); 0.4 (0.1–2.1)-0.3 (0–1.1)	
Borrelia miyamotoi				1 (1F); 0.4 (0.1–2.1)-0.3 (0–1.1)	
Rickettsia kotlanii		1 (1M); 4.4 (0.1–21.9)-0.3 (0–1.1)			
Rickettsia raoultii	29 (13M,16F); 34.1 (24.0–44.0)-8.3 (5.7–11.4)			1 (1F); 0.4 (0.1–2.1)-8.3 (5.7–11.4)	
Francisella sp.	31 (13M,18F);				
MIR: Minimum infectious rate; EPP: Maximum-likelihood Estimate of Pooled Prevalence; CI: 95% Confidence Interval; F: Female; M: Male; N: Nymph; L: Larvae; ¹ : Detected only with a single molecular marker; therefore, co-feeding transmission cannot be excluded					

Microorganism	Tick species. No. of positive pools (tick stage); MIR% (CI)-EPP% (CI)				
	<i>Dermacentor reticulatus</i>	<i>Haemaphysalis concinna</i>	<i>Ixodes hexagonus</i>	<i>Ixodes ricinus</i>	<i>Rhipicephalus bursa</i>
	36.5 (26.2–47.6)-8.5 (6–11.6)				
Babesia caballi	5 (3M,2F); 5.9 (0.9–11.0)-5.9 (2.5–13.0)				
Babesia capreoli		3 (2N,1M); 12.5 (2.8–33.6)-0.8 (0.2–1.2)			
Hepatozoon canis			2 (2F); 0.8 (0.1–2.7)-0.3 (0–1.1)	1 (1M); 14.3 (0.3–57.9)-0.3 (0–1.1)	
Hepatozoon martis				1 (1F); 14.3 (0.3–57.9)-0.3 (0–1.1)	
MIR: Minimum infectious rate; EPP: Maximum-likelihood Estimate of Pooled Prevalence; CI: 95% Confidence Interval; F: Female; M: Male; N: Nymph; L: Larvae; ¹ : Detected only with a single molecular marker; therefore, co-feeding transmission cannot be excluded					

Viruses

Three sequences obtained from *Rh. bursa* pools using the PCR originally designed to detect viruses of the family *Nairoviridae* belonged to viruses of the family *Chuviridae* (Supplementary Table 1). Sequence analysis identified the virus as *Mivirus boleense* (Bólè tick virus 3), based on similarity with public sequences of viruses detected in *Rh. bursa* from Türkiye¹⁵ (Table 2; Supplementary Tables 2 and 3). Nevertheless, the phylogenetic analysis showed that the sequences obtained from *Rh. bursa* clustered independently of those retrieved from *Hyalomma asiaticum*, *Hyalomma anatolicum* or *Dermacentor nuttalli* from China, which correspond to the original strains of *M. boleense* (Fig. 2)¹⁶. Phenuiviruses were detected in 34.4% (32/93) tick pools, specifically in pools of *D. reticulatus* (n = 23), *I. hexagonus* (n = 2), *I. ricinus* (n = 4) and *Rh. bursa* (n = 3). Phylogenetic analysis including representative tick-borne *Phenuiviridae* species supported the classification of these viruses to the genera *Hudivirus*, *Ixovirus* and *Uukuvirus* of this family (Fig. 3).

The inferred phylogenies of the two studied fragments of the L segment supported that a single *I. ricinus* pool (Tick-Wolf *Hudivirus* IR39) harbored a phenuivirus of the genus *Hudivirus* (Fig. 3; Table 2; Supplementary Tables 2 and 3). Due to the low identities reached with available sequences and according to the species demarcation criteria of this family¹⁷, the amplified virus did not correspond to the single recognized species of this group, *Hudivirus muscae* (Supplementary Table 3). We tentatively proposed the name *Hudivirus luzmelaense* for this potential novel species. In addition, *Ixovirus norvergiae* was identified in the remaining *I. ricinus* positive pools (n = 3), according to the analysis of the obtained sequences of one fragment of the screened L segment (Table 2; Supplementary Tables 2 and 3).

Two of the four *I. hexagonus* pools showed infection with *Ixovirus* species (Tick-Wolf *Ixovirus* IH9, Tick-Wolf *Ixovirus* IH8) (Fig. 2; Table 2; Supplementary Tables 2 and 3). Tick-Wolf *Ixovirus* IH9 corresponded to the recently proposed species *Ixovirus beronensis* (GenBank acc. No. PQ150398 and XEN41024-6), previously detected from *I. hexagonus* removed from mink in the studied area¹⁰. In contrast, the sequence retrieved in Tick-Wolf *Ixovirus* IH8 could not be identified to the species level due to the low identities reached with available sequences and according to the species demarcation criteria¹⁷, suggesting that it could represent a novel *Ixovirus* species. This potential new species was tentatively named *Ixovirus borragani*.

Uukuvirus tachengense was detected in 23 of the 32 *D. reticulatus* pools according to nucleotide and amino acid sequence analysis. All deduced amino acid sequences presented high identities (99.4–100%) with those from *U. tachengense* (syn. Stavropol virus, Tacheng Tick virus 2, Changping Tick Virus 1 or *Dermacentor reticulatus* Uukuvirus) available on GenBank (Table 2; Supplementary Tables 2 and 3). Additionally, an unclassified *Uukuvirus* species was detected in three out of four *Rh. bursa* pools (Table 2; Supplementary Tables 2 and 3). The obtained sequences showed high identities with an unclassified uukuvirus detected in *Rh. bursa* specimens, among other *Rhipicephalus* species (Fig. 3; Supplementary Tables 2 and 3). We tentatively proposed the name *Uukuvirus lebanense* for this potential novel species.

Bacteria

The analysis of the 16S rRNA amplicons obtained using the Anaplasmataceae-specific PCR assay revealed the presence of *Anaplasma phagocytophilum*, *Ehrlichia canis*, *Allocryptoplasma* sp., *Midichloria mitochondrii* and *Wolbachia* sp. (Table 2, Supplementary Table 2). *A. phagocytophilum* was detected in seven *I. ricinus* pools (Table 2). The analysis of the respective *groELS* sequences identified three different nucleotide genotypes encoding the same amino acid sequence, which were identical to those available from *A. phagocytophilum* belonging to ecotype 1 (Supplementary Table 2). The *E. canis* infection, found in a *Rh. bursa* pool using the 16S rRNA Anaplasmataceae-specific PCR assay, was confirmed by amplification and sequencing of a fragment of the *gltA* gene. In addition, the *Neoehrlichia*-specific PCR assay unexpectedly amplified a longer fragment of the *E. canis* 16S rRNA gene (Table 2; Supplementary Tables 1 and 2). Likewise, *Allocryptoplasma* sp. was unexpectedly amplified in an *I. ricinus* pool using the *Neoehrlichia*-specific PCR assay (Tables 1 and 2; Supplementary Table 2). *M. mitochondrii* and *Wolbachia*

sp. were detected in 11 *I. ricinus* and one *I. hexagonus* pools, respectively, based on analysis of a 16S rRNA gene fragment of anaplasmataceae (Table 2; Supplementary Table 2).

Two single *I. ricinus* pools gave positive results using the specific PCR assays performed for *Borrelia* of the Lyme (*flaB*) and the relapsing fever (*glpQ*) group detection (Table 2, Supplementary Tables 1 and 2). They were identified as *Borrelia lusitaniae* and *Borrelia miyamotoi*, respectively, based on the analysis of the corresponding sequences (Supplementary Table 2).

Two *Rickettsia* species were detected in 31 pools. Specifically, *Rickettsia kotlanii* was identified in a single *H. concinna* pool (Table 2). The strain was characterized by the analysis of the *ompA*, *ompB* and *sca4* genes, reaching highest identities with available public sequences of *R. kotlanii* amplified from *Haemaphysalis* spp. specimens (Supplementary Table 2). Additionally, *Rickettsia raoultii* was amplified in 29 *D. reticulatus* pools and one *I. ricinus* pool (Table 2). Fragments of the *ompA* and *ompB* genes were obtained from all the *D. reticulatus* pools, whereas only the *ompA* gene was amplified from the *I. ricinus* sample. Sequence analysis showed that the obtained *ompA* and *ompB* sequences were identical within each gene and shared highest identities with publicly available *R. raoultii* sequences (Supplementary Table 2).

Francisella DNA was detected using the *tu/4* PCR assay from 31 out of the 32 *D. reticulatus* pools analyzed. The obtained sequences were identical among them and to those available in GenBank previously amplified from *D. reticulatus* specimens (Supplementary Table 2).

Protozoa

Babesia caballi, *Babesia capreoli*, *Hepatozoon canis*, and *Hepatozoon martis* were detected by analysis of 18S rRNA gene fragments obtained with at least one of the selected PCR assays (Table 2; Supplementary Table 2). Specifically, *B. caballi* was amplified in five *D. reticulatus* pools, *B. capreoli* was detected from three *H. concinna* samples, *H. canis* was found in two *I. ricinus* and one *Rh. bursa* tick pools, while *H. martis* was identified in a pool of *Rh. bursa* (Table 2; Supplementary Table 2).

DISCUSSION

In this work, a comprehensive study of the ticks retrieved from an Iberian wolf population and their associated microorganisms is presented. Previous studies have described the arthropod ectoparasites infecting wild and domestic carnivores in Europe, as well as the microorganisms found in them. Some of these studies included wolves amongst the host species examined^{5,14,18}. However, to the best of our knowledge, this is the first study focused exclusively on wolf ticks and based on a significant number of these parasites, allowing for an in-depth study of the tick species infesting this host and the microorganisms associated with them. Our findings are also discussed in relation to our previous work¹¹, in which we investigated the prevalence of selected tick-borne microorganisms in the same wolf population.

All the tick species identified in this study, with the exception of *Rh. bursa* were expected to be present both in the host and in the study area. To our knowledge, this is the first description of *Rh. bursa* in wolves. Nevertheless, this tick species has occasionally been reported on other canids such as dogs and occurs in the study area¹⁹. Two other species not detected in this study have been reported in Iberian wolves: *Rhipicephalus pusillus*, and *Dermacentor marginatus*²⁰. These species occur in the study area but are not the most prevalent tick species. Predominant parasitism by *I. ricinus* and *D. reticulatus* in Iberian wolves has been described previously^{18,20}. *I. ricinus* is the tick species that most frequently bites humans in Europe, including the study area, and is the major vector for the transmission of a wide range of pathogens that cause diseases such as Lyme Borreliosis, anaplasmosis, neorickettsiosis, tick-borne encephalitis, babesiosis, etc.¹. *D. reticulatus* is widespread across Europe but restricted to the northern Iberian Peninsula, and absent from the southern and drier Mediterranean regions²¹. Adult specimens have a high affinity for canid hosts¹⁹. This species is vector of pathogenic species of *Babesia* and is associated with the maintenance and transmission of *R. raoultii* and *Francisella tularensis*²¹. *H. concinna* is a common exophilic tick in the Eurasian continent, including the study area, and it is expanding southwest^{22,23}. This expansion is concerning because this tick species has been associated to at least 40 human pathogens, although the vector competence was not proven for most of them²². It is a proven vector of *F. tularensis*, tick-borne encephalitis virus, and the novel Orthonairovirus Xue-Cheng Virus recently described²⁴. *I. hexagonus* is an endophilic hard tick widespread through temperate Europe and North Africa. It is mainly associated to small, nest-dwelling carnivore mammals, especially hedgehogs, but also parasitizes foxes, otters, badgers or mink^{10,19}. It is the second most common tick on domestic animals and a known vector of numerous bacterial and protozoan parasitic pathogens such as *Borrelia burgdorferi* sensu lato among others¹⁹.

A huge diversity of tick-associated viruses has been reported worldwide, some of them considered priority by the World Health Organization (WHO) because of their high potential to cause Public Health Emergencies of International Concern²⁵. The number of TBD is expected to increase exponentially. In fact, 16 of them (38%) have been described for the first time in the last seven years, and a wider geographical distribution has been demonstrated recently for others that were previously known^{7,8}. In the present study, the circulation of the human pathogen *U. tachengense* has been demonstrated in Spain. In addition, the circulation of *M. boleense* and *I. norvegicae*, both with a highly pathogenic potential, have been proved for the first time in the Iberian Peninsula. Finally, novel tick-associated viruses have been partially characterized. According to the partial genetic characterization and phylogeny inferred in this work, we have proposed the following names for the potential new virus species: *I. borragani* for the Tick-Wolf *Ixovirus* IH8; *U. lebanense* for the Tick-Wolf *Uukuvirus* RB11, RB12 and RB92; and *H. luzmelaense* for the Tick-Wolf *Hudivirus* IR39. The virus name *I. borragani* has been chosen in honor of the veterinarian from Cantabria Santiago Borragán, in recognition to his contributions to wildlife health and research. The virus name *H. luzmelaense* refers to Luzmela, the literary name historically associated with Mazcuerras, the Cantabrian locality where the virus has been identified. Finally, the virus name *U.*

lebanense refers to Liébana, a unique valley of Cantabria with a rich biodiversity that is enclosed by the Picos de Europa mountains, where this virus has been detected.

M. boleense (family *Chuviridae*) was first isolated in 2012 from *Hy. asiaticum* ticks collected in China¹⁶. Closely related viruses have subsequently been detected in other tick species, including *Rh. bursa*, both in Asia and in Europe¹⁵. The sequences obtained in the present study clustered with those previously detected in *Rh. bursa*, and formed a distinct lineage from those obtained from *Hy. asiaticum*, *D. nuttalli* and *Hy. anatolicum*. Therefore, we provisionally classified our sequences as *M. boleense*. However, the available complete L segment sequences of the virus amplified from *Rh. bursa* showed less than 90% amino acid identity to the recognized *M. boleense* sequences. This identity is below the species demarcation threshold established for this group^{26,27}. Thus, the viruses associated with *Rh. bursa*, including those detected in the present study, may represent a distinct species. Complete genome sequencing and further phylogenetic analyses will be required to clarify their taxonomic status. Their pathogenic potential also remains unexplored.

The family *Phenuiviridae* is the most widely distributed tick-associated viral family, which includes recognized pathogens such as *Bandavirus dabieense*, WHO priority pathogen causing the Severe Fever with Thrombocytopenia Syndrome, or *U. tachengense*, among others^{8,17,25}. The virus *U. tachengense* was discovered in *D. marginatus* specimens in China in 2015, but it was not until 2019 that it was involved in a human infection^{16,28}. Subsequently, a retrospective study conducted in this country confirmed new human cases. In that study, half of the cases were co-infected with *Rickettsia* spp., and they had nonspecific symptoms such as headache, fever, asthenia, vomiting, myalgia, rash, and meningitis-like signs²⁹. The virus has been previously reported from *D. reticulatus* but not from Spain³⁰. Our finding of the circulation of this infectious agent in northern Spain supports its consideration in the differential diagnosis of suspected TBD of unknown origin. Moreover, the pathogenic potential of this virus for other vertebrates, such as wolves, and the role of these in the epidemiologic cycle of the pathogen should be further investigated. Our findings also confirm the circulation of *Uukuvirus* species in *Rh. bursa* ticks from the Iberian Peninsula, as previously described by Pimentel et al.³¹. Closely related unclassified phenuiviruses have been amplified from this tick species in Asia and Europe, namely brown dog tick phlebovirus 2, Phlebovirus Strandja or Adagum virus, among others^{30,32}. To our knowledge, none of these viruses has been involved in human or animal diseases. However, their pathogenic potential should be monitored, taking into consideration that the tick *Rh. bursa* has both an anthropophilic behavior and affinity for wild and domestic ruminants^{19,33}.

The *Ixovirus* genus includes three species associated with *Ixodes* spp. ticks¹⁷. None of them has been involved as pathogens to date. However, all of them have a highly predicted probability to cause disease³⁴. *I. norvegiae* is the single species reported in Europe. Its circulation in northern Spain was expected because of its wide distribution, which includes neighboring France, and because *I. ricinus* is abundant in the area³⁵. Other related strains have been described in the study area in *I. hexagonus* and *I. acuminatus* specimens collected from mink¹⁰. Our data corroborates the presence of one of these

strains, which was provisionally named *I. beronensis*, in the tick species *I. hexagonus*. This finding also supports the role of *I. hexagonus* in the epidemiology of the new potential virus. We have also detected a novel potential *Ixovirus* species proposed as *I. borragani* in *I. hexagonus* ticks. However, more studies are necessary to ascertain the role of this tick species as vectors and the pathogenicity of these potential novel viruses. Finally, we retrieved an unclassified phenuivirus, proposed as *H. luzmelaense*, that clustered in a monophyletic clade with the single species of the genus *Hudivirus* (*H. muscae*; Húběi diptera virus 4) and the related virus named Santiago Bunya-like¹⁷. Due to this phylogeny, we concluded that the virus belonged to the *Hudivirus* genus. Little is known about this group, so further studies including the virus isolation and the tick-vector competence should be performed. Currently there is no cultured hudivirus isolate. Furthermore, *H. muscae* and closely related viruses have been amplified from insects (flies and rabbit fleas) but not from ticks³⁶. Since flies and fleas are common wolf ectoparasites, the detected virus could be in the blood meal of the ticks instead of in the arthropod itself.

Regarding the presence of gram-negative bacteria of the family Anaplasmataceae, the most relevant species found was *A. phagocytophilum*, with three very similar strains detected in *I. ricinus* pools. These strains belong to the Ecotype 1, which includes the pathogenic variants³⁷. *A. phagocytophilum* infects granulocytes and is the causative agent of anaplasmosis in domestic animals and of human granulocytic anaplasmosis³⁸. The bacterium is widespread and ubiquitous, but not all of the *A. phagocytophilum* strains have pathogenic potential³⁷. Remarkably, most strains isolated from domestic animals, wild canids (including wolves) and wild boar in Europe fall into the subcluster of the Ecotype 1 that has been defined as zoonotic³⁹. Herein, one of the strains was almost identical to the *A. phagocytophilum* Norway variant-1, the virulent genotype causative of the tick-borne fever in ruminants, identified as a major threat to sheep herds in Norway⁴⁰. The other two strains have been previously detected in questing and feeding ticks from red deer in northern Spain⁴¹, and their pathogenicity remains unclear. Granulocytic anaplasmosis has been described in a captive timber wolf and timber wolf crossbreeds and is a worldwide emerging disease in dogs with a self-limiting course^{42,43}. Therefore, free-ranging wolves would be expected to be vulnerable to the disease. Interestingly, despite the detection of infected ticks, *A. phagocytophilum* DNA was not detected in blood samples from these wolves in our previous study¹¹. Another gram-negative bacteria of the family Anaplasmataceae, *E. canis*, was identified in *Rh. bursa* ticks. *E. canis* is a major canine pathogen that infects circulating monocytes, and it is the causative agent of the canine monocytic ehrlichiosis (CME)⁴⁴. *E. canis* can cause serious disease in wild and domestic animals but is also an emerging zoonotic agent, as human cases are reported with increasing frequency^{44,45}. *Rhipicephalus sanguineus* is the main recognized vector, but the bacteria has been detected in several tick species with unproven vector competence, such as *Rh. bursa*^{44,46}. Wild wolves are likely susceptible to CME. However, diagnosing a clinical case of disease in a free-ranging gray wolf is an extremely rare event^{11,43}. *E. canis* was investigated in the wolf population studied herein and all of the wolves turned out negative¹¹. Even though *E. canis* has never been detected molecularly in the Iberian wolf, it was described in grey wolves from Italy⁴⁷. Also belonging to the family Anaplasmataceae, we found several tick endosymbionts in the wolf ticks. First, we detected the

endosymbiont *Ca. M. mitochondrii* in *I. ricinus* pools. It is a common intracellular bacterial endosymbiont in this tick species that colonizes the mitochondria inside their cells⁴⁸. Then, *Wolbachia* spp. was detected in *I. hexagonus* ticks. This endosymbiont is suspected to infect several species of insects, such as the tick parasitoid wasp *Ixodiphagus hookeri*, rather than being a natural endosymbiont of the tick itself⁴⁹. Lastly, we have found *Allocryptoplasma* spp. in *I. ricinus* ticks. *Allocryptoplasma* species belong to a putative genus of the family Anaplasmataceae. This bacterium has been detected worldwide in multiple tick species and various vertebrate hosts. The genetic diversity and pathogenic potential of this microorganism is complex and remains under investigation⁵⁰. To our knowledge, this finding represents the first description of *Allocryptoplasma* sp. in the Iberian Peninsula.

The surveillance and detection of *Borrelia* spp. is of critical importance, especially in Northern Spain, where the diagnosis of Lyme borreliosis in human hospitals has increased substantially in the last two decades⁹. We have found two *Borrelia* species in *I. ricinus* pools from the Iberian wolves: *B. lusitaniae* and *B. miyamotoi*, that are classified into the *B. burgdorferi* s.l. and the relapsing fever complex, respectively. *B. lusitaniae* is not the genospecies usually isolated from Lyme borreliosis cases, however, it has been anecdotally involved in atypical cases of borreliosis⁵¹. The absence of genospecies commonly associated with Lyme borreliosis in the North of Spain⁹ could be explained by the dilution effect described for geographical areas with a high biodiversity⁵². Furthermore, the wolf is considered an incompetent reservoir for the pathogen although they it can suffer the disease⁵³. The *Borrelia* species that cause tick-borne relapsing fever are predominantly transmitted by soft ticks; however, some of the species of this complex, such as *B. miyamotoi*, are transmitted by hard ticks. *B. miyamotoi* disease (BMD) is an emerging disease of the Northern Hemisphere with a systemic course, characterized by relapsing fever, headache, myalgia, arthralgia, elevated liver enzymes, neutropenia and thrombocytopenia⁵⁴. BMD may also result in meningoencephalitis. This condition has been described both in immunocompromised and immunocompetent patients^{54,55}. In Spain, although *B. miyamotoi* has been detected in questing *I. ricinus* ticks collected in 2009, no cases of infection have been notified yet⁵⁶.

As far as rickettsial agents, we found *R. raoultii* in most of the pools of *D. reticulatus*, which is a recognized vector. *R. raoultii* is one of the agents involved in Dermacentor-Borne-Necrosis-Erythema-Lymphadenopathy (DEBONEL), also known as Tick-borne Lymphadenopathy (TIBOLA), and is also the cause of the Scalp Eschar Neck Lymphadenopathy (SENLAT) syndrome. The disease is endemic in the study area, and its incidence is not negligible⁵⁷. *R. kotlanii*, whose pathogenic potential remains unknown, was also isolated from a *H. concinna* pool. To the best of our knowledge, this is the first report of *R. kotlanii* in Spain, and the first time both *R. raoultii* and *R. kotlanii* are detected in ticks retrieved from wolves.

Finally, *Francisella* spp. has been isolated from 96.9% of the *D. reticulatus* pools. Our sequence belongs to the *Francisella*-Like Endosymbiont group (FLE) and, although it does not belong to any of the previously validated *Francisella* species including the pathogenic species *F. tularensis*, it is identical to others FLE sequences described in *D. reticulatus* in Portugal and elsewhere⁵⁸. FLE is a common

ancestral nutritional symbiont of ticks that contains in its genome, among others, the biosynthesis pathways of B vitamins⁵⁹.

Regarding protozoa, our data corroborate the circulation of *B. caballi*, *B. capreoli*, and *H. canis* in Spain. To our best knowledge, this is the first report of *B. caballi* in ticks from wolves, although the sequence type of *B. caballi* found in this study, responsible for equine piroplasmosis disease, is identical to that described in equids worldwide. A *B. caballi*-like sequence was described in crab-eating foxes from the Brazilian Pantanal⁶⁰. On the contrary, *B. capreoli* does not pose health concern so far. Its occurrence is known in the study area²³. Moreover, infection by *B. capreoli* has been recently described in grey wolves from a neighboring area⁶¹. *H. canis* is a major canine pathogen with a worldwide distribution. In our previous work, we found that 96% of the wolves in this same wolf population tested positive for *H. canis*¹¹. The sequence identified in the wolves is identical to the one we have now found in their ticks. Thus, even though *Rhipicephalus* spp. is a recognized tick vector for *Hepatozoon* spp., the presence of *H. canis* in the blood meal rather than in the ticks cannot be ruled out. In another study performed in Türkiye, *H. canis* was found in *Haemaphysalis parva* ticks from two wolves, and the haplotype of the protozoan in the ticks and in the wolves also coincided⁵. We have also found *H. martis* in *Rh. bursa* collected from wolves. The haplotype found is identical to the one reported recently in *I. hexagonus* ticks from mink and previously in Eurasian badgers in the North of Spain⁶. Our finding corroborates the circulation of this *H. martis* haplotype in this region and expands the knowledge about the tick species and carnivore hosts related to this microorganism.

CONCLUSIONS

Characterizing the microorganisms harboured by ticks is an essential first step towards assessing their potential relevance in public and veterinary health. This study highlights the value of tick surveillance in wild canids for the early detection of emerging or previously unrecognized pathogens. Such surveillance is particularly relevant for viruses of potential public health significance, for which major knowledge gaps remain regarding their host range, transmission dynamics, pathogenicity, clinical manifestations, diagnosis, prevention and treatment. Our findings provide an ecological and epidemiological basis for future experimental and clinical investigations and may help identify agents that warrant consideration in surveillance programmes and in the differential diagnosis of unexplained febrile illness following tick exposure. The detection of microorganisms associated with the Iberian wolf is not only relevant for public health but also for wildlife conservation. Our work helps deepen the knowledge of the diversity and epidemiology of the pathogens that circulate in the ecosystem, alerting wildlife veterinarians and stakeholders to potential disease outbreaks both in the Iberian wolf and in other sympatric species, including relevant endangered vertebrates such as the Cantabrian brown bear. More broadly, our work reinforces the value of a One Health approach integrating wildlife, veterinary and human health surveillance to improve preparedness for emerging tick-borne diseases.

MATERIALS AND METHODS

Study area and host species

Cantabria is a coastal region of the Atlantic northern Spain (southwestern Europe) with a surface of 5,326 km². It is a mountainous territory, situated in the Central Cantabrian Mountain range, with almost half of its surface 700 m above sea level. The climate is predominantly Atlantic, although the southern plains have continental influence and there are several patches of Mediterranean climate around the northeastern Picos de Europa Mountains. The mean annual precipitation ranges 1,200 to 1,400 mm. Approximately 70% of the region is covered by forests from the Cantabrian mixed forest ecoregion, belonging to the European temperate deciduous forest biome⁶². This biome can be considered a hotspot for tick species due to its bioclimatic characteristics: high humidity, mild temperatures and abundant vegetation and wildlife.

The Iberian wolf belongs to the European grey wolf population. Even though it reached historical low numbers in Spain in the 1970s, the species partially recovered the following decades to occupy its current distribution area, found primarily in the northwestern Iberian Peninsula⁶³. In the region of Cantabria, the last census of the species estimated 23 wolf packs (Order DES/08/2026, Regional Government of Cantabria). Its home range in this Spanish province is characterized by a human-dominated landscape combined with a rich biodiversity. This close relationship of the wolf population and the human activities has led to a long-standing conflict of coexistence due to the damage caused by the species to extensive livestock herds. The management of the species (e.g., culling, protection policies) is the subject of constant debate¹².

Tick collection and classification

Over a seven-year period (2017–2023), under the wildlife health surveillance program of the Government of Cantabria, all wolves culled or found dead in the province underwent a complete postmortem investigation, starting with a necropsy at the Wildlife Rehabilitation Center of Cantabria (WRCC), as described by Fayos et al.¹¹. During the external examination, the carcasses were systematically inspected for ectoparasites for at least 5 minutes. The ticks found were introduced into tubes, labelled, and stored at -80 °C until analysis. Stored ticks were morphologically identified according to taxonomic keys^{19,64}. In incomplete immature specimens, the identification was performed using a PCR that partially amplifies the mitochondrial 16S rRNA gene and subsequently sequenced (Supplementary Table 1).

Microorganism analysis

Ticks were pooled (from one to 14 specimens; whole immature and halves of adult specimens) according to species and development stage. Frozen ticks were surface-sterilized, homogenized and subjected to nucleic acid extraction as described in Palomar et al.¹⁰. Successful nucleic acid extraction and the absence of PCR inhibitors of all the samples was confirmed amplifying the tick mitochondrial 16S rRNA gene fragment (Supplementary Table 1). All pool extracts were analyzed using specific PCRs for the detection of: i) viruses: orthonairoviruses, flaviviruses (including tick-borne encephalitis virus) and phenuiviruses; ii) bacteria: *Anaplasma* spp., *Ehrlichia* spp., *Neoehrlichia* spp., *Borrelia* spp., *Rickettsia*

spp., and *Francisella* spp.; and iii) protozoa: *Babesia* spp., *Theileria* spp. and *Hepatozoon* spp. (Supplementary Table 1). Two negative controls (one for extraction and one for PCR) and the appropriate positive controls were included in the PCR assays. The positive controls were extracts of: 1) *D. marginatus*, *A. phagocytophilum*, *Ehrlichia* sp., *Neoehrlichia mikurensis*, *B. miyamotoi*, *Rickettsia amblyommatis* or *Babesia bigemina* obtained from the “Zoonosis collection” registered in the National Registry of Biobanks from Carlos III Health Institute (Reference: C.0006409), located at CRETAV-CIBIR (Biomedical Research Center of La Rioja), Spain; 2) synthetic DNA from *F. tularensis* or *Orthoflavivirus encephalitidis* (AMPLIRUN®, Vircell, Spain); or 3) *Borrelia spielmanii*, *Orthoflavivirus japonicum*, *Uukuniemi uukuniemiense*, or *Orthonairovirus haemorrhagiae*, kindly provided by colleagues (please see acknowledgements section).

PCR products were visualised by 1% agarose gel electrophoresis. Amplicons of the expected size were sequenced by Sanger at the Molecular Diagnostic Department (CIBIR, Logroño, Spain). Nucleotide sequences were then analysed by BLAST search (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>), and new sequences were submitted to GenBank (<https://submit.ncbi.nlm.nih.gov/>). The Clustal Omega online software (<https://www.ebi.ac.uk/Tools/msa/clustalo/>) was used for multiple sequence alignment. Phylogenetic analyses were conducted with MEGA 12 (<http://www.megasoftware.net>) using the maximum likelihood method including all sites. The nucleotide substitution model was selected according to the Akaike information criterion implemented in MEGA 12. Confidence values for the individual branches of the resulting trees were determined by bootstrap analysis with 500 replicates.

The prevalence of infection was calculated using the two main estimation methods of pathogen prevalence in pooled ticks⁶⁵: Minimum Infection Rate (MIR) and Maximum-likelihood Estimate of Pooled Prevalence (EPP), as follows:

(i): MIR: assuming that each positive pool contained only one positive tick, as follows: $MIR (\%) = (\text{No. of positive pools} / \text{No. of ticks analyzed}) \times 100$.

(ii): EPP: infection rate most likely observed given the test results and an assumed probabilistic model (binomial distribution of infected individuals in a positive pool), assuming that each tick within a pool has an identical independent probability of being infected. EPP estimates were conducted in R (RStudio, 2023.12.1 + 402) using standard functions and the PoolTestR package⁶⁵ for pooled prevalence estimation.

Confidence Intervals (CI) (95%) were calculated for both MIR and EPP with EPITOOLS (<https://epitools.ausvet.com.au/ciproportion>).

Permits

All ectoparasites analyzed in this study were collected from necropsies and forensic examinations performed at the WRCC from animals legally culled under the Cantabrian livestock damage control

program or found dead in the region (e.g. road-killed) (Law 12/2006 and Order MED/5/2019, Regional Government of Cantabria). None of the animals were culled or euthanized for the purpose of this study.

Declarations

Competing interests

The authors declare no competing interests

Ethics declarations

No animals were killed for the purpose of this study. All of the samples were obtained opportunistically from wolf carcasses originating from legally culled individuals in accordance with regional wildlife management regulations (Law 12/2006 and Order MED/5/2019, Regional Government of Cantabria). No experimental procedures were performed on live animals. This study did not require therefore specific ethical approval under the current legislation.

Funding

This Project was partially funded by European Regional Development Funds (Programa FEDER de La Rioja 2021–2027; No. 32, 00860–2024/116698) at 60% in the Specific Objective 1.1 and 1.2 of Health-Advanced (HEALTH-A).

Author Contribution

MF, JAO and AMP conceptualized, designed and supervised the study, provided resources and drafting the manuscript. MF, AP, JAO and AMP performed the data analysis. MF, AP, CS, CCA, IS, RV, ACE, NS, PS and AMP conducted the research and helped with the data analysis. All authors reviewed and approved the final version of the manuscript.

Acknowledgement

We thank Sección de Caza and Sección de Fauna y Flora of the Dirección General de Montes y Conservación de la naturaleza, Gobierno de Cantabria. We also thank the Agentes del Medio Natural, Gobierno de Cantabria, for wolf population surveillance and carcass collection. We acknowledge A. de la Cuesta for his technical support and elaboration of the tick species map. We thank Volker Fingerle (German National Reference Centre for Borrelia), Matthias Niedrig (Centre for Biosafety, Robert Koch Institute, Germany), Luis M. Hernández-Triana (Wildlife Zoonoses and Vector-borne Diseases Research Group, Animal and Plant Health Agency, United Kingdom) and Houssam Attoui (UMR Virologie, National

Institute for Agriculture, Food, and Environment [INRAE], France), for providing positive controls. Ana Carolina Ewbank receives a Juan de la Cierva formación fellowship (JdC2022-048632-I) granted by Ministerio de Ciencia, Innovación y Universidades (MCIU)/Agencia Estatal de Investigación (AEI). The results of this study were partially presented at the 11th edition of the Tick and TickBorne Pathogen Conference held in September 2024 in Havana, Varadero, Cuba.

Data Availability

Sequences obtained in the present study were submitted to GenBank database under the accession numbers detailed in the Supplementary Table 2.

References

1. Oteo, J. A. & Palomar, A. M. More threats: The increase in tick-borne diseases and their spectrum. *Enferm Infecc Microbiol. Clin. (Engl Ed)*. **43**, 1–2. <https://doi.org/10.1016/j.eimce.2024.10.005> (2025).
2. Gortazar, C. et al. The wild side of disease control at the wildlife-livestock-human interface: a review. *Front. Vet. Sci.* **1**, 27. <https://doi.org/10.3389/fvets.2014.00027> (2015).
3. Aguirre, A. A. Wild canids as sentinels of ecological health: a conservation medicine perspective. *Parasit. Vectors.* **2** (Suppl 1). <https://doi.org/10.1186/1756-3305-2-S1-S7> (2009).
4. Tomassone, L., Portillo, A., Nováková, M., De Sousa, R. & Oteo, J. A. Neglected aspects of tick-borne rickettsioses. *Parasit. Vectors.* **11**, 263. <https://doi.org/10.1016/j.vetpar.2017.12.018> (2018).
5. Orkun, Ö. & Emir H Identification of tick-borne pathogens in ticks collected from wild animals in Turkey. *Parasitol. Res.* **119**, 3083–3091. <https://doi.org/10.1007/s00436-020-06812-2> (2020).
6. Rhyan, J. C. et al. Emergence of diseases from wildlife reservoirs. *Vet. Pathol.* **47**, 34–39. <https://doi.org/10.1177/0300985809354466jt90op> (2010).
7. Portillo, A., Palomar, A. M., Santibáñez, P. & Oteo, J. A. Epidemiological aspects of Crimean-Congo hemorrhagic fever in Western Europe: what about the future? *Microorganisms* **9**, 649. <https://doi.org/10.3390/microorganisms9030649> (2021).
8. Ye, R. Z., Li, Y. Y., Wang, X. Y. & Cao, W. C. Tick-borne viruses: discovery, clinical aspects, influencing factors and control. *Nat. Rev. Microbiol.* <https://doi.org/10.1038/s41579-026-01277-z> Erratum in: *Nat. Rev. Microbiol.* 2026 Mar 3. (2026). <https://doi.org/10.1038/s41579-026-01298-8>
9. Oteo, J. A., Santibáñez, S. & Portillo, A. Tick-borne diseases in Spain. *Med. Clin. (Barc)*. **164**, 106989. <https://doi.org/10.1016/j.medcli.2025.106989> (2025).
10. Palomar, A. M. et al. Ticks from mink and their associated microorganisms in Spain. *Transbound. Emerg. Dis.* 9995586 (2025). (2025). <https://doi.org/10.1155/tbed/9995586>
11. Fayos, M. et al. Fatal babesiosis in a free-ranging Iberian wolf co-infected with Hepatozoon canis and Leishmania infantum: Epidemiological implications for the cantabrian wolf population. *Int. J.*

- Parasitol. Parasites Wildl.* **27**, 101080. <https://doi.org/10.1016/j.ijppaw.2025.101080> (2025).
12. Ordiz, A., Canestrari, D. & Echegaray, J. Wolf conservation and management in Spain, an open debate. *Front. Environ. Sci.* **10**, 781169. <https://doi.org/10.3389/fenvs.2022.781169> (2022).
 13. Ebani, V. V., Nardoni, S. & Mancianti, F. Arthropod-borne pathogens in wild canids. *Vet. Sci.* **10**, 165. <https://doi.org/10.3390/vetsci10020165> (2023).
 14. Del Cerro, A. et al. Molecular identification of tick-borne pathogens (*Rickettsia* spp., *Anaplasma phagocytophilum*, *Borrelia burgdorferi sensu lato*, *Coxiella burnetii* and piroplasms) in questing and feeding hard ticks from North-Western Spain. *Ticks Tick. Borne Dis.* **13**, 101961. <https://doi.org/10.1016/j.ttbdis.2022.101961> (2022).
 15. Ergünay, K. et al. Multiple orthonairoviruses including Crimean-Congo hemorrhagic fever virus, Tamdy virus and the novel Meram virus in Anatolia. *Ticks Tick. Borne Dis.* **11**, 101448. <https://doi.org/10.1016/j.ttbdis.2020.101448> (2020).
 16. Li, C. X. et al. Unprecedented genomic diversity of RNA viruses in arthropods reveals the ancestry of negative-sense RNA viruses. *eLife* **4**, e05378. <https://doi.org/10.7554/eLife.05378> (2015).
 17. Sasaya, T. et al. ICTV virus taxonomy profile: Phenuiviridae 2023. *J. Gen. Virol.* **104**, 001893. <https://doi.org/10.1099/jgv.0.001893> (2023).
 18. Sobrino, R. et al. Ecological preferences of exophilic and endophilic ticks (Acari: Ixodidae) parasitizing wild carnivores in the Iberian Peninsula. *Vet. Parasitol.* **184**, 248–257. <https://doi.org/10.1016/j.vetpar.2011.09.003> (2012).
 19. Estrada-Peña, A., Mihalca, A. D. & Petney, T. N. (eds) *Ticks of Europe and North Africa: a guide to species identification* (Springer, 2017). <https://doi.org/10.1007/978-3-319-63760-0>
 20. Santos-Silva, M. M. et al. The hard-tick fauna of mainland Portugal (Acari: Ixodidae): an update on geographical distribution and known associations with hosts and pathogens. *Exp. Appl. Acarol.* **55**, 85–121. <https://doi.org/10.1007/s10493-011-9440-x> (2011).
 21. Földvári, G., Šíroký, P., Szekeres, S., Majoros, G. & Sprong, H. *Dermacentor reticulatus*: a vector on the rise. *Parasit. Vectors.* **9**, 314. <https://doi.org/10.1186/s13071-016-1599-x> (2016).
 22. Liu, J. et al. An integrated data analysis reveals distribution, hosts, and pathogen diversity of *Haemaphysalis concinna*. *Parasit. Vectors.* **17**, 92. <https://doi.org/10.1186/s13071-024-06152-5> (2024).
 23. Palomar, A. M. et al. Coinfection of *Anaplasma bovis*, *Anaplasma* sp. and *Theileria orientalis* complex in European bison (*Bison bonasus*). *TTP11: Scientific Program Abstract Book: TTP.11, 1st-6th September*, Havana, Varadero, Cuba (2024). (2024). https://books.google.es/books/about/11th_Tick_and_Tick_borne_Pathogen_Confer.html?id=8KYN0QEACAAJ&redir_esc=y
 24. Zhang, M. Z. et al. Human infection with a novel tickborne orthonairovirus species in China. *N Engl. J. Med.* **392**, 200–202. <https://doi.org/10.1056/NEJMc2410853> (2025).
 25. WHO. Vector Borne Diseases (Fact Sheets). (2024). <https://www.who.int/news-room/fact-sheets/detail/vector-borne-diseases>

26. Di Paola, N. et al. Jingchuvirales: a new taxonomical framework for a rapidly expanding order of unusual monjiviricete viruses broadly distributed among arthropod subphyla. *Appl. Environ. Microbiol.* **88**, e0195421. <https://doi.org/10.1128/aem.01954-21> (2022).
27. Ergunay, K. et al. Impact of nanopore-based metagenome sequencing on tick-borne virus detection. *Front. Microbiol.* **14**, 1177651. 10.3389/fmicb.2023.1177651 (2023a). <https://doi.org/10.3389/fmicb.2023.1177651>
28. Dong, Z. et al. Human Tacheng tick virus 2 infection, China, *Emerg. Infect. Dis.* **27**, 594 (2021). (2019). <https://doi.org/10.3201/eid2702.191486>
29. Jia, Y. et al. Clinical and historical infection of Tacheng tick virus 2: A retrospective investigation. *PloS Negl. Trop. Dis.* **18**, e0012168. <https://doi.org/10.1371/journal.pntd.0012168> (2024).
30. Bratuleanu, B. E. et al. The virome of Rhipicephalus, Dermacentor and Haemaphysalis ticks from Eastern Romania includes novel viruses with potential relevance for public health. *Transbound. Emerg. Dis.* **69**, 1387–1403. <https://doi.org/10.1111/tbed.14105> (2022).
31. Pimentel, V. et al. Geographic dispersal and genetic diversity of tick-borne phleboviruses (Phenuiviridae, Phlebovirus) as revealed by the analysis of L segment sequences. *Ticks Tick. Borne Dis.* **10**, 942–948. <https://doi.org/10.1016/j.ttbdis.2019.05.001> (2019).
32. Klimentov, A. S. et al. Phlebovirus sequences detected in ticks collected in Russia: Novel phleboviruses, distinguishing criteria and high tick specificity. *Infect. Genet. Evol.* **85**, 104524. <https://doi.org/10.1016/j.meegid.2020.104524> (2020).
33. Beltran, A. et al. MALDI-TOF MS as a tick identification tool in a tertiary hospital in Spain. *Acta Trop.* **242**, 106868. <https://doi.org/10.1016/j.actatropica.2023.106868> (2023).
34. Lin, Y. & Pascall, D. J. Characterisation of putative novel tick viruses and zoonotic risk prediction. *Ecol. Evol.* **14**, e10814. <https://doi.org/10.1002/ece3.10814> (2024).
35. Bratuleanu, B. E. et al. Diversity of Viruses in *Ixodes ricinus* in Europe including Novel and Potential Arboviruses. *Transbound. Emerg. Dis.* **21**, 2023:6661723. <https://doi.org/10.1155/2023/6661723> (2023).
36. Mahar, J. E., Shi, M., Hall, R. N., Strive, T. & Holmes, E. C. Comparative analysis of RNA virome composition in rabbits and associated ectoparasites. *J. Virol.* **94**, e02119–e02119. <https://doi.org/10.1128/jvi.02119-19> (2020).
37. Rar, V., Tkachev, S. & Tikunova, N. Genetic diversity of Anaplasma bacteria: Twenty years later. *Infect. Genet. Evol.* **91**, 104833. <https://doi.org/10.1016/j.meegid.2021.104833> (2021).
38. Oteo, J. A. & Brouqui, P. Ehrlichiosis y anaplasmosis humana. *Enferm Infecc Microbiol.* **23**, 375–380. <https://doi.org/10.1157/13076178> (2005).
39. Lesiczka, P. M. et al. European human granulocytic anaplasmosis is caused by a subcluster of Anaplasma phagocytophilum Ecotype I. *Curr. Res Parasitol. Vector Borne Dis.* **5**, 100324. <https://doi.org/10.1016/j.crpvbd.2025.100324> (2025).
40. Crosby, F. L. et al. Comparative whole genome analysis of an Anaplasma phagocytophilum strain isolated from Norwegian sheep. *Pathogens* **11**, 601. <https://doi.org/10.3390/pathogens11050601> (2022).

41. Portillo, A. et al. Anaplasma spp. in wild mammals and Ixodes ricinus from the north of Spain. *Vector Borne Zoonotic Dis.* **11**, 3–8. <https://doi.org/10.1089/vbz.2009.0214> (2011).
42. Leschnik, M., Kirtz, G., Virányi, Z., Wille-Piazzai, W. & Duscher, G. Acute granulocytic anaplasmosis in a captive timber wolf (Canis lupus occidentalis). *J. Zoo Wildl. Med.* **43**, 645–648. <https://doi.org/10.1638/2011-0224R.1> (2012).
43. Widyasanti, N. W. H., Putra, I. P. C. & Suwiti, N. K. Case Report: Ehrlichiosis and Anaplasmosis in Timber Wolf Crossbreed (Canis lupus) in Bali, Indonesia. *Media Kedokteran Hewan.* **36**, 88–99. <https://doi.org/10.20473/mkh.v36i1.2025.88-99> (2025).
44. Ferrolho, J. et al. The Complexities of Canine Monocytic Ehrlichiosis: Insights into Ehrlichia canis and Its Vector Rhipicephalus sanguineus. *Microbiol. Res.* **16**, 85. <https://doi.org/10.3390/microbiolres16040085> (2025).
45. Sgroi, G. et al. Ehrlichia canis in human and tick, Italy, *Emerg. Infect. Dis.* **30**, 2651 (2024). (2023). <https://doi.org/10.3201/eid3012.240339>
46. Masala, G. et al. First detection of Ehrlichia canis in Rhipicephalus bursa ticks in Sardinia, Italy. *Ticks Tick. Borne Dis.* **3**, 396–397. <https://doi.org/10.1016/j.ttbdis.2012.10.006> (2012).
47. Santoro, M. et al. Molecular survey of Ehrlichia canis and Coxiella burnetii infections in wild mammals of southern Italy. *Parasitol. Res.* **115**, 4427–4431. <https://doi.org/10.1007/s00436-016-5213-0> (2016).
48. Floriano, A. M. et al. The evolution of intramitochondriality in Midichloria bacteria. *Environ. Microbiol.* **25**, 2102–2117. <https://doi.org/10.1111/1462-2920.16446> (2023).
49. Luu, L. et al. Bacterial pathogens and symbionts harboured by Ixodes ricinus ticks parasitising red squirrels in the United Kingdom. *Pathogens* **10**, 458. <https://doi.org/10.3390/pathogens10040458> (2021).
50. Uessler, F. et al. Transcriptomic detection of Candidatus Allocryptoplasma (Anaplasmataceae) in Galápagos marine iguanas (Amblyrhynchus cristatus, Iguanidae). *Parasit. Vectors.* **18**, 492. <https://doi.org/10.1186/s13071-025-07138-7> (2025).
51. Veinović, G. et al. Isolation and cultivation of Borrelia lusitaniae from the blood of a patient with multiple erythema migrans. *J. Infect. Dev. Ctries.* **19**, 630–635. <https://doi.org/10.3855/jidc.20497> (2025).
52. LoGiudice, K., Ostfeld, R. S., Schmidt, K. A. & Keesing, F. The ecology of infectious disease: effects of host diversity and community composition on Lyme disease risk. *Proc. Natl. Acad. Sci. USA.* **100**, 567–571 (2003). <https://doi.org/10.1073/pnas.0233733100>
53. Levi, T., Keesing, F., Holt, R. D., Barfield, M. & Ostfeld, R. S. Quantifying dilution and amplification in a community of hosts for tick-borne pathogens. *Ecol. Appl.* **26**, 484–498. <https://doi.org/10.1890/15-0122> (2016).
54. Henningsson, A. J. et al. Two cases of Borrelia miyamotoi meningitis, Sweden, *Emerg. Infect. Dis.* **25**, 1965 (2019). (2018). <https://doi.org/10.3201/eid2510.190416>

55. Gugliotta, J. L., Goethert, H. K., Berardi, V. P. & Telford, S. R. III Meningoencephalitis from *Borrelia miyamotoi* in an immunocompromised patient. *N Engl. J. Med.* **368**, 240–245. <https://doi.org/10.1056/NEJMoa1209039> (2013).
56. Palomar, A. M., Portillo, A., Santibáñez, P., Santibáñez, S. & Oteo, J. A. *Borrelia miyamotoi*: Should this pathogen be considered for the diagnosis of tick-borne infectious diseases in Spain? *Enferm Infecc Microbiol. Clin. (Engl Ed)*. **36**, 568–571. <https://doi.org/10.1016/j.eimce.2017.10.020> (2018).
57. Santibáñez, S. et al. Epidemiological, clinical, and microbiological characteristics in a large series of patients affected by Dermacentor-borne-necrosis-erythema-lymphadenopathy from a unique centre from Spain. *Pathogens* **11**, 528. <https://doi.org/10.3390/pathogens11050528> (2022).
58. De Carvalho, I., Santos, N., Soares, T., Zè-Zè, L. & Núncio, M. Francisella-like endosymbiont in *Dermacentor reticulatus* Collected in Portugal. *Vector Borne Zoonotic Dis.* **11**, 185–188. <https://doi.org/10.1089/vbz.2010.0014> (2011).
59. Buysse, M. et al. A dual endosymbiosis supports nutritional adaptation to hematophagy in the invasive tick *Hyalomma marginatum*. *eLife*. **10**, e72747 (2021). <https://doi.org/10.7554/eLife.72747>
60. De Sousa, K. C. M. et al. Diversity of piroplasmids among wild and domestic mammals and ectoparasites in Pantanal wetland, Brazil. *Ticks Tick. Borne Dis.* **9**, 245–253. <https://doi.org/10.1016/j.ttbdis.2017.09.010> (2018).
61. Remesar, S. et al. Prevalence of tick-borne pathogens in grey wolves (*Canis lupus*) in southwestern Europe. *Vet. Microbiol.* **307**, 110579. <https://doi.org/10.1016/j.vetmic.2025.110579> (2025).
62. González-García, V., Fernández-Pascual, E., Font, X. & Jiménez-Alfaro, B. Forest habitat diversity in the Cantabrian Mixed Forests ecoregion (NW Iberian Peninsula), a climatic refugium in western Europe. *Appl. Veg. Sci.* **27**, e12793. <https://doi.org/10.1111/avsc.12793> (2024).
63. Clavero, M., García-Reyes, A., Fernández-Gil, A., Revilla, E. & Fernández, N. Where wolves were: setting historical baselines for wolf recovery in Spain. *Anim. Conserv.* **26**, 239–249 <https://doi.org/10.1111/acv.12814> (2023).
64. Manilla, G. *Acari, Ixodida (Fauna d'Italia 36)* (Edizioni Calderoni, 1998).
65. Fracasso, G. et al. Effective methods of estimation of pathogen prevalence in pooled ticks. *Pathogens* **12**, 557. <https://doi.org/10.3390/pathogens12040557> (2023).

Figures

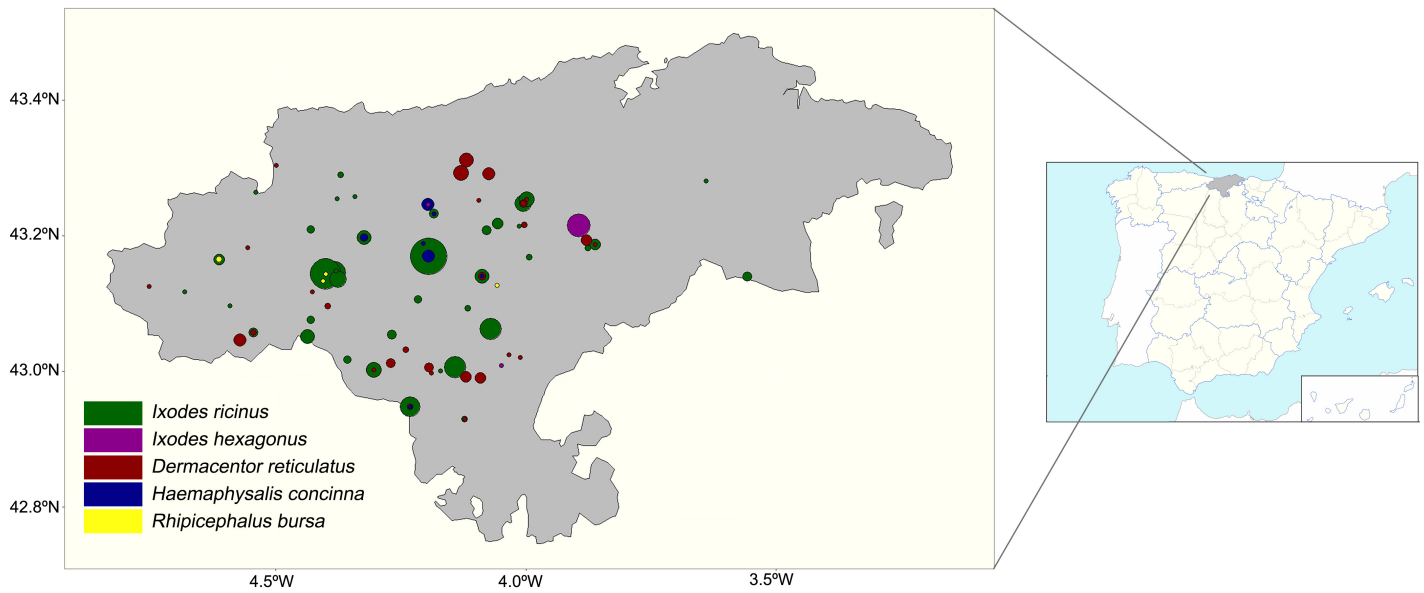


Figure 1

Map showing the study area (Cantabria) and the corresponding tick species collected from Iberian wolves (*Canis lupus signatus*) from 2017 to 2023. The circle size is proportional to the number of ticks collected from each species.

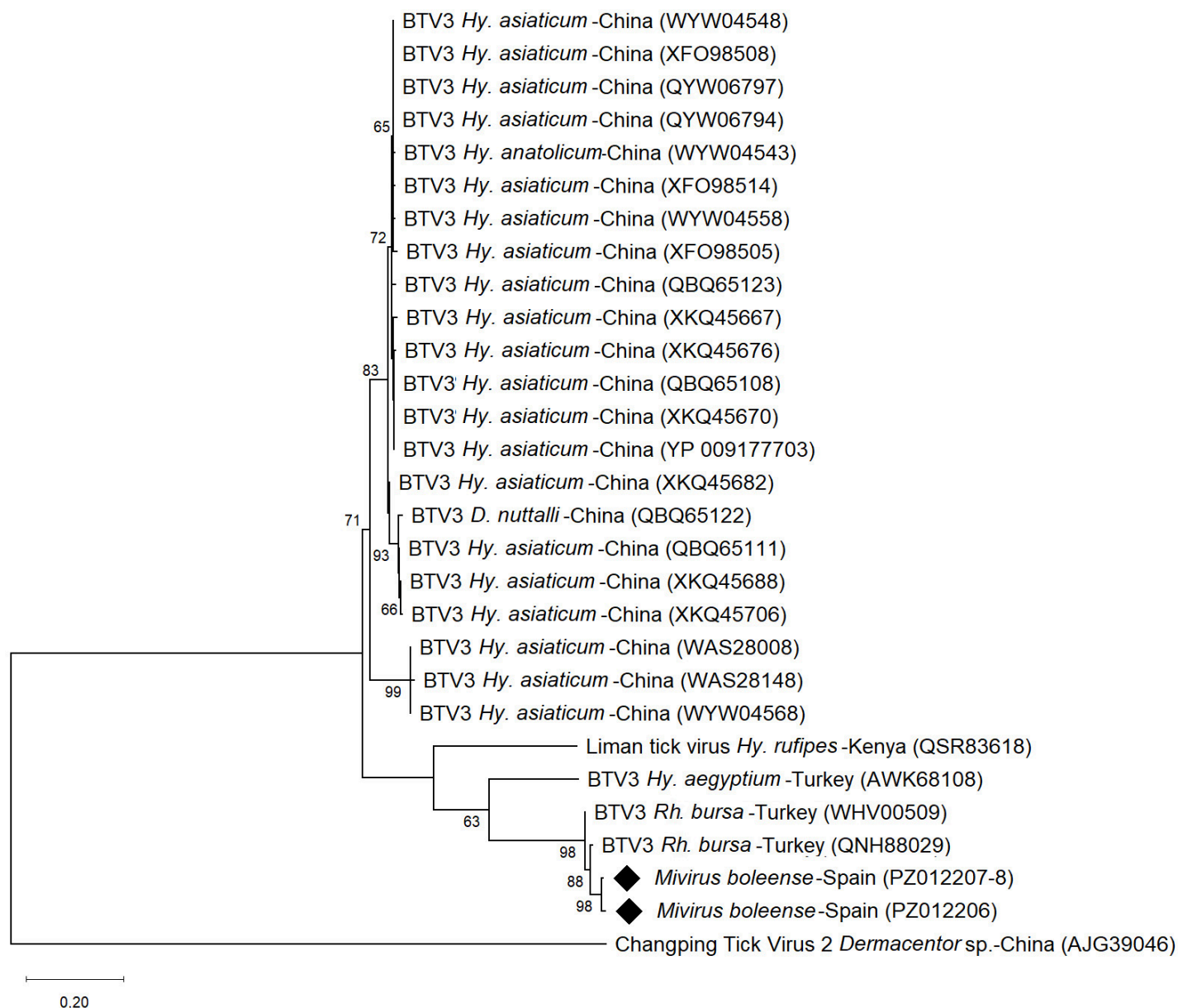


Figure 2

Unrooted dendrogram inferred using MEGA 12 showing the phylogenetic position of Bólè tick virus 3 (BTV3; *Mivirus boleense*, Chuviridae) detected in the present study (◆) in ticks removed from Iberian wolves (*Canis lupus signatus*) of Spain among other isolates and related viruses. Phylogeny was inferred from comparison of nucleoprotein amino acid sequences by the neighbor-joining method and Jones-Taylor-Thornton (500 replicates) with a discrete gamma distribution. This analysis involved 29 amino acid sequences and gaps and missing data were included in the analysis, with a total of 286 positions in the final dataset. GenBank accession numbers of the sequences used in the comparison are shown in brackets following each species and area in which they have been amplified. Bootstrap values <60% are not shown.

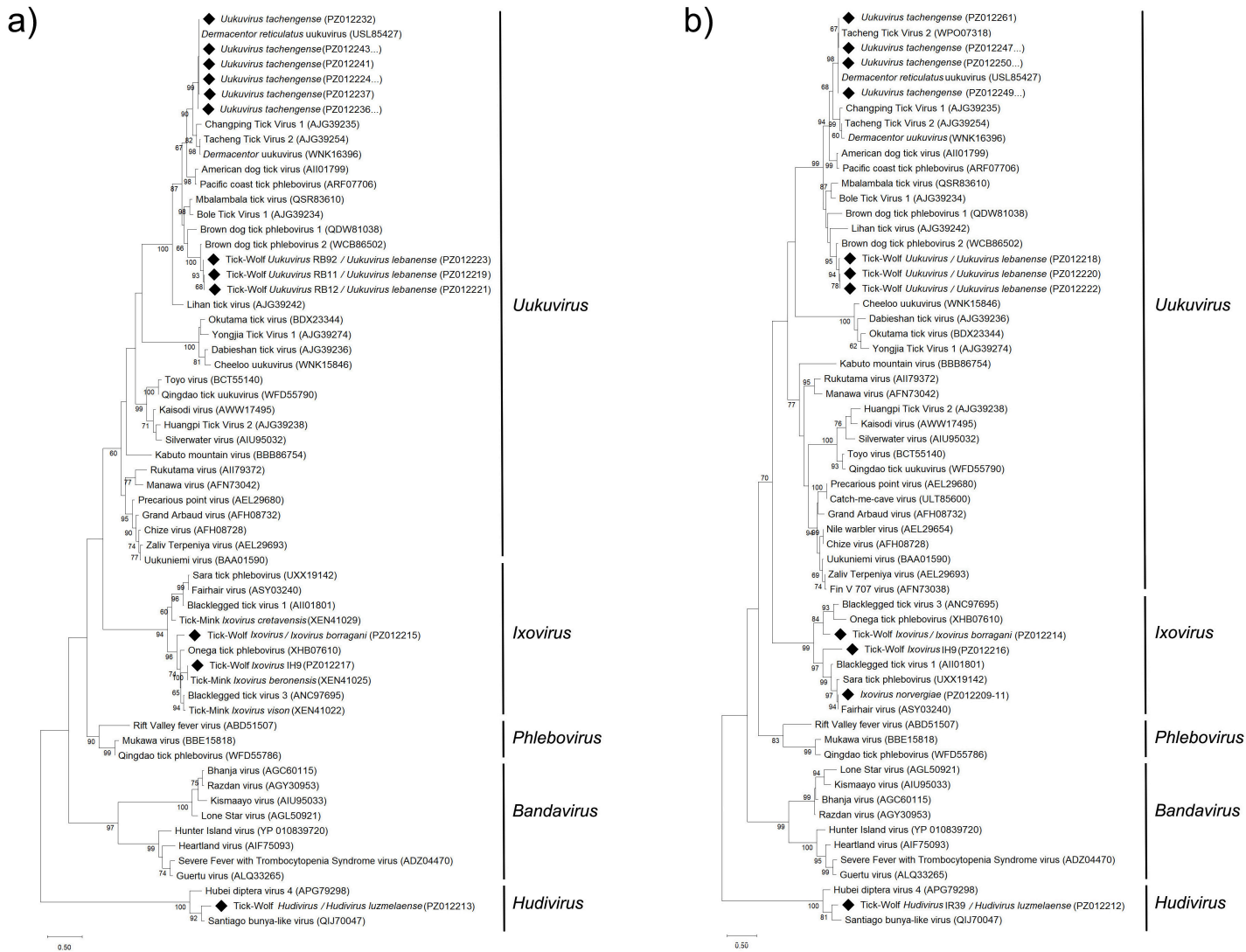


Figure 3

Unrooted dendrogram generated using MEGA 12 showing the phylogenetic position of phenuiviruses detected in the present study (◆) in ticks removed from Iberian wolves (*Canis lupus signatus*), together with other tick-associated phenuiviruses belonging to the genera *Uukuvirus*, *Ixovirus*, *Bandavirus* and *Phlebovirus* or related viruses of the genus *Hudivirus*. Phylogenetic relationships were inferred based on the comparison of partial amino acid sequences of the RNA-dependent RNA polymerase gene [L segment, a: Fragment 1; b: Fragment 2 (Supplementary Table 1)] using the neighbor-joining method and Le-Gascuel model (500 replicates). Evolutionary rate differences were modeled using a discrete gamma distribution across five categories, with <8.5% of sites deemed evolutionarily invariant. This analysis involved 61 amino acid sequences, as well as missing data were included in the analysis, with a total of 164 and 191 positions in the final dataset. GenBank accession numbers of the sequences used in the comparison are shown in parenthesis following each species and area in which they have been amplified. Bootstrap values <60% are not shown.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementarytablesSCFayos2026.pdf](#)