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Ticks and tick-borne diseases from Mallorca Island, Spain

Lidia Chitimia-Dobler[1,2], Michael Bröker[3], Silke Wölfel[4], Gerhard Dobler[1,5,6], Sabine Schaper[1], Katharina Müller[1], Anna Obiegala[7], Lara Maas[7], Ben J. Mans[8,9,10] and Heiner von Buttlar[1]

[1]Bundeswehr Institute of Microbiology, Munich, Germany; [2]Fraunhofer Institute of Immunology, Infection and Pandemic Research, Penzberg, Germany; [3]Global Health Press, Singapore/Marburg, Germany; [4]amedes MVZ for Laboratory Medicine and Microbiology, Fuerstenfeldbruck, Germany; [5]Department of Parasitology, Institute of Biology, University of Hohenheim, Stuttgart, Germany; [6]Dept. of Infectious Diseases and Tropical Medicine, Ludwig-Maximilians-University Munich, Munich, Germany; [7]Institute of Animal Hygiene and Veterinary Public Health, University of Leipzig, Leipzig, Germany; [8]Epidemiology, Parasites and Vectors, Agricultural Research Council-Onderstepoort Veterinary Research, Onderstepoort, South Africa; [9]Department of Life and Consumer Sciences, University of South Africa, Pretoria, South Africa and [10]Department of Zoology and Entomology, University of the Free State, Bloemfontein 9301, South Africa

Research Article

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Corresponding author:
Lidia Chitimia-Dobler;
Email: lydiachitimia@gmail.com

Abstract

Ixodid ticks are obligate blood-feeding arthropods and important vectors of pathogens. In Mallorca, almost no data on the tick fauna are available. Herein, we investigated ticks and tick-borne pathogens in ticks collected from dogs, a cat and humans in Mallorca as result of a citizen science project. A total of 91 ticks were received from German tourists and residents in Mallorca. Ticks were collected from March to October 2023 from dogs, cat and humans, morphologically and genetically identified and tested for pathogens by PCRs. Six tick species could be identified: *Ixodes ricinus* ($n = 2$), *Ixodes ventalloi* ($n = 1$), *Hyalomma lusitanicum* ($n = 7$), *Hyalomma marginatum* ($n = 1$), *Rhipicephalus sanguineus* s.l. ($n = 71$) and *Rhipicephalus pusillus* ($n = 9$). *Rhipicephalus sanguineus* s.l. adults were collected from dogs and four females from a cat and the 16S rDNA sequences identified it as *Rh. sanguineus* s.s. *Hyalomma lusitanicum* was collected from 1 human, 1 dog and 5 specimens were collected from the ground in the community of Santanyi, together with one *H. marginatum* male. This is the first report of *Hyalomma marginatum* in Mallorca. Both *I. ricinus* were collected from humans and *I. ventalloi* female was collected from a dog. All ticks tested negative for *Anaplasma phagocytophilum*, *Coxiella* spp., *Francisella* spp., and piroplasms. In 32/71 (45%) specimens of *Rh. sanguineus* s.s., *Rickettsia* spp. could be detected and in 18/32 (56.2%) sequenced tick DNAs *R. massiliae* was identified. *Ixodes ventalloi* female and both *I. ricinus* tested positive in the screening PCR, but the sequencing for the identification of the *Rickettsia* sp. failed.

Introduction

The Balearic Mallorca Island, belonging to Spain, is a popular holiday destination with more than 17 million tourists in 2023, of which about 5 million tourists are Germans. Touristic and public life has adapted to German speaking tourists (Germany, Austria, Switzerland), and nowadays, about 20 000 Germans are permanent residents with more than 60 000 Germans spending at least 3 months per year in Mallorca [Spanish Statistical Office (ine.es)].

Ixodid ticks are important obligate blood-feeding arthropod vectors of pathogens, and human parasitism by these ticks is a common event in the world (Sonenshine *et al*., 2002). In the family Ixodidae, there are currently 762 recognized species, divided into 2 groups, the Prostriata and the Metastriata, with 15 extant genera and 2 extinct genera (Guglielmone *et al*., 2023). In Europe, ixodid tick species belong to 5 genera: *Ixodes* (Prostriata) as well as *Dermacentor*, *Haemaphysalis*, *Hyalomma* and *Rhipicephalus* (Metastriata) (Nowak-Chmura, 2013). Ticks are hosting a large variety of microorganisms in their microbiome, among them pathogens, like rickettsiae and microorganisms, which form a crucial element in the various physiological processes as nutrition, development, reproduction, vector capacity and immunity (Stich *et al*., 2008; Bonnet *et al*., 2017). Certain microorganisms serve as endosymbionts, which may be essential or facultative for tick physiology (*Francisella*-like and *Coxiella*-like endosymbionts). Other microorganisms may be harmful pathogens for vertebrates, like *Francisella tularensis* and *Coxiella burnetii*, although they have not been identified to cause disease in their tick vectors. Different factors have influence on the tick microbiota composition, such as tick species, life stage and environment (Ponnusamy *et al*., 2014; Van Treuren *et al*., 2015; Aivelo *et al*., 2019).

While on the Spanish mainland 22 ixodid tick species are known (Guglielmone *et al*., 2023), data on the tick fauna on the Spanish islands are much less known. Guglielmone *et al*. (2023) did not include Mallorca as a separate entity in the last list of ixodid ticks in the world. Recently, a study reported 12 tick species in Mallorca (Monerris Mascaro and

del Mar Colom Noguera, 2020). However, the recent study did not investigate the potential pathogens in Mallorcan ticks. Therefore, the aim of the current study was to investigate tick fauna and tick-borne pathogens in the ticks collected from dogs, a cat and humans in Mallorca.

Materials and methods

Ticks were collected based on a citizen science project. One of the authors (M.B.) released a call, based on an interview article published in a German speaking Mallorca journal (www.mallorcazeitung.es) in August 2021. Tourists and residents were asked to send in any ticks they could find or collect in Mallorca. Along with the ticks, citizens were asked to provide information on the date and location of collection, and the host. To enhance participant engagement, citizens received feedback and were informed about the tick species that they had submitted and which pathogens the respective ticks carried. Ticks were received at irregular intervals, from March to October 2023. All data on collected ticks and their respective information are summarized in Table 1 and shown on a map (Fig. 1).

Ticks were identified based on morphological identification keys (Walker *et al.*, 2000; Pérez-Eid, 2007; Nava *et al.*, 2018), using a Keyence VHX-900F microscope (Itasca, IL, USA). DNA was extracted from individual ticks using the QIAamp Mini DNA extraction kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The 16S rRNA gene of ticks was amplified according to Halos *et al.* (2004), visualized in 1.5% agarose gel, purified using QIAquick® PCR Purification Kit (250) (Qiagen, Hilden, Germany), and subsequently bi-directionally sequenced, consensus sequences derived and submitted to GenBank. Additionally, DNA was analysed using pan-*Rickettsia* real-time PCR to amplify part of the *gltA* gene (Wölfel *et al.*, 2008), followed by PCR amplification of 23S-5S intergenic spacer region (Chitimia-Dobler *et al.*, 2018), *ompB* (Roux and Raoult, 2000), and *gltA* (Nilsson *et al.*, 1999), followed by Sanger sequencing to identify the *Rickettsia* species. Furthermore, the extracted tick DNA was analysed for the presence of *Francisella* spp. and *Francisella*-like endosymbionts (Gehringer *et al.*, 2013) using LightMix® *F. tularensis* 16S rRNA gene according to the manufacturer's instructions (TibMolBiol, Berlin, Germany). To detect genomic DNA from *Coxiella burnetii* and *Coxiella*-like endosymbionts the method described by Frangoulidis *et al.* (2021) was applied. The screening for *Anaplasma phagocytophilum* was performed with real-time PCR using a protocol by Courtney *et al.* (2004). A conventional PCR targeting a fragment (411–452 bp) of the 18S rRNA of piroplasms was performed using a protocol by Casati *et al.* (2006). All PCRs included a positive control (Table 2) and purified water as negative control. Moreover, *Francisella* specific PCR includes an internal control to rule out PCR inhibition by sample ingredients. Table 2 summarizes the information on PCR methods.

For ticks, the 16S rDNA sequences were screened with BLASTn analysis (Altschul *et al.*, 1990) and representative related sequences downloaded from GenBank (https://www.ncbi.nlm.nih.gov/nucleotide). Sequences were aligned using the online version of MAFFT (Katoh and Standley, 2013) with default parameters and maximum likelihood analyses performed with IQ-Tree2 v1.6.12 (Minh *et al.*, 2020). The optimal substitution model used was TIM2 + F + G4 and 10 000 bootstraps were performed to obtain nodal support values. The tree was rooted with *Ixodes* species.

The phylogenetical analysis of the *Rickettsia*-positive specimens subsequent to Sanger sequencing was conducted by an external contractor (Eurofins, Germany). Sequences were analysed using BioEdit Alignment Editor Version 7.1.1 (Hall, 1999) and compared with sequences deposited in the GenBank database of the National Centre for Biotechnology Information (NCBI) using the Basic Local Alignment Search Tool (BLAST) (Altschul *et al.*, 1990). Maximum likelihood analysis was performed in MEGA v7.0.14 (Kumar *et al.*, 2016) based on the

Table 1. Primers and probes used for molecular investigation of tick species and their pathogens

Date	Location	Host	Tick species	Number of life stage[a]			Total
				Female	Male	nymph	
23.3.2023	Ses Salines	Cat	*Rhipicephalus sanguineus* s.s.	3	–	–	3
2.4.2023	Es Llombards	Dog	*Ixodes ventalloi*	1(1)[b]	–	–	1
			Rhipicephalus sanguineus s.s.	5(1)[c]	3	–	8
			Rhipicephalus pusillus	3	3	–	6
18.4.2023	S'Illot	Human	*Ixodes ricinus*	1(1)[b]	–	–	1
24.4.2023	Cala Major	Human	*Hyalomma lusitanicum*	1	–	–	1
5.5.2023	Ses Salines	Cat	*Rhipicephalus sanguineus* s.s.	1	–	–	1
8.5.2023	S'Illot	Dog	*Hyalomma lusitanicum*	1	–	–	1
20.5.2023	Es Llombards	Dog	*Rhipicephalus sanguineus* s.s.	26(12)[c]	28(5)[c]	–	54
			Rhipicephalus pusillus	2	1	–	3
26.6.2023	Pina	Cat	*Rhipicephalus sanguineus* s.s.	2(2)[b]	–	–	2
26.6.2023	Es Llombards	Dog	*Rhipicephalus sanguineus* s.s.	2	1	–	3
5.8.2023	Palmanova	Human	*Ixodes ricinus*	–	–	1(1)[b]	1
8.10.2023	Santanyi	Ground	*Hyalomma marginatum*	–	1	–	1
			Hyalomma lusitanicum	2	3	–	5
				50	40	1	**91**

[a]In brackets are number of positive samples.
[b]*Rickettsia* sp., as could not be sequence due to low amount of DNA.
[c]*Rickettsia massilliae* was identified after sequencing.

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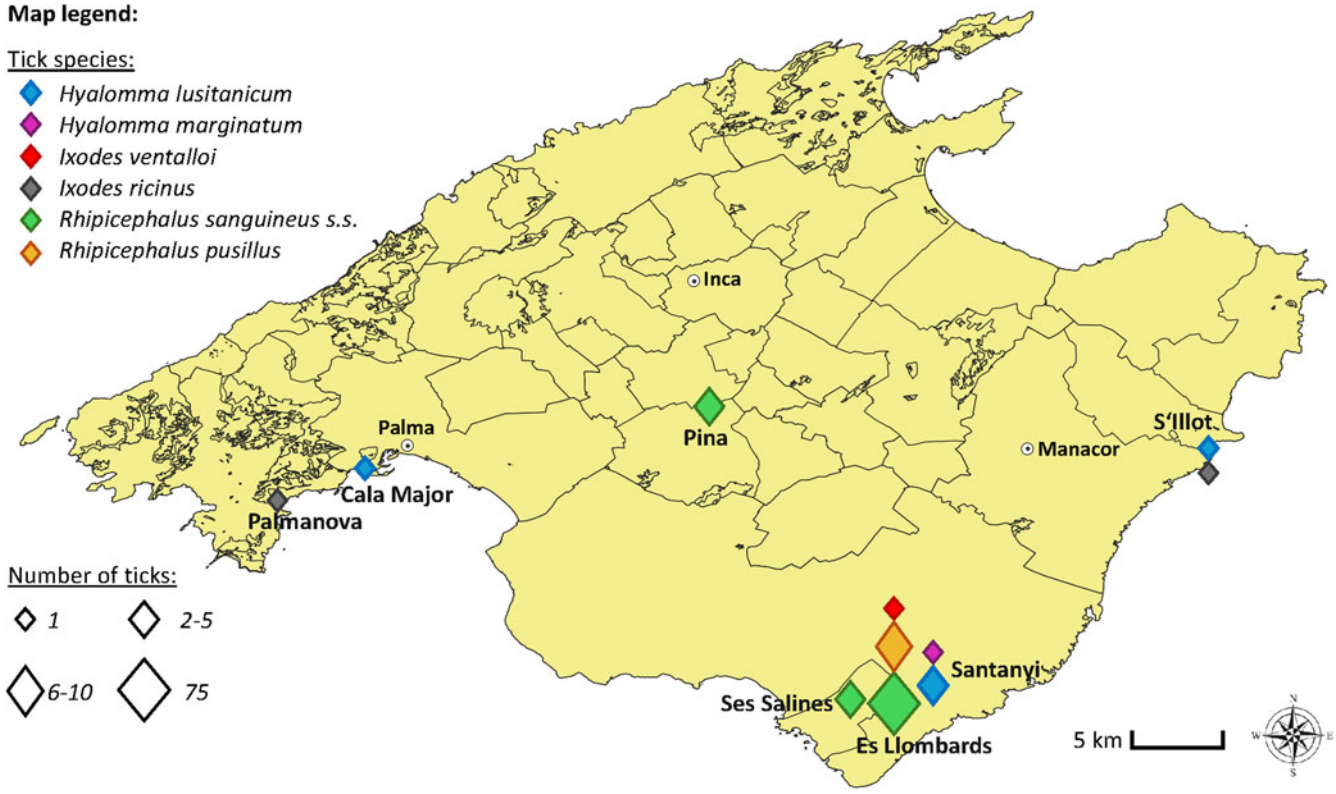

Figure 1. Map of the collection places created using QGis Version 3.34 Prizren, scale 1:220.000.

Tamura-Nei model (Tamura and Nei, 1993) with 1000 bootstraps.

Results

A total of 91 ticks were received from German tourists and residents in Mallorca. Six tick species could be identified: *Ixodes ricinus* ($n = 2$), *Ixodes ventalloi* ($n = 1$), *Hyalomma lusitanicum* ($n = 7$), *Hyalomma marginatum* ($n = 1$), *Rhipicephalus sanguineus* s.l. ($n = 71$) and *Rhipicephalus pusillus* ($n = 9$). Detailed information on the studied tick specimen presented in Table 1 and Fig. 1.

Sixty-seven specimens of *Rhipicephalus sanguineus* s.l., a three-host tick, were collected from dogs and 4 female specimens from a cat. All *Rhipicephalus*-ticks were adults (32 males and 39 females). The 16S rDNA sequences identified the species as *Rh. sanguineus* s.s. (temperate lineage, (accession numbers: PP227945–PP228018) (Fig. 2). All *Rh. pusillus* adults (4 males and 5 females) were collected from dogs, together with *Rh. sanguineus* s.s. Seven out of the 9 *Rh. pusillus* were confirmed amplifying the 16S rRNA gene and the sequences were submitted to GenBank (accession numbers: PP478783–PP478790), *Hyalomma lusitanicum* was collected from 1 human, 1 dog and 5 specimens were collected from the ground, together with a *H. marginatum* male in the community of Santanyi. Both *I. ricinus* (three-host tick, female and nymph) were collected from humans. *Ixodes ventalloi* female was found on a dog, together with *Rh. sanguineus* s.s.

All ticks tested negative for *Anaplasma phagocytophilum, Coxiella* spp., *Francisella* spp., and piroplasms (*Babesia*, *Theileria*, *Cytauxoon* spp.).

In 32 of 71 (45%) specimens of *Rh. sanguineus* s.s., *Rickettsia* spp. could be detected. However, only 18/32 (56.2%) PCR-positive *Rickettsia* spp. contained sufficient amount of DNA to enable sequencing and subsequent sequence analysis (Table 1). In all sequenced specimens *R. massiliae* was identified. The phylogenetic analysis of *R. massiliae* 23S-5S intergenic spacer region is shown in Fig. 3. All sequences were submitted in GenBank as follow: 23S-5S intergenic spacer region (accession numbers: PP263040–PP263054), *ompB* (accession numbers: PP263035–P263036), and *gltA* (accession numbers: PP263037–PP263039). The *I. ventalloi* female and the both *I. ricinus* tested positive in the *Rickettsia* screening PCR, but the DNA sequencing failed, precluding the identification of the *Rickettsia* sp. In total, DNA sequencing was unsuccessful for 14/32 (43.7%) samples due to the low amount of specific DNA.

Discussion

The Balearian Island of Mallorca is a main destiny of tourism. In 2023, a new record was observed with more than 12 million tourists visiting the island (http://www.mallorcamagazin.com/nachrichten/tourismus/2023/10/09/115575). The island in the Mediterranean Sea lies within the area of distribution of tick species with known major importance as vectors, e.g., *Rh. sanguineus* s.l., and pathogens of medical and veterinary importance, e.g., Mediterranean Spotted Fever and Crimean-Congo Haemorrhagic Fever. While several studies are available on ticks and tick-borne pathogens on the Spanish mainland, no data are available on the occurrence and prevalence of these pathogens in Mallorca. Also, since the discovery, that *Rh. sanguineus* s.l. as a complex of 3 species, no studies were conducted to clarify which of the 3 species might be present on the Balearian Islands and specifically on the Island of Mallorca. This knowledge about the tick fauna might be of importance for diagnostics and treatment of illnesses transmitted by ticks on the island.

Microclimatic conditions influence the tick species activity, abundance and survival (Bertrand and Wilson, 1996; Rynkiewicz and Clay, 2014). Ticks spend 90% of their life off-host in the environment where they quest for a suitable host and moult between life stages (Anderson, 2002), except for the one-host tick species which spends 90% on their host. During the last years, the

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Table 2. Overview of the collection dates and places of the different collected tick species and the number of Rickettia spp. positive specimens

Gene	Organism	Primers	Positive controls	Fragment length (bp)	PCR type	Reference
16S rRNA	Tick species	TQ16S + F1 5`-CTGCTCAATGATTTTTTAAATTGCTGTGG TQ-16S-2R 5`-ACGCTGTTATCCCTAGAG	No	320 bp	Conventional	Halos *et al.* (2004)
*glt*A	*Rickettsia*	RH314: 5′-AAACAGGTTGCTCATCATTC-3′ RH654: 5′-AGAGCATTTTTTATTATTGG-3′	*Rickettsia helvetica* AS 819 from cell culture	Not aplicable	Real time PCR	Wölfel *et al.* (2008)
23S-5S intergenic spacer region	*Rickettsia*	23S for (5′ -GATAGGTCGGGTGTGGAAGCAC-3′) 23S rev (5′-GGGATGGGATCGTGTGTTTCAC-3′)	*Rickettsia helvetica* AS 819 from cell culture	378–532 bp	Conventional	Chitimia-Dobler *et al.* (2018)
*glt*A	*Rickettsia*	RH314: 5′ -AAACAGGTTGCTCATCATTC-3′ RH654: 5′ -AGAGCATTTTTTATTATTGG-3′	*Rickettsia helvetica* AS 819 from cell culture	340 bp	Conventional	Nilsson *et al.* (1999)
*omp*B	*Rickettsia*	120-2788: 5′ -AAACAATAATCAAGGTACTGT-3′ 120-3599: 5′ -TACTTCCGGTTACAGCAAAGT-3′	*Rickettsia helvetica* AS 819 from cell culture	800 bp	Conventional	Roux and Raoult (2000)
16S rRNA	*Francisella* spp./ *Francisella*-like endosymbionts	Commercial Test Kit *Francisella* 16S (Tib-MolBiol, Berlin, Germany)	Positive control and internal control to rule out PCR inhibition included in the commercial test kit	Not aplicable	Real time PCR	Gehringer *et al.* (2013)
IS1111	*Coxiella burnetii* and *Coxiella*-like endosymbionts	Coxb_S: 5′ -GATAGCCCGATAAGCATCAAC; Coxb_A: 5′ -GCATTCGTATATCCGGCATC; Coxb_P: 5′ -6FAM-TCATCAAGGCACCAATGGTGGCCA-BBQ	Synthetic in-house positive control deduced from *C. burnetii* DNA	Not aplicable	Real time PCR	Frangoulidis *et al.* (2021)
Msp2	*Anaplasma phagocytophilum*	ApMSP2_ f : 5′-ATGGAAGGTAGTGTTGGTTATGGTATT-3′ ApMSP2_r: 5′-TTGGTCTTGAAGCGCTCGTA-3′ ApMSP2_p: FAM-TGGTGCCAGGGTTGAGCTTGAGATTG-BHQ1	*Anaplasma phagocytophilum* DNA from cell culture	77 bp	Real time PCR	Courtney *et al.* (2004)
18S rRNA	Piroplasms	BJ1: 5′-GTCTTGTAATTGGAATGATGG-3′ BN2: 5′-TAGTTTATGGTTAGGACTACG-3′	*Babesia microti*-DNA from positive *Clethrionomys glareolus*	411–452 bp	Conventional	Casati *et al.* (2006)

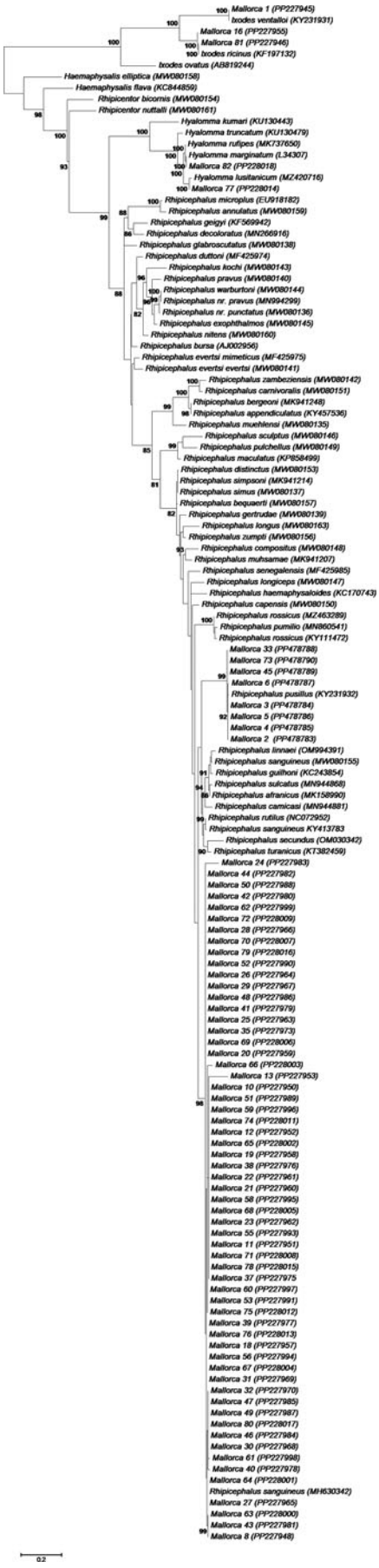

Figure 2. Phylogenetic analysis of the 16S rDNA sequences of ticks from Mallorca. The species name and accession numbers are indicated.

increasing expansion of the distribution of ticks and tick-borne diseases due to climatic changes have been observed (Gray and Ogden, 2021; Semenza and Paz, 2021; Semenza *et al.*, 2022). Studies as presented here are therefore also an important data basis for monitoring and surveillance of future developments in tick dispersal and expansion of distribution.

In this study, 91 ticks representing 6 species of hard ticks were collected by German tourists and residents living in Mallorca mainly from dogs, a cat, humans and from the ground. All hard tick species, except *H. marginatum*, found in this study had also been described by Monerris Mascaro and del Mar Colom Noguera (2020), who reported 12 tick species among more than 2000 ticks collected from sheep, wildlife and from vegetation by flagging in Mallorca. In this study on ticks in Mallorca, however, the presence of ticks on pet animals was not examined. Also, no exact differentiation of *Rh. sanguineus* s.l. was conducted. However, this differentiation becomes more and more important, as studies now have shown that the 3 accepted extant species of this tick species complex exhibit differing vector capacities for pathogens, e.g., rickettsiae (Chitimia-Dobler *et al.*, 2019*a*, 2019*b*).

In the study of Monerris Mascaro and del Mar Colom Noguera (2020), *Rh. turanicus* was reported in Mallorca. Another study found *Rh. turanicus* also in Menorca (Castella *et al.*, 2001). More recent genetic studies from the Canary Islands and from mainland of Spain and Portugal are not in agreement with these former data, as only *Rh sanguineus* s.s. has been identified in these studies (Nava *et al.*, 2018; Chitimia-Dobler *et al.*, 2019*a*, 2019*b*). There, *Rh. turanicus* could not be identified to confirm the old reports, but all investigated ticks were determined as *Rh. sanguineus* s.s. in analogy to *Rh. sanguineus* s.l. There is now a new classification of *Rh. turanicus* s.l., such as *Rh. turanicus* s.s., *Rh. afranicus* (Bakkes *et al.*, 2020), and the more recently described *Rh. secundus* (Mumcuoglu *et al.*, 2022). 1The possible presence of *Rh. turanicus* in Mallorca should be reconsidered and confirmed by further investigations. In the current study only *Rh. sanguineus* s.s. was found in Mallorca. *Rhipicephalus sanguineus* s.s. has a large distribution in Europe (including Canary Islands), U.S.A., parts of South America (Nava *et al.*, 2018; Chitimia-Dobler *et al.*, 2019*a*, 2019*b*), and in some regions in Algeria (Laatamna *et al.*, 2020). The Algerian study (Laatamna *et al.*, 2020) detected a large spectrum of pathogens in *Rh. sanguineus* s.s., such as *Hepatozoon canis, Babesia vogeli. Anaplasma platys, Ehrlichia canis*, *R. massiliae* and *Rickettsia conorii conorii*. In this study in Mallorca, only *R. massiliae* was detected. This might indicate, that *R. massiliae* predominantly circulates in Mallorca rather than *R. conorii*. One resident, who sent ticks collected from one of her cats, reported a history of a severe clinical rickettsiosis with long-term sequelae after a tick bite on her neck. Although *R. conorii* IgM was detected by laboratory diagnostics it cannot be ruled out that the causative agent was *R. massiliae*, as serological cross-reaction among SFG rickettsiae are common and a diagnostic differentiation between *R. conorii* and *R. massiliae* infections is difficult (Hechemy *et al.*, 1989; Raoult and Paddock, 2005). *Rhipicephalus sanguineus* s.l. feeds frequently on humans, especially in the adult stage (Guglielmone and Robbins, 2018). None of the four *Rh. sanguineus* s.s. collected from the cat tested positive for *R. massiliae*.

Rhipicephalus pusillus ticks are commonly found in southern Europe (Portugal, Spain and France) and northern Africa (Tunisia and Morocco). It is presumed a 3-host tick and has European rabbit as primary host, but has been reported from other hosts (Walker *et al.*, 2000). This tick species is also considered exclusively endophilic (Osácar, 1992 cited in Estrada-Peña

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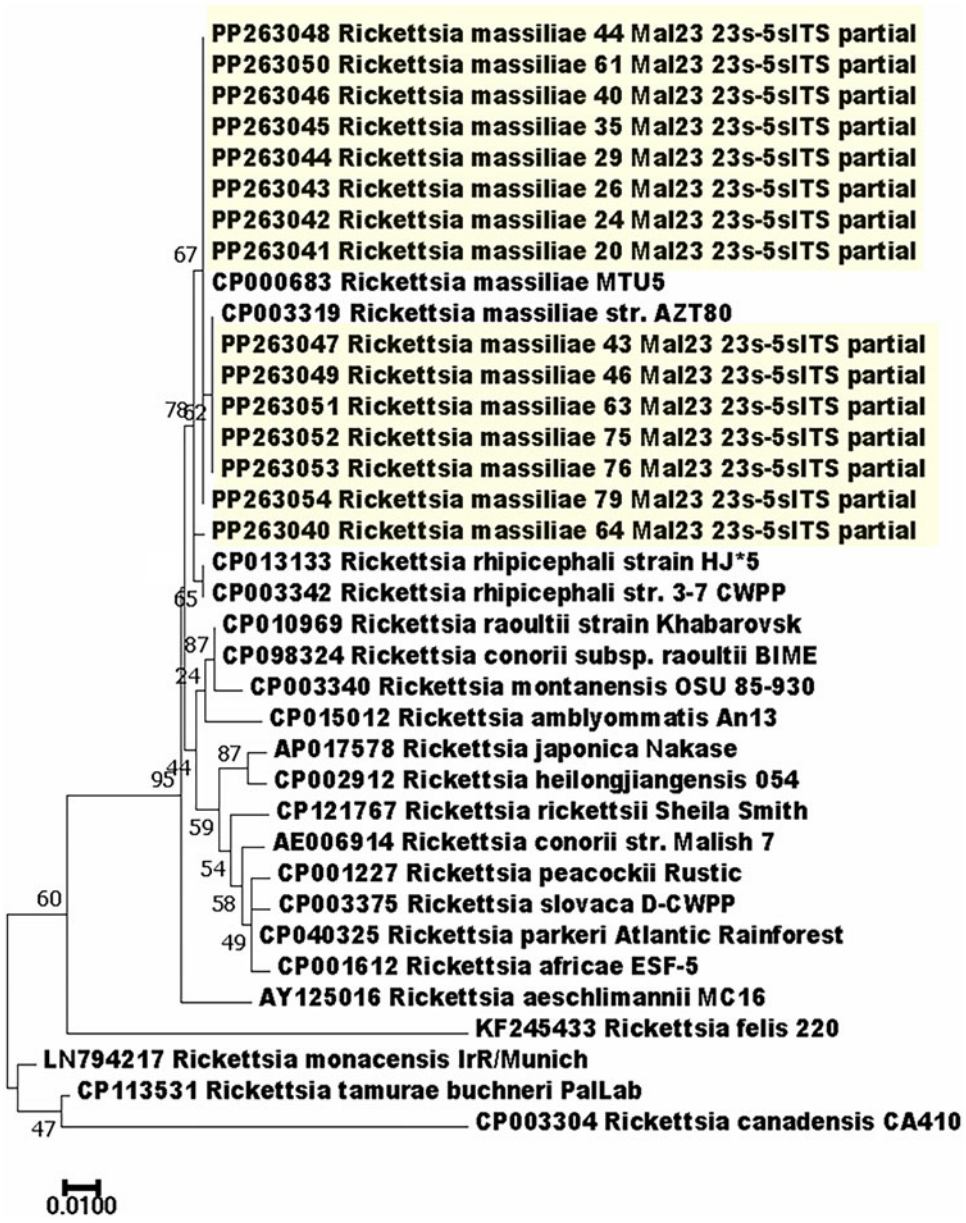

Figure 3. Phylogenetic analysis of the 23S-5S intergenic spacer region of *Rickettsia massiliae* in Mallorca.

et al., 2018) rarely parasitizing humans (Guglielmone and Robbins, 2018). *Rickettsia massiliae* was first isolated in 1992 from *Rh. sanguineus* ticks collected near Marseille, France (Beati and Raoult, 1993). *Rickettsia massiliae* has been identified in southern Spain (Marquez, 2008) and in the Canary Islands (Fernández de Mera *et al*., 2009), but not in Mallorca, so far. *Rhipicephalus pusillus* is considered vector of *R. massiliae* and the primary hosts are rabbits and hares. In Europe, *Lepus europaeus* (European hare) and rabbits are reservoirs of *Rickettsia conorii*, *Rickettsia slovaca*, *C. burnetiid* and *Francisella tularensis holarctica* (Rehácek *et al*., 1978; Pérez Castrillón *et al*., 2001; Fernández de Mera *et al*., 2009; Eremeeva and Dasch, 2015). All *Rh. pusillus* ($n = 9$) collected from dogs in Mallorca tested negative for *Rickettsia* spp. and *Coxiella* spp. Considering the result that almost 50% of the *Rh. sanguineus* s.s. ticks carried *R. massiliae* could be interpreted such that *Rh. pusillus* is not playing an important role for *R. massiliae* in Mallorca or at all.

Hyalomma lusitanicum is probably the most abundant exophilic tick species in the central and southern part of the Iberian Peninsula, but also in other European countries (France, Italy) and North Africa (Algeria and Morocco) (Válcarel *et al*., 2020). Hornok *et al*. (2020) reported this species from Malta, collected from rabbits and cats, which is supported by a personal report of a resident in Malta to one author (LCD), who collected many *H. lusitanicum* adults from the ground in his garden and sent them for identification and further analyses. *Hyalomma lusitanicum* is a 3-host tick species, immatures are endo- and exophilic, while adults are exophilic. Wild rabbits and hares are considered as the main hosts and many other wild and domestic animals as secondary hosts. It can sometimes also be found on humans, but humans are not the preferred host and thus, it is only a sporadic parasite of humans (Guglielmone and Robbins, 2018; Válcarel *et al*., 2020). On the other hand, it has been reported that attachments to humans have increased in recent years, and more frequent human infestation has been reported in Portugal (Valcárcel *et al*., 2023). One *H. lusitanicum* female was found attached to a human head, which is the first report of a human *H. lusitanicum* infestation in Mallorca. The patient did not develop any disease, but a local reaction to the bite on her head was visible for more than a week. In the present study, all *H. lusitanicum* ticks tested negative for any of the investigated pathogens. However, *H. lusitanicum* is a known vector for *C.*

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burnetii, Theileria equi and *Theileria annulata*. It may be involved in the cycle of other pathogens such as Crimean-Congo Haemorrhagic Fever (CCHF) virus, *A. phagocytophilum*, *F. tularensis* and *R. aeschlimannii* (Válcarel *et al.*, 2020).

Hyalomma marginatum is a 2-host tick species. It has a large distribution in North Africa, Asia and many European countries including Spain (Válcarel *et al.*, 2020). According to the ECDC map, *H. marginatum* has not been observed in Mallorca hitherto, but on the other neighbouring small islands (ECDC, 2023). In the present study, a male was collected from the ground together with 5 specimens of *H. lusitanicum*. It is important to know the geographical distribution and potential introduction of *H. marginatum* into new areas, concerning its vector competence for CCHF virus (Válcarel *et al.*, 2020) and *R. aeschlimannii* (Beati *et al.* (1997). The found male tested negative for all investigated pathogens. Nevertheless, the occurrence of *H. marginatum* must be considered a risk for public and animal health and should be monitored closely. Migratory birds play an important role in the epizootiology and epidemiology of ticks and tick-borne pathogens and have received increased attention in recent years (Chitimia-Dobler *et al.*, 2019*a*, 2019*b*; Grandi *et al.*, 2020). One prominent example is the introduction of *H. marginatum* and *H. rufipes* into Germany and the fact that 50% of the specimens carried *R. aeschlimannii* (Chitimia-Dobler *et al.*, 2019*a*, 2019*b*).

Two *I. ricinus* (a female and a nymph) were removed from humans. This tick species is very common parasite of humans, despite not being specifically reported from humans in Mallorca (Guglielmone and Robbins, 2018). Both *I. ricinus* tested positive for *Rickettsia* spp., however, the species identification was not successful. *Ixodes ricinus* is both vector and reservoir for 2 *Rickettsia* species from the Spotted Fever Group, *Rickettsia helvetica* and *Rickettsia monacensis* (Simser *et al.*, 2002; Parola *et al.*, 2013). Interestingly, the research of Maitre *et al.* (2022) showed that a *R. helvetica* infection in *I. ricinus* reduces significantly the diversity of the microbiota and the connectivity of the co-occurrence network.

In this study we report for the first time *I. ventalloi* feeding on a dog. The *I. ventalloi* female was collected feeding at the same time from that respective dog together with 8 *Rh. sanguineus* s.s. (3 males and 5 females). *Ixodes ventalloi* has been already reported from Spain (including Mallorca), Portugal, southern part of France and Italy, Cyprus and North Africa. Lagomorphs, carnivores, and rodents are hosts for all life stages (Estrada-Peña *et al.*, 2018). In the study of Estrada-Peña *et al.* (2018) the carnivores from which this tick species was collected are listed in detail, but it was never found on dogs so far. Additionally, to the mentioned birds in the study of Estrada-Peña *et al.* (2018) *I. ventalloi* nymphs were found on European robin (*Erithacus rubecula*) and Black redstart (*Phoenicurus ochruros*) in Ponza, Italy (Rollins *et al.*, 2021). A summary of *I. ventalloi* collections from different hosts was done by Santos and Santos-Silva (2018), which also outlined the fact that this species can be collected by dragging over the grassy ground. In our study the *Rickettsia* screening PCR was positive, but subsequent identification *via* sequencing failed due to the low amount of DNA (CT 36.9). *Ixodes ventalloi* was collected from a dog, which was concomitantly infested with 8 *Rh. sanguineus* s.s. adults. Only *I. ventalloi* and 1 *Rh. sanguineus* female tested positive for *Rickettsia* spp., which could be identified as *R. massiliae* only in *Rh. sanguineus* s.s.. This finding could indicate that the dog was not the source of infection, but the ticks had already acquired the infection during the larva or nymph stages. Several pathogens, including *C. burnetii*, *Rickettsia* spp., *Anaplasma* spp. and *Borrelia* spp. or protozoa, were detected in *I. ventalloi* collected from different animals, humans or from vegetation (Santos and Santos-Silva, 2018).

Conclusion

Mallorca Island is a main tourist destination in the Mediterranean. The presence of *R. massiliae* on the island constitutes a risk for human infection and should be considered in clinical diagnostics. Also, the detection of *H. marginatum* poses a potential public health risk and the occurrence and distribution as well as the carrier status for certain pathogens, especially CCHF virus should be monitored closely. The detection of unusual tick species, e.g., *H. lusitanicum*, infesting humans shows that under specific conditions rare tick species may infest humans and therefore, also may serve as vectors of unusual pathogens to humans. The results emphasize specific risks associated with ticks and tick-borne pathogens on the Island of Mallorca and appeal for more intensive surveillance, and also for intensified vector control on pets.

Data availability. All the sequences were submitted in GenBank and are available for further studies.

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Author contributions. LCD identified the tick species and wrote the manuscript; LCD, GD and SS tested the ticks for *Rickettsia* including species identification, MB organized tick collection and wrote the first draft of the manuscript. HB tested the ticks for *Francisella* spp; KM tested the ticks for *Coxiella burnetii*; AO tested the ticks for *Babesia* and *Anaplasma* species. LM made the map. SW did the *Rickettsia* phylogenetic analysis; BJM did the tick phylogenetic analysis and submitted the sequences in GenBank. All authors read and approved the final version of the manuscript.

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References

Aivelo T, Norberg A and Tschirren B (2019) Bacterial microbiota composition of *Ixodes ricinus* ticks: the role of environmental variation, tick characteristics and microbial interactions. *PeerJ* **7**, e8217.

Altschul SF, Gish W, Miller W, Myers EW and Lipman DJ (1990) Basic local alignment search tool. *Journal of Molecular Biology* **215**, 403–410.

Anderson JF (2002) The natural history of ticks. *Medical Clinics of North America* **86**, 205–218.

Bakkes DK, Chitimia-Dobler L, Matloa D, Oosthuysen M, Mumcuoglu KY, Mans BJ and Matthee CA (2020) Integrative taxonomy and species delimitation of *Rhipicephalus turanicus* (Acari: Ixodida: Ixodidae). *International Journal of Parasitology* **50**, 577–594.

Beati L and Raoult L (1993) *Rickettsiae massiliae* sp. *nov*., a new spotted fever group rickettsia. *International Journal Systematic Bacteriology* **43**, 839–840.

Beati L, Meskini M, Thiers B and Raoult D (1997) *Rickettsia aeschlimannii* sp. nov., a new spotted fever group rickettsia associated with *Hyalomma marginatum* ticks. *International Journal of Systematic Bacteriology* **47**, 548–554.

Bertrand MR and Wilson ML (1996) Microclimate-dependent survival of unfed adult *Ixodes scapularis* (Acari:Ixodidae) in nature: life cycle and study design implications. *Journal of Medical Entomology* **33**, 619–627.

Bonnet SI, Binetruy F, Hernández-Jarguín AM and Duron O (2017) The tick microbiome: why non-pathogenic microorganisms matter in tick biology and pathogen transmission. *Frontiers in Cellular and Infection Microbiology* **7**, 236.

Casati S, Sager H, Gern L and Piffaretti JC (2006) Presence of potentially pathogenic *Babesia* sp. for human in *Ixodes ricinus* in Switzerland. *Annals of Agricultural and Environmental Medicine* **13**, 65–70.

https://doi.org/10.1017/S0031182024000544 Published online by Cambridge University Press

Castellà J, Estrada-Peña A, Almeria S, Ferrer D, Gutiérrez JF and Ortuño A (2001) A survey of ticks (Acari: Ixodidae) on dairy cattle on the island of Menorca in Spain. *Experimental Applied of Acarology* **25**, 899–908.

Chitimia-Dobler L, Rieß R, Kahl O, Wölfel S, Dobler G, Nava S and Estrada-Peña A (2018) *Ixodes inopinatus* – Occurring also outside the Mediterranean region. *Ticks and Tick-Borne Diseases* **9**, 196–200.

Chitimia-Dobler L, Kurzrock L, Molčányi T, Rieß R, Ute Mackenstedt U and Nava S (2019a) Genetic analysis of *Rhipicephalus sanguineus* sensu lato ticks, parasites of dogs in the Canary Islands, Cyprus, and Croatia, based on mitochondrial 16S rRNA gene sequences. *Parasitology Research* **118**, 1067–1071.

Chitimia-Dobler L, Schaper S, Rieß R, Bitterwolf K, Frangoulidis D, Bestehorn M, Springer A, Oehme R, Drehmann M, Lindau A, Mackenstedt U, Strube C and Dobler G (2019b) Imported *Hyalomma* ticks in Germany in 2018. *Parasites and Vectors* **12**, 134.

Courtney JW, Kostelnik LM, Zeidner NS and Massung RF (2004) Multiplex real-time PCR for detection of *Anaplasma phagocytophilum* and *Borrelia burgdorferi*. *Journal of Clinical Microbiology* **42**, 3164–3168.

Eremeeva ME and Dasch AD (2015) Challenges posed by tick-borne rickettsiae: eco-epidemiology and public health implications. *Frontiers in Public Health* **3**, 1–17.

Estrada-Peña A, Venzal JM and Nava S (2018) Redescription, molecular features, and neotype deposition of *Rhipicephalus pusillus* Gil Collado and *Ixodes ventalloi* Gil Collado (Acari, Ixodidae). *Zootaxa* **4442**, 262–276.

European Centre for Disease Prevention and Control and European Food Safety Authority (2023) *Tick Maps Internet*. Stockholm: ECDC. Available at https://www.ecdc.europa.eu/en/disease-vectors/surveillance-and-disease-data/tick-maps (consulted 2024.01.10).

Fernández de Mera IG, Zivkovic Z, Bolanños M, Carranza C, Pérez-Arellano JL, Gutiérrez C and de la Fuente J (2009) *Rickettsia massiliae* in the Canary Islands. *Emerging Infectious Diseases* **15**, 1869–1870.

Frangoulidis D, Kahlhofer C, Said AS, Osman AY, Chitimia-Dobler L and Shuaib YA (2021) High prevalence and new genotype of *Coxiella burnetiid* in ticks infesting camels in Somalia. *Pathogens (Basel, Switzerland)* **10**, 741.

Gehringer H, Schacht E, Maylaender N, Zeman E, Kaysser P, Oehme R, Pluta S and Splettstoesser WD (2013) Presence of an emerging subclone of *Francisella tularensis holarctica* in *Ixodes ricinus* ticks from south-western Germany. *Ticks and Tick-Borne Diseases* **4**, 93–100.

Grandi G, Chitimia-Dobler L, Choklikitumnuey P, Strube C, Springer A, Albihna A, Jaenson TGT and Omazic A (2020) First records of adult *Hyalomma marginatum* and *H. rufipes* ticks (Acari: Ixodidae) in Sweden. *Ticks and Tick-Borne Diseases* **11**, 101403.

Gray JS and Ogden NH (2021) Ticks, human babesiosis and climate change. *Pathogens (Basel, Switzerland)* **10**, 1430.

Guglielmone AA and Robbins RG (2018) *Hard Ticks (Acari: Ixodida: Ixodidae) Parasitizing Humans*. Cham, Switzerland: Springer, 314 pp.

Guglielmone AA, Nava S and Robbins RG (2023) Geographic distribution of the hard ticks (Acari: Ixodida: Ixodidae) of the world by countries and territories. *Zootaxa* **5251**, 001–274.

Hall TA (1999) BioEdit: a user friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41**, 95–98.

Halos L, Jamal T, Vial L, Maillard R, Suau A, Le Menach A, Boulouis H-J and Vayssier-Taussat M (2004) Determination of an efficient and reliable method for DNA extraction from ticks. *Veterinary Research* **35**, 709–713.

Hechemy KE, Raoult D, Fox J, Han Y, Elliott LB and Rawlings J (1989) Cross-reaction of immune sera from patients with rickettsial diseases. *Journal of Medical Entomology* **29**, 199–202.

Hornok S, Grima A, Takács N, Szekeres S and Kontschán J (2020) First records and molecular-phylogenetic analyses of three tick species (*Ixodes kaiseri*, *Hyalomma lusitanicum* and *Ornithodoros coniceps*) from Malta. *Ticks and Tick-Borne Diseases* **11**, 101379.

Katoh K and Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* **30**, 772–780.

Kumar S, Stecher G and Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for bigger datasets. *Molecular Biology and Evolution* **33**, 1870–1874.

Laatamna A, Oswald B, Chitimia-Dobler L and Bakkes DK (2020) Mitochondrial 16S rRNA gene analysis reveals occurrence of *Rhipicephalus sanguineus* sensu stricto from steppe and high plateaus regions, Algeria. *Parasitology Research* **119**, 2085–2091.

Maitre A, Wu-Chuang A, Mateos-Hernández L, Foucault-Simonin A, Moutailler S, Paoli J-C, Falchi A, Diaz-Sánchez AA, Banović P, Obregón D and Cabezas-Cruz A (2022) *Rickettsia helvetica* infection is associated with microbiome modulation in *Ixodes ricinus* collected from humans in Serbia. *Scientific Reports* **12**, 11464.

Marquez FJ (2008) Spotted fever group *Rickettsia* in ticks from southeastern Spain natural parks. *Experimental and Applied Acarology* **45**, 185–194.

Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, von Haeseler A and Lanfear R (2020) IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Molecular Biology and Evolution* **37**, 1530–1534.

Monerris Mascaró M and del Mar Colom Noguera M (2020) Estudi de la fauna d'Ixodida a Mallorca (Illes Baleares). *NEMUS* **10**, 37–46.

Mumcuoglu KY, Estrada-Peña A, Tarragona EL, Sebastian PS, Guglielmone AA and Nava S (2022) Reestablishment of *Rhipicephalus secundus* Feldman-Muhsam, 1952 (Acari: Ixodidae). *Ticks and Tick-Borne Diseases* **13**, 101897.

Nava S, Beati L, Venzal JM, Labruna MB, Szabó MPJ, Petney T, Saracho-Bottero MN, Tarragona EL, Dantas-Torres F, Silva MMS, Mangold AJ, Guglielmone AA and Estrada-Peña A (2018) *Rhipicephalus sanguineus* (Latreille, 1806): neotype designation, morphological re-description of all parasitic stages and molecular characterization. *Ticks and Tick-Borne Diseases* **9**, 1573–1585.

Nilsson K, Lindquist O and Pahlson C (1999) Association of *Rickettsia helvetica* with chronic perimyocarditis in sudden cardiac death. *Lancet (London, England)* **354**, 1169–1173.

Nowak-Chmura M (2013) *Tick Fauna (Ixodida) of Central Europe*. Kraków, Poland: Wyd Nauk Uniw Ped, pp. 88–211.

Parola P, Paddock CD, Socolovschi C, Labruna MB, Mediannikov O, Kernif T, Abdad MY, Stenos J, Bitam I, Fournier PE and Raoult D (2013) Update on tick-borne rickettsioses around the world: a geographic approach. *Clinical Microbiology Reviews* **26**, 657–702.

Pérez-Castrillón JL, Bachiller-Luque P, Martín-Luquero M, Mena-Martín FJ and Herreros V (2001) Tularemia epidemic in Northwestern Spain: clinical description and therapeutic response. *Clinical Infectious Diseases* **33**, 573–576.

Pérez-Eid C (2007) Les tiques. Identification, biologie, importance médicale et vétérinaire. Levoisier, p. 278.

Ponnusamy L, Gonzalez A, Van Treuren W, Weiss S, Parobek CM, Juliano JJ, Knight R, Roe RM, Apperson CS and Meshnick SR (2014) Diversity of Rickettsiales in the microbiome of the lone star tick, *Amblyomma americanum*. *Applied and Environmental Microbiology* **80**, 354–359.

Raoult D and Paddock CD (2005) *Rickettsia parkeri* infection and other spotted fevers in the United States. *The New England Journal of Medicine* **353**, 626–627.

Rehácek J, Urvölgyi J, Brezina R, Kazár J and Kovácová E (1978) Experimental infection of hare (*Lepus europaeus*) with *Coxiella burnetii* and *Rickettsia slovaca*. *Acta Virologica* **22**, 417–425.

Rollins R, Schaper S, Kahlhofer C, Frangoulidis D, Strauß AFT, Cardinale M, Springer A, Strube C, Bakkes DK, Becker NS and Chitimia-Dobler L (2021) Ticks (Acari: Ixodidae) on birds migrating to the island of Ponza, Italy, and the tick-borne pathogens they carry. *Ticks and Tick-Borne Diseases* **12**, 101590.

Roux V and Raoult D (2000) Phylogenetic analysis of members of the genus *Rickettsia* using the gene encoding the outer-membrane protein rOmpB (ompB). *International Journal of Systematic and Evolutionary Microbiology* **50**, 1449–1455.

Rynkiewicz EC and Clay K (2014) Tick community composition in Midwestern US habitats in relation to sampling method and environmental conditions. *Experimental and Applied Acarology* **64**, 109–119.

Santos AS and Santos-Silva MM (2018) *Ixodes ventalloi* Gil Collado, 1936: a vector role to be explored. *Vectors and Vector-Borne Zoonotic Diseases*, 1–15. http://dx.doi.org/10.5772/intechopen.81615

Semenza JC and Paz S (2021) Climate change and infectious diseases in Europe: impact, projection and adaptation. *Lancet Reg Health Eur* **9**, 100230.

Semenza JC, Rocklöv J and Ebi KL (2022) Climate change and cascading risks from infectious diseases. *Infectious Diseases and Therapy* **11**, 1371–1390.

Simser JA, Palmer AT, Fingerle V, Wilske B, Kurtti TJ and Munderloh UG (2002) *Rickettsia monacensis* sp. nov., a spotted fever group *Rickettsia*, from ticks (*Ixodes ricinus*) collected in a European city park. *Applied and Environmental Microbiology* **68**, 4559–4466.

https://doi.org/10.1017/S0031182024000544 Published online by Cambridge University Press

Sonenshine DE, Lane RS and Nicholson WL (2002) Ticks (Ixodida). In Mullen GR and Mullen LA (eds), *Medical and Veterinary Entomology*. San Diego, CA: Academic Press, pp. 517–558.

Stich RW, Schaefer JJ, Bremer WG, Needham GR and Jittapalapong S (2008) Host surveys, ixodid tick biology and transmission scenarios as related to the tick-borne pathogen, *Ehrlichia canis*. *Veterinary Parasitology* **158**, 256–273.

Tamura K and Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution* **10**, 512–552.

Válcarcel F, Elhachini L, Vilá M, Tomassone L, Sánchez M, Selles SMA, Kouidri M, González MG, Martin-Hernández R, Valcárcel Á, Fernández N, Tercero JM, Sanchis J, Bellido-Blasco J, González-Coloma A and Olmeda AS (2023) Emerging *Hyalomma lusitanicum*: from identification to vectorial role and integrated control. *Medical and Veterinary Entomology* **37**, 425–459.

Válcarel F, González J, González MG, Sánchez M, Tercero JM, Elhachimi L, Carbonell JD and Olmeda AS (2020) Comparative ecology of *Hyalomma lusitanicum* and *Hyalomma marginatum* Koch, 1944 (Acarina: Ixodidae). *Insects* **11**, 303.

Van Treuren W, Ponnusamy L, Brinkerhoff RJ, Gonzalez A, Parobek CM, Juliano JJ, Andreadis TG, Falco RC, Ziegler LB, Hathaway N, Keeler C, Emch M, Bailey JA, Roe RM, Apperson CS, Knight R and Meshnick SR (2015) Variation in the microbiota of Ixodes ticks with regard to geography, species. and sex. *Applied and Experimental Acarology* **81**, 6200–6209.

Walker JB, Keirans JE and Horak IG (2000) *The Genus Rhipicephalus (Acari, Ixodidae): A Guide to the Brown Ticks of the World*. Cambridge, UK: Cambridge University Press.

Wölfel R, Essbauer S and Dobler G (2008) Diagnostics of tick-borne rickettsioses in Germany: a modern concept for a neglected disease. *International Journal of Medical Microbiology* **298**, 368–374.

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