



# First molecular detection of the human pathogen *Rickettsia raoultii* and other spotted fever group rickettsiae in Ixodid ticks from wild and domestic mammals

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## Abstract

Tick-borne rickettsioses are recognized as emerging vector-borne infections capable of infecting both human and animal hosts worldwide. This study focuses on the detection and molecular identification of species belonging to the genus *Rickettsia* in ticks sampled from human, vegetation, and domestic and wild vertebrates in Sardinia. Ticks were tested by PCR targeting *gltA*, *ompA*, and *ompB* genes, followed by sequencing analysis. The results provide evidences of a great variety of *Rickettsia* species of the Spotted fever group in Ixodid ticks and allow establishing for the first time the presence of *R. raoultii* in *Rhipicephalus sanguineus* s.l. and *Dermacentor marginatus* ticks in Sardinia island. *Rickettsia massiliae* was detected on *R. sanguineus* s.l. and *R. aeschlimannii* in *Hyalomma marginatum* and *Hy. lusitanicum* ticks. In addition, eight *D. marginatus* ticks were positive for *R. slovaca*. This study provides further evidence that different *Rickettsia* species are widespread in Sardinian ticks and that detailed investigations are required to understand the role these tick species play on spotted fever group rickettsiae circulation. More studies will provide new background on molecular epidemiology of zoonotic rickettsiae, the geographical distribution of tick-transmitted rickettsial pathogens, and the involvement of vertebrate hosts in propagation and maintenance of these bacteria in nature.

**Keywords** Ticks · Tick-borne rickettsioses · Rickettsiosis

## Introduction

The importance of ticks (Acari: Ixodida) has long been recognized due to their ability to feed on a large range of host species and to transmit microorganisms capable of infecting both human and animal hosts (de la Fuente et al. 2017; Brites-Neto et al. 2015). Identification and characterization of these circulating agents are crucial for the development of preventive measures in response to the gradually increasing exposure of humans to tick vectors. In Italy, the incidence of tick-borne rickettsioses (TBD) has increased over the last decade with 3170 clinical cases and 18 deaths documented by the Health

Ministry from 2009 to 2013. The average incidence of Rickettsiosis for this period was 21.17 to 176.88 cases per million persons ([http://old.iss.it/binary/publ/cont/16\\_1\\_web.pdf](http://old.iss.it/binary/publ/cont/16_1_web.pdf)). *Rickettsia* species are the causative agents of human or animal diseases, including spotted fevers and murine or epidemic typhus which can cause a range of mild to fatal diseases, mostly through arthropod bites (El Karkouri et al. 2016).

This genus encompasses at least 27 *Rickettsia* species with validated and published names, and a number of putative novel *Rickettsia* species that have not been fully characterized, but were continually isolated from or detected in ticks (Parola et al. 2013; Merhej et al. 2014). The members of *Rickettsia* have been classified into four different groups, including the well-defined spotted fever group (SFG) and typhus group (TG), the *Rickettsia bellii* group, and the *Rickettsia canadensis* group (Merhej et al. 2014). Currently, several studies have revealed the extensive diversity of SFG rickettsiae in different tick species and geographic locations (Merhej et al. 2014). In Sardinia, the second largest island in the Mediterranean Sea,

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cases of notifiable tick-borne diseases are increasing (Madeddu et al. 2016) and Mediterranean Spotted Fever (MSF) rickettsiosis continues to be endemic with an incidence of 10/10,000 inhabitants per year (<http://www.epicentro.iss.it/problemi/zecche/rickettsiosi.asp>). Members of the spotted fever group, capable of causing disease, have been detected in Sardinian ticks, such as *R. aeschlimannii*, *R. massiliae*, *R. conorii israeliensis*, *R. slovaca*, *R. helvetica*, and *R. monacensis*. In addition, two *Rickettsiae* of unknown pathogenicity namely *Rickettsia hoogstralii* and *Candidatus Rickettsia barbariae* have been previously identified (Chisu et al. 2018; Madeddu et al. 2016). Even if some of these spotted fever group *rickettsia* seem to have low pathogenicity, namely *R. massiliae*, *R. monacensis*, and *R. raoultii*, they were found to be involved in human disease (Parola et al. 2009; Jado et al. 2007; Vitale et al. 2006). The objective of this study was to determine the presence of *Rickettsia* species in ticks collected from several sites and to update the knowledge on tick-borne rickettsiae in Sardinia.

## Material and methods

### Tick sampling

Between March and November 2017, ticks were collected from humans, domestic mammals, and wildlife in Sardinia, Italy. Three ticks were also collected from vegetation in the areas grazed by vertebrate hosts. Domestic mammals (21 dogs, 4 goats, 7 cattle, and 1 cat) were reared in small farms, and owners provided the ticks in 15-ml tubes. Wild vertebrates sampled during the study included 1 fox (*Cynotherium sardous*), 3 mouflons (*Ovis orientalis musimon*), 7 wild boars (*Sus scrofa meridionalis*), 1 marten (*Martes martes*), 3 deer (*Cervus elaphus corsicanus*), and 3 birds (*Corvus corone*). They were brought dead to our laboratories for necropsy analyses and tick collection. All of the ticks removed from animals were adult and partially or completely engorged.

Ticks were carefully removed from their hosts by using fine-tipped tweezers and placed in vials with 70% ethanol at room temperature. Ticks were then identified to species level, developmental stage, and sex under a dissecting microscope using conventional taxonomic keys (Manilla 1998).

### DNA extraction and amplification

Ticks were rinsed twice in distilled sterile water for 10 min and dried on sterile filter paper. Each sample was then longitudinally incised using an individual scalpel into two parts, where only one piece was crushed using unique scalpel in sterile tubes (Eppendorf; Hamburg, Germany). The remaining portion of each tick was kept at  $-80\text{ C}$  for further control. All experiments and handling of blood and ticks were conducted

in a laminar flow biosafety hood. A total of 1000  $\mu\text{L}$  of homogenized ticks was transferred to a specific tubes prior to their extraction using **QIAgen columns** (QIAamp tissue kit, Qiagen, Hilden, Germany), according to the manufacturer's instructions to obtain the final **elution volume** of 100  $\mu\text{L}$ . A negative control was included every 10 DNA extractions to monitor the occurrence of false-positives. All biological materials including ticks DNA were stored at  $-20\text{ C}$  until further use.

To investigate the presence of *Rickettsia* species in ticks, the samples were initially tested by PCR oligonucleotide primers Rp CS. 409p and Rp CS.1258n (Eurogentec, Seraing, Belgium), which amplify a 750-bp fragment of the citrate synthase gene (*gltA*) of *Rickettsia*, as previously reported (Roux et al. 1997). The conventional PCR assay amplification reactions were performed with a DNA thermal cycler under the following conditions: an initial denaturation step at 95 C for 15 min, followed by 40 cycles consisting of 1 min denaturation at 94 C, 1 min annealing at 60 C, and a 1 min extension at 72 C. A final extension cycle at 72 °C for 5 min was performed, and the reactions were cooled at 15 C. Next, the positive samples were amplified by using primers Rr 190.70, Rr 190.180, and Rr 190.701, which amplify a 629–632-bp fragment of the gene for outer membrane protein A (*ompA*) (Fournier et al. 1998). Finally, one more PCR reaction using primers 120-M59 and 120–807 that amplify a 865 bp of the outer membrane protein B (*ompB*) gene fragment was used (Roux and Raoult 2000). For primers targeting the *ompA* and *ompB* genes, the amplification conditions were as described above. A negative control of DNA extracted from non-infected laboratory ticks and a positive control of *R. rickettsii* DNA (one negative control for every 10 tested ticks) were included in each test. All laboratory procedures used in this study, including the amplification and sequencing of sample, have contributed to achieve the higher levels of *Rickettsia* species diversity, as evidenced in this study.

### DNA purification, sequencing, and phylogenetic analyses

The *gltA*, *ompA*, and *ompB* PCR products were then purified using QIAquick PCR purification kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Purified products were then directly sequenced by using an ABI Prism BigDye terminator cycle sequencing ready reaction kit (Life Technologies, Italy) on an ABI 377 DNA sequencer, according to the manufacturer's recommendations. The sequencing reactions were performed with the forward and reverse primers used for the PCR amplifications. Sequences generated with *gltA*, *ompA*, and *ompB* primers were edited with Chromas 2.2 (Technelysium, Helensvale, Australia), aligned with CLUSTALX (Larkin et al. 2007) in order to assign them to unique sequence types, and then

checked against the GenBank database with nucleotide blast BLASTN (Altschul et al. 1990). Pairwise/multiple sequence alignments and sequence similarities were calculated using the CLUSTALW (Thompson et al. 1994) and the identity matrix options of Bioedit (Hall 1999), respectively.

The evolutionary history was inferred by using the maximum likelihood method based on the protein-coding *gltA*, *ompA*, and *ompB* genes, in the MEGA6 (Tamura et al. 2013) program (<http://megasoftware.net/>). Statistical support for internal branches of the trees was evaluated by bootstrapping with 1000 iterations (Felsenstein 1985). Initial trees for the heuristic search were obtained by applying the neighbor-joining method to a matrix of pairwise distances estimated using the maximum composite likelihood (MCL) approach. All positions containing gaps and missing data were eliminated.

The sequence types generated with *gltA* primers were aligned with a set of 27 sequences representing *gltA* variability of the different species belonging to the genus *Rickettsia*. The reference sequences were as follows: *R. conorii* (U59728), *R. bellii* (CP000849), *R. prowazekii* (CP003394), *R. typhi* (CP003398), *R. hoogstralii* (KF791209), *R. felis* (CP000053), *R. canadensis* (CP000409), *R. australis* (CP003338), *R. monacensis* (LN794217), *R. helvetica* (KP866150), *R. montanensis* (U74756), *R. massiliae* (KU498299), *R. rhipicephali* (U59721), *R. aeschlimannii* (HQ335150), *R. raoultii* (KT899090), *R. amblyommatis* (KY273595), *R. japonica* (AP017602), *R. heilongjiangensis* (AB473812), *R. slovacica* (AY129301), *R. parkeri* (JN126320), *R. rickettsii* (HM446474), *R. philipii* (CP003308), *R. africae* (JN043505), *R. israeli* tick typhus (U59727), *R. sibirica* (KM288711), *R. mongolotimonae* (DQ097081), *R. akari* (CP000847).

The *ompA* sequence types generated during this study were also aligned with a set of 22 *ompA* sequences available in the GenBank database and representative of the *Rickettsia* genus. The *ompA* reference sequences were as follows: *R. conorii* (KT368818), *R. felis* (KY172882), *R. canadensis* (CP000409), *R. australis* (AF149108), *R. monacensis* (EU665233), *R. montanensis* (CP003340), *R. massiliae* (KR401145), *R. rhipicephali* (CP003342), *R. aeschlimannii* (HQ335157), *R. raoultii* (MF511255), *R. amblyommatis* (CP015012), *R. japonica* (KY484160), *R. heilongjiangensis* (AF179362), *R. slovacica* (KX506735), *R. parkeri* (EU715288), *R. rickettsii* (CP018914), *R. philipii* (CP003308), *R. africae* (KT633262), *R. israeliensis* (KF245449), *R. sibirica* (MF098408), and *R. akari* (CP000847).

Finally, sequence types obtained with primer targeting the the *ompB* genes were similarly processed and used to reconstruct evolutionary history. The dataset included 18 sequences: *R. slovacica* (KX506741), *R. massiliae* (MF098412), *R. raoultii* (KU961542), *R. sibirica* (AF123722), *R.*

*rhipicephali* (KX018051), *R. mongolotimonae* (AF123715), *R. africae* (KF660535; AF123706), *R. aeschlimannii* (KU961544), *R. rickettsii* (GU723475), *R. parkeri* (KX018050), *R. israeliensis* (AF123712), *R. conorii* subsp. *caspia* (AY643093), *R. honei* (AF123711), *R. amblyommatis* (MG674591), *R. montanensis* (AF123716), *R. japonica* (KY364904), and *R. hulinensis* (AY260452).

## Sequence accession numbers

Sequences of the rickettsial *gltA*, *ompA*, and *ompB* genes were deposited in the GenBank using the National Center for Biotechnology Information (NCBI; Bethesda, MD) BankIt v3.0 submission tool (<http://www3.ncbi.nlm.nih.gov/BankIt/>). Accession numbers are: MH064440-MH064462 (*Rickettsia gltA* gene), MH532235-MH532257 (*Rickettsia ompA* gene), MH532258-MH532280 (*Rickettsia ompB* gene).

## Results

A total of 185 adult ticks from wild and domestic animals, human and vegetation, were collected from 23 collection sites and identified morphologically as: *Rhipicephalus sanguineus* s.l., *R. bursa*, *R. annulatus*, *Hyalomma marginatum*, *Hy. lusitanicum*, *Haemaphysalis punctata*, *R. pusillus*, and *Dermacentor marginatus* (Table 1). One exemplar of the soft tick *Ornithodoros maritimus* was also detected.

By using *gltA*, *ompA*, and *ompB* PCR, the DNA of *Rickettsia* spp. was detected in 29 out of 185 (16%) of ticks removed from different wild and domestic hosts (dog, cattle, deer, wildboar) and from the vegetation (Table 1). The sequence analyses of 23 PCR products obtained by *gltA*, *ompA*, and *ompB* PCR primers returned clear sequencing signals, but the quality of sequencing of six samples was of low quality (presumably due to the presence of more than one bacterial species in the sample material) and they were excluded from all subsequent analysis.

Sequence analyses of *gltA*-positive amplicons showed that three *R. sanguineus* s.l. ticks shared 100% sequence identity with *gltA* sequences of *R. massiliae*; one *Hy. lusitanicum* and two *Hy. marginatum* positive ticks shared the 100% nucleotide identity with the *gltA* gene of *R. aeschlimannii* strains. Eight out of 29 *D. marginatus* ticks (28%) contained DNA of *Rickettsia*, which showed 100% sequence identity with the 750-bp fragment of the *Ri. slovacica gltA* gene. Three *R. sanguineus* s.l. and six *D. marginatus* ticks were positive for the presence of rickettsial DNA that shared 99–100% identity with that of *R. raoultii* strains isolated from ticks worldwide, which were identified as the closest match by nBLAST.

Ticks positive for rickettsial *gltA* were also positive when tested with the primers targeting the outer surface protein rOmpA (*ompA*) and the outer-membrane protein rOmpB

**Table 1** Tick species analyzed in this study including the collection sites, host, number, stage and sex, and the total of *gltA*, *ompA*, and *ompB* positive PCR ticks

Tick species (number of specimens)	Collection site	Host (number)	No, stage, sex			PCR		
			♀	♂	Nymph	<i>gltA</i>	<i>ompA</i>	<i>ompB</i>
<i>R. bursa</i> (22)	Urzulei	Human (1)	2	–	–	–	–	–
	Aritzto	Human (1)	1	–	–	–	–	–
	Talana	Fox (1)	1	2	–	–	–	–
	S. Nicolò Gerrei	Goat (1)	–	1	–	–	–	–
	Perdasdefogu	Goat (1)	8	3	1	–	–	–
	Asinara	Mouflon (1)	1	–	–	–	–	–
	Santadi	Deer (1)	1	–	–	–	–	–
	Uta	Cattle (1)	–	1	–	–	–	–
<i>R. sanguineus</i> s.l. (97)	Baunei	Goat (1)	2	–	–	–	–	–
	Borutta	Dog (4)	2	6	4	4	4	–
	Urzulei	Dog (2)	3	1	–	1	1	–
		Marten (1)	1	4	–	–	–	–
	Cardedu	Dog (1)	1	–	–	–	–	–
	Barisardo	Dog (2)	4	2	–	2	2	–
	Sassari	Dog (3)	4	4	6	1	1	1
		Cat (1)	1	–	–	–	–	–
	Alghero	Dog (1)	1	–	–	–	–	–
	Ozieri	Dog (7)	20	16	8	6	6	6
	Triei	Dog (1)	1	–	–	–	–	–
	Tortoli	Soil (2)	2	–	–	–	–	–
	Dorgali	Mouflon (1)	–	3	–	–	–	–
Pattada	Deer (1)	–	–	1	–	–	–	
<i>Ornithodoros maritimus</i> (1)	Alghero	Human (1)	1	–	–	–	–	–
<i>Hy. lusitanicum</i> (1)	Alghero	Soil (1)	–	–	1	1	1	1
<i>R. annulatus</i> (13)	S. Anna Arresi	Cattle (1)	3	5	–	–	–	–
	Uta	Cattle (1)	2	3	–	–	–	–
<i>Hy. marginatum</i> (16)	Orgosolo	Human (1)	1	–	–	–	–	–
	Uta	Cattle (4)	–	4	3	2	2	2
	Alghero	Wildboar (1)	4	–	–	–	–	–
	Asinara	Bird (2)	2	2	–	–	–	–
<i>Hae. punctata</i> (6)	Talana	Mouflon (1)	1	–	–	–	–	–
	Tortoli	Crow (1)	–	1	–	–	–	–
	Oschiri	Cattle (2)	1	3	–	–	–	–
<i>D. marginatus</i> (29)	Santadi	Deer (1)	–	2	3	4	4	4
	Oschiri	Cattle (2)	5	2	3	2	2	2
		Wildboar (1)	–	3	2	5	5	5
	Talana	Wildboar (2)	6	3	–	1	1	1
Total (185)			82	71	32	29	29	29

(*ompB*) genes. Upon sequencing and ClustalX alignment, the comparative analysis of *Rickettsia* sequences obtained from the *ompA* gene was 100% identical with *R. massiliae* (sequence type Rm-*ompA1* detected from three *R. sanguineus* s. l.), *R. aeschlimannii* (sequence type Ra-*ompA2* from one *Hy. lusitanicum* and two *Hy. marginatum* ticks), *R. raoultii* (sequence type Rr-*ompA4* from six *D. marginatus* and three

*R. sanguineus* s. l. ticks), and *R. slovaca* (sequence type Rs-*ompA3*) strains. The *ompB* sequences obtained from three *R. sanguineus* s. l. showed that the closest sequences available in GenBank were those for *R. massiliae* (sequence type Rm-*ompB1*). Two sequences from one *Hy. lusitanicum* and two *Hy. marginatum* ticks showed 100% identity with *R. aeschlimannii* (sequence type Ra-*ompB2*); six *D. marginatus*

and three *R. sanguineus* s. l. ticks were 100% identical with *R. raoultii* (sequence type Rr-ompB3). Finally, eight *D. marginatus* ticks shared a *Rickettsia* sequence that was 100% similar to *R. slovaca* (Rs-ompB4) as detailed in Table 2.

Phylogenetic analysis based on the alignment of the five *gltA* sequence types with the rickettsial reference sequences allowed to identify two main groups: the spotted fever group including 26 sequences and the typhus group comprising *R. prowazekii* and *R. typhi* (Fig. 1). The phylogenetic tree indicated that Rm-*gltA1* (obtained from 3 *R. sanguineus* s.l.) and Ra-*gltA2* (from two *Hy. marginatum* and one *Hy. lusitanicum*) sequence types grouped with reference strains representative of *R. aeschlimannii* and *R. massiliae*, respectively. The two sequence types named Rr-*gltA3* (derived from three *R. sanguineus* s.l.) and Rr-*gltA4* (derived from six *D. marginatum*) were closely related to the *R. raoultii* sequence reference strain. Finally, the Rs-*gltA5* (obtained from eight *D. marginatus*) sequence type formed a single clade with *R. slovaca* reference strain. The five sequence types detected in this study can be classified within the SFG *rickettsiae*. The main sequence clusters were statistically supported by bootstrap analyses.

Phylogenetic analyses conducted on the alignment of *ompA* and *ompB* sequence types obtained in this study with selected *Rickettsia* sequences found in GenBank are shown in Figs. 2 and 3.

## Discussion

Sardinian ticks have been reported to infest wild and domestic hosts and to be a rich reservoir of well-known families of

bacterial pathogens (Chisu et al. 2018; Masala et al. 2012a; Satta et al. 2011). The results of this study confirm that Sardinian ticks carry a wide range of bacteria from the *Rickettsia* genus and provide for the first time molecular evidence on the occurrence of *R. raoultii* in *R. sanguineus* s.l. and *D. marginatus* ticks collected from domestic (dogs and cattle) and wild vertebrates (wild boar and deer). *Rhipicephalus* ticks were the most abundant species detected in this study. The phylogenetic analyses showed that the *Rickettsia* species obtained in this study were closely related to representative SFG rickettsiae with known pathogenicity, which can cause human diseases (Fig. 1). Since the ticks have been collected directly from the hosts, no conclusion can be drawn regarding the circulation of the pathogens within the tick population, as every detection can be the result of ingesting infected blood.

The identification of *R. massiliae* in *R. sanguineus* s.l. ticks is consistent with previous reports where the potential role for *Rhipicephalus* ticks in the transmission of these pathogens has been postulated (Chisu et al. 2018, 2017; Parola et al. 2013). *Rickettsia massiliae* is recognized as a pathogenic species causing spotted fever in human (Parola et al. 2013) and where it has been putatively linked to mild to moderately severe illnesses in dogs in California (Beeler et al. 2011). Human cases of *R. massiliae* have been documented in patients from Sicily, Italy (Cascio et al. 2013; Vitale et al. 2006).

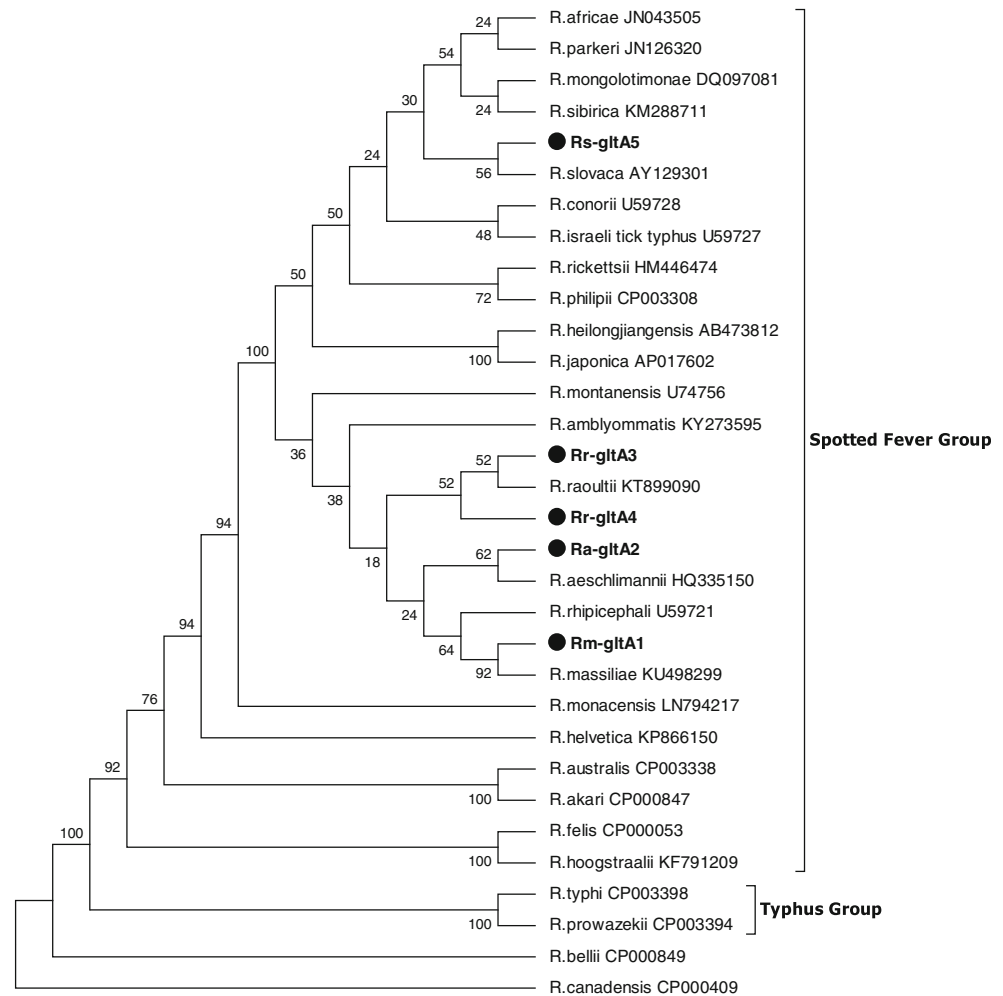
Detection of *R. slovaca* in *D. marginatus* confirms the role played by these tick species in *R. slovaca* transmission, as previously reported in other studies (Masala et al. 2012b; Chisu et al. 2017, 2018).

*Rickettsia slovaca* is associated with a syndrome characterized by scalp eschars and neck lymphadenopathy following tick bites. This syndrome was named TIBOLA (tick-borne

**Table 2** Detection and BLASTN maximum identities of *Rickettsia* spp. identified from different tick species by *gltA*, *ompA*, and *ompB* genes

Tick species (n. of tick positive)	Host	Sequence type	BLAST analyses ( <i>gltA</i> gene)	Sequence type	BLAST analyses ( <i>ompA</i> gene)	Sequence type	BLAST analyses ( <i>ompB</i> gene)
<i>R. sanguineus</i> s.l. (3)	Dog	Rm- <i>gltA1</i>	100% <i>R. massiliae</i> (detected in ticks from Guinea and Argentina)	Rm- <i>ompA1</i>	100% <i>R. massiliae</i> (detected in ticks from China)	Rm- <i>ompB1</i>	100% <i>R. massiliae</i> (detected in ticks from Argentina, and USA)
<i>Hy. lusitanicum</i> (1)	Soil	Ra- <i>gltA2</i>	100% <i>R. aeschlimannii</i> (detected in ticks in Italy, Russia and Senegal)	Ra- <i>ompA2</i>	100% <i>R. aeschlimannii</i> (detected in ticks from Turkey, and Egypt)	Ra- <i>ompB2</i>	100% <i>R. aeschlimannii</i> (detected in ticks from Russia)
<i>Hy. marginatum</i> (2)	Cattle						
<i>R. sanguineus</i> s.l. (3)	Dog	Rr- <i>gltA3</i>	100% <i>R. raoultii</i> (detected in ticks from China)	Rr- <i>ompA3</i>	100% <i>R. raoultii</i> (detected in louse flies, ticks from China, and Italy)	Rr- <i>ompB3</i>	100% <i>R. raoultii</i> (detected in ticks from Russia, China, and France)
<i>D. marginatus</i> (6)	Deer, Wildboar	Rr- <i>gltA4</i>	99% <i>R. raoultii</i> (detected in ticks from Russia and China)				
<i>D. marginatus</i> (8)	Deer, Cattle, Wildboar	Rs- <i>gltA5</i>	100% <i>R. slovaca</i> (detected in ticks from France, Russia and China)	Rs- <i>ompA4</i>	100% <i>R. slovaca</i> (detected in wild boar, louse flies, and ticks from Turkey, China and Italy)	Rs- <i>ompB4</i>	100% <i>R. slovaca</i> (detected in louse flies from China)

**Fig. 1** Phylogenetic tree inferred with partial sequences of the *gltA* gene of *Rickettsia* species generated in this study and other sequences representative of the different species of the genus *Rickettsia*. The evolutionary history was inferred using the maximum likelihood method. Numbers next the branches indicate bootstrap values based on 1000 replicates. The *Rickettsia* sequences obtained in this study are represented in bold and marked with a circle



lymphadenopathy) or DEBONEL (*Dermacentor*-borne necrotic erythema and lymphadenopathy). The term “SENLAT” (scalp eschar and neck lymphadenopathy after a tick bite) has also been recently proposed (Parola et al. 2013; Angelakis et al. 2010).

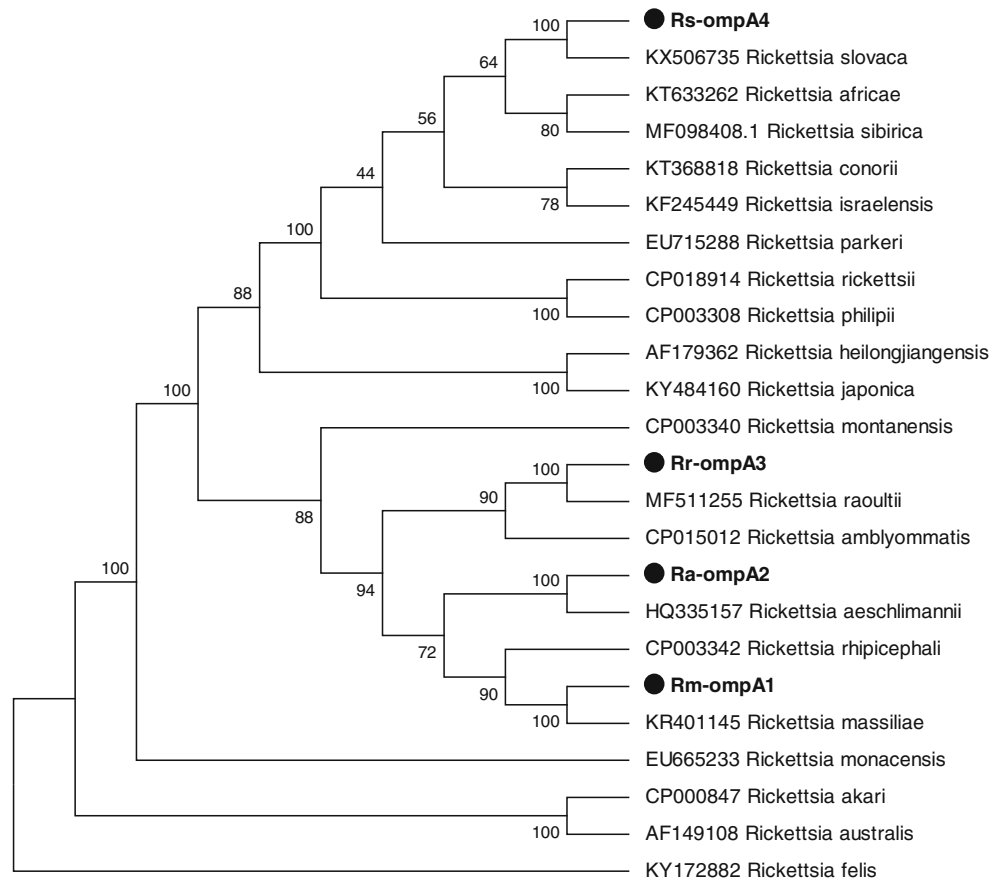
In this study, the human pathogen *R. aeschlimannii* was identified from *Hy. lusitanicum* and *Hy. marginatum* ticks collected from the vegetation and cattle, respectively. The presence of *R. aeschlimannii* in *Hy. marginatum* ticks was consistent with previous studies reported in Sardinia and in other countries (Chisu et al. 2017; Santos-Silva et al. 2006; Wallménus et al. 2014). Recently, *D. marginatus* and *Hy. lusitanicum* have been indicated as potentially new tick vectors of *R. aeschlimannii* (Parola et al. 2013). The pathogenicity of this bacterium to humans is not well understood, although a clinical picture similar to MSF-like lesions was reported. Infections in humans have been previously confirmed in Europe, South Africa, Algeria, and Tunisia (Blanda et al. 2017; Portillo et al. 2015; Germanakis et al.

2013; Demoncheaux et al. 2012; Pretorius and Birtles 2002; Raoult et al. 2002).

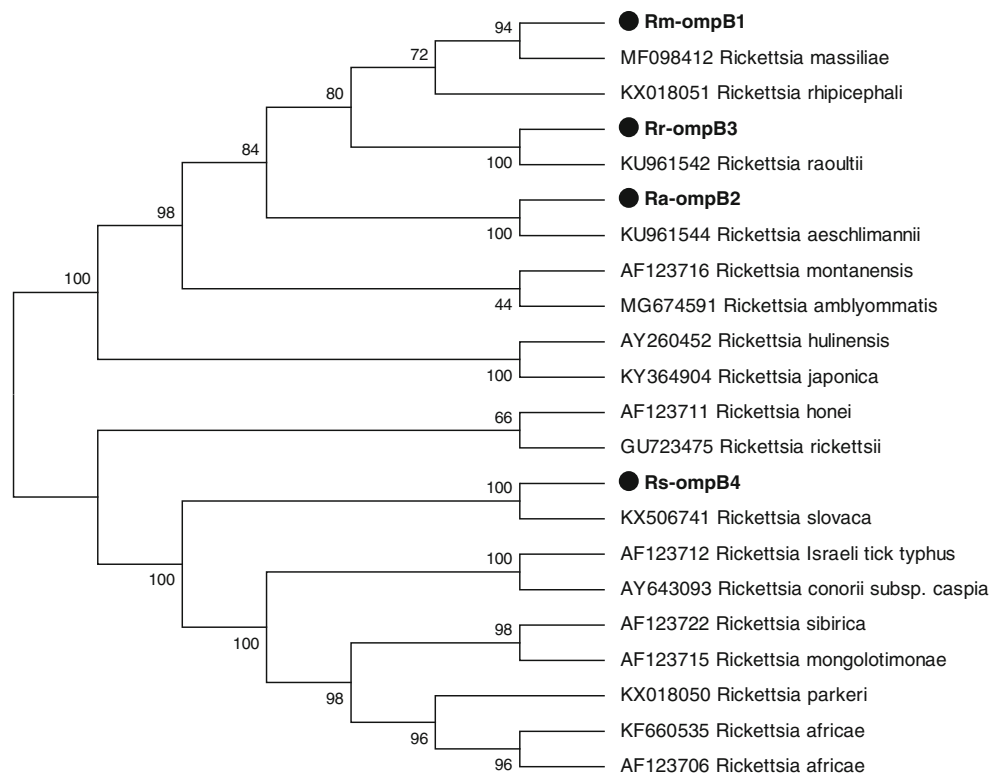
In this study, *gltA*, *ompA*, *ompB*-based molecular diagnosis allowed to identify the presence of *R. raoultii* sequences in *D. marginatus* and *R. sanguineus* s.l. ticks from vertebrate hosts. *R. raoultii* is a member of the spotted fever group rickettsiae and has been implicated in cases of DEBONEL/TIBOLA/SENLAT (Mediannikov et al. 2008). *Rickettsia raoultii* has been reported in many European and Asian countries in *Dermacentor* ticks. Other hard ticks, such as *Haemaphysalis*, *Rhipicephalus*, *Hyalomma*, and *Amblyomma* ticks, were also involved (Blanda et al. 2017).

These data implicate the broad and ubiquitous distribution of *Rickettsia* species in Sardinian ticks. The high percentage of *R. sanguineus* s.l. and *D. marginatus* ticks infected with *R. slovaca* and *R. raoultii* strongly indicates an increase in the prospect of medical intervention for persons in localities where these ticks occur. Clinicians should be aware that patients with tick-borne lymphadenopathy may be on the island.

**Fig. 2** Phylogenetic tree inferred with partial sequences of the *ompA* gene of *Rickettsia* species generated in this study and other sequences representative of the different species of the genus *Rickettsia*. The evolutionary history was inferred using the maximum likelihood method. Numbers next the branches indicate bootstrap values based on 1000 replicates. The *Rickettsia* sequences obtained in this study are represented in bold and marked with a circle



**Fig. 3** Phylogenetic tree inferred with partial sequences of the *ompB* gene of *Rickettsia* species generated in this study and other sequences representative of the different species of the genus *Rickettsia*. The evolutionary history was inferred using the maximum likelihood method. Numbers next the branches indicate bootstrap values based on 1000 replicates. The *Rickettsia* sequences obtained in this study are represented in bold and marked with a circle



## Compliance with ethical standards

**Ethical approval** All applicable international, national, and/or institutional guidelines for the care and use of animal were followed. All procedures performed in studies involving human participant were in accordance with the ethical standards of the institutional and/or national ethic committee. A written informed consent was obtained from patients at the time of hospitalization. The Istituto Zooprofilattico Sperimentale of Sardinia was authorized by the ethics committee of the Local Health Authority of Sassari (Comitato di Bioetica, ASL N. 1, Sassari) Prot N. 1136, to analyze human sera following the request of the National Health Service doctors, since 03/26/2013.

**Conflict of interest** The authors declare that they have no conflict of interest.

## References

- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. *J Mol Biol* 215:403–410. [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2)
- Angelakis E, Pulcini C, Waton J, Imbert P, Socolovschi C, Edouard S, Dellamonica P, Raoult D (2010) Scalp eschar and neck lymphadenopathy caused by *Bartonella henselae* after tick bite. *Clin Infect Dis* 50:549–551. <https://doi.org/10.1086/650172>
- Beeler E, Abramowicz KF, Zambrano ML, Sturgeon MM, Khalaf N, Hu R, Dasch GA, Ereemeeva ME (2011) A focus of dogs and *Rickettsia massiliae*-infected *Rhipicephalus sanguineus* in California. *Am J Trop Med Hyg* 84:244–249. <https://doi.org/10.4269/ajtmh.2011.10-0355>
- Blanda V, Torina A, La Russa F, D'Agostino R, Randazzo K, Scimeca S, Giudice E, Caracappa S, Cascio A, de la Fuente J (2017) A retrospective study of the characterization of *Rickettsia* species in ticks collected from humans. *Ticks Tick Borne Dis* 8:610–614. <https://doi.org/10.1016/j.ttbdis.2017.04.005>
- Brites-Neto J, Duarte KMR, Martins TF (2015) Tick-borne infections in human and animal population worldwide. *Vet world* 8:301–315. <https://doi.org/10.14202/vetworld.2015.301-315>
- Cascio A, Torina A, Valenzise M, Blanda V, Camarda N, Bombaci S, Iaria C, De Luca F, Wasniewska M (2013) Scalp eschar and neck lymphadenopathy caused by *Rickettsia massiliae*. *Emerging Infect Dis* 19:836–837. <https://doi.org/10.3201/eid1905.121169>
- Chisu V, Leulmi H, Masala G, Piredda M, Foxi C, Parola P (2017) Detection of *Rickettsia hoogstraalii*, *Rickettsia helvetica*, *Rickettsia massiliae*, *Rickettsia slovaca* and *Rickettsia aeschlimannii* in ticks from Sardinia, Italy. *Ticks Tick Borne Dis* 8:347–352. <https://doi.org/10.1016/j.ttbdis.2016.12.007>
- Chisu V, Foxi C, Mannu R, Satta G, Masala G (2018) A five-year survey of tick species and identification of tick-borne bacteria in Sardinia, Italy. *Ticks Tick Borne Dis* 9:678–681. <https://doi.org/10.1016/j.ttbdis.2018.02.008>
- Demoncheaux J-P, Socolovschi C, Davoust B, Haddad S, Raoult D, Parola P (2012) First detection of *Rickettsia aeschlimannii* in *Hyalomma dromedarii* ticks from Tunisia. *Ticks Tick Borne Dis* 3:398–402. <https://doi.org/10.1016/j.ttbdis.2012.10.003>
- El Karkouri K, Mediannikov O, Robert C, Raoult D, Fournier PE (2016) Genome sequence of the tick-borne pathogen *Rickettsia raoultii*. *Genome Announc* 4. doi: <https://doi.org/10.1128/genomeA.00157-16>
- Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39:783–791. <https://doi.org/10.1111/j.1558-5646.1985.tb00420.x>
- Fournier PE, Roux V, Raoult D (1998) Phylogenetic analysis of spotted fever group rickettsiae by study of the outer surface protein rOmpA. *Int J Syst Bacteriol* 48:839–849
- Germanakis A, Chochlakos D, Angelakis E, Tselentis Y, Psaroulaki A (2013) *Rickettsia aeschlimannii* infection in a man, Greece. *Emerging Infect Dis* 19:1176–1177. <https://doi.org/10.3201/eid1907.130232>
- Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* 41: 95–98
- Jado I, Oteo JA, Aldámiz M, Escudero R, Ibarra V, Portu J, Portillo A, Lezaun MJ, García-Amil C, Rodríguez-Moreno I, Anda P (2007) *Rickettsia monacensis* and human disease, Spain. *Emerging Infect Dis* 13:1405–1407. <https://doi.org/10.3201/eid1309.060186>
- de la Fuente J, Antunes S, Bonnet S, Cabezas-Cruz A, Domingos AG, Estrada-Peña A, Johnson N, Kocan KM, Mansfield KL, Nijhof AM, Papa A, Rudenko N, Villar M, Alberdi P, Torina A, Ayllón N, Vancova M, Golovchenko M, Grubhoffer L, Caracappa S, Fooks AR, Gortazar C, Rego ROM (2017) Tick-pathogen interactions and vector competence: identification of molecular drivers for tick-borne diseases. *Front Cell Infect Microbiol* 7:114. <https://doi.org/10.3389/fcimb.2017.00114>
- Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG (2007) Clustal W and Clustal X version 2.0. *Bioinformatics* 23:2947–2948. <https://doi.org/10.1093/bioinformatics/btm404>
- Madeddu G, Fiore V, Mancini F, Caddeo A, Ciervo A, Babudieri S, Masala G, Bagella P, Nunnari G, Rezza G, Mura MS (2016) Mediterranean spotted fever-like illness in Sardinia, Italy: a clinical and microbiological study. *Infection* 44:733–738. <https://doi.org/10.1007/s15010-016-0921-z>
- Manilla G (1998) Acari, Ixodida. *Fauna d'Italia* 36. Edizioni Calderini, Bologna
- Masala G, Chisu V, Foxi C, Socolovschi C, Raoult D, Parola P (2012a) 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>
- Masala G, Chisu V, Satta G, Socolovschi C, Raoult D, Parola P (2012b) *Rickettsia slovaca* from *Dermacentor marginatus* ticks in Sardinia, Italy. *Ticks Tick Borne Dis* 3:393–395. <https://doi.org/10.1016/j.ttbdis.2012.10.007>
- Mediannikov O, Matsumoto K, Samoylenko I, Drancourt M, Roux V, Rydkina E, Davoust B, Tarasevich I, Brouqui P, Fournier PE (2008) *Rickettsia raoultii* sp. nov., a spotted fever group rickettsia associated with *Dermacentor* ticks in Europe and Russia. *Int J Syst Evol Microbiol* 58:1635–1639. <https://doi.org/10.1099/ijs.0.64952-0>
- Merhej V, Angelakis E, Socolovschi C, Raoult D (2014) Genotyping, evolution and epidemiological findings of *Rickettsia* species. *Infect Genet Evol* 25:122–137. <https://doi.org/10.1016/j.meegid.2014.03.014>
- Parola P, Roveery C, Rolain JM, Brouqui P, Davoust B, Raoult D (2009) *Rickettsia slovaca* and *R. raoultii* in tick-borne Rickettsioses. *Emerging Infect Dis* 15:1105–1108. <https://doi.org/10.3201/eid1507.081449>
- Parola P, Paddock CD, Socolovschi C, Labruna MB, Mediannikov O, Kernif T, Abdad MY, Stenos J, Bitam I, Fournier PE, Raoult D (2013) Update on tick-borne rickettsioses around the world: a geographic approach. *Clin Microbiol Rev* 26:657–702. <https://doi.org/10.1128/CMR.00032-13>
- Portillo A, Santibañez S, García-Álvarez L, Palomar AM, Oteo JA (2015) Rickettsioses in Europe. *Microbes Infect* 17:834–838. <https://doi.org/10.1016/j.micinf.2015.09.009>
- Pretorius A-M, Birtles RJ (2002) *Rickettsia aeschlimannii*: a new pathogenic spotted fever group rickettsia, South Africa. *Emerging Infect Dis* 8:874. <https://doi.org/10.3201/eid0805.020199>



- Raoult D, Fournier P-E, Abboud P, Caron F (2002) First documented human *Rickettsia aeschlimannii* infection. *Emerging Infect Dis* 8: 748–749. <https://doi.org/10.3201/eid0807.010480>
- Roux V, Raoult D (2000) Phylogenetic analysis of members of the genus *Rickettsia* using the gene encoding the outer-membrane protein rOmpB (ompB). *Int J Syst Evol Microbiol* 50:1449–5145
- Roux V, Rydkina E, Ereemeeva M, Raoult D (1997) Citrate synthase gene comparison, a new tool for phylogenetic analysis, and its application for the *rickettsiae*. *Int J Syst Bacteriol* 47:252–261
- Santos-Silva MM, Sousa R, Santos AS, Melo P, Encarnação V, Bacellar F (2006) Ticks parasitizing wild birds in Portugal: detection of *Rickettsia aeschlimannii*, *R. helvetica* and *R. massiliae*. *Exp Appl Acarol* 39:331–338. <https://doi.org/10.1007/s10493-006-9008-3>
- Satta G, Chisu V, Cabras P, Fois F, Masala G (2011) Pathogens and symbionts in ticks: a survey on tick species distribution and presence of tick-transmitted micro-organisms in Sardinia, Italy. *J Med Microbiol* 60:63–68. <https://doi.org/10.1099/jmm.0.021543-0>
- Tamura K, Stecher G, Peterson D, Filipowski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* 30:2725–2729. <https://doi.org/10.1093/molbev/mst197>
- Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 22:4673–4680
- Vitale G, Mansuelo S, Rolain J-M, Raoult D (2006) *Rickettsia massiliae* human isolation. *Emerging Infect Dis* 12:174–175. <https://doi.org/10.3201/eid1201.050850>
- Wallménius K, Barbutis C, Fransson T, Jaenson TG, Lindgren PE, Nyström F, Olsen B, Salaneck E, Nilsson K (2014) Spotted fever *Rickettsia* species in *Hyalomma* and *Ixodes* ticks infesting migratory birds in the European Mediterranean area. *Parasit Vectors* 7:318. <https://doi.org/10.1186/1756-3305-7-318>