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Short communication

# Diversity of *Rickettsia* spp. in ticks from wild mammals of Morocco and Mauritania

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SUMMARY

Ticks are known as vectors and reservoirs of rickettsiae and, wildlife vertebrate hosts as suitable dispersers of ticks contributing to the life cycle of rickettsial agents in nature. In the herein study, the presence of rickettsiae was investigated in ticks from wild mammals (*Gerbillus* and *Jaculus, Vulpes rueppellii, Canis anthus, Felis lybica* and *Felis margarita*) in Mauritania and Morocco. Morphological and molecular analysis of ticks allowed their identification as *Rhipicephalus sanguineus* sensu lato and *Hyalomma impeltatum*. A total of 126 partially engorged adult ticks, collected from 40 animals, were screened for the presence of rickettsial DNA by conventional PCR targeting the *ompB* gene, followed by *ompA* and *gltA* targets and bidirectional sequencing. As a result of the sequence analyses, that at least three different species of pathogenic spotted fever group rickettsiae were detected. *Rickettsia parkeri*-like was detected in a *R. sanguineus* s.l. (n=1) collected from an African wildcat from Morocco. *Rickettsia aeschlimannii* was detected in a *H. impeltatum* (n=1) collected from a gerbil rodent. *Rickettsia massiliae* was detected in *R. sanguineus* s.l. ticks (n=5) collected from two Ruppells' foxes. The herein study demonstrates that pathogenic *Rickettsia* species are circulating in Morocco and Mauritania wildlife.

## 1. Introduction

Rickettsiae are zoonotic obligate intracellular bacteria, phylogenetically classified into four groups: the spotted fever group (SFG), the typhus group, transitional group and ancestral group (Gillespie et al., 2007). Rickettsioses are considered one of the important emerging diseases with a worldwide distribution (Parola et al., 2013), mainly associated with ticks and known as causing infection in animals and humans (Eremeeva and Dasch, 2015).

In northern Africa, several *Rickettsia* species associated with disease in humans have been detected in ticks and vertebrate animals (Abdel-Shafy et al., 2012). These include *Rickettsia conorii* and *Rickettsia massiliae* detected in *Rhipicephalus sanguineus* sensu lato (s.l.) collected from hedgehogs in Algeria (Bitam et al., 2006), *Rickettsia aeschlimannii* detected in *Hyalomma marginatum* collected from cattle and migratory birds in Morocco and from cattle in Algeria (Beati et al., 1997; Bitam et al., 2006; Palomar et al., 2016), *Rickettsia sibirica mongolitimonae* detected in *Hyalomma* truncatum collected from domestic animals in Senegal (Mediannikov et al., 2010), and *Rickettsia africae* detected in *Amblyomma variegatum* collected from cattle in Huambo (Barradas et al., 2021a). In addition, other reports detected *Rickettsia helvetica* and *Rickettsia monacensis* in *Ixodes ricinus* from Tunisia (Sfar et al., 2008), *Rickettsia slovaca* and *Rickettsia raoultii* in *Dermacentor marginatus* from Morocco (Sarih et al., 2008), and '*Candidatus* Rickettsia barbariae' in ticks from Algeria (Abdelkadir et al., 2019).

Due to the free-roaming habits, wildlife animals are of particular interest in the context of the ecology of tick-borne diseases as these are highly exposed to ticks serving as suitable hosts (Orkun and Çakmak, 2019). Consequently, due to the use of wild areas as pastures, sylvatic animals pose a threat to humans and domestic animals. As such, the

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present study aimed to investigate the overall diversity of SFG rickettsiae in tick species collected from wild animals in Morocco and Mauritania.

## 2. Materials and methods

### 2.1. Study area

This study was conducted in Morocco and Mauritania. Morocco is located on the northwestern Africa and stretches from the Atlantic Ocean to Mauritania. It is one of the most sparsely populated territories in the world, presenting a wide variety of agricultural ecosystems that include rain-fed Atlantic plains, Mediterranean mountainous zones and desert areas.

The desert flatlands are characterized by a hot desert climate (Köppen climate classification BWh). Along the Atlantic coast, average temperatures are relatively constant and moderated throughout the year. However, summertime is long and hot, and wintertime is short and warm.

Mauritania is a country located in northwestern Africa. It is the largest country worldwide lying entirely below an altitude of 1000 meters and is bordered by the northern Atlantic Ocean. It is characterized by a tropical and subtropical desert climate (Köppen: BWh) with extremes in temperature and by sparse and irregular rainfall.

### 2.2. Tick sampling

The ticks processed in this study were removed from 40 trapped animals, during collector trips performed between 2015 and 2020 in both countries as previously reported (Barros et al., 2018). A total of 126 ticks were collected from trapped mammals in Mauritania (n=33) and Morocco (n= 87). In brief, from Mauritania, 29 ticks were removed from 16 individuals of the genus *Gerbillus*, and four ticks were collected from four individuals of the genus *Jaculus*. From Morocco, 66 ticks were collected from 12 Rüppell's foxes (*Vulpes rueppellii*), two ticks from an African golden wolf (*Canis anthus*), five ticks from an African wildcat (*Felis lybica*), three ticks from a sand cat (*Felis margarita*) and 17 ticks from five rodents (*Gerbillus* spp.) (Supplementary material S1). The tick burden varied between 1 and 27 ticks per animal. All tick specimens were stored in 95% ethanol at room temperature until further processing.

### 2.3. Tick identification and DNA extraction

Ticks were identified to genus level based on the morphological characters and using taxonomic keys (Estrada-Peña et al., 2017). DNA of partially engorged ticks was extracted and processed individually. Each tick was washed in 200 µl of a 10% bleach solution for five minutes and then rinsed three times in deionized water to remove residual bleach. Arthropods were dried on filter paper, transferred to 1.5 ml tubes, and stored at -80 °C until further processing.

A modification of the QIAamp® DNA Mini Kit (Qiagen, Valencia, CA, USA) was used to extract DNA from the ticks, following previously described methods for nucleic extraction in ticks (Crowder et al., 2010).

In brief, each frozen tick was disrupted mechanically with a mortar and a pestle. The tubes were filled with 420-µl of lysis buffer and 25 µl proteinase K solution. The tubes were briefly mixed by vortexing for 30 s and then centrifuged for 2 min at 6000 × g. A 350 µl aliquot of the recoverable supernatant was transferred to a fresh microcentrifuge tube and 350 µl of RTL buffer was added. The tubes were briefly mixed by vortexing for 30 s, pulse centrifuged, and incubated at 37°C for 10 min. The next steps followed the QIAamp® DNAMini Kit (Qiagen, Valencia, CA, USA) using an automated QIAcube (Qiagen GmbH, Germany).

A negative extraction control was processed along with each batch of arthropods (12 samples).

To confirm tick morphological identification, conventional PCR reactions targeting a partial region of the mitochondrial 16S rDNA (Black and Piesman, 1994)were performed on a randomly selected sample of ticks from each genus (n=14 *Rhipicephalus* and n=4 *Hyalomma*; 10% of samples).

### 2.4. Detection of rickettsial DNA in ticks

Tick DNA specimens were initially screened for the presence of SFG rickettsiae using a conventional PCR targeting a broad spectrum 511 bp fragment of the outer membrane protein B (*ompB*) gene, as previously described (Choi et al., 2005). To confirm positive results and genetically characterize *Rickettsia* spp., ticks were further tested for a 532 bp fragment of the outer membrane protein A (*ompA*) gene (Regnery et al., 1991) and the near-complete (806 bp) of the citrate synthase (*gltA*) gene (de Sousa et al., 2005). For all reactions, a total of 3 µl of genomic DNA was added to 5.6 µl KAPA Taq DNA Polymerase mix (KAPA Biosystems, Woburn, MA, USA), 14.4-µl of deionized sterile water and 1-µl (10 µM) of the primers in a 25.0 µl final volume of the reaction mixture. The reactions were carried out in an automatic DNA thermal cycler 100 (Bio-Rad), including negative (water) and positive (DNA of *R. africae*) controls. The PCR amplification products were visualized by Xpert green (Grisp, Porto, Portugal) fluorescence after electrophoresis in a 1.5% agarose gel at 100 V for 40 min.

### 2.5. Sequencing and phylogenetic analysis

All *Rickettsia*-positive and 16S rDNA amplicons of the expected size were sequenced for genetic characterization. Briefly, amplicons were purified with GRS PCR & Gel Band Purification Kit (Grisp, Porto, Portugal), and bidirectional sequencing was performed by the Sanger method, using the respective primers of the different target genes. Sequences were manually corrected using the BioEdit Sequence Alignment Editor v 7.1.9 software package, version 2.1 (Ibis Biosciences) and further analysis were performed by comparison with the sequences available in the NCBI (GenBank) nucleotide database (http://blast.ncbi.nlm.nih.gov/Blast) (Altschul et al., 1990). Phylogenetic analysis was performed using MEGA version 6.0 software (Tamura et al., 2013). The *ompA* gene and *gltA* gene sequences identified in this study and representative sequences for the *R. parkeri, R. aeschlimannii* and *R. massiliae* obtained from GenBank were used for the phylogenetic analysis. A maximum-likelihood (ML) method was applied (Kumar et al., 2018; Tamura, 1992). The ML bootstrap values were estimated using 1000 replicates with Tamura 3-parameter as the correction model. Tamura 3-parameter model was estimated as the best substitution model by MEGA version 6.0 software. We deposited 16S rDNA tick and *ompB, ompA* and *gltA Rickettsia* sequences recovered in this study in GenBank.

## 3. Results

### 3.1. Morphological and molecular identification of ticks

From the total of 126 adult ticks collected, 85 (85/126; 67%) were morphologically identified at genus level as *Rhipicephalus* sp. and 41 (41/126; 33 %) as *Hyalomma* sp. Analysis of the 16S rDNA mitochondrial gene for molecular identification of the tick species was performed. BLAST analysis of the 16S segments obtained from *R. sanguineus* s.l. demonstrated that these tick species are identical, sharing 99% nucleotide identity with *R. sanguineus* s.l. (accession no. MK159005) from South Africa and with *R. sanguineus* s.l. (accession no. JX195174) from South America. BLAST analysis of the 16S segments obtained from *H. impeltatum* ticks showed 99.31% identity with *H. impeltatum* (accession no. MN960583) collected from camels from Tunisia (Supplemental material S1).

### 3.2. Identification of rickettsiae in the examined ticks

Of the total number of ticks (n = 126) screened for *Rickettsia*, seven (7/126, 6%) showed to be positive for *ompB* gene. Further characterization of the BLAST analyses of the partial *ompB* gene indicated one *R. parkeri* sequence obtained from a *R. sanguineus* s.l. tick collected in an African wildcat, having 100% identity with a *R. parkeri* sequence obtained from *Amblyomma aureolatum* ticks from Brazil (accession no. KY113111). One *R. aeschlimannii* sequence was obtained from a *H. impeltatum* adult tick collected from a gerbil rodent, having 100% identity with a *R. aeschlimannii* sequence obtained from *H. marginatum* ticks from Egypt (accession no. HQ335156). Five *R. massiliae* sequences were obtained from *R. sanguineus* s.l. ticks collected from two Rüppells' foxes, having 100% identity with a *R. massiliae* sequence obtained from *R. sanguineus* s.l. from Italy (accession no. KJ663754) and Portugal (accession no. MN853114).

To confirm positive results by *ompB* gene, ticks were further studied for the *ompA* and *gltA* gene regions. BLAST analyses of the partial *ompA* gene indicated that the *R. parkeri* sequence had 99.56% identity with a *R. parkeri* sequence obtained from *Amblyomma ovale* in Brazil (accession no. MK962699), *R. aeschlimannii* sequence had 99.79% identity with a *R. aeschlimannii* sequence obtained from *H. marginatum* from Turkey (accession no. MK922658) and *R. massiliae* sequences presented between 99.79% and 100% identity with *R. massiliae* sequences obtained from *Rhipicephalus microplus* from Pakistan (accession no. MH990860).

BLAST analyses of the partial *gltA* gene indicated that *R. parkeri* sequence had 99.87% identity with a *R. parkeri* sequence obtained from *A. ovale* from Colombia (accession no. CP040325), *R. aeschlimannii* sequence had 100% identity with a *R. aeschlimannii* sequence obtained from *H. marginatum* from China (accession no. MH267736) and *R. massiliae* sequences had 100% identity with an *R. massiliae* sequence from France (accession no. CP000683).

Phylogenetic analysis was performed for both partial *ompA* (Fig. 1) and *gltA* gene (Fig. 2) sequences in order to obtain information about their genetic relatedness with other *Rickettsia* species reference sequences.

The GenBank accession numbers for the sequences of *R. sanguineus* s. l. and *H. impeltatum* 16S rDNA gene fragments recovered in this study are described in Supplementary material S1 and the *Rickettsia* gene fragments for *ompB, ompA and gltA* sequences are described in Table 1.

## 4. Discussion

The present study investigated the occurrence of SFG rickettsiae in ticks collected from *Gerbillus* and *Jaculus* rodents, Rüppell's foxes, an African golden wolf, an African wildcat and a sand cat, in Morocco and Mauritania. Combined morphological and molecular characterization of the ticks identified them as *R. sanguineus* s.l. and *H. impeltatum* These tick species have already been reported in neighboring regions from north-eastern Algeria (Sadeddine et al., 2020) or in West Bank, Palestinian territories (Ereqat et al., 2016), both collected from domestic and wild animals. Additionally, *R. sanguineus* s.l. and *H. impeltatum* were also found parasitizing camels in Northern Sudan (Elghali and Hassan, 2009).

Although strongly associated with domestic dogs and feeding primarily on them, *R. sanguineus* (s.l.) can survive in a wide range of ecological niches and parasitize synanthropic and wild animals such as golden jackals (*Canis aureus*) (D'Amico et al., 2017), wildcats (*Felis silvestris*) (Sobrino et al., 2012), European hedgehogs (*Erinaceus europaeus*) (Barradas et al., 2021b) or stone marten (*Martes foina*) (Dumitrache et al., 2014). *Rhipicephalus sanguineus* s.l. is a cosmopolitan, three-host tick species (Dantas-Torres, 2010), playing an important role as a

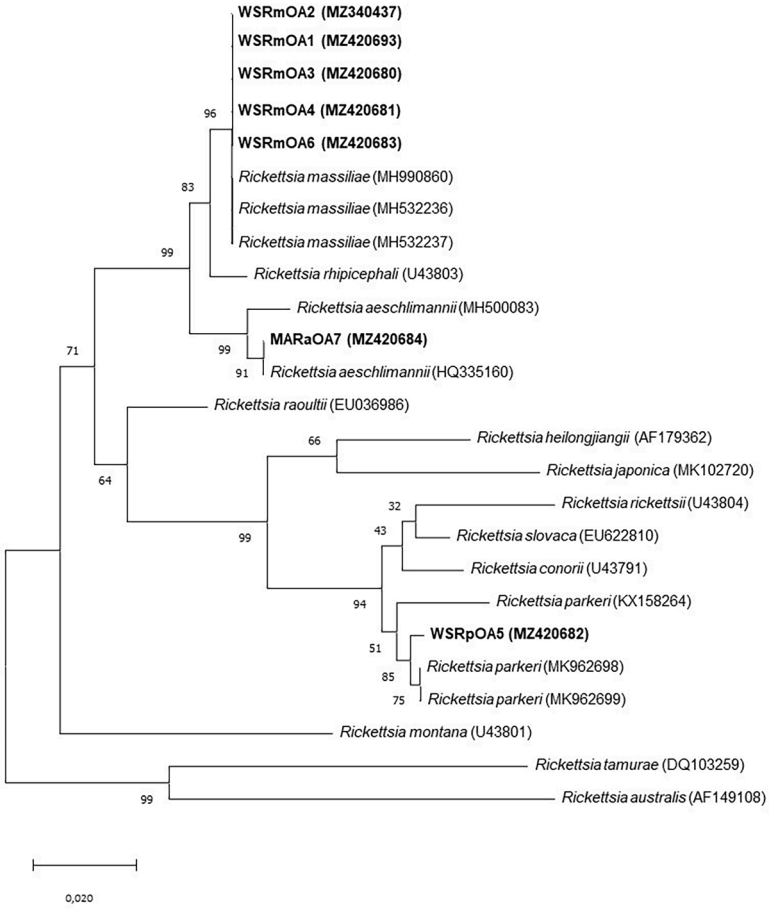


**Fig. 1.** Phylogenetic analysis of *Rickettsia* spp. identified in *Rhipicephalus sanguineus* s.l. and *Hyalomma impeltatum* ticks. A maximum likelihood method based on the Tamura 3-parameter model phylogenetic tree was constructed based on *Rickettsia ompA* DNA sequences. Reliability of internal branches was assessed using the bootstrapping method (1000 replicates). *Rickettsia* spp. characterized in this study are shown as country/*Rickettsia* species/gene.
WS: Morocco; MA: Mauritania; Rp: *Rickettsia parkeri*; Rm: *Rickettsia massiliae*, Ra: *Rickettsia aeschlimannii*; OA: *ompA*.

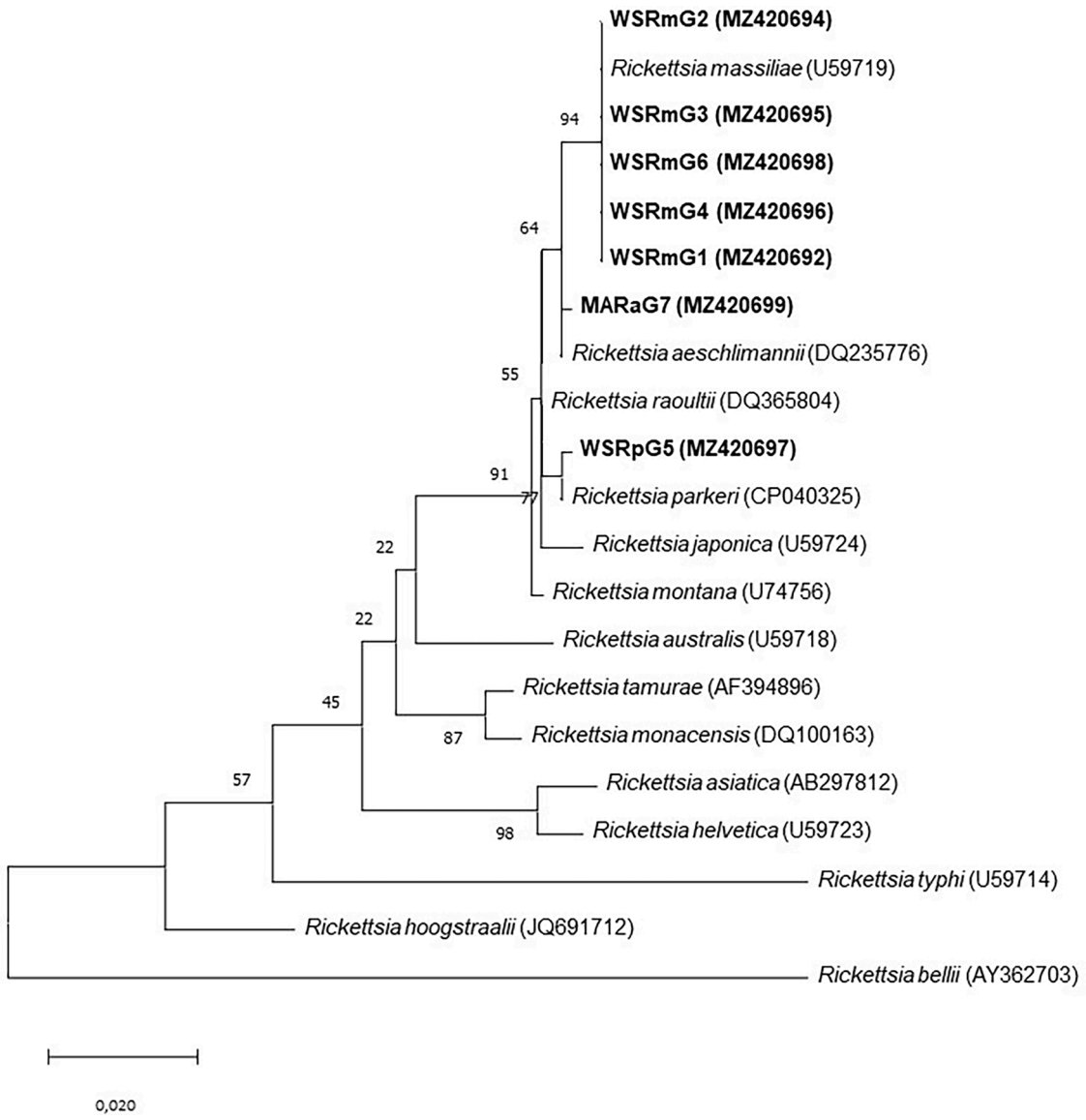


**Fig. 2.** Phylogenetic analysis of *Rickettsia* spp. identified in *Rhipicephalus sanguineus* and *Hyalomma impeltatum* ticks. A maximum likelihood method based on the Tamura 3-parameter model phylogenetic tree was constructed based on *Rickettsia gltA* DNA sequences. Reliability of internal branches was assessed using the bootstrapping method (1000 replicates).
*Rickettsia* spp. characterized in this study are shown as country/*Rickettsia* species/gene.
WS: Morocco; MA: Mauritania; Rp: *Rickettsia parkeri*; Rm: *Rickettsia massiliae*, Ra: *Rickettsia aeschlimannii*; G: *gltA*.

**Table 1**
Detection of *Rickettsia* spp. in ticks collected from wild animals in Morocco and Mauritania.

| Host (ID, genus/ species) | COUNTRY | Tick species (Accession number) | *Rickettsia* spp. (*ompB*) (Accession number) | *Rickettsia* spp. (*gltA*) (Accession number) | *Rickettsia* spp. (*ompA*) (Accession number) |
|---|---|---|---|---|---|
| (FL 01, *Felis lybica)* | Morocco | *Rhipicephalus sanguineus* (MZ190323; MZ314890) | *Rickettsia parkeri* (MZ420689) | *Rickettsia parkeri* (MZ420697) | *Rickettsia parkeri* (MZ420682) |
| (VR 12, *Vulpes ruepellii)* | Morocco | *Rhipicephalus sanguineus* (MZ322670) | *Rickettsia massiliae* (MZ420690) | *Rickettsia massiliae* (MZ420698) | *Rickettsia massiliae* (MZ420683) |
| (VR 13, *Vulpes ruepellii*) | Morocco | *Rhipicephalus sanguineus* (MZ322664 - MZ322667) | *Rickettsia massiliae* (MZ420685 - MZ420688) | *Rickettsia massiliae* (MZ420692; MZ420694 - MZ420696) | *Rickettsia massiliae* (MZ420693; MZ340437; MZ420680; MZ420681) |
| (ZBSC 1177, Gerbillus) | Mauritania | *Hyalomma impeltatum* (MZ314893) | *Rickettsia aeschlimannii* (MZ420691) | *Rickettsia aeschlimannii* (MZ420699) | *Rickettsia aeschlimannii* (MZ420684) |

vector of numerous humans and animals' infectious pathogens (Aktas and Özübek, 2017; Cabezas-Cruz et al., 2019; Körner et al., 2021; Solano-Gallego et al., 2016) some of these of zoonotic concern such as *R. conorii* and *R. massiliae* (Cabezas-Cruz et al., 2019).

*Hyalomma impeltatum* is an Afrotropical and Palearctic species whose immature stages feed on small animals like rodents, hares and ground birds during summer and autumn and adults parasitize large domestic animals throughout the year (Estrada-Peña et al., 2017). Several tick surveys conducted on camels confirmed that *H. impeltatum* and *H. dromedarii*, are the most common and dominant tick species infesting

camels in arid areas from different countries in northern and eastern Africa (Elghali and Hassan, 2009). Since camels graze together with livestock there is a risk of ticks finding alternative hosts and contributing to the spread of pathogens in these animal species (Perveen et al., 2020). *Hyalomma impeltatum* has been reported as capable of transmitting zoonotic agents such as Crimean-Congo haemorrhagic fever virus (Spengler and Estrada-Peña, 2018) and the pathogenic *R. aeschlimannii* (Onyiche et al., 2020).

Vector-borne rickettsioses are considered emerging zoonoses, being increasingly recognized in many countries worldwide as a cause of significant morbidity among infected individuals (Parola et al., 2013).

Although the presence of rickettsiae in ticks has already been reported in Africa (Barradas et al., 2021a; Beati et al., 1997; Magaia et al., 2020; Selmi et al., 2020; Vanegas et al., 2018), scarce information exists regarding the role of wild mammals in the ecology dynamics of these bacteria.

In the herein study, rickettsiae DNA was amplified in 6% of the ticks for the *ompB, ompA* and *gltA* genes. The sequence analyses revealed the presence of three pathogenic *Rickettsia* species, *R. parkeri*-like, *R. aeschlimannii and R. massiliae.* In particular, *R. parkeri*-like was amplified from a *R. sanguineus* s.l. with *ompB* and *ompA* nucleotide sequences sharing highest identities with sequences from Brazil (Faccini-Martínez et al., 2020) and *gltA* nucleotide sequence sharing the highest identity with a sequence from Colombia (Londoño et al., 2019). *Rickettsia parkeri* is an emerging pathogen that causes human spotted fever group rickettsiosis (Paddock et al., 2004; Venzal et al., 2004) and has also been described as causing clinical disease in dogs (Grasperge et al., 2012). It is primarily transmitted by *Amblyomma* spp. (Paddock et al., 2004). Notwithstanding, *R. parkeri* has also been amplified in other hard ticks such as *Dermacentor parumapertus* (Sánchez-Montes et al., 2018), *Ixodes scapularis* (Parola et al., 2013) and *R. sanguineus* s.l. (Williamson et al., 2010; Henning et al, 2014).The presence of *R. parkeri*-like DNA in *R. sanguineus* s.l. might be a result of cofeeding and subsequent spillover of this bacterium (Lee et al., 2018). As, *R. parkeri sensu stricto* and *R. parkeri* strain Atlantic rainforest are human pathogens (Paddock et al., 2004; Krawczak et al., 2016), the potential of *R. parkeri*-like to be human pathogens should not be discarded.*Rickettsia aeschlimannii* was also amplified in this study, from an *H. impeltatum* with *ompB, ompA* and *gltA* nucleotide sequences sharing the highest identities with sequences from Egypt, Turkey and China, respectively. Our findings agree with previous studies that have reported the presence of *R. aeschlimannii* in *Hyalomma* ticks collected in various countries namely, Algeria, Nigeria, Tunisia and Morocco (Aquino et al., 2016; Kamani et al., 2015; Palomar et al., 2016). This SFG *Rickettsia* has been detected in H. marginatum in southern Europe and northern Africa (Bitam et al., 2006), in *H. rufipes* in sub-Saharan Africa (Parola et al., 2013) and *H. impeltatum* from Nigeria (Moshaverinia and Moghaddas, 2015). *Hyalomma* tick species seems to be both vectors and reservoirs of *R. aeschlimannii* and as such, the eco-epidemiology of this *Rickettsia* sp. in specific geographic regions correlates with their geographic distribution.

In the present study, *R. massiliae* was clearly identified in five out of 41 *R. sanguineus* s.l. ticks (12%) with *ompB, ompA* and *gltA* nucleotide sequences sharing the highest identities with sequences from Italy and Pakistan. Our results are in accordance with previous studies reporting *R. massiliae* molecular detection from *R. sanguineus* s.l. ticks collected from domestic and wild animals (Barradas et al., 2020; Barradas et al., 2021b; Cicculli et al., 2019). *Rickettsia massiliae* is considered an etiological agent of Mediterranean Spotted Fever-like illness prevalent worldwide and transmitted by and isolated from *R. sanguineus* s.l. (Eremeeva et al., 2006; Parola et al., 2013). Noteworthy, *R. sanguineus* s. l. has been pointed as the reservoir of *R. massiliae*, with transovarial passage rates up to 100 % (Matsumoto et al., 2005). It is identified as an agent causing human disease (Vitale et al., 2006) and has also been suggested as causing disease in dogs (Beeler et al., 2011).

As far as we know, this is the first molecular study reporting the presence of *R. parkeri*-like in *R. sanguineus* s.l. collected from wild animals from Morocco contributing to an extent of the geographic location of these rickettsiae. Data regarding wild animals and their ticks are important to determine the dynamics of zoonotic agents amongst the wildlife in northern Africa.

This study demonstrated that pathogenic *Rickettsia* species are circulating in ticks from northern Africa wildlife and these zoonotic agents can pose a threat to human and animal health. Also, it has been determined that the investigated wild animals play a role as maintenance hosts for vector ticks; therefore, these animals must also be considered in the ecology of the mentioned rickettsiae.

## Ethical approval

Capturing and handling of animals adhered to the guidelines and regulations approved by the local authorities (the Haut Commissariat aux Eaux et Forêts et à la Lutte Contre la Désertification of Morocco, decisions 20/2013, 41/2014, 42/2014, and the Ministère de l'Environnement et du Développement Durable of Mauritania, decision 227/08.11.2012).

## Data availability statement

Raw data were generated at Virology Lab of Faculty of Pharmacy of the University of Porto. Derived data supporting the findings of this study are available from the corresponding author [PB] on request.

## CRediT authorship contribution statement

**Sérgio Santos-Silva:** Data curation, Formal analysis. **Nuno Santos:** Writing – review & editing. **Zbyszek Boratyński:** Writing – review & editing. **João R. Mesquita:** Conceptualization, Methodology, Resources, Investigation, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Patrícia F. Barradas:** Conceptualization, Methodology, Resources, Investigation, Data curation, Writing – original draft, Writing – review & editing.

## Declaration of Competing Interest

The authors declare that they have no conflict of interest.

## Data availability

Data will be made available on request.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ttbdis.2023.102235.

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